MASSACHUSETTS GENERAL HOSPITAL

Center for Skeletal Research

8th Annual Skeletal Research Symposium

Poster Presentations

MGH Main Campus: Thier 1 Conference Room

Monday, May 8, 2023 11:15-12:45 (odd posters: 11:15-12:00; even posters: 12:00-12:45)

Posters For Oral Presentations		
Poster Number	**Presenter**	**Oral Presentation Title**
47	Xiaomeng You	*Gut microbiome promotion of bone growth in early life is mediated by GPR43*
48	Chilan B. G. Leite	*Physiological production of specialized pro-resolving mediators does not prevent posttraumatic osteoarthritis after anterior cruciate ligament transection*
49	Petra Simic	*1,25-dihydroxy vitamin D regulates furin-mediated FGF23 cleavage*
50	Emily Moore	*The role of BMP signaling in appositional bone growth*
51	Yizhong Hu	*Piezo1 Regulates cGMP/PKG Signaling in Osteocyte Mechanotransduction*
52	Sung-Hee Seanna Yoon	*SIK2/SIK3 inhibitor increases cortical bone mass in a mouse model of CKD-MBD*
53	Vineel Kondiboyina	*Calcium signaling in in-situ chondrocytes under dynamic compressive loading*
54	Rosanne Raftery	*Stability and regenerative capacity of articular cartilage tissue derived from human pluripotent stem cells*
55	Courtney M. Mazur	*Subcellular transcriptomics reveals selective mRNA trafficking to osteocyte dendrites*
56	Fjola Johannesdottir	*Femoral Neck Bone Density and Structure in Older Adults with Longstanding Type 1 Diabetes: A Case-Control Study*

Poster Session		
Poster Number	**First Author**	**Abstract Title**
1	Supriya Jagga	*Impaired 1,25 dihydroxyvitamin D3 action and hypophosphatemia underlie the altered lacuno-canalicular remodeling observed in the Hyp mouse model of XLH*
2	Maisa Da Silva	*"Mmp13 Deletion Modulates Osteocytic Perilacunar Remodeling and Prevents Lactation-Induced Bone Loss: Insights into Osteocytic Osteolysis and Bone Homeostasis"*
3	Alex Goraltchouk	*FGF18 Gene Therapy Treatment for Osteoarthritis Promotes Regenerative Chondroanabolic and Anti- Catabolic Activity*
4	Ignacio Portales-Castillo	*ALTERED DESENSITIZATION OF pthrp SIGNALING AS A NOVEL MECHANISM FOR DELAYED OSSIFICATION IN EIKEN SYNDROME.*
5	Tadatoshi Sato	*In vitro evaluation of CRISPR/Cas9-mediated gene therapy for Osteogenesis Imperfecta*
6	Nora Renthal	*Genome-wide CRISPR Screening of Chondrocyte Maturation Newly Implicates Multiple Genes in Longitudinal Skeletal Growth and Height-GWAS Associated Loci*
7	Brent Hawkins	*Vav2/Wasl signaling is a novel pathway regulating the vertebrate skeleton*
8	Prem Yadav	*Phosphate induced activation of VEGFR2 is required for Caspase 9 mediated apoptosis of hypertrophic chondrocytes during endochondral bone formation.*
9	Jakob Hoppner	*Skeletal phenotype in mice with a truncating PTH1R mutation resembles skeletal abnormalities in subjects with Eiken Syndrome*
10	Gabrielle Gallant	*Involvement of morphogenetic protein 2 in osteogenesis and angiogenesis during distraction osteogenesis*
11	Adina E. Draghici	*Mechanisms of tibial bone blood flow regulation in humans*
12	Vikram Khedgikar	*Mouse LGR6 regulates osteogenesis in vitro and in vivo through differential ligand use*
13	Katelyn DeMaio	*Cfp1 and chondrocyte maturation: analysis of phenotypic changes in the context of gene deletion in embryonic mice*
14	Jasmine M. Nutakki	*Effects of Increasing LRP5 Signaling During Chondrocyte Transdifferentiation to Osteoblast Development On Osteogenesis Imperfecta*
15	Yorihiro Iwasaki	*GNAS AS2 DMR Methylation Depends on STX16/NESP55 icrs and Has Diagnostic Potential in AD-PHP1B*

16	Christodoulos K. Constantinou	*In-Vitro Differentiation of Human Acetabular Mesenchymal Stem Cells as a Potential Model to Study Osteoarthritic Risk Factors*
17	Grace Jung	*Risk of Wrist Fracture in Gastric Bypass Compared to Gastric Banding as Estimated by the Load-to-Strength Ratio*
18	Shannon R. Emerzian	*Total body irradia,on results in long-term deficits in vertebral but not femoral bone structure in male rhesus macaques*
19	David E. Maridas	*Smad1/5/8 signaling in osteoblasts: early observations from a triple knockout mouse model*
20	Yaser Peymanfar	*Roles of osteoblast dopamine D2 receptor in type 1 diabetes-induced bone loss*
21	Jingting Yao	*Characterization of Bone Mineralization and Vascular Calcification Using Ion-Selective Phosphorus-31 Solid State Magnetic Resonance Spectroscopy*
22	Kira Fagerstrom	*Developing genetic tools to analyze skeletal homeostasis in aging*
23	Yiwei Kong	*Bony bar formation v. Cartilage healing in a vertical Salter-Harris type4 (SH4) GP fracture*
24	Sara Monaci	*Role of Tyrosine Phosphorylation in Yes-Associated Protein 1 Function in Bone Marrow Stromal Cells*
25	Vineel Kondiboyina	*Estimation of Material Properties of Regenerating Axolotl Limbs Using Inverse* *Finite Element Analysis*
26	Kathryn M. Yammine	*Elucidating collagen-II proteostasis defects in human chondrodysplasia using induced pluripotent stem cells*
27	Austin Baacke	*Movement As a Marker of Pain in Severe Osteogenesis Imperfecta*
28	Katherine C. Woronowicz	*Dissecting the Role of an Ultraconserved Cis-Regulatory Hub in Modulating Jaw Size*
29	Anthony N. Aggouras	*Earlier Proteoglycan Turnover Promotes Higher Efficiency Matrix Remodeling in MRL/mpj Tendons*
30	Emma J. Stowe	*Senescence Contributes to Death Resistance of Aged Tenocytes in a Model of* *Secondary Joint Damage*
31	Susan MacLauchlan	*Tonic interferon signaling in osteoclast formation and clonal hematopoiesis*
32	Giullia Montagna	*Rationale for Improving Existing OI Mouse Models*
33	Stephen Treaster	*Insights from the edge: Genetic analysis of exceptionally long- and short-lived species to reveal the regulation of human aging*

34	John M. Baronas	*Exploring the Function of Protein Inhibitor of Activated STAT 1 (Pias1) to Maintain Chondrocyte Immaturity in Growth Plate Chondrocytes*
35	Yu Liu	*Prx1 Muscle Cell Population needs to be Activated*
36	Shannon R. Emerzian	*Cortical Bone Post-Yield Energy Absorption is Reduced in Older Adults with Long-Duration Type 1 Diabetes*
37	Neilesh Frings	*Risk of vertebral endplate failure during vertebral fracture*
38	Sherr-Ann Burnett-Bowie	*Racial and Ethnic Disparities in Metabolic Bone Disease*
39	Christine Lary	*A Meta-analysis of the association between bone density measures and the risk of dementia*
40	Soha Ben Tahar	*Use of 3D Turing patterns to model skeletal development.*
41	Milica Sipovac	*Neonatal line width in primary teeth from the Early Neolithic and Modern ages*
42	Divya Venkatasubramanian	*Lineage-Specific Differences and Inference of Regulatory Networks Governing Human Chondrocyte Development*
43	Nicola Blum	*Running the red light – defining signals driving progressive growth in skeletal disorders*
44	Stephanie L. Tsai	*Endogenous Tenocyte Activation Underlies the Regenerative Capacity of Adult Zebrafish Tendon*
45	Anna Wadhwa	*Space-like radiation leads to deficits in vertebral bone density and microarchitecture in male Alzheimer's-like transgenic mice*
46	André F. Gutiérrez	*Age-related mechanical degradation of cortical bone is driven by microstructural changes in addition to porosity*

Poster Abstracts

Presenter: Supriya Jagga
Institution: Harvard Medical School **Department:**

Poster Number: 1

Title: Impaired 1,25 dihydroxyvitamin D3 action and hypophosphatemia underlie the altered lacunocanalicular
remodeling observed in the Hyp mouse model of XLH
Authors: Supriya Jagga, Ye Yuan, Rakshya Rana, Paola Divieti Pajevic, and Eva S. Liu

Abstract:
Osteocytes remodel their surrounding perilacunar matrix and canalicular network. X-linked hypophosphatemia (XLH) is characterized by elevated serum levels of fibroblast growth factor 23 (FGF23), impaired production of 1,25 dihydroxyvitamin D3(1,25D), and hypophosphatemia. Bones from XLH (Hyp) mice show enlarged osteocyte lacunae and impaired lacuno-canalicular (LCN) structure. XLH mice treated with 1,25D or anti-FGF23blocking antibody improves LCN organization, suggesting roles for 1,25D and phosphate in regulating LCN remodeling. To address whether impaired 1,25D action affects LCN remodeling, LCN organization was characterized in mice lacking the vitamin D receptor (VDR) in osteocytes (VDRf/f;DMP1Cre+), which have normal mineral ion/hormone levels. To determine if increased 1,25D compensates for hypophosphatemia in regulating LCN remodeling, the LCN phenotype was analyzed in mice lacking the sodium phosphate transporter 2a (NPT2aKO), which have hypophosphatemia and high serum 1,25D levels. MicroCT analyses showed no significant alterations in cortical microarchitecture of either mouse, thus allowing for the dissection of 1,25D and phosphate-specific effects on LCN remodeling. Histomorphometric analyses show that osteocytes in the tibial cortices and calvariae of VDRf/f;DMP1Cre+ and NPT2aKO mice have enlarged lacunae, which corresponds with enhanced osteocyte
mRNA expression of matrix resorption genes classically expressed by osteoclasts, such as Cathepsin-k and ATPase H+ transporting lysosomal V0 subunit. Treatment of Ocy454 osteocytes with 1,25D or phosphate inhibits expression of these genes, indicating that 1,25D and phosphate each act directly on osteocytes to regulate perilacunar matrix resorption. Silver and phalloidin staining of the osteocyte canaliculi
in VDRf/f;DMP1Cre+ and NPT2aKO tibiae and calvariae showed impaired canalicular organization similar to the Hyp mice. These studies demonstrate that 1,25D acts directly on osteocytes to regulate LCN remodeling. Hypophosphatemia also stimulates perilacunar matrix resorption and impairs canalicular structure. Impaired 1,25D action and low phosphate levels contribute to the abnormal LCN phenotype observed in XLH.

Presenter: Maisa Da Silva
Institution: Harvard School of Dental Medicine

Poster Number: 2

Title: Mmp13 Deletion Modulates Osteocytic Perilacunar Remodeling and Prevents Lactation-Induced Bone Loss: Insights into Osteocytic Osteolysis and Bone Homeostasis

Abstract:
Lactation elicits bone resorption to supply calcium in milk, via osteoclastic bone resorption and perilacunar remodeling (PLR), driven by osteocytes (Ocy). Ocy orchestrate the bone's response to both hormonal and mechanical cues. Embedded within the matrix, Ocy exert their influence by modulating their micro-environment, engaging in both matrix resorption and deposition, altering their lacunar space. The complex interplay between osteocytes and their microenvironment and the molecular mechanisms underlying osteocytic PLR regulation are not fully elucidated. Mmp13, a matrix-degrading enzyme, is a downstream target gene of various skeletal regulating factors and is upregulated during lactation. To investigate Mmp13's role in PLR, we used the 10 kb-Dmp1-cre mouse to generate Mmp13ocy mice. BSEM showed, counter-

intuitively, that cortical osteocyte lacunar area (LcA) was significantly larger (22.68±0.47μm2 vs 26.68±1.14 μm2, p=0.006) in virgin Mmp13ocy mice compared to control virgin mice, suggesting that Mmp13 deletion affects PLR homeostasis at steady state. However, Mmp13 deletion prevented LcA enlargement during lactation, pointing to a critical role for Mmp13 in lactation-induced PLR. Furthermore, virgin Mmp13ocy exhibited a significant increase in trabecular bone mass (+43.12%, p<0.0001) compared to control virgin mice and did not lose bone during lactation. Bulk RNAseq analysis of enriched Ocy preparation revealed up-regulation of resorption-related genes, including Ctsk, Acp5, Oscar, and Ocstamp in Ocy and identified several signaling pathways related to osteocytic osteolysis, (lysosome and protein digestion/absorption). Notably, the amplified resorption activity by Ocy was independent of osteoclast contamination, as the expression of Tnfsf11 in our Ocy-enriched preparations remained unchanged. In conclusion, deletion of Mmp13 in osteocytes leads, counter-intuitively to osteocytic osteolysis by shifting the Ocy transcriptome towards a “resorbing” mode, increasing in particular Ctsk. However, during lactation, the deletion of Mmp13 prevented the expected bone loss, highlighting the critical role of Ocy Mmp13 in PLR and skeletal homeostasis.

Presenter: Alex Goraltchouk

Institution: Remedium Bio

Poster Number: 3

Title: FGF18 Gene Therapy Treatment for Osteoarthritis Promotes Regenerative Chondroanabolic and Anti- Catabolic Activity

Authors: Alex Goraltchouk, Judith M Hollander, Francesco Luppino, Li Zeng, Alexey Seregin

Abstract:
Background: Osteoarthritis (OA) affects more than 500 million people around the globe, with increasing prevalence driven by an aging population and rising obesity. Despite significant efforts, there are still no approved disease modifying treatments. Recent advances with chondroanabolics appear promising, with the placebo-controlled trial of the rhFGF18 protein analog, Sprifermin, demonstrating a dose-dependent increase in cartilage thickness and reduction in the rate of progression to joint replacement in the high-risk subgroup.
Methods: We evaluated the ability of a single injection AAV2-FGF18 gene therapy treatment to reverse progression of OA in a Sprague-Dawley rat, destabilized medial meniscus model (DMM), relative to a series of 6 bi-weekly injections of rhFGF18. Expression durability was assessed by bioluminescence imaging of an AAV2-nLuc reporter. Cartilage thickness in the defect zone, defect width at cartilage surface, and overall cartilage thickness of the tibial plateau were measured in Safranin-O-stained histological sections. Mechanistic analysis was performed by RNA-seq and 2D cell culture on primary human chondrocytes and synoviocytes.
Results: Studies demonstrated optimal cytocompatibility of the AAV2 vector, high transducibility of chondrocytes in culture, and the ability of AAV2-hFGF18 to upregulate a number of chondroanabolic genes (PRG4, COL2A1, HAS2), while downregulating catabolic transcripts (MMP2, ADAMTS-1/5/15, Lox). In vivo studies demonstrated a treatment dose response, reducing the defect width by 76% relative to PBS; at the same time, cartilage thickness in the thinnest zone increased by 106% relative to PBS. Vehicle-treated joints demonstrated significant cartilage erosion, presence of bone marrow lesions, sub-chondral bone remodeling, and even instances of complete subchondral bone collapse. In contrast, joints treated with AAV2-hFGF18 demonstrated nearly normal cartilage architecture with only moderate regional glycosaminoglycan loss, and occasional marginal cartilage erosion.
Conclusion: The results of this study suggest that AAV2-hFGF18 gene therapy has significant regenerative potential in arthritic joints via both chondroanabolic and anti-catabolic mechanisms.

Presenter: Ignacio Portales-Castillo

Institution: Washington University in St. Louis, St. Louis, MO **Department:** Department of Medicine

Poster Number: 4

Title: ALTERED DESENSITIZATION OF pthrp SIGNALING AS A NOVEL MECHANISM FOR DELAYED OSSIFICATION IN EIKEN SYNDROME.

Authors: Ignacio Portales-Castillo, Jakob Hoeppner, Patrick Hanna, Thomas Dean, Monica Reyes, Brendan Creemer, Ross Cheloha, Harald Jüppner, and Thomas J Gardella

Abstract:
During endochondral bone formation, parathyroid hormone related peptide (PTHrP) is secreted by cells in the perichondrium and acts via the parathyroid hormone receptor (PTH1R) in nearby chondrocytes to promote cell proliferation and prevent premature cell differentiation and ossification. In addition to mediating responses to PTHrP, the PTH1R acts as the receptor for parathyroid hormone (PTH) to maintain calcium homeostasis. Eiken syndrome is a very rare disease characterized by delayed bone formation without hypercalcemia and is associated with homozygous mutations in the PTH1R. Three different mutations have been reported. The mutation R485X truncates the cytoplasmic C-terminal tail of the receptor, while the mutations E35K and Y134S alter residues in the extracellular N-terminal domain. We performed a series of in vitro studies to understand how these mutations impact PTH1R function. For the R485X mutant we found elevated rates of basal cAMP signaling and prolonged cAMP responses to PTHrP. For the E35K and Y134S mutants we found normal rates of basal and initial PTHrP-stimulated cAMP signaling, but blunted capacities to desensitize the cAMP response to a rechallenge with PTHrP. Each PTH1R mutant exhibited a defect in recruiting βarrestin2 to endosomes in response to PTHrP; these defects paralleled the impairment in either terminating the initial PTHrP-induced cAMP signaling response (R485X) or desensitizing the response to PTHrP-rechallenge (E35K, Y134S). To extend these in vitro studies, we generated new KI mice homozygous for the R485X mutation. The studies are still preliminary, but these mice exhibit short tails and a delay in bone mineralization, which is remarkably similar to the phenotype seen in Col2A-PTHrP transgenic mice overexpressing PTHrP in chondrocytes (Weir et al 1996 PNAS). Our studies thus support enhanced PTHrP-mediated signaling as a new pathobiological mechanism underlying delayed ossification in Eiken syndrome.

Presenter: Tadatoshi Sato

Institution: UMass Chan Medical School **Department:** Department of Medicine, Rheumatology

Poster Number: 5

Title: In vitro evaluation of CRISPR/Cas9-mediated gene therapy for Osteogenesis Imperfecta

Authors: Tadatoshi Sato, Yen Yang, Agustina Rodriguez, Chen Zhihao, Sachin Chaugule, Jun Xie, Guangping Gao, Jae-Hyuck Shim

Abstract:
Osteogenesis Imperfecta (OI) is the most common rare skeletal disease characterized by bone fragility. The incidence is approximately 1 in 25,000-50,000 in the US. Up to 85% of OI patients have autosomal dominant mutations in either the COL1A1 or COL1A2 gene. The treatments of OI are improving bone strength, reducing fracture risk and pain, and preventing long-term complications. However, drug treatments show limited success because they cannot alter the causes of collagen mutations. To develop novel therapeutics to correct collagen mutations in OI, we recently developed a bone-tropic AAV capsid by grafting bone-homing peptides ((AspSerSer)6, DSS) to the VP2 capsid protein (rAAV9.DSS) and confirmed that rAAV9.DSS was highly effective for the transduction of bone-forming osteoblasts (OB) in vitro and in vivo. We also used OIM mice harboring the deletion of a single-nucleotide (G) at 3983 in the Col1a2 gene as a mouse model of the dominant form of human OI (type 3). As a result, OIM mice displayed smaller body sizes, multiple non-union bone fractures, and pelvic bone deformity. μCT analysis demonstrated significant decrease in trabecular bone mass in the long bones of these mice. CRISPR-Cas9 is one of the most

powerful gene editing tools for skeletal rare diseases. We examined CRISPR-Cas9-mediated editing efficiency of the Col1a2 gene in immortalized OIM OB line, generated by expressing the heat-sensitive SV40T antigen. For gene replacement, we designed the optimized Condon templates for avoiding OIM mutation gRNA targeting, and for enhancing repaired mouse Col1a2 expression to accelerate therapeutic efficacy. Sanger sequencing analysis showed 31±4 % gene correction of OIM mutation in OBs when treated with CRISPR-Cas9. However, further study will be required to test gene correcting efficacy of the CRISPR-Cas9 in bone-lining cells of OIM mice when administered with intravenous injection of AAV vector.

Presenter: Nora Renthal

Institution: Boston Children's Hospital **Department:** Pediatrics

Poster Number: 6

Title: Genome-wide CRISPR Screening of Chondrocyte Maturation Newly Implicates Multiple Genes in Longitudinal Skeletal Growth and Height-GWAS Associated Loci

Authors: John M. Baronas, Eric Bartell, Henry M. Kronenberg, Joel N. Hirschhorn, Nora E. Renthal

Abstract:
Disorders of skeletal growth often display altered chondrocyte proliferation and maturation, typically resulting in altered final height, yet our understanding of the genetic pathways that regulate chondrocytes within the growth plate is incomplete. Prior and ongoing genome-wide association studies (GWAS) have identified variants associated with height, but challenges remain in connecting associated variants to the specific genes mediating effects on skeletal growth. The current study pairs human height GWAS data with CRISPR-based genome-wide knock out (KO) screens using an in vitro assay of chondrocyte proliferation and maturation to identify genes and gene pathways of biological relevance to human growth plate maturation. Our CRISPR screen uncovered 162 genes that significantly alter chondrocyte proliferation and maturation at early and/or late time points in culture, of which 90% validated in a secondary screening assay. Genes whose KO altered chondrocyte maturation were significantly enriched for heritability in height GWAS and for genes that underlie monogenic disorders of skeletal growth. Top KEGG pathways among the KOs significantly affecting chondrocyte maturation include those known to be critical for endochondral ossification (BMP/TGF-beta, Wnt, and hedgehog signaling), further validating the results from our screening assay. Thus, our CRISPR screen detects relevant candidates for regulating human growth plate maturation, illustrating the value of functional studies in tissues of biological relevance as orthogonal data sets to refine likely causal genes from large-scale human genetic studies.

Presenter: Brent Hawkins

Institution: Boston Children's Hospital **Department:** Orthopedic Research

Poster Number: 7

Title: Vav2/Wasl signaling is a novel pathway regulating the vertebrate skeleton

Authors: M. Brent Hawkins, Katrin Henke, Xiaotian Feng, Radhika Atit, Matthew P. Harris

Abstract:
The zebrafish is a powerful model system to investigate the genetic basis of development and disease. Using an unbiased forward genetic approach we discovered novel key regulators of skeletal development that are conserved across vertebrates. We identified two zebrafish mutants by this approach that unexpectedly transform the simple fin skeleton into a complex limb-like structure, including synovial joints, new muscle attachments, and articulation. We identified these mutant phenotypes are caused by gain-of-function mutations in two previously unrecognized skeletal regulators, Vav2 and Wasl, and further that they

function together in a common pathway to foster the creation of unique novel skeletal elements. This pathway is conserved, as we find that conditional inactivation of Wasl in the mouse causes myriad skeletal defects, including cleft palate, agenesis of the skull roof, and loss and fusions of appendage bones. Following our discovery of the Vav2/Wasl pathway, we are using genetic approaches in mouse and zebrafish to parse the molecular mechanisms of Wasl regulation of the skeleton. Wasl is a nucleator of F-actin and has known roles in cell migration, cell signaling, and transcription. We find that Wasl gain-of-function causes increased Hox gene expression in the zebrafish fin, suggesting Wasl acts as a transcriptional regulator in the skeleton. However, we also find that Wasl gain-of-function increases F-actin foci in the cytoplasm, indicating that changes to cell migration or interaction with the extracellular matrix may also mediate the effects of Wasl. Our continued studies will define the mechanistic action of the Vav2/Wasl pathway in the skeleton.

Presenter: Prem Yadav

Institution: Massachusetts General Hospital **Department:** Endocrine Unit, Department of Medicine

Poster Number: 8

Title: Phosphate induced activation of VEGFR2 is required for Caspase 9 mediated apoptosis of hypertrophic chondrocytes during endochondral bone formation.

Authors: Prem Swaroop Yadav, Garyfallia Papaioannou, Margaret M Kobelski, Marie B. Demay

Abstract:
Phosphate (Pi) is critical for skeletal development and maturation. Low circulating phosphate impairs skeletal mineralization and leads to rickets, characterized by expansion of the hypertrophic chondrocyte (HC) layer of the growth plate due to impaired HC apoptosis. Studies in cultured HCs demonstrate that Pi-induced activation of the Raf/MEK/ERK1/2 pathway activates the mitochondrial apoptotic pathway. To determine how Pi activates this signaling pathway, an unbiased small molecule kinase inhibitor screen was undertaken to identify inhibitors of phosphate induced ERK1/2 phosphorylation in HCs. VEGFR2 was identified as a target by these screens.
In vitro studies in HCs demonstrate that VEGFR2 inhibitors block Pi-induced pERK1/2 and Caspase-9 cleavage. Like Pi, rhVEGF phosphorylates ERK1/2 and induces caspase-9 cleavage in HCs. Both Pi and rhVEGF induce rapid phosphorylation of VEGFR2 in HCs, confirming that Pi activates the VEGFR2 signaling pathway in these cells. Chondrocyte-specific depletion of VEGFR2 *in vivo* leads to an increase in the number of Coll-X expressing HCs during development, associated with impaired vascular invasion and a decrease in HC apoptosis, which is further exacerbated by dietary phosphate restriction. Thus, VEGFR2 mediates the actions of extracellular Pi in the developing and maturing growth plate.

Presenter: Jakob Hoppner

Institution: Massachusetts General Hospital **Department:** Endocrine Unit, Department of Medicine

Poster Number: 9

Title: Skeletal phenotype in mice with a truncating PTH1R mutation resembles skeletal abnormalities in subjects with Eiken Syndrome

Authors: Jakob Höppner, Thomas Gardella, Monica Reyes, Patrick Hanna, Harald Jüppner, Ignacio Portales-Castillo

Abstract:
Background: Eiken syndrome is an ultra-rare disease characterized by delayed bone ossification that is caused by homozygous mutations in the parathyroid hormone/parathyroid hormone-related protein receptor (PTHR1). In-vitro, the first Eiken mutant receptor, R485X-PTH1R, showed 1) increased basal cAMP

signaling, 2) enhanced PTH- and PTHrP-stimulated cAMP formation, 3) prolonged cAMP formation in response to PTHrP, but not to PTH, and 4) increased intracellular Ca2+ response to PTH and PTHrP. Additionally, β-arrestin2 over-expression suppressed the constitutive signaling activity of R485X-PTH1R and diminished its sustained cAMP response to PTHrP.
The aim of this study was to generate a R485X-PTH1R knock-in mouse model of Eiken disease to gain further insights into how the mutant receptor impacts bone mineralization.
Methods: Mutant mice (R485X-PTH1R) were generated using iGONAD (improved genome editing via oviductal nucleic acids delivery) in "humanized" PTH1R mice, which express the human PTH1R.. Histological, radiographic and laboratory results of homozygous and heterozygous mutant mice at different ages were compared with age-matched control mice.
Results: Mice homozygous for the R485X mutation had short broad tails but otherwise appeared normal. Heterozygous mice were indistinguishable from WT mice. Skeletal analysis at postnatal day 1-6 revealed reduced mineralization in the long bones and carpals of the homozygous mice. Histological analysis of the growth plates in H&E-stained tibial sections revealed increased zones of proliferative as well as hypertrophic chondrocytes. Beyond day 21, bone mineralization and growth plate histology appeared similar in mutants and WT mice, however, the mutant tarsal bones and vertebrae were misshaped. Homozygous R485X mice exhibited normocalcemia but serum PTH levels tended to be elevated, suggesting moderate PTH-resistance.
Conclusion: The R485X mutant mice recapitulates the main findings of patients with Eiken Syndrome. Our data suggests that this Eiken mutant receptor leads to increased sensitivity to PTHrP in vivo and thus enhanced proliferation of chondrocytes in the growth plates. They further suggest a previously unrecognized role for β-arrestins in regulating PTH1R signaling during bone development.

Presenter: Gabrielle Gallant

Institution: Boston University **Department:** Orthopedic surgery

Poster Number: 10

Title: Involvement of morphogenetic protein 2 in osteogenesis and angiogenesis during distraction osteogenesis

Authors: Gabrielle Gallant

Abstract:
Bone is one of the only tissues in the body capable of full regeneration after a posttraumatic fracture. For bone repair to occur, osteogenesis must be coupled to angiogenesis to properly nourish the injured area. Bone morphogenetic protein 2 (BMP2) is a growth factor required for the initiation of bone repair and is synthesized by the vessels. In distraction osteogenesis, a surgical ostomy is made and the separation of the bone fragments is mechanically made by the incremental separation of the two bone fragments. The mechanical stimulation primarily drives bone regeneration to use an intramembranous ossification process of direct bone formation without a cartilage intermediary. To study the role of BMP2 in coupling osteogenesis and angiogenesis, transgenic mice were used to express Cre recombinase that was driven from a tamoxifen-inducible smooth muscle actin (SMA) promoter. These mice were crossed with those containing BMP2 floxed genes allowing for the conditional deletion of the BMP2 gene in SMAexpressing cells. Tissue samples of the femur were examined histologically on post-operative days 17 (end of active distraction) and day 31 (end of the consolidation period). Histological data indicated that the deletion of BMP2 in vascular tissue resulted in a reduction of angiogenesis and subsequent reduction in bone development. Safranin-O with a fast green stain revealed the control group at day 17 had more cartilage formation within the gap compared to the BMP2 knockout. Both respective experimental groups showed a larger number of osteoclasts within the distraction gap at the beginning of the consolidation phase at the end, showing an early remodeling of bone. This study helps to provide a basis for how BMP2 is useful for fracture repair by increasing both osteogenesis and angiogenesis post-fracture in the postembryotic state.

Presenter: Adina E. Draghici

Institution: Harvard Medical School **Department:** Physical Medicine and Rehabilitation

Poster Number: 11

Title: Mechanisms of tibial bone blood flow regulation in humans

Authors: Adina E. Draghici, Matthew R. Ely, J. Andrew Taylor

Abstract:
Regulation of bone blood flow is critical for skeletal health but remains poorly understood in humans due to the lack non-invasive tools. To overcome this, we are using a custommade near infrared spectroscopy (NIRS) device to non-invasively assess bone blood flow regulation in humans. We recently showed that sympathetic innervation actively controls bone vasculature in young adults. This furthers our findings by investigating additional key mechanisms – myogenic vasodilation and vasoconstriction, and nitric oxide (NO) vasodilation. In young adults, we characterized myogenic vasodilation and vasoconstriction (N=10), and NO-vasodilation (N=8) of tibial blood flow. We employed reactive hyperemia (via cuff occlusion), leg dependency (via 2-levels), and sublingual nitroglycerin to assess myogenic vasodilation, vasoconstriction, and NO-vasodilation, respectively, in the tibia and calf. For all stimuli, we monitored tibial blood flow (total hemoglobin, totalHb) via NIRS and whole leg blood flow via Doppler ultrasound. During cuff occlusion, tibial totalHb did not change, but increased rapidly, far surpassing baseline when flow was restored; a similar overshoot was seen in whole leg flow at cuff release. During leg dependency, with increased perfusion pressure at both levels (26.3+6.07; 41.0+6.44mmHg), whole leg flow decreased (-7.67+3.69; 12.0+3.68cm/s), while tibial flow did not change (-1.00+7.19; 1.65+13.0μM). Unchanged tibial flow despite increased pressure indicates a compensatory vasoconstriction which is smaller and saturates faster compared to the whole leg. In response to nitroglycerin, tibial totalHb increased rapidly to 1.61+0.69μM after 3.5min; afterwards, it steadily declined, returning to baseline by 10min. Whole leg flow increased at a slower rate reaching 7.12cm/s after 4.5min; however, it remained elevated throughout 10min. Our results indicate that bone vasculature has robust myogenic control and NOvasodilation, but with different magnitudes and time-course responses compared to whole leg vasculature. This work provides a foundation of bone blood flow regulation and sets the stage for exploring its contribution to osteoporosis.

Presenter: Vikram Khedgikar

Institution: Brigham and Women's Hospital **Department:** Department of Orthopedic Surgery

Poster Number: 12

Title: Mouse LGR6 regulates osteogenesis in vitro and in vivo through differential ligand use

Authors: Vikram Khedgikar, Julia F. Charles, and Jessica A. Lehoczky

Abstract: Leucine-rich repeat containing G-protein-coupled receptor 6 (LGR6) is a marker of osteoprogenitor cells and is dynamically expressed during in vitro osteodifferentation of mouse and human mesenchymal stem cells (MSCs). While the Lgr6 genomic locus has been associated with osteoporosis in human cohorts, the precise molecular function of LGR6 in osteogenesis and maintenance of bone mass are not yet known. In this study, we performed in vitro Lgr6 knockdown and overexpression experiments in murine osteoblastic cells and find decreased Lgr6 levels results in reduced osteoblast proliferation, differentiation, and mineralization. Consistent with these data, overexpression of Lgr6 in these cells leads to significantly increased proliferation and osteodifferentiation. To determine whether these findings are recapitulated in vivo, we performed microCT and ex vivo osteodifferentiation analyses using our newly generated CRISPR-Cas9 mediated Lgr6 mouse knockout allele (Lgr6-KO). We find that ex vivo osteodifferentiation of Lgr6-KO primary MSCs is significantly reduced, and 8 week- old Lgr6-KO mice have less trabecular bone mass as compared to Lgr6 wildtype controls, indicating that Lgr6 is necessary for

normal osteogenesis and bone mass. Toward mechanism, we analyzed in vitro signaling in the context of two LGR6 ligands, RSPO2 and MaR1. We find that RSPO2 stimulates LGR6-mediated WNT/B-catenin signaling whereas MaR1 stimulates LGR6-mediated cAMP activity, suggesting two ligand-dependent functions for LGR6 receptor signaling during osteogenesis. Collectively, this study reveals that Lgr6 is necessary for wildtype levels of proliferation and differentiation of osteoblasts, and achieving normal bone mass.

Presenter: Katelyn DeMaio

Institution: Boston Children's Hospital **Department:** Pediatrics

Poster Number: 13

Title: Cfp1 and chondrocyte maturation: analysis of phenotypic changes in the context of gene deletion in embryonic mice

Authors: Katelyn DeMaio, Mausam Patel, Molly Persky, Lijie Jiang, Diana L. Carlone

Abstract:
Bone dysplasia's affect every 1 in 5,000 babies; most of these dysplasia's are incurable, and some are even lethal. Hundreds of skeletal dysplasia's are heritable, yet the genes involved are not well defined. Most of the skeleton forms through a process called endochondral ossification (EO). There are three parts of EO: chondrogenesis, maturation, and ossification. During chondrogenesis, mesenchymal progenitor cells condense and then differentiate into chondrocytes. After differentiation, chondrocytes will elongate, then proliferate and mature to set up for primary ossification. We know that this process happens through many activated genes, but the sequential steps through which this is achieved has yet to be elucidated. In order to understand the cause of skeletal dysplasia's and find new treatments, the molecular mechanisms controlling EO requires further investigation. This study focuses on one protein, CXXC Finger Protein 1, Cfp1, and its role in chondrocyte maturation during skeletal mouse development. Earlier studies showed that loss of Cfp1 in limb bud progenitor cells disrupted chondrocyte maturation and primary ossification. To investigate if this was due to Cfp1 action in chondrocytes, we used Col2a1-Cre to specifically delete Cfp1 in chondrocytes and the resultant effects on cartilage and bone were analyzed. cKOCol2 mice die at birth and exhibit an overall mild skeletal phenotype. No phenotypic differences were observed in the ribs, sternum, or trachea, but we did observe reduced ossification in the vertebrae. In addition, mutant long bones were bowed and shorter than controls with a corresponding decrease in growth plate length. Surprisingly, both metatarsals and metacarpals exhibited a complete lack of ossification, which upon further analysis showed was due to a disruption in chondrocyte maturation. Together our data demonstrate that Cfp1 is necessary for normal chondrocyte maturation and growth plate function.

Presenter: Jasmine M. Nutakki

Institution: Boston Children's Hospital **Department:** Orthopedic Research

Poster Number: 14

Title: Effects of Increasing LRP5 Signaling During Chondrocyte Transdifferentiation to Osteoblast Development On Osteogenesis Imperfecta

Authors: Jasmine M. Nutakki, Daniel Osorio, Emily Rodgers, Alexander Robling, Christina M. Jacobsen

Abstract:
Osteogenesis Imperfecta (OI) is a genetic disorder caused by the mutations primarily in type I collagen genes, COL1A1 and COL1A2. We have previously shown that a dominant, gain of function mutation in the

low-density lipoprotein receptor-related protein 5 (LRP5) receptor leads to increased bone mass and bone strength in mice with OI, but did not decrease long bone fractures when active only in mid to late-stage osteoblasts. We hypothesized that increasing LRP5 signaling during osteoblast development could improve bone properties in OI through transdifferentiation of chondrocytes into osteoblasts.
Mice with a conditional high bone mass (HBM) Lrp5 allele (HBM-Neo) were crossed with a model of severe OI, Col1a1Aga2/+ (Aga2), that spontaneously fractures. Offspring were bred to a Cre driver, Col10A1:Cre expressed in late stage chondrocytes. Offspring had four resulting genotypes: Cre/WT/WT, Cre/ HBM-Neo/WT, Cre/WT/Aga2, and Cre/ HBM-Neo/Aga2.
At 12 weeks of age, mice were sacrificed, and we performed whole body DXA, microCT and faxitron X-rays to measure fracture rate. For the Col10A1:Cre/WT/Aga2 mice compared to Cre/HBM-Neo/Aga2 littermate controls, there was no significant difference in the BMD by DXA between female mice and a small but significant difference ($p<0.05$) in the male mice. There were no significant differences in the microCT values or ultimate force to failure. As expected, there was no clinically significant difference in long bone or pelvic fracture rates. There was a significant difference found between Cre/WT/WT and Cre/ HBM-Neo/WT mice in all assays ($p<0.05$). (N=6 mice per group for all experiments.)
These results demonstrate that activation of LRP5 signaling in late chondrocytes is not sufficient to improve phenotype in severe OI. However, it does lead to an increase high bone mass in WT mice. This suggests that OI affects the transdifferentiation of chondrocytes to osteoblasts. Further studies are needed to determine the mechanism behind this phenotype.

Presenter: Yorihiro Iwasaki

Institution: Massachusetts General Hospital **Department:** Endocrine Unit, Department of Medicine

Poster Number: 15

Title: GNAS AS2 DMR Methylation Depends on STX16/NESP55 icrs and Has Diagnostic Potential in AD-PHP1B

Authors: Yorihiro Iwasaki, Monica Reyes, Harald Jüppner, Murat Bastepe

Abstract:
[BACKGROUND] Gsα is a ubiquitous signaling protein essential for mediating the actions of numerous endogenous agonists. It is encoded by the GNAS gene, an imprinted locus giving rise to the mRNA encoding Gsα through use of a unique first exon and to alternatively spliced messages through the use of additional first exons, such as NESP55, AS, XL, and A/B, that reside within differentially methylated regions (DMRs). Parent-specific methylation of these GNAS DMRs and thus expression from the non-methylated allele alone plays a key role in Gsα expression and the pathogenesis of diseases caused by GNAS mutations. Perturbed methylation at the GNAS DMRs, especially hypomethylation at A/B DMR, leads to pseudohypoparathyroidism type 1B (PHP1B), a disorder of hormone resistance primarily affecting the parathyroid hormone. We have recently generated human embryonic stem cell (hESC) models of autosomal dominant PHP1B (AD-PHP1B) by deleting either of the two GNAS imprinting control regions (ICRs), namely STX16-ICR and NESP55-ICR. These cell models revealed that interaction between the two GNAS ICRs critically regulates methylation at A/B DMR during the early postzygotic period. [MATERIALS and METHODS] Using our AD-PHP1B hESC models, we analyzed methylation levels at another recently described DMR, termed AS2, located between the AS and XL DMRs. We used methylation-sensitive restriction enzyme digestion followed by qPCR to measure AS2 methylation levels in wild-type (WT), STX16-ICR-deleted, and NESP55-ICR-deleted hESCs. Parental origins of deleted alleles were determined by genotyping informative single nucleotide polymorphisms in the region. [RESULTS] In WT hESCs, methylation levels at AS2 DMR were low (~3.5%) compared with other GNAS DMRs, such as A/B, which showed ~30-50% methylation. Deleting either STX16- or NESP55-ICRs resulted in further, significant hypomethylation at AS2 DMR, but only when the deletion was on the maternal allele (3.6% vs. 0.03%, $p<0.0001$ for STX16-ICR deletion, and 3.5% vs. 0.6%, $p=0.0015$ for NESP-ICR deletion). Methylation levels at conventionally analyzed nearby DMRs, AS and XL, were not significantly reduced by maternal deletion of either of these two GNAS ICRs. [DISCUSSION] In this study, we found that methylation at AS2 DMR depends in hESCs on intact STX16- and NESP55-ICRs. These results suggest that AS2 DMR, unlike AS

and XL DMRs, is critically regulated by GNAS ICRs in the early embryo via a mechanism that may be similar to that governing A/B DMR methylation. Our cell-based experimental data thus provide further rationale for analyzing AS2 DMR to define in patients the disease-causing PHP1B variant.

Presenter: Christodoulos K. Constantinou

Institution: Boston University School of Medicine. **Department:** Orthopedics Research

Poster Number: 16

Title: In-Vitro Differentiation of Human Acetabular Mesenchymal Stem Cells as a Potential Model to Study Osteoarthritic Risk Factors

Authors: Christodoulos K. Constantinou, Giulia Montagna, Yuhei Uda, Milica Šipovac, Paola Divieti Pajevic, Louis C. Gerstenfeld

Abstract:
Osteoarthritis (OA) is a degenerative joint disease characterized by the deterioration of articular cartilage protecting bone tissue, resulting in pain and loss of mobility. In the US, OA affects over 32.5 million people, and often requires invasive surgical treatment. Ongoing research targets the prevention and treatment of OA, primarily through clinical trials. On the other hand, cellular models are utilized less, in part due to the gap in knowledge between molecular-level interactions in joint tissue and clinical manifestations of OA. Additionally, the use of in-vitro cell cultures as models to investigate bone pathologies remains controversial, as mesenchymal stem cells (MSCs) lose their local and systemic interactions once removed from their microenvironment. In this study, we devised a process of isolating, passaging, differentiating, and characterizing human MSCs from resected tissue during total hip arthroplasty, as well as paired molecular characteristics of the differentiated cells to demographic and clinical information. Cells were characterized using histology and RT-qPCR analyses. Using data from MSCs of six patients, we found that the differentiation groups expressed several adipogenic, chondrogenic, and osteoblastogenic gene markers significantly more than non-differentiated cells. Additionally, certain patients expressed gene markers in one or more lineages at higher rates than others. Our data suggests that the cell population isolated from acetabular reamings contains MSCs, and that these MSCs are able to differentiate into all three cell lineages. Furthermore, we demonstrated that differentiated MSCs from individual patients contain intrinsic variability in lineage commitment, and potentially follow trends in molecular characteristics when correlated to demographics and comorbidities. This data therefore validates this procedure as an effective way to isolate and culture human MSCs for further investigation, and paves a path for future correlational studies to test the effectiveness of passaged MSCs as a model to investigate molecular interactions that differ between osteoarthritic patients' cells.

Presenter: Grace Jung

Institution: Massachusetts General Hospital **Department:** Endocrine Unit, Department of Medicine

Poster Number: 17

Title: Risk of Wrist Fracture in Gastric Bypass Compared to Gastric Banding as Estimated by the Load-to-Strength Ratio

Authors: Grace H. Jung, Bita Zahedi, Katherine Lindeman, Claire C. Rushin, Michael C. Cheney, Mary L. Bouxsein, Elaine W. Yu

Abstract:

Bariatric surgeries such as Roux-en-Y gastric bypass (RYGB) and adjustable gastric banding (AGB) lead to long-term deficits in bone density but are also accompanied by decreased weight, which may lower the impact force with falls. The aim of this study is to compare the long-term skeletal impact of RYGB and AGB using a biomechanical evaluation of load-to-strength ratio as a surrogate for fracture risk.
We examined a cohort of adults who received RYGB and AGB surgery ≥ 10 years ago (RYGB: n=25; AGB: n=25). We computed the load-to-strength ratio at the distal radius as a ratio of impact force (F) to bone strength (estimated via high-resolution peripheral quantitative CT). Differences in bone outcomes between RYGB and AGB groups were compared using generalized linear modeling and adjusted for age, sex/menopausal status, and race/ethnicity.
Compared to AGB, the RYGB group was younger (56 vs 62 years), had more premenopausal women (41% vs 9%), more individuals who identified as non-White (40% vs 12%) and longer duration since surgery (13 vs 11 years) ($p<0.05$). In multivariate analysis, the RYGB group demonstrated higher bone turnover markers ($p<0.01$), lower aBMD Z-scores at the hip, femoral neck, and spine, as well as lower vBMD at the distal radius ($p<0.01$). HR-pQCT-derived failure load at the distal radius, or estimated bone strength, was lower in RYGB compared to AGB (3694 N vs. 4162 N, $p=0.01$). Load-to-strength ratio was higher in RYGB as compared with AGB (0.82 ± 0.04 vs 0.73 ± 0.05, $p=0.08$), suggestive of higher fracture risk at the wrist, although this did not reach statistical significance.
These results indicate the long-term deleterious skeletal effects are more concerning with RYGB than AGB.

Presenter: Shannon R. Emerzian

Institution: Beth Israel Deaconess Medical Center

Poster Number: 18

Title: Total body irradia,on results in long-term deficits in vertebral but not femoral bone structure in male rhesus macaques

Authors: Shannon R. Emerzian, Isabel Barnet, Trinity Tedtsen, Sun Park, Joseph Moore, John D. Olson, Mary L. Bouxsein, J. Mark Cline, Jeffrey S. Willey

Abstract:
Skeletal fractures are a late effect of high-dose clinical radiaTon therapy (RT) for cancer treatment, parTcularly in normal bone that absorbs dose during RT. However, liLle is known about the effects of lower-dose, total body irradiaTon (TBI) on skeletally-mature bone. The objecTve of this study was to quanTfy late effects of TBI on vertebral and femoral bone microstructure from rhesus macaque (Macaca mula(a) non-human primates (NHPs).
Femora (n=7) and lumbar vertebrae (LV; n=5) were obtained post-mortem from irradiated (IRR) male NHPs; femora (n=6) and LV (n=5) from age- and sex-matched non-irradiated controls were also obtained. Skeletally-mature NHPs received an acute dose of 6.0-6.75Gy TBI and Tssues harvested an average of 10yrs a`er TBI. High-resoluTon computed tomography scans were used to assess trabecular (Tb) bone structure of the LV and distal femur; femoral corTcal bone was assessed mid-diaphysis. Urinary Nterminal telopepTde crosslinks (NTX), a marker of osteoclasTc resorpTon of bone, was quanTfied at sacrifice. T-test assessed group effects; data reported as mean±SD; α $p<0.05$.
Mean age (19±2.2yrs) and body weight (14±3.3kg) were similar in IRR and controls ($p>0.5$ for both). In the LV, IRR NHPs had 27% lower Tb bone volume fracTon (Figure, 19.1±5.1%vs.26.3±2.0%; $p=0.032$) and number (1.6±0.4mm-1vs.2.2±0.3mm-1; $p=0.042$) vs. control. LV BMD was also lower in IRR vs. control (Figure; -20%; $p=0.051$). In contrast, femoral Tb and corTcal bone outcomes in IRR were similar to control (Figure). NTX levels did not differ between groups ($p=0.71$).
TBI resulted in long-term Tb bone loss in the axial skeleton of adult NHPs when comparing age- and sexmatched
non-irradiated controls. NTX was similar between groups, suggesTng bone turnover rate was similar and bone deficits from TBI persisted from earlier atrophy of bone. These results indicate that survivable TBI from a radiologic event could result in weakening of vertebrae and predispose fracture among adult survivors.

Presenter: David E. Maridas

Institution: Harvard School of Dental Medicine

Poster Number: 19

Title: Smad1/5/8 signaling in osteoblasts: early observations from a triple knockout mouse model

Authors: David E. Maridas, Laura Gamer, Emily Moore, Vicki Rosen

Abstract:
The TGFb superfamily of multifunctional cytokines can be traced back over 500 million years in the earliest known metazoan fossils of the Cambrian period. Today, TGFb superfamily ligands are recognized as fundamental regulators of cell function in all tissues, having central roles in development, postnatal growth, immunity, and healing. In bone, research spanning decades demonstrated that the Smad1/5/8 signaling pathway, activated by certain TGFb ligands, is crucial for initiation of skeletogenesis and for the differentiation of skeletal progenitors to osteoblasts. Yet, it remains unclear if signaling through Smad1/5/8 is required for the function of mature osteoblasts in the adult skeleton. Here, we use mice carrying conditional alleles for Smad1, 5, and 8 crossed with Cre deleter strains (Prx1-Cre, Osx-Cre ERt, Dmp1-Cre) to target different stages of osteoblast maturation. We observed that mice carrying only a single copy of Smad1 with Prx1-Cre had thin, bent, and disjointed long bones at 3 weeks of age. Mice lacking Smad1/5/8 in Dmp1+ cells exhibited long bone fractures and died before skeletal maturity. Finally, mice with deletion of Smad1/5/8 in Osx-Cre ERt+ cells showed decreased in trabecular bone volume a month after recombination. Collectively, our early observations suggest that Smad1/5/8 signaling has a physiological role in postnatal osteoblasts. In future studies we will characterize serial allelic deletions of our knockout models.

Presenter: Yaser Peymanfar

Institution: The Forsyth Institute, Cambridge, MA **Department:** Mineralized biology

Poster Number: 20

Title: Roles of osteoblast dopamine D2 receptor in type 1 diabetes-induced bone loss

Authors: Yaser Peymanfar, Philip Trackman

Abstract:
Dopamine is best known as a brain chemical and neurotransmitter that controls mood. However, dopamine is also produced by the gut and directly regulates cells and tissues outside of the brain in the body's periphery. Here, the hypothesis, based on our previous study is that gut-derived dopamine occurs at abnormally elevated levels in type 1 diabetes that may directly inhibits bone forming cells (osteoblasts). Our data points to a mechanism in which the ability of another gut hormone known as Glucose-dependent Insulinotropic Polypeptide or GIP to stimulate bone formation is normally balanced by dopamine. When peripheral dopamine levels become too high in diabetes, we propose that bone formation becomes abnormally low.
Recently our published data showed that diabetes-induced bone disease is associated with diminished LOX levels. Diabetes was induced in 8-week-old male and female C57BL6/J mice using the MLD-STZ method. Eight weeks following induction of diabetes (glucose >250 mg/dl), mice were sacrificed, and femurs were collected for micro-computed tomography (μCT) or histology, and tibia were used to isolate RNA for qPCR. μCT analyses illustrated bone loss with diminished trabecular parameters including bone volume fraction (BV/TV). Data shows very significant diminished collagen structure and remarkable downregulation of lysyl

oxidase mRNA level in diabetic mice compared to control. Amisulpride attenuated dopamine receptor D2R and preserved bone structure in mice, possibly by increasing the LOX expression level and recovery of collagen structure as observed.
Since the systemic administration of Amisulpride may trigger secondary effects on metabolism and bone turnover, we generated heterozygous mice with osteoblast-specific partial deletion of D2r using Col1-2.3-cre+/- and D2rfl/fl and control mice in the C57BL6/J background. Surprisingly but interestingly our preliminary results exhibited a high degree of embryonic lethality in homozygous KO mice. Our findings suggest that D2R signaling may interact with other pathways, and that attenuation of, rather than full knockout is sufficient to rescue diabetic bone phenotype.

Presenter: Jingting Yao

Institution: MGH Martinos Center for Biomedical Imaging **Department:** Department of Radiology

Poster Number: 21

Title: Characterization of Bone Mineralization and Vascular Calcification Using Ion-Selective Phosphorus-31 Solid State Magnetic Resonance Spectroscopy

Authors: Jingting Yao, Christian T. Farrar, Elena Aikawa, David E. Sosnovik, Aditi Kulkarni, Jerome L. Ackerman

Abstract:
Background: Bone mineralization and vascular calcification share similarities in terms of composition, cell types, molecular mechanisms and risk factors. The same proteins and cells associated with bone growth and remodeling are often found in calcified vascular tissues. Evidence suggests an interrelationship between bone diseases and cardiovascular calcifications, and that pharmacological interventions for the treatment of one would affect the other. Understanding the relationship between bone mineralization and vascular calcification is important for the development of effective treatments and diagnostic tools. The goal of this study is to identify compositional features of calcium phosphates in bone and calcified vascular tissues with the novel use of ion-selective phosphorus-31 solid state magnetic resonance (MR) spectroscopy.
Methods: Experiments were performed on biological specimens of human trabecular bone (n = 1), human plaque (n = 1) and calcified aortic valves (n = 4), as well as calcified aortic tissues of apolipoprotein E-deficient mouse (n = 1) fed with high cholesterol diet. Synthetic compounds including hydroxyapatite ($Ca10(OH)2(PO4)6$) and brushite ($CaHPO4{\cdot}2H2O$) were used as reference models, based on the knowledge that mature minerals are dominated by $PO4^{-3}$ and have a lower $HPO4^{-2}$ concentration.
Results: The MR spectral patterns of calcification from the human trabecular bone, human plaque and aortic valves resemble a dense, well-organized material characteristic of the mature mineral. In contrast, calcification in the mouse aorta resembles that of immature or embryonic bone investigated in our previous studies, exhibiting a more disorganized pattern of mineralization that likely models early preclinical calcification of human aortic valves. These observations were consistent with mineral maturity quantified by the cross-polarization time constants and chemical shift tensors derived from the MR spectra.
Conclusion: The ion-selective MR spectroscopy method is sensitive to compositional differences, especially mineral maturity, in bone and cardiovascular minerals, providing complementary information unattainable by existing non-destructive methods.

Presenter: Kira Fagerstrom

Institution: Boston Children's Hospital **Department:** Department of Orthopaedic Research

Poster Number: 22

Title: Developing genetic tools to analyze skeletal homeostasis in aging

Authors: Kira Fagerstrom, Joao Castro, Matthew Harris

Abstract:
Aging can be defined as the progressive loss of tissue homeostasis with time. Keeping deterioration at bay is thus key to retaining normal healthy tissue function. Zebrafish with defective celsr1a function provide a novel aging model displaying a systemic early aging phenotype at just 10-12 weeks of age. Previous work has shown that this progeric phenotype arises due to loss of maintenance of tissue-resident stem cells. Consistent with early aging phenotypes, celsr1a mutants display low skeletal mineral density. As celsr1 is also linked with low mineral density in humans, we set out to investigate the role of celsr1a in bone. Using published ATAC-seq data, we identified 37 putative enhancers of celsr1a. The C1a-A enhancer was active in the notochord and skull in 3dpf zebrafish embryos, but later in development is also evident in the endochondral bones of the fins and vertebrae. Fin clip and ray fracture assays did not result in upregulated C1a-A expression, possibly indicating that C1a-A is responsive in endochondral-derived bones, not dermoskeletal bones. To determine if C1a-A regulates celsr1a expression in bone, we crossed the C1a-A-GFP enhancer-reporter line to a celsr1a-RFP reporter line to assess colocalization in the adult zebrafish. Preliminary analysis of C1a-A and celsr1a expression in endochondral bone shows a small, diffuse C1a-A and celsr1a-positive cell population in the hypural bones and caudal vertebrae, and in neuromast cells of the lateral line. Current and future experiments include analyzing C1a-A and celsr1a expression upon hypural bone injury, targeted deletion of the C1a-A enhancer to evaluate its effects on stem cell-mediated bone regeneration and homeostatic maintenance, and Cre-driven ablation of celsr1a in bone. Taking advantage of this potential celsr1a regulatory element may provide an efficient and highly specific method to model aging and stem cell activity in zebrafish bone.

Presenter: Yiwei Kong

Institution: Northeastern University **Department:** Biology

Poster Number: 23

Title: Bony bar formation v. Cartilage healing in a vertical Salter-Harris type4 (SH4) GP fracture

Authors: Yiwei Kong, Shanmugam Muruganandan, Arina Ovchinnikova, Rachel Pierce, Tommy Nguyen, Maya Kanj, Lilian Bergin, Angela Lee, Andreia M. Ionescu

Abstract:
We previously mapped the hierarchy of the growth plate (GP) stem cells in FoxA2+LTSSC (longterm stem cells) and PTHrP+STSSC (short-term stem cells). FoxA2+LTSSCs expand and repair the GP cartilage in response to a self-healing, horizontal, Salter-Harris type1 (SH1) fracture. We seek to understand why FoxA2+LTSSC cannot repair a vertical SH4 fracture, which leads to a bony bar formation and growth arrest. We performed SH4 surgery on Col.1(2.3kb)-GFP mice using a large 21G needle (0.819mm) and a small 30G needle (0.311mm). SH4 21G-injured mice present a large lesion, which persists at D7 post-op and develops into a col.1+bony bar. SH4 30Ginjured mice heal efficiently with hyaline cartilage by D7 post-op, and no signs of col.1+cells accumulation. This suggests that SH4 fractures can heal if the defect size is small enough. To determine if the SH4 lesion develops into a bony bar because FoxA2+LTSSC are outcompeted by LepR+mesenchymal cells, Osx+osteoprogenitors, or col.1+osteoblasts migrating in the defect, we operated SH4 on FoxA2CreER/+;ZsGreenfl+/, LepRCre;Tomatofl/+;Col.1(2.3kb)-GFP, OsxCreER/+; Tomatofl/fl;Col.1(2.3kb)-GFP, and Col.1CreER/+;ZsGreenfl/+ mice. We found that: 1)LepR+cells migrate first (D1), while Osx+cells and col.1+cells follow later (D3), 2)LepR+cells migrate in the large(21G) lesion, but not the small(30G) lesion, although neutrophils can migrate in both, 3)Similar with SH1 injury, FoxA2+cells expand with a delay (D3), outside the large(21G) lesion, but inside the small(30G) defect. To address if osteoprogenitors create a physical barrier or a signaling niche that prevent FoxA2+LTSSC from healing the large SH4 defect, we created FoxA2DreER/+;Tomatorox/+ mice to be crossed with LepRCre;DTRfl/fl mice, to

assess the timeline/pattern of FoxA2+cell expansion in response to DTA LepR+cell ablation. Preliminary single-cell transcriptomics shows that, as FoxA2+LTSSC turn into PTHrP+STSSC, TGFβ signaling inhibits FoxA2 and induces PTHrP expression in PTHrP+descendants. After SH1 surgery, increased TGF-β signaling and reduced FoxA2 expression in the injured niche is necessary for healing the SH1 defect.

Presenter: Sara Monaci

Institution: Harvard University **Department:** School of Dental Medicine

Poster Number: 24

Title: Role of Tyrosine Phosphorylation in Yes-Associated Protein 1 Function in Bone Marrow Stromal Cells

Authors: Sara Monaci, Anna Bollag, Mengrui Wu, Francesca Gori, Roland Baron

Abstract:
The Hippo pathway is a highly conserved signaling cascade that plays a critical role in controlling a variety of biological processes, including organ size, cell proliferation, and skeletal development. The Hippo pathway functions as a serine/threonine kinase cascade via the transcriptional coactivators yes-associated protein 1 (YAP1) and Tafazzin (TAZ). Studies have shown that phosphorylation of YAP1 at specific serine residues (S381 and four other sites) causes it to be degraded in the cytoplasm so it cannot translocate to the nucleus to activate target genes. YAP1 can also be phosphorylated at tyrosine residues, but the role of tyrosine phosphorylation in YAP1 function has yet to be determined. We identified two novel tyrosine phosphorylation sites (Y375, Y428) and investigated their role in YAP1 transcriptional activity and function in osteoblast and adipocyte differentiation. YAP1 levels were increased in the overexpressed YAP1, the stabilized YAP1, the mutant Y375F, and the mutant Y428D cells. Only in the non-phosphorylatable Y428F cells were YAP1 levels decreased significantly compared to the other mutant cells. The expression of Hippo signaling target genes (CTGF, CYR61, and MYC) showed an increase relative to the GFP cells in the YAP1 OX, stabilized YAP1, mutant Y375F, and mutant Y428D cells. The mutant Y428F cells showed a significant decrease in the expression of target genes relative to the other mutant cells which parallels the trend seen in YAP1 levels. ALP staining demonstrates that ALP activity was significantly decreased in the non-phosphorylatable Y428F cells; the expression of osteoblast-differentiation markers (Col1a and Runx2) was also significantly decreased in the Y428F cells relative to the Y428D cells indicating that tyrosine phosphorylation plays a role in regulating osteoblastogenesis. A similar trend was also observed in both the oil red staining and in the expression of adipocyte-specific markers with Y428F cells showing decreased lipid deposition and decreased expression of ADIPOQ and PPARγ which suggests that YAP1 stability impacts adipocyte differentiation. These results suggest that YAP1 tyrosine phosphorylation at specific residues (namely Y428) may protect YAP1 from degradation and lead to increased YAP1 transcriptional activity and stability. These results also suggest a potential role for tyrosine phosphorylation in the regulation of osteoblast and adipocyte differentiation via the Hippo signaling pathway.
Further investigation into the effects of phosphorylation of specific tyrosine residues on YAP1 stability and function, particularly in response to relevant stimulation, is clearly warranted.

Presenter: Vineel Kondiboyina

Institution: Northeastern University **Department:** Bioengineering

Poster Number: 25

Title: Estimation of Material Properties of Regenerating Axolotl Limbs Using Inverse Finite Element Analysis

Authors: Vineel Kondiboyina, Timothy Duerr , James Monaghan, Sandra J Shefelbine

Abstract:
Background: The extracellular mechanical environment plays an important role in the skeletal development process. Characterization of the material properties of regenerating tissues that recapitulate development, provides insights into the mechanical environment experienced by the cells and the maturation of the matrix. In this study, we estimated the viscoelastic material properties of regenerating forelimbs in the axolotl (Ambystoma mexicanum) at three different regeneration stages: 27 days post-amputation (mid-late bud) and 41 days post-amputation (palette stage), and fully-grown time points. A stress-relaxation indentation test followed by two-term Prony series viscoelastic inverse finite element analysis was used to obtain material parameters. Glycosaminoglycan (GAG) content was estimated using a 1,9- dimethyl methylene blue assay.
Results: The instantaneous (GO) and equilibrium shear moduli significantly increased with regeneration while the short-term stress relaxation time significantly decreased with limb regeneration. The long-term stress relaxation time in the fully-grown time point was significantly lower than 27 and 41 DPA groups. The GAG content was not significantly different between 27 and 41 DPA but the GAG content of cartilage in the fully-grown group was significantly greater than in 27 and 41 DPA. There is a positive correlation between GAG content and instantaneous shear modulus.
Conclusions: The mechanical environment of the proliferating cells changes drastically during limb regeneration. Understanding how the tissue's mechanical properties change during limb regeneration is critical for linking molecular-level matrix production of the cells to tissue-level behavior and mechanical signals.

Presenter: Kathryn M. Yammine

Institution: Massachusetts Institute of Technology **Department:** Chemistry

Poster Number: 26

Title: Elucidating collagen-II proteostasis defects in human chondrodysplasia using induced pluripotent stem cells

Authors: Kathryn M. Yammine, Sophia Mirda, John F. Bateman, Shireen R. Lamandé, Matthew D. Shoulders

Abstract:
Type II collagenopathies are most commonly caused by missense mutations in collagen type-II, the most abundant protein in cartilage. These protein misfolding-related diseases present with various symptoms, including pathologic joint structure, cartilage properties, and skeletal development. Currently, they have no cure. Patients must rely on palliative treatments as symptoms arise. Two examples of these diseases are the chondrodysplasias caused by the G1170S or R719C substitutions in COL2A1. Patients present with one or more of the following symptoms: precocious osteoarthritis, avascular necrosis of the femoral head, and Legg-Calvé-Perthes disease. Previous cell and mouse models of these diseases only poorly recapitulate key features of the human phenotype. Moreover, they provide limited adaptability for detailed biochemical and mechanistic experiments required to attain a molecular-level understanding of these disorders, given the innate challenges in studying collagen folding. To elucidate the underlying collagen production and misfolding defects that cause these chondrodysplasias, we developed a chondronoid model system leveraging induced pluripotent stem cell (iPSC) lines capable of being differentiated into cartilage-producing chondrocytes. We generated otherwise isogenic iPSC lines harboring the G1170S or R719C substitutions in COL2A1, along with a wild-type control iPSC line. Upon differentiating into chondrocytes and inducing cartilage-like matrix deposition, we were able to characterize, in detail, how these mutations impact cartilage properties. Here, we deploy a suite of assays aimed at characterizing the pathologic phenotype and understanding its underlying molecular cause. For example, we discovered accumulation of intracellular collagen-II in disease variants using immunohistochemistry, as well as a deficient extracellular matrix by transmission electron microscopy. These results deepen our understanding of collagen-II homeostasis in health and disease, and begin to lay a foundation for identifying proteostasis network-based therapeutic targets for the collagenopathies.

Presenter: Austin Baacke

Institution: Boston Children's Hospital **Department:** Orthopedic Research

Poster Number: 27

Title: Movement As a Marker of Pain in Severe Osteogenesis Imperfecta

Authors: Austin Baacke, Stephanie Lee, Matthew L. Warman, Christina M. Jacobsen

Abstract:
Osteogenesis Imperfecta (OI) is a genetic disorder characterized by skeletal fragility. Nearly all adult OI patients report chronic pain that interferes with everyday activities and quality of life; however, it remains unclear how different genetic mutations and medical therapies affect pain outcomes. We have previously shown that a dominant, gain of function mutation in the low-density lipoprotein receptor-related protein 5 (LRP5) receptor leads to increased bone mass and bone strength in mice with OI. We hypothesized that mice with both OI and an LRP5 high bone mass (HBM) allele would have decreased fractures and pain. To test this, in collaboration with the Children's Hospital Pain and Behavioral Physiology Core, we administered validated pain perception and activity assays to mice resulting from crosses of a severe OI mouse model (Col1a1Aga2/+) (Aga) and HBM (Lrp5A214V/+) (AV) mice. Aga2 mice fracture spontaneously in our colony. We administered holeboard assays to measure different forms of movement to 16 mice with the following genotypes: (WT/WT); (Aga/WT), (Aga/AV) and (WT/AV) at 5, 9, and 13 weeks of age. Faxitron was done at each time-point to measure bone fracture and deformity.
Aga/WT littermate did have decreased holeboard basic movement compared to WT/WT littermates (p=0.000250). Compared to Aga/AV littermates, the AV/OI mice did exhibit decreased fracture rate on Faxitron but did not show a difference in basic movement (p=0.570). Interestingly, mice with AV/WT genotype exhibited greater activity throughout the holeboard assay when compared to WT/WT mice at week 5, but not at older ages. (p=.0008). Based on this data, we conclude that Aga2 mice show decreased movement compared to WT mice, suggesting they are experiencing pain. However, it is unclear if this decreased movement is due to their fractures or chronic pain. Further studies are needed to determine the difference.

Presenter: Katherine C. Woronowicz

Institution: Boston Children's Hospital **Department:** Department of Orthopaedic Surgery

Poster Number: 28

Title: Dissecting the Role of an Ultraconserved Cis-Regulatory Hub in Modulating Jaw Size

Authors: Katherine C. Woronowicz, Jacob M. Daane, Matthew P. Harris

Abstract:
Human craniofacial malformations are among the most common congenital defects. Fortuitously, jaw development is orchestrated by exceptionally well conserved genetic programs. Such extensive conservation allows us to make discoveries that are pertinent to human development and malformations through studying the variation of naturally occurring craniofacial forms. Here, we mine genome-wide variation among 39 species of Beloniformes - an order that exhibits exceptional variability in upper and lower jaw lengths including representatives of flying fishes, halfbeaks, and needlefishes. Through this, I have identified an exceptionally well conserved interval of the genome which has been maintained since our last common vertebrate ancestor more than 400 million years ago. This microsyntenic interval contains a cluster of ultraconserved noncoding elements (UCNEs) and a region which has been implicated in humanspecific
evolution. Variation within this region is implicated in human craniofacial malformations including hemifacial microsomia and suggests that this region may constitute an essential regulatory hub of

craniofacial development. This work is centered on the largest and best conserved UCNE from the cluster. I found that this UCNE is sufficient to drive fluorescent reporter expression proximolaterally in both the upper and lower jaws as well as in other embryonic tissues in a pattern which forms a sharp boundary with putative target gene, gbx2. These findings suggest that the UCNE may function to spatially restrict gbx2 expression. Targeted deletions in the UCNE, created to mirror the loss of sequence identity within flying fishes, drive a dramatic distomedial shift in reporter expression. Modulation of enhancer expression domains may disrupt the normal expression pattern of putative target gene, gbx2, and ultimately impede jaw elongation during development. Currently, we are integrating these results with tissues collected during mandibular distraction osteogenesis procedures in children with microsomia to shed light on the etiology of human skeletal disorders.

Presenter: Anthony N. Aggouras

Institution: Boston University **Department:** Department of Biomedical Engineering

Poster Number: 29

Title: Earlier Proteoglycan Turnover Promotes Higher Efficiency Matrix Remodeling in MRL/mpj Tendons

Authors: Anthony N. Aggouras, Brianne K. Connizzo

Abstract:
INTRODUCTION: While most mammalian tissue regeneration is limited, the Murphy Roths Large (MRL/MpJ) mouse can regenerate multiple tissues, including tendon [1]. Recent studies have indicated that this regenerative response is innate to the tendon tissue and not reliant on a systemic inflammatory response [2]. Previously, we showed that MRL/MpJ tendons have greater matrix turnover in response to stress deprivation, with a higher rate of collagen synthesis and greater matrix metalloproteinase (MMP) activity [3]. The objective of the current study was to elucidate the differences in the mechanisms of matrix turnover and the subsequent functional outcomes of this improved turnover.
METHODS: MRL/MpJ and C57BL/6J flexor digitorum longus tendon explants were subjected to stress-deprived conditions in vitro for up to 14 days, as described previously [4]. Explant biosynthesis, matrix metalloproteinase activity, gene expression, and tendon biomechanics were assessed periodically.

Presenter: Emma J. Stowe

Institution: Boston University **Department:** BME

Poster Number: 30

Title: Senescence Contributes to Death Resistance of Aged Tenocytes in a Model of Secondary Joint Damage

Authors: Emma J. Stowe, Brianne K. Connizzo

Abstract:
INTRODUCTION: Rotator cuff injuries disproportionately affect aging populations, yet the pathogenesis is unknown. We developed an in vitro model of secondary joint damage in the murine rotator cuff [1]. In this model, injuries to muscle and bone result in cell death and matrix degeneration in the tendon. Surprisingly, aged explants show delayed tenocyte death compared to young tissues [2]. The goal of this study was to investigate whether this prolonged viability is due to altered chemical mediators or innate differences in aged cells.
METHODS: Bone-tendon-muscle (BTM) and flexor digitorum longus (FDL) tendon explants were harvested from young (4m) and aged (22-24m) male C57BL/6J mice [1,3]. Conditioned medium (CM) from young and aged BTMs was added to young and aged FDLs (Fig. 1a). Viability was evaluated via live/dead staining and

metabolic activity. Apoptosis was induced with staurosporine. Senescence was induced with doxorubicin and irradiation and assessed with SA-βgal [4]. Statistics were performed using one-way ANOVAs with post-hoc t-tests.

Presenter: Susan MacLauchlan

Institution: BWH **Department:** Rheumatology

Poster Number: 31

Title: Tonic interferon signaling in osteoclast formation and clonal hematopoiesis

Authors: Susan MacLauchlan, Catherine A Manning, Albert Tai, Maria Zuriaga, Jose Fuster, Elizabeth Faudoa, Sally Lakis, Kenneth Walsh, Christian Jacome-Galarza, Shruti Sharma, and Ellen Gravallese

Abstract:
Purpose: Interferon (IFN) and Interferon Stimulated Genes (ISGs) potently regulate hematopoiesis and bone homeostasis. Induced and tonic IFN, that is the IFN produced in the absence of external stimuli, shape bone turnover through their inhibition of osteoclastogenesis. Ten-Eleven Translocase 2 (TET2) is an epigenetic modifier, an important determinant of myeloid development, and one of the most frequently mutated genes in myelodysplastic syndrome and clonal hematopoiesis of indeterminant potential. Our studies evaluate whether TET2 regulates tonic and RANKL-induced IFN signaling and its impact on myeloid and osteoclast development.
Methods: TET2 germline deletion (TET2 KO) mice and littermate controls were used in aging studies and in surgical ovariectomy. Ovariectomy or sham surgeries were performed in 8 week old mice and samples collected 8 weeks following surgery. Trabecular bone in the distal end of the femur of aging mice was evaluated by microCT (Scanco 35). Bone marrow osteoclast precursors were quantified by FACS. Osteoclast fusion was induced by RANKL in mCSF-expanded bone marrow derived precursors in vitro and samples collected for ELISA and qPCR.
Results: TET2 KO mice showed a marked reduction in RANKL-induced osteoclast fusion in vitro and failure to induce OCSTAMP, a gene essential for osteoclast fusion. The limited osteoclastogenesis is recapitulated in vivo, as female TET2 KO mice exhibit slight increase in bone volume and protection from ovariectomy-induced bone loss. Interestingly, TET2 KO mice exhibit increased osteoclast precursors, indicating that the osteoclast formation defect is due to a failure in differentiation and not precursor availability. We characterized the kinetics of tonic and inducible ISGs following RANKL-stimulated osteoclast differentiation, and identified CCL5 and CXCL10 as IFN-dependent RANKL-induced genes. TET2 KO osteoclast precursors exhibit enhanced IFN signaling, indicated by increased CCL5 and CXCL10 production in response to RANKL, which may limit their conversion to osteoclasts while promoting myeloid skewing.
Conclusion: TET2 deficiency limits osteoclast formation by inhibiting OCSTAMP and may have previously unrecognized roles in promoting RANKL-induced IFN signaling. Regulation of IFN and ISGs by TET2 may represent a critical mechanistic node shaping the myeloid-bias of the TET2 KO and may reveal insight into clinical management of TET2-driven clonal hematopoiesis.

Presenter: Giullia Montagna

Institution: Boston Children's Hospital **Department:** Department of Orthopedic Surgery

Poster Number: 32

Title: Rationale for Improving Existing OI Mouse Models

Authors: Giulia Montagna, Stephanie Lee, Austin Baacke, Christina Jacobsen, Matthew Warman

Abstract:

Osteogenesis Imperfecta (OI) is a genetic disease characterized by skeletal fragility. 85% of patients have dominant mutations in COL1A1 or COL1A2. We use OI mouse models to understand disease pathophysiology and test preclinical therapies. Col1a1Aga2/+ mouse model of progressive deforming OI presents with multiple fractures and scoliosis and, here, we used this model to test whether anabolic bone therapies reduce fracture incidence and improve mobility.

We crossed Aga2 mice with High Bone Mass (HBM) mice, the latter have an Lrp5 mutation that increases bone mass, and then counted fractures radiographically and measured mobility via a Holeboard assay at 5, 9 and 13 weeks of age in the WT, Aga2, and Aga2;HBM offspring.

We found that as early as 5 weeks of age Aga2;HBM mice had 33% fewer fractures and less scoliosis than Aga2 mice ($p<0.0005$; $p=0.02$, respectively). However, we observed no difference in mobility between the Aga2;HBM and Aga2, probably because both mice still had multiple fractures by the time the first Holeboard assay was conducted.

To circumvent confounding by pre-existing fractures we are making a conditional allele by inserting a floxed gene trap into the Aga2 allele. This conditional allele will allow us to temporally induce mutant collagen expression, so we can study whether decreased mobility is a consequence of the fractures or due to soft tissue or bony pain that precedes fractures. It will also allow us to tissue-specifically express mutant collagen so we can study the effect on other organ systems without confounding caused by skeletal deformity.

Presenter: Stephen Treaster

Institution: Harvard Medical School

Poster Number: 33

Title: Insights from the edge: Genetic analysis of exceptionally long- and short-lived species to reveal the regulation of human aging

Authors: Stephen Treaster[1,2], Joris Deelen[3], Jacob Daane[4], David Karasik[5], Matthew Harris[1,2]

Abstract:

Longevity is a defining trait that varies dramatically across vertebrates. Inherent to this trait are the means to maintain health over time, the knowledge of which would have untold medical value. To decipher these mechanisms that set both lifespan and healthspan, we have mined genomic variation at the evolutionary limits of natural longevity, including Rockfishes, surviving up to 205 years, and Dwarf Gobies, surviving only 59 days in nature. We also capitalize on longevity variation across mammals, for which diverse genomic resources are available from well-characterized lineages. Using TRACCER, a new tool to detect evolutionary rate convergence, we identify genes and pathways evolving with shifts in longevity. Canonical pathways associated with aging, such as insulin-signaling, are prominent in these analyses, but we also identify other, less orthodox gene sets. Our data include “flavonoid metabolism” underlying rockfish longevity, as well as “chromatoid body” underlying pan-mammalian longevity, the latter of which may have an unappreciated role in maintaining somatic genomic integrity. We directly compared these evolutionary gene sets with human variation data and identified the “flavonoid metabolism” as significantly associated with human survival to the 99th percentile; the same gene set is underlying the evolution of longevity in both rockfish and humans. These genes have broad impacts on detoxification and hormonal signaling, with intriguing implications in the regulation of aging and natural history traits. Guided by this data, we are targeting genetic interventions to model this pathway’s effects on aging phenotypes in both wild-type and accelerated aging mutant zebrafish. In this experimental model, we assess age-related epigenetic changes, deterioration of gut integrity, stem-cell maintenance, senescence-associated histochemical marks, and lifespan itself. Just as rockfish can survive for two centuries while maintaining neural, immune, and musculoskeletal function, the variations we have uncovered could have conserved roles to delay or prevent age-related diseases in people.

Presenter: John M. Baronas

Institution: Massachusetts General Hospital

Poster Number: 34

Title: Exploring the Function of Protein Inhibitor of Activated STAT 1 (Pias1) to Maintain Chondrocyte Immaturity in Growth Plate Chondrocytes

Authors: John M. Baronas, BS, Henry M. Kronenberg, MD, Joel N. Hirschhorn, MD, PhD, Nora E. Renthal, MD, PhD

Abstract:

Protein Inhibitor of Activated STAT 1 (PIAS1) is a member of the PIAS family of proteins that function as SUMO E3 ligases in the post-translational regulation of gene expression. PIAS1 is involved in the regulation of multiple cellular processes, including transcriptional regulation, cell cycle control, and DNA damage response, however, the function of PIAS1 during chondrocyte maturation and growth plate development has not been studied. Our project focuses on investigating the role of PIAS1 in growth plate chondrocytes. Using a CRISPR-based genome-wide knockout (KO) screens, our laboratory previously identified 145 genes that significantly alter chondrocyte proliferation and maturation, with PIAS1 KO showing a marked effect on chondrocyte maturation, namely that loss of PIAS1 drove early maturation of chondrocytes. Our findings suggest that PIAS1 plays a crucial role in the growth plate by prolonging growth and delaying hypertrophy. Studies are ongoing to examine the molecular mechanisms by which PIAS1 regulates chondrocyte maturation and growth plate development. Our study aims to provide a better understanding of the regulatory pathways involved in growth plate development and contribute to the development of novel therapies for growth-related disorders, such as achondroplasia and hypochondroplasia.

Presenter: Yu Liu

Institution: Boston University School of Medicine **Department:** Department of Orthopaedic Surgery

Poster Number: 35

Title: Prx1 Muscle Cell Population needs to be Activated

Authors: Yu Liu, Louis C. Gerstenfeld, Beth Bragdon

Abstract:

Prx1 expression connotes a postnatal stem/progenitor cell population contributing to bone homeostasis, fracture, and ectopic bone and is located in various tissues. Questions remain as to heterogeneity and functional differences of these Prx1 cell populations. All procedures were approved by IACUC. The tamoxifen inducible Prx1 reporter mouse was used to fluorescently tag the Prx1 population. Male and female mice, 8-12 weeks, were used and received two doses of tamoxifen. Gelatin sponges with BMP2 (0 µg – 5.0 µg) were implanted at the femoral periosteal surface or within a muscular pouch for three days, followed by transplantation to a wild type mouse for bone formation assay. Transplants were to the periosteum, muscle, or fracture site. Radiographs detected mineralization. In a second set of animals, the Prx1 cells were isolated from the bone marrow, periosteum and muscle. Antibodies were used to isolate three subpopulations (P1: CD105-CD200+CD45-; P2: CD105-CD200-CD45-; and P3: CD105+CD200variableCD45-). Bulk RNA sequencing was performed. Stem cell activation analysis was completed by labeling of Ki67 and ATP quantification. The muscle Prx1 cells were only able to induce ectopic bone with increased levels of BMP2 (5.0 µg), low levels of BMP2 could not activate bone formation. Bulk RNA sequencing showed the muscle Prx1 cells were quiescent while periosteal Prx1 cells had varying levels of cell cycling. This was confirmed with Ki67 labeling. The muscle and periosteal Prx1 cells were

transplanted to a fracture at time of injury resulting in activation of both Prx1 cell populations. These results showed the periosteal Prx1 cells is proliferative and able to induce bone formation, however during homeostasis the muscle can inhibit this ability to form ectopic bone. The muscle derived Prx1 cells are highly quiescent and stimuli such as high levels of BMP2 or injury is needed to activate the muscle Prx1 cells to induce bone formation.

Presenter: Shannon R. Emerzian

Institution: Beth Israel Deaconess Medical Center

Poster Number: 36

Title: Cortical Bone Post-Yield Energy Absorption is Reduced in Older Adults with Long-Duration Type 1 Diabetes

Authors: Shannon R. Emerzian[1], Jarred Chow[1], Ramina Behzad[2], Daniel J. Brooks[1], I-Hsien Wu[3], John Gauthier[3], Surya Vishva Teja Jangolla[3], Marc Gregory Yu[3,4], Hetal S. Shah[3,4], George L. King[3,4], Lamya Karim[2], Fjola Johannesdottir[1], Elaine W. Yu[5], Mary L. Bouxsein

Abstract:

People with type 1 diabetes (T1D) have up to a 7-fold increased risk for hip fracture compared to non-diabetics, but the factors contributing to skeletal fragility in T1D are not well understood. The objective of this study was to quantify cortical bone material properties and accumulation of fluorescent advanced glycation end-products (fAGEs) in femoral bone from older adults with T1D.

Whole femora were acquired post-mortem from the Joslin Medalist Study, a cohort of individuals with T1D ≥ 50 years (n=21); femora of age- and sex-matched non-diabetic controls were also obtained (n=14). Cortical beams were extracted from the midshaft, polished to exact dimensions (2x2x40mm), and assessed via 4-point bending. Force, displacement, and geometry data were used to calculate the apparent cortical bone material properties. Collagen crosslinks via total fAGEs were quantified. T-test assessed group effects; mean±SD; α $p<0.05$.

The T1D cohort was 57% women with disease duration of 67±6 years and age at death of 80±8 years; age and sex distribution were similar in controls ($p>0.7$ for both). Energy absorption was diminished in T1D (Table), with toughness to fracture (-25%, $p=0.033$, Fig1A) and toughness to maximum force (-22%, $p=0.038$, Fig1B) lower in T1D versus control. In contrast, elastic properties such as bending modulus were not different between groups ($p=0.85$, Fig1C). fAGE content was similar in T1D and controls ($p=0.12$, Fig1D).

The presented data suggest that the mechanical implications of T1D manifest in post-yield energy absorption deficits in cortical bone, which may contribute to increased fracture risk in T1D. Although reduced toughness and post-yield behavior are attributed to altered matrix collagen such as increased AGEs, here fAGEs were similar in T1D and controls. Further investigation is necessary to determine the cause of the significant decrease in post-yield properties in T1D.

Presenter: Neilesh Frings

Institution: Boston University **Department:** Biomedical Engineering Department

Poster Number: 37

Title: Risk of vertebral endplate failure during vertebral fracture

Authors: Neilesh Frings, Elise Morgan

Abstract:

Vertebral fracture (VF) is the most common type of osteoporotic fracture[1]. The *vertebral endplate region*, located at the superior and inferior end of each vertebra, is made up of the cartilage endplate, vertebral endplate (VEP), and underlying subchondral trabecular bone (STB). This region plays a critical role in spinal loading and is frequently involved in VF. VF does not always include fracture of the VEP[2,3], but those that do are associated with a higher risk of future disc degeneration and worsening of the fracture over time[3-5]. The conditions responsible for these different modes of failure are unclear; their identification can aid in evaluation of injury risk and need for preventative treatment[6]. This study aimed to use high-resolution micro-finite element (μFE) models to evaluate relative risk of failure in the VEP, STB, and other portions of the vertebral body at the point of onset of VF.

Models were built from micro-CT scans (0.074mm resolution) of L2 vertebrae previously scanned and mechanically tested[2]. Superior and inferior boundaries of the models were prescribed experimentally matched displacements corresponding to the yield point of the compression test. Volumes of interest (VOIs) were defined on coronal image slices and mapped to the model (Figure 1).

High strains in the vertebrae were frequently observed in the superior endplate and cortical shell (Figure 2). The proportion of elements that yielded varied among vertebrae (Figure 3A), and was not different across VOIs. When normalizing VOI yield fraction to each sample, however, the VEP exhibited greater risk of failure (Figure 3B). These results indicated that at the onset of vertebral fracture, tissue-level yield typically occurs in the VEP, and to a lesser extent, the rest of the vertebral body. These results are consistent with prior observations that VF frequently involve the superior endplate region[7,8].

Presenter: Sherri-Ann M. Burnett-Bowie

Institution: Massachusetts General Hospital **Department:** Department of Medicine

Poster Number: 38

Title: Racial and Ethnic Disparities in Metabolic Bone Disease

Authors: Lauren Y. Maldonado, MD, MPH,[1,2]* Linette Bosques, MD, PhD,[1]* Sara J. Cromer, MD,[3] Sharl S. Azar, MD,[4] Elaine W. Yu, MD, MMSc,[3] Sherri-Ann M. Burnett-Bowie, MD, MPH[3]

Abstract:

Background: Racial and ethnic disparities impact the prevalence and management of metabolic bone disease (MBD) in osteoporosis, metastatic cancer, and sickle cell disease (SCD). Osteoporosis remains under-screened and undertreated in Asian, Black, Indigenous, and Latinx populations. Skeletal-related events (SREs) in metastatic cancer reduce survival and are associated with loss of social functioning, decreased quality of life, and significant medical cost. MBD contributes to morbidity in SCD; however, clinical guidelines for screening and treatment do not exist.

Purpose: (1) Highlight disparities in osteoporosis screening and treatment and in SREs management. (2) Discuss major gaps in bone health management in SCD. (3) Provide clinical recommendations to decrease disparities and guide future research efforts.

Methods: The following keywords were searched in PubMed: MBD, osteoporosis, metastatic bone disease, SCD, health disparities. We identified a total of 90 articles and reviewed and summarized the pertinent data.

Findings: Screening tools and safe, effective therapies for osteoporosis are available; however, screening and treatment rates are lower and disease morbidity is higher in minoritized populations. Risk calculators and scoring mechanisms worsen diagnostic and treatment disparities in osteoporosis. Racial and ethnic minority groups are disproportionately affected by SREs due to metastatic bone disease yet experience greater delays in treatment and are more likely to present with advanced disease. Patients with SCD, who are predominantly Black, experience SCD-related complications including avascular necrosis and low bone mineral density. The role of bisphosphonates in optimizing SCD-related bone health is poorly understood. Given the high prevalence of vitamin D deficiency among SCD patients, routine screening, and empiric vitamin D supplementation higher than RDA is recommended.

Conclusion: Clinical recommendations (Table) are provided to raise awareness and close healthcare gaps. Further studies should clarify the impact of these diseases on Asian, Black, Indigenous, and Latinx populations and solidify recommendations for treatment and management.

Presenter: Christine Lary

Institution: Roux Institute at Northeastern University

Poster Number: 39

Title: A Meta-analysis of the association between bone density measures and the risk of dementia

Authors: Christine W. Larya,b PhD, Samuel Ghatanc BS, Meghan Geretyd BS, Alexandra Hintonb MPH, Archana Nagarajana,b,e BS, Clifford Rosenb MD, Ryan D. Rossf PhD, David A. Bennettg MD, Anita L. DeStefanoh PhD, Mohammad A. Ikramc MD, PhD, Fernando Rivadeneirac MD, PhD, Douglas P. Kieli,j MD, Sudha Seshadrik,l MD, Alexa Beiserh,l PhD

Abstract:
We sought to measure the association between bone mineral density (BMD) or annualized decline in BMD (prior bone loss) and incident dementia in the Framingham Heart Study (FHS), the Rotterdam Study (RS), and the Rush Memory and Aging Project (MAP). We included individuals with one or two BMD assessments aged ≥60 years and free of dementia at baseline with follow-up available. BMD was measured at the hip femoral neck using dual-energy X-ray absorptiometry (DXA) in FHS and RS and at the heel calcaneus using quantitative ultrasound to calculate eBMD (estimated BMD) in MAP. Cox proportional hazards models were used to examine the associations between baseline BMD or prior bone loss (annualized percentage change in BMD prior to baseline) and incident dementia or AD adjusting for age, sex, body mass index (BMI), ApoE4 genotype, and educational attainment, and family structure in FHS as a random effect. We performed a meta- analysis of baseline BMD across all three studies and prior bone loss in FHS and RS using fixed and random effects models using the inverse variance method to pool results. Our combined sample size was 4,436 participants in three studies with 606 incident dementia diagnoses, 498 of which were AD. We found that higher BMD in cognitively intact adults is associated with a lower risk of ADRD diagnosis, accounting for confounders, with a hazard ratio of 0.47 or 53% reduced risk per g/cm2 (95% CI: 0.24-0.96, p=0.038), but that prior bone loss did not show a consistent association across studies. In future work we propose to bring together several longitudinal aging studies with detailed bone and dementia assessments to determine how the magnitude of these associations differ according to age, sex, and race and ethnicity. This work may lead to novel screening approaches for each disease and novel treatment targets and mechanisms.

Presenter: Soha Ben Tahar

Institution: Northeastern University

Poster Number: 40

Title: Use of 3D Turing patterns to model skeletal development.

Authors: Soha Ben Tahar, Sandra Shefelbine

Abstract:
Summary: Embryonic development remains a fascinating puzzle that has captivated researchers for over a century. Understanding how these patterns emerge is crucial for unraveling the mysteries of embryogenesis. One key factor in this process is the role of morphogens, chemical signals that cells use to communicate with each other, which play a crucial role in determining cell fate and tissue specialization. Alan Turing proposed a model for how morphogens work, known as the reaction- diffusion system, which has been used to model patterning in a variety of biological applications. However, the emergence of patterns in 3D domains has been sparsely investigated, and this gap in the literature highlights the need for further investigation on Turing pattern formation in 3D growing domains and how it can be linked to the skeleton formation.
In this abstract, we investigate the pattern evolution in 3D growing domains using finite element analysis. Additionally, we identify patterns during growth using light sheet imaging of morphogens during the axolotl's limb regeneration. By exploring both numerically and experimentally patterns, we aim to gain insights into how morphogens play a role in shaping the complex 3D morphology of skeletal tissue.

Presenter: Milica Sipovac

Institution: University of Novi Sad Faculty of Medicine

Poster Number: 41

Title: Neonatal line width in primary teeth from the Early Neolithic and Modern ages

Authors: Milica Sipovac

Abstract:

The effect of physiological and pathological stress factors on the organism during the process of amelogenesis leaves visible traces in the enamel in the form of macroscopically visible hypoplasia and microscopically visible accentuated lines. Due to the organism's adaptation to extrauterine life conditions, biological stress causes the formation of the first incremental line in the enamel - the neonatal line. The purpose of this study was to determine whether children from Early Neolithic Age (5900-5500 BC) and modern populations differ in terms of the width of the neonatal line. The sample (N=22) consisted of two groups: 11 deciduous incisors removed from the jaws of children (skeletal age range from 38-40 gestational weeks) whose skeletal remains were found in Early Neolithic archaeological graves in Lepenski Vir, Serbia, and 11 present-day exfoliated deciduous incisors from 6–11-year-old children. The analysis was carried out on ground sections with a scanning electron microscope. Two clinicians measured the width of the neonatal line. Neonatal line was visualized in most of the samples ranging between 2 μm and 30 μm. The Mann-Whitney U test confirmed that there was a statistically significant difference between the children from the Early Neolithic (Median=11.56) and Modern ages (Median=5.95) with respect to the width of the neonatal line ($U=129$, $Z=-4.63$, $p< .0001$). This study is the first to investigate the width of the neonatal line in teeth of Early Neolithic children and Serbian children from the Modern age. The wider neonatal line of children from the Early Neolithic age indicates the possibility that they have experienced more overall stress in perinatal life.

Presenter: Divya Venkatasubramanian

Institution: Boston Children's Hospital

Poster Number: 42

Title: Lineage-Specific Differences and Inference of Regulatory Networks Governing Human Chondrocyte Development

Authors: Divya Venkatasubramanian, Daniel Richard, Steven Pregizer, Rosanne Raftery, Terence Capellini, April Craft

Abstract:
To address large gaps in our understanding of the molecular regulation of articular and growth plate cartilage development in humans, we used our directed differentiation approach to generate distinct cartilage tissues from human embryonic stem cells (hESCs). The resulting transcriptomic profiles of hESC-derived articular and growth plate chondrocytes were similar to fetal epiphyseal and growth plate chondrocytes, with respect to genes both known and previously unknown to cartilage biology. With the goal to characterize the regulatory landscapes accompanying these respective transcriptomes, we mapped chromatin accessibility in hESC- derived chondrocyte lineages, and mouse embryonic chondrocytes, using ATAC-sequencing. Integration of the expression dataset with the differentially accessible genomic regions revealed lineage-specific gene regulatory networks. A closer analysis of how gene expression variance was controlled by epigenetic accessibility revealed more biologically relevant information, including several TFs and TF families previously unknown in cartilage biology, whose role in chondrogenesis can now be further investigated. Transcriptomic profiles of hESC-derived cartilage across developmental time also revealed TFs that may play a role in specification of chondrocytes to these distinct lineages. We functionally validated binding of two transcription factors (RUNX2 in growth plate chondrocytes and RELA in articular chondrocytes) with their predicted genomic targets. The maps we provide thus represent a framework for probing regulatory interactions governing chondrocyte differentiation. This work constitutes a substantial step towards comprehensive and comparative molecular characterizations of distinct chondrogenic lineages, and sheds new light on human cartilage development and biology.

Presenter: Nicola Blum

Institution: Boston Children's Hospital **Department:** Department of Orthopedics

Poster Number: 43

Title: Running the red light – defining signals driving progressive growth in skeletal disorders

Authors: Nicola Blum and Matthew P. Harris

Abstract:
How tissues and organs control size and prevent overgrowth is a fundamental yet poorly understood question. We study mosaic overgrowth disorders to understand the mechanisms that act as a brake to stop growth. Probably the most bizarre mosaic disorder is Proteus syndrome characterized by progressive focal bone overgrowth. The most famous case of what some suggest could be Proteus syndrome is that of Joseph Merrick, known as the Elephant Man. Patients with Proteus syndrome carry clones with a well-known somatic oncogenic mutation in AKT1 scattered throughout their tissues and organs. However, while overgrowth occurs, it is unknown whether the mutation is sufficient to drive progressive skeletal overgrowth that is a hallmark of this disorder. We developed a zebrafish model for Proteus syndrome, that allow in vivo imaging of AKT1 mutant clones throughout life, to explore how mutant clones overcome growth control mechanisms leading to progressive overgrowth. Of note, our model is the first animal model that recapitulates bone phenotypes seen in patients. Surprisingly, our experiments reveal differential growth dynamics of clones. While all clones cause an initial burst in bone formation, only a small subset of clones cause progressive bone overgrowth. These findings indicate that oncogenic AKT1 is necessary but not sufficient to cause progressive overgrowth and

demonstrates the importance of modifying factors in Proteus syndrome. We have begun to identify these factors and testing whether these attributes can be leveraged to design interventions or treatments.

Presenter: Stephanie L. Tsai

Institution: Massachusetts General Hospital **Department:** Department of Orthopaedic Surgery

Poster Number: 44

Title: Endogenous Tenocyte Activation Underlies the Regenerative Capacity of Adult Zebrafish Tendon

Authors: Stephanie L. Tsai1, Steffany Villasenor1, Rishita Shah2, Jenna L. Galloway1,3*

Abstract:
Tendons are essential, frequently injured connective tissues that transmit forces from muscle to bone. Their unique highly ordered, matrix-rich structure is critical for proper function. While adult mammalian tendons heal after acute injuries, endogenous tendon cells, or tenocytes, fail to respond appropriately, resulting in the formation of disorganized fibrovascular scar tissue with impaired function and increased propensity for re-injury. Here, we show that unlike their mammalian counterparts, adult zebrafish tenocytes activate upon injury and fully regenerate the tendon. Using a full tear injury model in the adult zebrafish tendon, we define the hallmark stages and cellular basis of tendon regeneration through multiphoton imaging, lineage tracing, and transmission electron microscopy approaches. Remarkably, we observe that the zebrafish tendon can regenerate and restore normal collagen matrix ultrastructure by 6 months post-injury (mpi). We show that regeneration progresses in three main phases: inflammation within 1 day post-injury (dpi), proliferation and formation of a cellular bridge between the severed tendon ends at 3-5 dpi, and differentiation/matrix remodeling beginning from 5 dpi to 6 mpi. Foremost, we demonstrate that pre-existing tenocytes are the main cellular source of regeneration. Ongoing research is centered on utilizing cross-species comparative approaches to identify divergent mechanisms driving tendon regeneration versus fibrosis at single cell resolution. Collectively, our work debuts the zebrafish tendon as one of the only reported adult tendon regenerative models, thereby positioning it as an invaluable comparative system that may be leveraged to elucidate mechanisms required for regeneration that may inspire new treatments in the clinic.

Presenter: Anna Wadhwa

Institution: Beth Israel Deaconess Medical Center **Department:** Harvard Medical School

Poster Number: 45

Title: Space-like radiation leads to deficits in vertebral bone density and microarchitecture in male Alzheimer's-like transgenic mice

Authors: Anna Wadhwa[1*]; Shannon R. Emerzian, PhD[1*]; Takaomi C. Saido, PhD[2]; David M. Holtzman, MD[3]; Mary L. Bouxsein, PhD[1]; Cynthia A. Lemere, PhD[4].

Abstract:
Irradiation and Alzheimer's are both associated with diminished bone health, though their combined effects are not fully understood. This is of consideration for long-duration space exploration missions, where chronic radiation exposure and age-associated comorbidities—such as Alzheimer's—are of great concern. The objectives of this study were to compare murine bone microstructure in an Alzheimer's model to wild type, and to quantify late effects of radiation exposure on bone microstructure in the Alzheimer's model. APPNL-F/NL-F knock-in mice were crossed with human APOE3 floxed targeted replacement mice on a C57BL/6 background (ALZ). Male ALZ mice were exposed to 0.75Gy 5-ion mixed field beam irradiation (GCRsim) (n=9) or 2Gy gamma irradiation (n=7) at 7 months of age. Sham-treated male ALZ (n=9) or WT mice (n=6) were used as controls. Mice were sacrificed at 17 months of age; lumbar vertebral trabecular bone structure was assessed using micro-computed tomography. ANOVA with Tukey post-hoc assessed group effects; data reported as mean±2 SD; α $p<0.05$.

ALZ-Sham mice had greater bone mineral density (198±10 mgHA/ccm vs. 171±14 mgHA/ccm; p=0.048) and trabecular number (4.10±0.2 vs. 3.7±0.2 mm-1; p=0.004) than WT-Sham. ALZ-GCRsim mice had lower bone mineral density (-16%, p=0.008), bone volume fraction (-23%, p=0.005), trabecular number (-9%, p=0.001), trabecular thickness (-11%, p=0.023), connectivity density (-21%, p=0.011), and greater trabecular separation (+11%, p=0.001) compared to ALZ-Sham. In contrast, bone microstructure in ALZ-Gamma mice was not significantly different from ALZ-Sham.
In this ALZ model, gamma radiation did not influence bone microstructure, whereas GCRsim induced significant deficits in bone density and microstructure that likely increase fracture risk. While this ALZ model did not have adverse effects on lumbar vertebral bone structure compared to WT, the possible compound effects of GCRsim and ALZ should be further explored in a more severe ALZ model (APOE4 replacement).

Presenter: Name

Institution: Boston University
Department: Department of Mechanical Engineering and Center for Multiscale and Translational Mechanobiology

Poster Number: 46

Title: Age-related mechanical degradation of cortical bone is driven by microstructural changes in addition to porosity

Authors: André F. Gutiérrez Marty, Paul E. Barbone, Elise F. Morgan

Abstract:
This study aims to gain mechanistic understanding of how aging-related changes in the microstructure of cortical bone drive mechanical consequences at the macroscale. To that end, cortical bone was modeled as a bundle of elastic-plastic, parallel fibers loaded in uniaxial tension, which comprised osteons and interstitial tissue. Distinct material properties were assigned to each fiber in either the osteon or interstitial fiber "families." Models representative of mature (20-60 yrs.) bone, and elderly (60+) bone were created. Aging-related changes were modeled along three independent dimensions: (i) increased porosity, (ii) increased ratio of osteon fibers relative to interstitial fibers, and (iii) a change in fiber material properties.
The model captured decreases in modulus, yield stress, yield strain, ultimate stress, ultimate strain, and toughness with age of 14%, 11%, 8%, 6%, 20%, and 30%, respectively. In both mature and elderly bundles, rupture of the interstitial fibers drove the initial loss of strength following the ultimate point. Plasticity and more gradual rupture of the osteons drove the remainder of the response. Both the onset and completion of interstitial fiber rupture occurred at lower strains in the elderly vs. mature case.
Changes along all three dimensions were required for the model to capture aging-related decline in the strength, ductility, and toughness of cortical bone. These findings point to the importance of studying microstructural changes beyond porosity, such as the area fraction of osteons and the microconstituent material properties of osteon and interstitial tissue, in order to further our understanding of aging-related changes in bone.

Presenter: Xiaomeng You

Institution: Brigham and Women's Hospital **Department:** Depar tment of Orthopedic Surgery

Poster Number: 47

Title: Gut microbiome promotion of bone growth in early life is mediated by GPR43

Authors: Xiaomeng You[1], Cole Hodys[1], Kelly Tsang[1], Julia F. Charles[1,2]

Abstract:
G protein coupled receptor 41 and 43 (GPR41 and GPR43) are primarily activated by microbiome derived short-chain fatty acids (SCFA) and play an important role in mediating host-microbiome interaction. Emerging evidence suggests an important role of gut microbiome in bone homeostasis, though most studies

focus on adult or aging subjects. The impact on skeletal growth in early life is not well characterized. In this study, we tested the hypothesis that gut microbiome would promote bone growth in weaning age mice in a GPR41/43 dependent manner.
Murine gut microbiota was depleted by 2 weeks of vancomycin supplemented drinking water. We found that vancomycin treatment significantly reduced femur longitudinal growth in weaning age (3.5-week-old) wild-type (WT) mice. In contrast, skeletal growth in young adult (5.5-week-old) WT mice was not sensitive to vancomycin treatment. Colon expression of Gpr41 and Gpr43 was significantly reduced by vancomycin treatment. Gut microbiota effects are likely mediated by SCFA, as 3 weeks of propionate supplementation to vancomycin treated mice stimulated bone growth and increased bone marrow Runx2 and Wnt10b gene expression. The negative effect of microbiota depletion by vancomycin on skeletal growth was abrogated in Gpr43-/- mice but indistinguishable from WT in Gpr41-/- mice. Growth plate thickness was significantly reduced by vancomycin in WT mice but not in Gpr43-/- mice. Moreover, both WT and Gpr43-/- mice showed decreased fecal microbial diversities and SCFA production in response to vancomycin treatment. No fundamental difference in microbial compositions, functions and SCFA production was found between WT and Gpr43-/- mice. Thus, GPR43 primarily mediates host response to microbiome during bone growth.
In conclusion, early life is a critical time window during which gut microbiome has a significant impact on skeletal growth in mice. This effect appears to be mediated by SCFA and their receptor GPR43 but independent of GPR41.

Presenter: Chilan B. G. Leite

Institution: Brigham and Women's Hospital **Department:** Department of Orthopedic Surgery

Poster Number: 48

Title: Physiological production of specialized pro-resolving mediators does not prevent posttraumatic osteoarthritis after anterior cruciate ligament transection

Authors: Chilan B. G. Leite1, Luciana P. Tavares2, Julie Mekhail1, Gergo Merkely1, Jessica Lehoczky1, Janey Whalen1, Julia F. Charles1, Christian Lattermann1

Abstract:
INTRODUCTION: Anterior cruciate ligament (ACL) tear results in immediate activation of inflammatory responses that, if unresolved, can cause culminate in posttraumatic osteoarthritis (PTOA). Resolution of inflammation is an active process modulated by the specialized pro-resolving mediators (SPMs), omega-3/6 fatty-acid-derivatives with anti-inflammatory and regenerative properties. Although SPMs have been detected in arthritis, their role in PTOA remains unclear. This study aimed to determine the levels of inflammatory and proresolutive markers overtime in a mice model of ACL transection (ACLT). We hypothesize that SPMs, particularly maresin1 (MaR1) and resolvinD1 (RvD1), are physiologically produced after ACLT; however, this production does not completely resolve the robust inflammation that leads to PTOA.
METHODS: Eight-weeks-old male and female mice underwent ACLT. At days 1, 7, 14, 21, 28, 56 post-injury (n=6/time-point), euthanasia was performed for collection of synovial fluid and the tibiofemoral joint. The contralateral (unoperated) knee served as control. Synovial fluid was used for measuring pro- inflammatory cytokines (TNF-α, IL-1β, IL-6) and SPMs (MaR1, RvD1) by ELISA. Tibiofemoral joint was used for PTOA evaluation through histology (Safranin-O; OARSI score) and microCT analyses.
RESULTS: ACLT induced an acute inflammation characterized by increased levels of pro-inflammatory cytokines, that peaked in the first week post-injury and declined afterwards, although evidence of chronic inflammation persisted overtime. Additionally, a proresolutive response was also induced, with increased levels of MaR1 and RvD1 peaking at day 7. Yet, significant findings of PTOA were observed in both histology and microCT ($p<0.0001$).
CONCLUSION: ACLT results in acute inflammation that declines overtime although tends to be sustained for long period post-ACLT. Additionally, as shown for the first time, SPMs (Mar1, RvD1) are produced in the joint after ACLT. Thus, physiological proresolutive response is triggered concomitantly with the inflammation

onset. However, the endogenous inflammation resolution post-injury is insufficient to joint tissues homeostasis return, leading to PTOA.

Presenter: Petra Simic

Institution: Massachusetts General Hospital **Department:** Nephrology Division

Poster Number: 49

Title: 1,25-dihydroxy vitamin D regulates furin-mediated FGF23 cleavage

Authors: Xie H[1,2], Bastepe I[1,2], Zhou W[1,2], Ay B[2], Ceraj Z[1,2], Portales Castillo I[1,2], Burnett-Bowie S[2], Jüppner H[2,3], Rhee EP[1,2], Bastepe M[2], Simic P[1,2*]

Abstract:
Fibroblast growth factor-23 (FGF23) is a phosphaturic hormone cleaved by furin from active intact FGF23 (iFGF23) into inactive N-terminal and C-terminal fragments. Several studies have implicated vitamin D in regulating furin in infections. Thus, we investigated the effect of 1,25-dihydroxy vitamin D (1,25(OH)2D) and the vitamin D receptor (VDR) on furin-mediated FGF23 cleavage. Mice lacking VDR (Vdr-/-) had a 25-fold increase in FGF23 cleavage, with increased furin levels and activity compared to wild-type (WT) littermates. Inhibition of furin activity blocked the increase in FGF23 cleavage in Vdr-/- animals and in a Vdr knock-down osteocyte OCY454 cell line. Chromatin immunoprecipitation revealed VDR binding to DNA upstream of the Furin gene, with more transcription in the absence of VDR. In WT mice, furin inhibition reduced FGF23 cleavage, increased iFGF23, and reduced serum phosphate levels. Similarly, 1,25(OH)2D reduced furin activity, decreased FGF23 cleavage, and increased total FGF23. In a post-hoc analysis of a randomized clinical trial, we found that ergocalciferol treatment significantly increased serum 1,25(OH)2D and decreased serum furin activity and FGF23 cleavage, compared to placebo. Thus, 1,25(OH)2D inhibits FGF23 cleavage via VDR-mediated suppression of Furin expression, thus providing a mechanism by which vitamin D signaling can augment biologically active, iFGF23 levels.

Presenter: Emily Moore

Institution: Harvard School of Dental Medicine

Poster Number: 50

Title: The role of BMP signaling in appositional bone growth

Authors: *Emily R. Moore, Gavin Chen, David Maridas, Kate Burton, Laura Gamer, Vicki Rosen*

Abstract:
The periosteum contains progenitor cells capable of differentiating into osteoblasts and chondrocytes that contribute to bone growth and repair. Bone morphogenetic protein (BMP) signaling is central to the activation and differentiation of periosteal cells, and mice lacking periosteal BMP2 (Prx1Cre;Bmp2fl/fl) have significantly thinner bones and exhibit a complete lack of fracture healing due to decreased periosteal osteogenesis. Here, we investigate the unique periosteal phenotype resulting from removal of BMP2 in periosteal lineage cells.
Periosteal tissue was collected from newborn Prx1Cre;Bmp2fl/fl mutants and Bmp2fl/fl littermate controls for bulk RNAseq analysis. Multiple markers for osteogenesis and BMP signaling were downregulated in mutants, suggesting Prx1Cre;Bmp2fl/fl mice have reduced periosteal activity. To further interrogate periosteal activity in appositional growth, we designed an ex vivo growth model. Briefly, femurs from P7 littermates were exposed to oscillatory fluid flow (OFF) for 5 consecutive days. Bmp2fl/fl femurs were slightly longer compared to Prx1Cre;Bmp2fl/fl femurs under static conditions and OFF did not influence length. Femurs from control Bmp2fl/fl mice widened significantly when OFF was applied, but this behavior was lost in Prx1Cre;Bmp2fl/fl explants. Histological analysis revealed the periosteum expanded and bone formation

was enhanced in the mid-diaphysis with OFF in control femurs. In contrast, the periosteal surface was comparatively inactive in Prx1Cre;Bmp2fl/fl femurs.
The study of periosteal cells is currently limited due to a lack of an immortalized cell line for in vitro experiments. Thus, we generated a periosteum-derived cell line in order to better study the role of BMP signaling in the periosteum. This cell line expresses established periosteal markers, engages in BMP signaling, and differentiates into chondrocytes, osteoblasts, and adipocytes. We further determined these cells respond to mechanical stimulation using a custom chamber system to apply OFF.
Collectively, we anticipate our bulk dataset and novel experimental tools will reveal important insights into periosteal cell activity and function.

Presenter: Yizhong Hu

Institution: Harvard School of Dental Medicine **Department:** Department of Developmental Biology

Poster Number: 51

Title: Piezo1 Regulates cGMP/PKG Signaling in Osteocyte Mechanotransduction

Authors: Yizhong Hu, Xinchen Wu, Yingzi Yang

Abstract:
Mechanical stimuli and osteocyte mechanotransduction are critical for maintenance of healthy adult bone. Following mechanical stimulation, increased [Ca2+]in initiates downstream signaling activities modulating bone anabolic responses. Enhanced cGMP/PKG signaling resulting from increased [Ca2+]in promotes osteoblast and osteocyte differentiation. Recent works from our lab and others have demonstrated that the force-gated calcium ion channel Piezo 1 at the cell membrane is required for load-induced bone adaptation. RNA-seq analysis of Piezo1-deficient mouse bones further demonstrated reduced cGMP/PKG signaling. We therefore hypothesize that Piezo1 regulates early mechanotransduction events through the cGMP/PKG pathway.
To test this hypothesis, we generated a Piezo1-/- OCY454 osteocyte cell line using CRISPR-Cas9. After 21-day differentiation, Piezo1-/- OCY454 expressed lower levels of osteocyte genes (DMP1, SOST, PHEX, TNFSF11) but higher levels of osteoblast genes (SP7, RUNX2, BGLAP) than WT OCY454, suggesting that Piezo1 deficiency impedes osteocyte differentiation. Furthermore, bulk RNA seq analysis of the differentiated Piezo1-/- and WT OCY454 revealed downregulation of hippo signaling and upregulation of Wnt signaling.
In WT OCY454, stimulation by fluid shear stress and Yoda1 treatment, a Piezo1 activator, induced PKG activation measured by increased VASP Ser239 phosphorylation. These effects were abolished in Piezo1-/- OCY454, suggesting that mechanically stimulated Ca2+-dependent PKG activation acts through Piezo1. Furthermore, mRNA and protein levels of PKG1 and PKG2 decreased significantly in Piezo1-/- OCY454. Pharmacological activation of YAP, a downstream effector of Piezo1, increased prkg1/2 mRNA and PKG1/2 protein levels, suggesting that Piezo1 may transcriptionally modulate PKG signaling via YAP. Using primary BMSCs isolated from Piezo1f/f mouse, deletion of Piezo1 by Ad-Cre infection reduced osteogenic differentiation measured by ALP staining, consistent with published studies. Importantly, PKG activation by 8-pCPT-cGMP treatment rescued osteogenic differentiation defects in Ad-Cre-infected Piezo1f/f BMSCs measured by the number of ALP-positive CFUs. Taken together, these results indicate that Piezo1 regulates cGMP/PKG signaling in osteocyte differentiation and mechanotransduction.

Presenter: Sung-Hee Seanna Yoon

Institution: Massachusetts General Hospital **Department:** Endocrine Unit

Poster Number: 52

Title: SIK2/SIK3 inhibitor increases cortical bone mass in a mouse model of CKD-MBD

Authors: Sung-Hee Seanna Yoon, Han Xie, Michael Mannstadt, Marc Wein

Abstract:
Chronic Kidney Disease (CKD) is defined as a decrease in renal function with low glomerular filtration rate, albuminuria, and/or structural abnormalities in kidney1. Compromised renal function results in inadequate filtration of waste products and minerals and is associated with markedly increased risk of bone and mineral disease (CKD-MBD). With secondary hyperparathyroidism, some CKD patients show high bone turnover associated bone loss, while some patients suffer from adynamic bone, which PTH resistance/hypo-responsiveness could be responsible for. Salt-Inducible Kinases (SIKs) were identified in bone and kidney to work as an important downstream mediator of PTH receptor signaling2,3. SIK inhibition by cAMP/PKA signaling upon PTH receptor activation results in increased renal *Cyp27b1* expression thus 1,25- vitamin-D production, showing PTH-like effects3. Similarly in bone, SIK inhibition increases *Rankl* and decreases *Sost* expression, thus stimulating bone turnover and net bone mass gain2. These PTH-like effects may be desirable in CKD-MBD with renal osteodystrophy and low 1,25-vitamin-D levels. Thus, we hypothesized that targeting SIKs, downstream of PTH receptor, would bypass PTH resistance and bring PTH-like effects. To test this, we examined the effects of SIK inhibitor (SK-124) in 0.2% adenine diet induced CKD model in 8-week old CD1 female mice. After 6 weeks of adenine diet, mice showed expected CKD-MBD changes including hyperphosphatemia, increased BUN, hyperparathyroidism, increased CTx bone resorption marker, and decreased trabecular thickness as well as cortical bone area and thickness in femurs. Daily SK-1244 treatment (40mg/kg) in the presence of adenine diet significantly alleviated hyperphosphatemia and hyperparathyroidism, and increased only the bone formation marker P1NP without changing CTx levels. This net bone formation stimulated by SK-124 led to complete rescue of CKD-associated bone changes including improved trabecular thickness and cortical bone area. Thus, daily SK-124 SIK2/3 inhibitor administration in adenine diet induced CKD mouse model showed beneficial effects in bone health and mineral homeostasis.

Presenter: Vineel Kondiboyina

Institution: Northeastern University. **Department:** Dept. of Bioengineering

Poster Number: 53

Title: *Calcium signaling in in-situ chondrocytes under dynamic compressive loading*

Authors: Vineel Kondiboyina1, Timothy Boyer1, Sandra J. Shefelbine1,2

Abstract:
Introduction: Chondrocytes respond to mechanical stimuli by increasing their intracellular calcium concentration. The response depends on the cellular environment. Previous studies have investigated chondrocytes under non-physiological strain rates or cells embedded in hydrogels, but the response of chondrocytes in their native environment under physiologically relevant cyclic loads has not been studied. This study aimed to investigate the calcium signaling response of in-situ chondrocytes under physiological cyclic compressive loads with varying frequency and strain rates.
Methods: Bovine cartilage explants were stained with a fluorescent calcium indicator dye and subjected to physiologically relevant cyclic compressive loads using a custom-built loading device secured on a confocal microscope. Calcium fluorescence intensities of the cells were tracked and analyzed using one- way ANOVA followed by a posthoc test with Tukey correction (α=0.05).
Results: The percentage of cells signaling increased in all loading conditions compared to the no-load baseline. The percentage of cells responding under 1Hz load was significantly greater than the slow ramp and 0.1Hz group. Similarly, the number of cycles had no effect on the calcium signaling response. The width and time between consecutive peaks were also not different between different loading conditions.
Conclusions: In-situ chondrocytes respond to physiological loads in a strain rate-dependent manner with an increased number of responsive cells and unaltered temporal characteristics.

Presenter: Rosanne Raftery

Institution: Boston Children's Hospital **Department:** Department of Orthopedic Surgery

Poster Number: 54

Title: *Stability and regenerative capacity of articular cartilage tissue derived from human pluripotent stem cells*

Authors: Rosanne M. Raftery, Steven K. Preziger, Sophia Kocher, Suyash Raj, Divya Venkatasubramanian, and April M. Craft

Abstract:
The ideal cell source for cartilage repair remains elusive, in part due to an incomplete understanding of articular chondrocyte specification during human joint development. We pioneered directed differentiation protocols to generate articular, or growth plate cartilage tissues, from human pluripotent stem cells (hPSCs) facilitating in depth investigation into human cartilage development. We first compared the transcriptomic profiles of hPSC-derived chondrocytes isolated from TGFB3-treated articular cartilage or BMP4-treated growth plate tissues to cells isolated from the developing epiphysis and growth plate of the human femur. Like the fetal epiphyseal cartilage, in vitro articular cartilage was zonally organized with both superficial zone chondrocytes that express *PRG4*, encoding lubricin, and intermediate zone-like chondrocytes that express COL2A1 and CNMD. In contrast, chondrocytes from the BMP4 treated tissues were highly proliferative and in the process of undergoing hypertrophy, like those in the fetal growth plate. With the knowledge that the hPSC-derived cartilage tissues behaved similarly to developing cartilage, we next wanted to determine if they can function as permanent cartilage, an important characteristic for clinical translation. To test the stability of TGFB3-treated articular chondrocytes to resist hypertrophy, such as that observed in osteoarthritis and in the growth plate, we challenged the tissues with BMP4 and found that they gained resistance to hypertrophy after 8-10 weeks. Importantly, they retained their ability to respond to TGFβ3, indicating a commitment to the articular chondrocyte lineage, and suggesting that the cells will respond favorably to compressive loads experienced in weight-bearing joints. Thus, the newly identified differentially expressed genes between articular and growth plate-like chondrocytes during differentiation likely contain important players in cell fate decisions and lineage commitment, which may translate as critical quality attributes clinically. The results presented here highlight the importance of this hPSCs model of human articular cartilage development and their immense translational potential for cartilage repair.

Presenter: Courtney M. Mazur

Institution: Massachusetts General Hospital **Department:** Endocrine Department

Poster Number: 55

Title: *Subcellular transcriptomics reveals selective mRNA trafficking to osteocyte dendrites*

Authors: Courtney M. Mazur, Christian D. Castro Andrade, Parthena Kotsalidis, J. Matthew Taliaferro, Marc N. Wein

Abstract:
To bring together the proteins that define each subcellular compartment, some cells traffic mRNA for local translation. This results in a local transcriptome that contributes to specialized morphology and function in subcellular regions like neuronal dendrites and fibroblast protrusions. Since osteocyte dendritic projections share some morphologic characteristics with neurons, and genes linked to osteocyte dendrite formation are also involved in neuron cell projections, we hypothesized that osteocytes traffic mRNA to dendrites as a regulatory mechanism controlling dendrite formation and maintenance.
Osteocyte-like Ocy454 cells were grown on transwell membranes, which support the cell body while allowing dendritic projections to grow through 1 μm pores. To fractionate cells, the membranes are gently scraped to collect cell bodies, and then dendrites are extracted from the membranes using protein or mRNA lysis buffer.

3D imaging of membranes confirms that scraping depletes DAPI-stained nuclei but leaves phalloidin-stained dendrites. mRNA sequencing of fractionated osteocytes showed that dendrites contain thousands of mRNA transcripts, with 420 mRNAs significantly enriched in dendrites versus cell bodies (log2FC31, padj<0.01). We confirmed dendrite enrichment of 15 genes in independent samples, as well as cell body enrichment of Pth1r.
To ask if osteocytes can translate mRNA into protein in dendrites, we first measured expression of ribosomal RNA and proteins. rRNAs 18s, 28s, and 5.8s were detected in osteocyte dendrite fractions by RT-qPCR, and ribosomal protein Rpl26 was detected by Western immunoblotting. Short term (10-30 minute) puromycin labeling of osteocyte dendrites in transwell membranes immediately after scraping away cell bodies revealed puromycylated proteins, suggesting that protein synthesis occurs locally in dendrites.
Together this work suggests that mRNA is trafficked to osteocyte dendrites and locally translated. Investigation of this molecular regulatory mechanism may lead to improved understanding of osteocyte dendrite formation and maintenance, as well as novel therapeutic targets for bone diseases involving osteocytes.

Presenter: Fjola Johannesdottir

Institution: Beth Israel Deaconess **Department:** Center for Advanced Orthopaedic Studies

Poster Number: 56

Title: Femoral Neck Bone Density and Structure in Older Adults with Longstanding Type 1 Diabetes: A Case-Control Study

Authors: Fjola Johannesdottir1,2, Trinity Tedtsen1, Laura Michelle Cooke3, Meng Zhang1, Sarah Mahar1, Jordan Nustad1, Margaret Ann Garrahan3, Sarah E. Gehman3, Elaine W. Yu2,3, Mary L. Bouxsein1,2,3

Abstract:
Adults with type 1 diabetes (T1D) have increased hip fracture risk, yet no studies have assessed bone density or structure at the hip in older adults with T1D. We used previously collected CT scans of the proximal femur from older adults with T1D and non-diabetic controls (CON) to identify bone deficits that may contribute to hip fracture in T1D.
In this retrospective case-control study, we identified 101 adults with T1D and 185 age-, sex- and race-matched CON who had underwent CT exams. Exclusion criteria included history of malignancy, CKD (stage 4 or higher) and history of abnormalities in bone metabolism other than osteoporosis. We used the CT scans to measure areal BMD (aBMD); total (Tt), trabecular (Tb) and cortical (Ct) volumetric BMD (vBMD); and cross-sectional area (CSA) and CtCSA at the femoral neck (FN) using validated phantomless calibration (CliniQCT, Mindways, Austin, TX, USA). We employed linear models to estimate the differences in bone outcomes between T1D and matched CON with adjustment for age,
height, weight and sex.
Among T1D, 34% had nephropathy, 62% had neuropathy and 70% had retinopathy. Within the whole cohort, T1D tended to have lower FN densities, though differences did not reach statistical significance (Figure). The subset of T1D who were diagnosed before age 20 had lower FN aBMD (-7.2%), lower TbvBMD (-7.6%), and smaller CtCSA (-10%) than their matched CON ($p<0.05$). T1D who were diagnosed at a later age did not differ from CON ($p>0.64$). T1D with nephropathy had lower FN aBMD (-9.9%), lower TtvBMD (-8.0%) and smaller CtCSA (- 13%) compared to matched CON ($p<0.05$). Bone outcomes in subgroups of T1D with neuropathy and retinopathy did not differ from CON ($p>0.12$).
These novel observations highlight the longstanding detrimental impact of T1D when present during bone accrual and the importance of preventing nephropathy to inhibit bone complications.