4th Annual Symposium on Musculoskeletal and Neuromuscular Disorders

University of Missouri-Kansas City November 22, 2019

Below are some of the abstracts and the agenda for the 4th Annual Symposium on Musculoskeletal and Neuromuscular Disorders which was held on the University of Missouri-Kansas City campus on November 22, 2019. Also, some pictures I took on my cell phone. This is a consortium of four universities: University of Kansas Medical Center; University of Missouri-Kansas City; University of Missouri-Columbia; and Kansas City University (the regions Osteopathic medical school. The Vice Chancellors of Research of the respective universities formed the consortium in 2015 to promote faculty at each institution who are interested in musculoskeletal disorders to work together. Musculoskeletal disorders for the purposes of KCMD included disorders or biology of bone, joints, cartilage, muscle, peripheral nerve, neuromuscular junction and anterior horn cell and other motor neuron related disorders. So, it is a broad group of conditions and investigators. We have an annual meeting in the fall where investigators from all the universities meet and mingle and present their research. In January, we put out an RFA for grants that would be done by faculty on at least two campuses on a research project. We fund two or three a year with funding between $30,000 and $50,000 per project. We then have the teams present their findings at subsequent KCMD meetings.

Richard J. Barohn, MD
Kansas City Musculoskeletal Diseases Consortium
4th Annual Symposium on Musculoskeletal and Neuromuscular Diseases
November 22, 2019
Pierson Auditorium, Atterbury Student Success Center
University of Missouri Kansas City
5000 Holmes, Kansas City, MO 64110

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<td>10:00 am - 10:15 am</td>
<td>Welcome and Opening Remarks: Richard J. Barohn, MD, Vice Chancellor for Research, The University of Kansas Medical Center, Executive Director, KCMD Consortium</td>
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<td>10:15 am – 10:30 am</td>
<td>Pamela Tran, KUMC, and Erin Bumann, UMKC, “Role of genetic interaction between ciliary paralogs, Thm2 and Thm1, in postnatal skeletogenesis” – 2019 Pilot Grant Award Winner Presentation</td>
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<td>10:30 am – 10:45 am</td>
<td>Charlotte Phillips, MU, and Sarah Dallas, UMKC, “Compromised Mitochondrial Function in the Pathogenesis of Osteogenesis Imperfecta” – 2019 Pilot Grant Award Winner Presentation</td>
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<td>10:45 am – 11:00 am</td>
<td>David Upchurch, KSU, &quot;Administration of Adipose-derived Stromal Vascular Fraction and Platelet Rich Plasma in Dogs with Coxofemoral Osteoarthritis&quot;</td>
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<td>11:00 am – 11:15 am</td>
<td>Group Speaker Q&amp;A – Rick Barohn</td>
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<td>11:15 am – 11:30 am</td>
<td>Joan Coates, MU, “Canine Degenerative Myelopathy in Translation to ALS”</td>
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<td>11:30 am – 11:45 am</td>
<td>A. Baki Agbas, KCU, “Plasma Extracellular Vesicles TDP-43 assessment in ALS”</td>
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<td>11:45 am – 12:00 pm</td>
<td>Mark Johnson, UMKC, “Estrogen Action in Bone and Muscle Crosstalk”</td>
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<td>12:00 pm – 12:15 pm</td>
<td>Group Q&amp;A – Ed O’Connor</td>
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<td>12:15 pm – 1:45 pm</td>
<td>Lunch and Posters</td>
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<td>1:45 pm – 2:00 pm</td>
<td>Douglas Haase, KUMC, “Association between cartilage NFAT1 levels and severity of radiographic osteoarthritis in human hips”</td>
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<td>2:00 pm – 2:15 pm</td>
<td>Nicole Nichols, MU, and Teresa Lever, MU,” Upregulating neuroplasticity in surviving motor neurons to preserve upper airway function in a novel motor neuron disease model”</td>
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<td>Patty Kluding, KUMC, “Sedentary behavior and diabetic neuropathy”</td>
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<td>Group Q&amp;A – Chris Liu</td>
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2:45 pm – 3:00pm  BREAK and Posters

3:00 pm – 3:15 pm  Timothy Cox, UMKC, “Understanding clinical variability in craniofacial birth defects: opportunities to reduce the burden of reconstructive surgeries”

3:15pm – 3:30 pm  Steven Segal, MU, DDW Cornelison, MU, and Bret Ulery, MU, “Interactions among tissue components during skeletal muscle regeneration”

3:30 pm – 3:45 pm  Michael Wacker, UMKC, “Bone-Heart Crosstalk”

3:45 pm – 4:00 pm  Leslie Lyons, MU, and Gary Johnson, MU, “Precision/Genomic Medicine in Companion Animals – Rapid Identification of Musculoskeletal and Neuromuscular Disease Models”

4:00 pm – 4:15 pm  Duaa Jabari, KUMC, “Studying phenylbutyrate in inclusion body myositis – Rational and study design”

4:15 pm – 4:30 pm  Closing Remarks: Chris Liu, PhD, Vice Chancellor for Research Office of Research and Economic Development, UMKC, KCMD Executive Committee Member, KCMD Consortium

The Executive Committee of the Kansas City Musculoskeletal Diseases Consortium:

- Richard J. Barohn, M.D. Vice Chancellor for Research, The University of Kansas Medical Center and KCMD Executive Director

- Yusheng (Chris) Liu, PhD, Vice Chancellor for Research Office of Research and Economic Development, UMKC

- Mark A. McIntosh, Ph.D. Vice President for Research and Economic Development, UM System, and Vice Chancellor for Research, Graduate Studies and Economic Development, University of Missouri

- Edward R. O’Connor, Ph.D., MBA, FACHE, Provost and Executive Vice President for Academic, Research, and Student Affairs, Chief Academic Officer, Kansas City University
Symposium Speakers:

**Kansas City University (KCU)**

A. Baki Agbas, Professor of Biochemistry, Department of Basic Sciences

**Kansas State University (KSU)**

David Upchurch, Assistant Professor, Small Animal Soft Tissue Surgery

**University of Kansas Medical Center (KUMC)**

Douglas Haase, Resident, Department of Orthopedic Surgery

Duaa Jabari, Assistant Professor, Department of Neurology

Patty Kluding, Professor and Chair, Physical Therapy and Rehabilitation Science

Pamela Tran, Associate Professor, Department of Anatomy and Cell Biology/Kidney Institute

**University of Missouri (MU)**

Joan Coates, Professor, Neurology and Neurosurgery; Director, Comparative Neurology Program, Department of Veterinary Medicine and Surgery

D Cornelison, Professor, Biological Sciences, Professor, Department of Molecular Microbiology and Immunology

Gary Johnson, Associate Professor, Veterinary Pathobiology, Veterinary Pathobiology

Teresa Lever, Associate Professor, Department of Otolaryngology

Leslie Lyons, Gilbreath-McLorn Endowed Professor, Comparative Medicine; Director, Feline Genetics and Comparative Medicine Laboratory, Department of Veterinary Pathobiology

Nichole Nichols, Assistant Professor, Department of Biomedical Sciences; Investigator, Dalton Cardiovascular Research Center

Charlotte Phillips, Professor, Department of Biochemistry and Child Health

Steven Segal, Curators Distinguished Professor; Margaret Proctor Mulligan Professor in Medical Research, Department of Medical Pharmacology and Physiology

Bret Ulery, Assistant Professor, Department of Biomedical, Biological, and Chemical Engineering
University of Missouri-Kansas City (UMKC)

Erin Bumann, Assistant Professor, Department of Oral and Craniofacial Sciences

Timothy Cox, Endowed Chair in Musculoskeletal Tissues, Department of Oral and Craniofacial Sciences, School of Dentistry

Sarah Dallas, Lee M. and William Lefkowitz Endowed Professor, Department of Oral and Craniofacial Sciences

Mark Johnson, Professor and Chair, Department of Oral and Craniofacial Sciences, School of Dentistry

Michael Wacker, Associate Dean of Academic Affairs, Associate Professor and Vice Chair Biomedical Sciences Department
**Poster Presentations:**

**University of Kansas (KU)**

Jonathan Brumberg, Multisession brain-computer interface performance using a switch-scanning augmentative and alternative communication device by individuals with ALS

**University of Kansas Medical Center (KUMC)**

Yomna Badawi, Degeneration of ALS mouse neuromuscular junctions analyzed using super resolution microscopy and ameliorated using human mesenchymal stem cells

Ryan Funk, Utilization of the Collagen-Induced Arthritis Mouse Model to Evaluate Molecular Biomarkers of Methotrexate Efficacy in Autoimmune Arthritis

Aaron LacKamp, Ileus and dysautonomia can contribute to significant morbidity in Guillain-Barre syndrome independent of peripheral motor involvement

Takashi Matsuda, Applying Human Umbilical Cord Derived Mesenchymal Stem Cells for the Treatment of Amyotrophic Lateral Sclerosis

Mehrdad Maz, The Prevalence and Patterns of Celiac Disease Associated Arthropathy and Coexistence of Celiac Disease with Rheumatic Disorders in a Single Tertiary Medical Center

Mehrdad Maz, Efficacy of Mycophenolate Mofetil in the Treatment of Rheumatoid Arthritis Associated Interstitial Lung Disease

Mehrdad Maz, Pattern of Arthropathy in Patients with Cystic Fibrosis

Peter Rowe, ASARM reverses hyperphosphatemia, prevents calciphylaxis-like lesions, corrects renal, bone brain and cardiovascular calcification in a rat model of chronic kidney disease

Peter Rowe, Nephrogenic Systemic Fibrosis is induced in high phosphate diet CKD rats exposed to Gd3+ Binding Contrast Agents (GBCA): Role of ASARM peptides

Jinxi Wang, Treatment of posttraumatic arthrofibrosis using high intensity ultrasound and laser in a novel rabbit model of knee contracture

Mingcai Zhang, Mast cell deficiency amplifies inflammatory response in a mouse model of Kawasaki’s disease

**University of Missouri (MU)**

Lauren Borkowski, Accessory inspiratory muscle (e.g., pectoralis minor) activity is increased in a rodent model of respiratory motor neuron loss

Joan Coates, Positron Emission Tomography Spinal Imaging in a Canine Model of ALS
Rebecca Dirkes, Voluntary Wheel Running Partially Compensates for the Effects of Global Estrogen Receptor-α Knockout on Cortical Bone in Young Male Mice

Brian Flesner, Multi-Modal Pain Assessment of Client-owned Dogs with Primary Bone Tumors

Ashley Kloepper, Developing Swallow-Evoked Potentials (SwEPs) to Identify Pathological Neural Generator Sources for Targeted Dysphagia Treatment

Kerry McDonald, Preload-induced ventricular dysfunction in isolated hearts from Duchenne Muscular Dystrophy (DMD<sup>mdx-4Cv</sup>) mice

Charlotte Phillips, Skeletal muscle mitochondrial dysfunction and whole body metabolic alterations in a mouse model of osteogenesis imperfecta

David Schulz, Neurostimulation of Bladder Efferents After Spinal Cord Injury to Preserve Autonomic Ganglion Function

University of Missouri-Kansas City (UMKC)

Thiagarajan Ganesh, Biomechanical Role and Strength of the Anterolateral Ligament in the Rotational Control of the Knee

Loretta Laughrey, Multi-scale finite element analysis of: 3D multiplexed images to examine bone mechanotransduction and heterogeneous activation of β-catenin signaling in osteocytes.

Hammad Mumtaz, Jaw morphogenesis: a budding role of neural crest in mineral density, microarchitecture, and calcification of bone

Joel Robinett, Regulation of Myofilament Force and Loaded Shortening by Skeletal Myosin Binding Protein-C

Kun Wang, Overlapping properties and functions of matrix vesicles and exosomes/extracellular vesicles in bone cells
**Plasma Exosomal TDP-43 Assessment in ALS**

**A. Baki Agbas, MSc, Ph.D., Yajaira Marin Esqueda, Edina Kosa**

*Department of Basic Sciences, Kansas City University of Medicine and Biosciences, Kansas City, MO*

**Abstract:** Blood-based biomarkers are in high demand for monitoring the prognosis and early diagnosis of the neurodegenerative diseases. Our purpose is to study the extracellular vesicles and their protein content that may be assigned as a surrogate biomarker for ALS. Extracellular vesicles (EV) are excreted from cells into surrounding media and can be found in many, if not all, body fluids. Due to their small size (10-1000 nanometer), EVs can penetrate through blood brain barrier that makes them great interest in the search for biomarkers. We are working on optimizing the isolation of extracellular vesicles and their sub-groups (i.e., microvesicles and exosomes) from serum/plasma samples obtained from human ALS patients and healthy subjects. In initial phase of this project, we have isolated the exosomes from human plasma by using a commercial kit procedure based on the capture of water molecules, which otherwise form the hydrate envelope of particles in suspension. This method is easy to use and does not require any specialized equipment. Our laboratory has developed an interest to validate TDP-43 and its derivatives as potential blood-based surrogate biomarker for ALS. We have demonstrated that isolated exosomes from human plasma contain TDP-43 and its chemical derivative, phospho-TDP-43. The results from these assays will provide a valuable information about the TDP-43 protein profile. The outcome of this pilot study will establish an optimized working assay protocol to isolate, verify, and analyze the profile of misfolded/aggregated proteins in extracellular vesicles that can be assigned for a potential surrogate biomarker for ALS.
Title: Administration of Adipose-derived Stromal Vascular Fraction and Platelet Rich Plasma in Dogs with Coxofemoral Osteoarthritis

Author: David Upchurch, DVM

Abstract: Objective: To evaluate the safety and effect of a single simultaneous intra-articular and intravenous injection of autologous adipose-derived stromal vascular fraction (SVF) and platelet rich plasma (PRP) on coxofemoral osteoarthritis (OA) in dogs.

Methods: This was a randomized, double-blind, placebo-controlled prospective trial of intra-articular and intravenous SVF and PRP for coxofemoral OA. Dogs with coxofemoral OA causing lameness or discomfort were evaluated by orthopedic exam, visual lameness score, Canine Brief Pain Inventory (CBPI), goniometry, visual analogue scale (VAS), and pressure-sensitive walkway (PSW) at week 0 (baseline), and 4, 8, 12 and 24 weeks after injection. Joint radiographs were scored at 0 and 24 weeks.

Results: Twenty-two client-owned dogs with OA of the coxofemoral joints were enrolled (12 placebo-control, 10 SVF-treated). CBPI pain severity scores were lower in the treatment group at 24 weeks compared to the placebo group (p=0.042). The VAS score for the treatment group was significantly greater at 0 weeks than at 4, 8, or 24 weeks (p<0.05). When dogs with low quartile baseline PVF were compared, the treatment group had statistically higher PVF at all post-injection time points when compared to the placebo group. After SVF injection, fewer dogs in the treated group were lame compared to the control group.

Clinical Significance: This study is the first to utilize objective data from PSW as an outcome measure for dogs treated with SVF and PRP for coxofemoral OA. No adverse events were noted. Improvements were noted in some measured parameters in the treated dogs.
University of Kansas Medical Center

Title: Decreases in articular cartilage NFAT1 levels associated with increased radiographic osteoarthritis severity in human hips

Author: Douglas R. Haase¹, MD, Resident in Orthopedic Surgery, University of Kansas Medical Center; Kevin Hodge¹, MD, Resident in Orthopedic Surgery, University of Kansas Medical Center; Perwaiz Nawabi², BS, Kansas City University of Medicine and Biosciences; Jinxi Wang¹, MD, Ph.D., Professor of Orthopedic Surgery, University of Kansas Medical Center

Abstract: Purpose: Nuclear factor of activated T cells (NFAT1) has been found to be integral to joint homeostasis. NFAT1 knockout mice develop premature osteoarthritis in young adults. The purpose of this study was to evaluate the association of human cartilage NFAT1 levels with radiographic osteoarthritic changes observed in hip joints.

Methods: 135 articular cartilage samples were collected from patients undergoing hip arthroplasty and their NFAT1 mRNA levels were measured by quantitative real-time RT-PCR. Three independent reviewers examined the preoperative hip radiographs in a blinded manner. Radiographic grading of osteoarthritis severity included the Kellgren and Lawrence (KL) classification and the Osteoarthritis Research Society International (OARSI) atlas. Patients were divided into five cohorts based on their NFAT1 levels compared to control patients with healthy cartilage.

Results: The cohort with moderately decreased NFAT1 had an average increase in KL score of 0.44 (p=0.008), while the cohort with severely decreased NFAT1 had an average increase of 0.66 (p=0.0001) compared to the control patient group. Additionally, the cohort with moderately decreased NFAT1 had an average increase in superior joint space narrowing of 0.77 (p=0.006), while the severely decreased cohort had an average increase of 1.23 (p=0.00002). Significant associations between decreased NFAT1 levels and elevated superior-sided osteophyte scores were also observed.

Conclusions: This study found that moderate and severe decreases in human articular cartilage NFAT1 levels are associated with significantly increased radiographic osteoarthritic changes. While this study has several limitations, it provides initial evidence regarding the association of decreased NFAT1 levels and osteoarthritis severity in humans.
Title: **Studying Phenylbutyrate in Inclusion Body Myositis- Rational and Study Design**

Author: *Duaa Jabari, M.D., Richard J. Barohn, M.D., Department of Neurology*

Abstract: Inclusion body myositis (IBM) is the most common acquired muscle disorder after age 50. It is a progressive debilitating disease with no available treatment. The muscle biopsy in IBM shows both inflammatory and degenerative features but the lack of response to immunomodulatory treatments suggest that IBM is a degenerative disorder with secondary inflammatory changes. This is supported by abnormal accumulation of amyloid-beta protein precursor, and its proteolytic fragment amyloid-beta along with other misfolded proteins. Many similar neurodegenerative disorders, termed “protein-misfolding disorders” are characterized by the accumulation of intracellular or extracellular protein aggregates. A highly conserved class of proteins called molecular chaperones has evolved to prevent such midfolding or repair or clear misfolded protein. Phenylbutyrate, an orally active chemical chaperone approved by the US Food and Drug Administration for treatment of urea cycle disorders, mimics the function of intracellular molecular chaperones in preventing protein aggregation and oligomerization. Phenylbutyrate showed positive effect on the muscle cell model of IBM by improving lysosomal activity, ameliorating consequences of impaired autophagy and decreasing vacuolization. This provides rational to study this medication in patients with IBM. We plan to conduct an open label study to evaluate the safety and tolerability of phenylbutyrate in IBM and to look for any early signal of effectiveness. With the preliminary data obtained from this study, we will proceed with larger randomized, placebo-controlled safety and efficacy study. If phenylbutyrate is found to be effective in treating IBM, it will be the only available treatment for this disease.
**Title:** Sedentary behavior and diabetic neuropathy  
**Author:** Patricia Kluding, Ph.D., Physical Therapy & Rehabilitation Science

**Abstract:** 

**Background:** Sedentary behavior is associated with multiple metabolic risk factors in people with type 2 diabetes (T2D); when sitting for a prolonged time, postural muscles are inactive, directly leading to impaired postprandial glucose and insulin action. People with diabetic peripheral neuropathy (DPN) may be more sedentary due to pain, sensory loss and fall risk. Exercise intervention studies have demonstrated improved neuropathy symptoms and function; however, it is unknown whether sedentary behavior interventions have a similar effect.

**Methods:** First, a cross sectional study of sedentary adults with T2D (n=59, mean age 64±7) assessed the relationship of sedentary behavior with HbA1C. Participants wore an activity monitor for 7 days to determine average daily sitting time. Second, a pilot study was completed in a subgroup (n=9) to assess a sedentary behavior intervention with actigraphy feedback, vibrotactile prompts, and personalized activity counseling.

**Results:** Participants were sedentary for 68% of waking hours or 11.08 ± 2.31 hours/day. Multiple linear regression analysis showed that lower HbA1C (β= 0.40; 95% CI: 14.43, 58.13) was associated with increased sedentary time, independent of time spent in moderate-vigorous physical activity. The pilot study showed a significant decrease in total sitting time and decreased HBA1C following the intervention.

**Conclusion:** A sedentary behavior intervention may address many barriers to traditional exercise programs for this population, as it induces minimal physiological stress or pain and is potentially more sustainable. Further research is necessary to study this type of intervention in a large randomized trial.
Title: Role of Genetic Interaction between Ciliary Paralogs, Thm2 and Thm1, in Postnatal Skeletogenesis

Author: Pamela Tran, Ph.D., University of Kansas Medical Center; Erin BUmann, DDS, Ph.D., University of Missouri-Kansas City. 2019 Pilot Grant Award Winner Presentation

Abstract: Skeletal dysplasias affect 1:5,000 births and range in severity from perinatal lethality to craniofacial dysmorphogenesis and short stature. These disorders are often heritable, and identification of the causative mutations has been instrumental in improving diagnosis, understanding mode of inheritance, and identifying therapeutic targets. The translational impact of finding causative loci underscores the need to continue to identify and study novel genes involved in skeletal development. Primary cilia are signaling organelles that receive mechanical and chemical cues, and are potent modifiers of skeletal growth. Mutation of ciliary genes causes syndromic disorders termed ciliopathies, which can manifest osteochondrodysplasias. One such ciliopathy is Jeune Syndrome, in which mutations in THM1, a cilia gene and Hedgehog (Hh) modulator, have been identified. We have generated a knock-out mouse of Thm2, which is a paralog of Thm1 and a gene that is largely uncharacterized. In contrast to Thm1-null mouse late embryos which show shortened long bones, Thm2-null mice are healthy and reach adulthood, suggesting that Thm2 alone is dispensable for development. Since paralogs can have redundant roles, we have generated Thm2-/-; Thm1+/-(triple allele) mutant mice. These triple allele mutant mice appear normal at birth, but are markedly smaller than their littermates by postnatal day (P)14, and some do not survive to weaning. We hypothesize that by regulating mechanosensing and Hh signaling, genetic interaction between Thm2 and Thm1 is critical for postnatal skeletal development. To examine our hypothesis, we propose to examine Thm2-/-; Thm1+/-(triple allele) skeletal and ciliary phenotype and to examine role of Thm2-/-; Thm1+/-(triple allele) in Hh signaling in skeletal phenotype. Investigations will include quantifying skeletal defects using microCT imaging, examining functional role of genetic downregulation of Hh signaling in triple allele mutant mice, and examining mechano- and chemotransductive capabilities of triple allele mutant primary cilia in chondrocytes. This project will provide new insights into genetic interactions underlying skeletal development, and will establish Thm2 as a novel locus important for cilia-mediated mechanosensation and signaling.
Canine degenerative myelopathy (DM) is an adult-onset, progressive neurodegenerative disease that shares important similarities to amyotrophic lateral sclerosis (ALS) including clinical presentation and progression, pathological features, and superoxide dismutase 1 gene (SOD1) mutations. The E40K SOD1 mutation is widespread among companion dogs and leads to enzymatically actively SOD1 aggregate accumulations within cells, the putative mechanism of disease in DM and in both familial and sporadic forms of human ALS. This shared pathologic mechanism provides an avenue to direct therapeutic approaches toward SOD1 silencing. The similarities between the canine and human nervous systems, the homogeneity in onset and clinical progression of disease, and the ability to longitudinally analyze and collect samples from affected dogs during treatment make companion dogs with DM a uniquely valuable large animal disease model for therapeutic development and optimization. Moreover, companion dogs with DM represent a clinical population confounded by complexities in diagnosis, comorbidity and environmental and genetic diversity similar to those encountered in a human clinical trial setting. While experimental models are vital for understanding biologic mechanisms and early screening of novel therapies, development of a clinically relevant, time efficient predictive model system is paramount to therapeutic advancements. Thus, incorporation of veterinary clinical trials into the ALS treatment development paradigm will enhance translational efficiency by identifying and optimizing those therapies most likely to generate clinical benefit. A multi-institutional network of veterinary clinician-scientists focused on DM can be refined and formalized to establish a veterinary platform trial for DM to serve as a “translational accelerator” for ALS and DM therapies.
Title: Interactions among tissue components during skeletal muscle regeneration

Author: Steven S. Segal¹, DDW Cornelison², Bret D. Ulery³, Medical Pharmacology and Physiology¹, Biological Sciences², Biomedical, Biological, and Chemical Engineering³, University of Missouri, Columbia MO

Abstract: Skeletal muscle is the largest tissue in the body and is necessary for motility, respiration, and energy generation. Contractile myofibers make up the bulk of muscle tissue, but are surrounded by blood vessels which supply oxygen and nutrients, motor nerves which control contraction, satellite cells which regenerate damaged myofibers, and fibroblasts/interstitial cells which interact biophysically and biochemically with the other resident cell types. In the context of both development and regeneration after injury, the constituent cell types tend to be studied as isolated systems, rather than as interconnected components of the same tissue. Our collaboration is designed to identify key interactions among resident tissue components that regulate coordinated regeneration, with the goal of identifying signaling pathways that can be targeted for biochemical and/or bioengineered interventions to improve muscle function and repair. Our laboratories have been developing physiological (Segal, intravital imaging), molecular (Cornelison, stem cell biology), and engineered (Ulery, biomaterials) approaches to investigate how muscle fibers and microvessels integrate their responses to injury and in regeneration. Current work is focused on understanding how loss of one tissue component (e.g., muscle fibers or blood vessels) affects regeneration of the others and on developing 3D coculture systems and cellularized scaffolds to recapitulate multicomponent interactions. Our preliminary data suggest that inhibiting angiogenesis disrupts myofiber regeneration after acute injury, while loss of regenerated myofibers results in respecification of microvessels from the morphology associated with myofibers to that seen in adipose. Inhibition of either angiogenesis or myogenesis negatively impacts reinnervation.
Title: **Precision/Genomic Medicine in Companion Animals- Rapid Identification of Musculoskeletal and Neuromuscular Disease Models**

Author: *Leslie Lyons, Ph.D., Joan Coates, DVM, Gary Johnson, DVM, PhD, College of Veterinary Medicine, University of Missouri*

Abstract: Precision/Genomic Medicine can be implemented in dogs and cats, which not only leads to improvements in their health care, but also expedites the identification of new biomedical models. Less than one drug is approved for each billion dollars of research and development. Large animal models are clearly an asset to drug development and gene therapies by providing a different biological system to evaluate physiological effects. Importantly, these disease models permit studies of therapy intervention using similar procedures as those in human patients. Demonstrating dosing and delivery paradigms and safety of therapy in the large animal disease models will provide key supportive data and improve the probability of clinical trial success. However, the model must exist before it can be used, including detailed genetic and clinical phenotyping. As part of the MU systems strategic initiative, Precision/Genomic Medicine, as a diagnostic opportunity, is being offered to veterinary patients that have clinical presentations likely to be caused by heritable DNA variants. Our research has had dedication and successes in the discovery of many musculoskeletal and neuromuscular diseases in cats and dogs. Researchers and veterinary clinicians at the University of Missouri (MU), College of Veterinary Medicine are world class in the genetic definition of canine and feline neuromuscular diseases. In cats, investigators have genetically defined several musculoskeletal diseases as well as several neuromuscular syndromes including, hypokalemia, myotonia congenita, a generalized myopathy, multiple systems degeneration, Niemann-Pick Type C, an inherited neurological syndrome with forebrain commissural malformations, ventriculomegaly and interhemispheric cysts. Canine degenerative myelopathy is now a disease model in companion dogs for amyotrophic lateral sclerosis. At least ten different forms of neuronal ceroid lipofuscinosi have been identified and one has impacted the translation of a therapy into children. MU has the bioinformatic skills, computational resources, clinical medicine, diagnostics, and investigative team to rapidly define new large animal biomedical models for musculoskeletal and neuromuscular disease in pets.
Title: **Upregulating neuroplasticity in surviving motor neurons to preserve upper airway function in a novel motor neuron disease model**

Author: *Teresa Lever, Ph.D., Department of Otolaryngology, Nicole Nichols, Ph.D., Department of Biomedical Sciences, University of Missouri*

Abstract: Motor neuron diseases (MNDs; *e.g.*, amyotrophic lateral sclerosis/ALS and primary lateral sclerosis/PLS) result in life-threatening alterations in upper airway function (*i.e.*, swallowing and breathing), primarily due to degeneration of the hypoglossal (XII) axis (*i.e.*, upper motor neurons/UMNs and lower motor neurons/LMNs controlling the tongue). Despite its critical importance, upper airway function has seldom been studied in MNDs; thus, effective treatments remain to be discovered. The fundamental goals of this study are to understand how XII axis degeneration impairs function and coordination of swallowing and breathing in a novel model of MND, and to utilize this model for translational treatment discovery. Specifically, intralingual injection of cholera toxin B conjugated to saporin (CTB-SAP) in rats mimics numerous aspects of dysphagia in MNDs including: 1) targeted death of XII LMNs and corresponding degenerative changes throughout the XII axis (XII nerve, UMNs, and tongue); 2) decreased XII motor output; 3) reduced tongue strength and motility; and 4) impaired swallowing. Here, we will use a multidisciplinary approach to test the central hypothesis that upper airway function/coordination can be preserved in the face of XII LMN degeneration by harnessing the therapeutic potential of tongue exercise to upregulate neuroplasticity *via* neurotrophic factor expression in spared XII axis motor neurons. Our pilot data suggest that tongue exercise in CTB-SAP rats preserves tongue strength and motility as well as swallowing and breathing patterns/coordination. This work may identify noninvasive, cost-effective strategies to preserve upper airway function and improve the quality and duration of life for patients with MNDs.
Compromised Mitochondrial Function in the Pathogenesis of Osteogenesis Imperfecta

Charlotte Phillips, Ph.D., Department of Biochemistry and Child Health, University of Missouri, Sarah Dallas, Ph.D., Department of Oral and Craniofacial Sciences, University of Missouri-Kansas City

Abstract: Osteogenesis imperfecta (OI) is a heritable disorder of skeletal fragility, ranging in severity from mild with few fractures to perinatal lethal with marked bone deformity and fragility. There is no cure; treatment is limited to surgical intervention and anti-resorptive drugs, both with limited success. Bone is mechanosensitive, responding and adapting to its mechanical environment to strengthen the skeleton, and contracting muscles are one of the largest physiological loads that bone experiences. Recent studies demonstrated that 80% of OI patients exhibit muscle weakness. To investigate the pathogenesis of OI muscle weakness, we examined the mitochondria, generators of the cellular energy needed for contraction, in the osteogenesis imperfecta mouse model (oim) with muscle weakness. We found oim skeletal muscle has severely compromised mitochondrial function. This opens up potential therapeutic targets to enhance muscle and bone weakness, but also raises the question as to whether bone cell mitochondrial function is similarly compromised. The goal of this pilot project is to test whether mitochondrial dysfunction is a major contributor to pathogenic muscle weakness and skeletal fragility in OI through investigation of three aims: 1) to determine if the compromised respiration rates in isolated oim skeletal muscle mitochondria are associated with altered Ca$^{2+}$ homeostasis and production reactive oxygen species, 2) determine whether cultured primary oim calvarial cells have compromised mitochondrial bioenergetics and metabolism, and 3) to determine how the dynamic, morphological and functional properties of mitochondria are altered in oim bone and muscle cells using live cell and intravital imaging approaches. This innovative hierarchical approach (from isolated mitochondria through to live cell/intravital imaging) will competitively position this unique interdisciplinary team for future external grant submissions to NIH/NIAMS.
Title: Role of Genetic Interaction between Ciliary Paralogs, Thm2 and Thm1, in Postnatal Skeletogenesis

Author: Erin Bumann, DDS, Ph.D., University of Missouri-Kansas City, Pamela Tran, Ph.D., University of Kansas Medical Center. 2019 Pilot Grant Award Winner Presentation

Abstract: Skeletal dysplasias affect 1:5,000 births and range in severity from perinatal lethality to craniofacial dysmorphogenesis and short stature. These disorders are often heritable, and identification of the causative mutations has been instrumental in improving diagnosis, understanding mode of inheritance, and identifying therapeutic targets. The translational impact of finding causative loci underscores the need to continue to identify and study novel genes involved in skeletal development. Primary cilia are signaling organelles that receive mechanical and chemical cues, and are potent modifiers of skeletal growth. Mutation of ciliary genes causes syndromic disorders termed ciliopathies, which can manifest osteochondrodysplasias. One such ciliopathy is Jeune Syndrome, in which mutations in THM1, a cilia gene and Hedgehog (Hh) modulator, have been identified. We have generated a knock-out mouse of Thm2, which is a paralog of Thm1 and a gene that is largely uncharacterized. In contrast to Thm1-null mouse late embryos which show shortened long bones, Thm2-null mice are healthy and reach adulthood, suggesting that Thm2 alone is dispensable for development. Since paralogs can have redundant roles, we have generated Thm2-/-; Thm1+/- (triple allele) mutant mice. These triple allele mutant mice appear normal at birth, but are markedly smaller than their littermates by postnatal day (P)14, and some do not survive to weaning. We hypothesize that by regulating mechanosensing and Hh signaling, genetic interaction between Thm2 and Thm1 is critical for postnatal skeletal development. To examine our hypothesis, we propose to examine Thm2-/-; Thm1+/- skeletal and ciliary phenotype and to examine role of Thm2-/-; Thm1+/- in Hh signaling in skeletal phenotype. Investigations will include quantifying skeletal defects using microCT imaging, examining functional role of genetic downregulation of Hh signaling in triple allele mutant mice, and examining mechano- and chemotransductive capabilities of triple allele mutant primary cilia in chondrocytes. This project will provide new insights into genetic interactions underlying skeletal development, and will establish Thm2 as a novel locus important for cilia-mediated mechanosensation and signaling.
Title: Understanding clinical variability in craniofacial birth defects: opportunities to reduce the burden of reconstructive surgeries.

Author: Timothy C. Cox, Department of Oral & Craniofacial Sciences, School of Dentistry, & Department of Pediatrics, School of Medicine, University of Missouri-Kansas City, Missouri.

Abstract: Non-syndromic cleft lip/palate (NS-CL/P) affects ~1 in 700 live births. It is widely considered to be a complex trait, encompassing a spectrum of clinical presentations. Despite the identification of many population-level genetic variants contributing to an individual’s risk of clefting, it remains impossible to predict whether a child will be born with NS-CL/P or how severe the presentation will be. As severity is the major determinant of surgical burden, there is a pressing need to understand additional risk factors.

Manipulation of the maternal diet is an attractive avenue to modify an embryo’s genetically-determined risk. We have initially focused on Vitamin A as it is solely acquired from our diet yet essential for early facial development. Furthermore, dietary vitamin A deficiency is among the most common nutritional deficiencies worldwide. We are exploiting a unique mouse model that facilitates control of dietary vitamin A availability to address whether embryos carrying defined genetic risk factors for CL/P are hypersensitive to normal fluctuations in maternal dietary vitamin A bioavailability.

Preliminary data will be presented on the impact of transient reductions in maternal vitamin A availability on cleft-labile embryos. Ongoing efforts are aimed at investigating different genetic sensitivities, the impact of timing and duration of the deficiency, and whether low level supplementation can reduce cleft severity and hence minimize the surgical burden for patients. We hope these studies will open up the prospect of simple interventional treatments for genetically at-risk individuals much like that of folate supplementation for reducing the risk of neural tube defects.

Funding: Endowment in Dental and Mineralized Tissue Research.
Title: Compromised Mitochondrial Function in the Pathogenesis of Osteogenesis Imperfecta

Author: Charlotte Phillips, Ph.D., Department of Biochemistry and Child Health, University of Missouri, Sarah Dallas, Ph.D., Department of Oral and Craniofacial Sciences, University of Missouri-Kansas City

Abstract: Osteogenesis imperfecta (OI) is a heritable disorder of skeletal fragility, ranging in severity from mild with few fractures to perinatal lethal with marked bone deformity and fragility. There is no cure; treatment is limited to surgical intervention and anti-resorptive drugs, both with limited success. Bone is mechanosensitive, responding and adapting to its mechanical environment to strengthen the skeleton, and contracting muscles are one of the largest physiological loads that bone experiences. Recent studies demonstrated that 80% of OI patients exhibit muscle weakness. To investigate the pathogenesis of OI muscle weakness, we examined the mitochondria, generators of the cellular energy needed for contraction, in the osteogenesis imperfecta mouse model (oim) with muscle weakness. We found oim skeletal muscle has severely compromised mitochondrial function. This opens up potential therapeutic targets to enhance muscle and bone weakness, but also raises the question as to whether bone cell mitochondrial function is similarly compromised. The goal of this pilot project is to test whether mitochondrial dysfunction is a major contributor to pathogenic muscle weakness and skeletal fragility in OI through investigation of three aims: 1) to determine if the compromised respiration rates in isolated oim skeletal muscle mitochondria are associated with altered Ca\(^{2+}\) homeostasis and production reactive oxygen species, 2) determine whether cultured primary oim calvarial cells have compromised mitochondrial bioenergetics and metabolism, and 3) to determine how the dynamic, morphological and functional properties of mitochondria are altered in oim bone and muscle cells using live cell and intravital imaging approaches. This innovative hierarchical approach (from isolated mitochondria through to live cell/intravital imaging) will competitively position this unique interdisciplinary team for future external grant submissions to NIH/NIAMS.
**Title:** Estrogen Action in Bone and Muscle Crosstalk  
**Author:** Mark L. Johnson, Nuria Lara-Castillo, Erica Jackson, Mark Dallas, Julian Vallejo, Jennifer Rosser, Ellie Ray, Derrick Nelson, Ganesh Thiagarajan and Michael Wacker

**Abstract:** Estrogens play important roles in bone metabolism and muscle mass and function throughout the lifespan in both females and males. Evidence has accumulated that beyond the mechanical coupling of the bone and muscle, these two tissue communicate with each other biochemically. We hypothesize that loss of estrogen mediated signaling with aging impairs crosstalk signaling between bone and muscle. Estrogen signals through both genomic (via estrogen receptors; ERα and ERβ) and non-genomic mechanisms. We are using several animal models that have targeted deletion of ERα and ERβ in bone (oy-cKO-ER) or skeletal muscle (sm-cKO-ER) to dissect the individual roles of these estrogen receptors in each tissue across aging and in bone-muscle crosstalk. Ovariectomy of TOPGAL mice (β-catenin reporter mice) attenuates load-induced activation of β-catenin signaling in osteocytes, which is a prerequisite for bone formation in response to mechanical loading. Osteocyte targeted deletion of ERβ (oy-cKO-ERβ) resulted in decreased trabecular BV/TV and trabecular number with increased trabecular separation in male mice compared to control littermates, but not females. It also increased the modulus of elasticity in male oy-cKO-ERβ mouse femurs, but decreased this property in female oy-cKO-ERβ mice. The elastic stiffness of male oy-cKO-ERβ femurs increased with no effect in females. Interestingly, male oy-cKO-ERβ soleus muscles recovered to a lesser extent following fatigue *ex vivo*, but female oy-cKO-ERβ mice muscles showed no changes. Preliminary data from the sm-cKO-ERβ mice demonstrated a left shifted load:strain relationship, implying a change in the biomechanical properties of those tibiae. Using an *in vitro* model (TOPflash-MLO-Y4 osteocyte like cells) to further dissect the role of estrogen and estrogen receptors in bone and muscle crosstalk, we have demonstrated that conditioned media from C2C12 myotubes, but not myoblasts, produces a factor that synergizes with Wnt signaling. Purification and identification of this factor is currently ongoing. Additionally, estrogen receptor inhibition resulted in attenuation of Wnt activation of the pathway *in vitro*. Collectively these studies have demonstrated a sex and estrogen receptor isoform specific role for estrogen action in bone and muscle. Furthermore, these studies demonstrated that estrogen receptor deletion in bone or muscle consequently alters the properties of the other tissue, implying crosstalk communication and its control is regulated differently in males and females.
Title: **Bone-Heart Crosstalk**

Author: *Michael Wacker, Julian Vallejo, Mark Gray, Derek Wang, Nuria Lara-Castillo, Mark Dallas, Mark Johnson*

Abstract: Bone has previously been thought of to be a static organ, however, its role as a significant endocrine organ is now fully established and appreciated. Our bone and muscle collaborative has identified several factors released by bone (PGE2 and Wnt3a) that can regulate skeletal muscle. Another factor released by bone, fibroblast growth factor 23 (FGF23), can regulate phosphate via interactions with the kidney and parathyroid. My laboratory has previously demonstrated that FGF23 can also directly increase calcium entry in cardiomyocytes and increase cardiac contractility. We have now shown that pathological levels of FGF23 experienced during end stage chronic kidney disease can induce arrhythmias in isolated heart preparations and prolong the QT interval on EKG measurements (n=10; P<0.01). These data along with clinical studies demonstrating an association between osteoporosis and heart health has led us to further hypothesize that there may be factors released by bone that modify cardiovascular function to match bone strain. We have conducted initial studies in which isolated, paced, Langendorff-perfused mouse hearts were treated with conditioned media from MLOY4 cultured cells (osteocyte-like cell line) that underwent fluid flow sheer stress. Applications of various percentages of media (0.075%-7.5%) induced on average greater than 30 premature contractions/min in paced, *ex vivo* mouse hearts (n=5; P<0.05), which may indicate there is a factor increasing the excitability of cardiac myocytes. The exact mechanism for this response remains to be determined. Alteration of cardiac function by bone response to mechanical loading is a new area of research focus.
Title: Multisession brain-computer interface performance using a switch-scanning augmentative and alternative communication device by individuals with ALS

Author: Jonathan Brumberg & Kevin Pitt

Abstract: In this study we examined the performance of a motor-based brain-computer interface (BCI) access method to a switch-scanning augmentative and alternative communication device by four individuals with amyotrophic lateral sclerosis and three control participants without any neurological disease or injury. Participants with ALS completed 12 sessions either in a lab setting (N=2) or at home (N=2) while control participants completed 3 sessions in a lab setting. In addition to BCI tests, participants with ALS also completed a comprehensive BCI screening and assessment tool for identifying sensory, cognitive and motor (imagery) skills likely important for successful BCI performance, as well as ratings on fatigue, satisfaction, frustration, and motivation before and after each session. The results of this study found that two of four participants with ALS were able to increase their performance over time. Performance and frustration levels influenced satisfaction, with performance being largely tracked by symptom severity. Participants without ALS also varied in their performance, though all were able to control the device at levels above those with ALS. This study demonstrates that BCI-based control of augmentative and alternative communication devices is possible, and future training programs for individuals who may use BCI access methods may be enhanced by collecting user feedback regarding fatigue, frustration, and satisfaction.
Title: **Degeneration of ALS mouse neuromuscular junctions analyzed using super-resolution microscopy and ameliorated using human mesenchymal stem cells**

Author: **Yomna Badawi¹**, **Sudheer Tungtur¹**, **Tomohiro Tanaka¹**, **Rupal Soder²**, **Richard Barohn³**, **Kazuhiro Shigemoto⁴**, and **Hiroshi Nishimune¹**

¹Department of Anatomy and Cell Biology, ²Midwest Stem Cell Therapy Center, ³Department of Neurology, University of Kansas Medical Center, Kansas City, KS, 66160, USA ⁴Geriatric Medicine, Tokyo Metropolitan Institute of Gerontology, Itabashi, Japan

Abstract: Presynaptic active zones play an essential role as synaptic vesicle release sites for synaptic transmission. In this study, stimulated emission depletion (STED) super resolution microscopy analysis supports our molecular mechanism suggesting that laminin β2 anchors presynaptic voltage-gated calcium channels (VGCC) in front of postsynaptic junctional folds. PQ-type VGCC can then function as a scaffolding protein for active zone–specific proteins. Based on the knowledge obtained from wild-type NMJs, we evaluated the active zone proteins in NMJs of amyotrophic lateral sclerosis (ALS) mouse models. ALS is a neurodegenerative disorder in which NMJ denervation occurs before the death of motor neuron cell bodies in the spinal cord, suggesting a “dying-back” neuropathy. The mechanisms underlying NMJ denervation in ALS remain unknown. The pathogenesis of ALS may involve changes in protein levels, which are important for the maintenance of NMJ active zones and regulation of neurotransmission. For this purpose, we analyzed active zone proteins in NMJs of SOD1G93A mice, at an early, pre-symptomatic stage (P85) and a symptomatic stage (P140). Interestingly, we found that the quantity of laminin β2 and active zone proteins Bassoon, Piccolo, and PQ-type VGCC decreased in innervated NMJs of ALS mice compared to age-matched wild-type mice.

As a therapeutic source of laminin β2, we evaluated stem cells as vectors to express and secrete proteins that promote NMJ maintenance and are neuroprotective. This could prove to be an efficient and a long-term delivery system for laminin β2 to reduce NMJ denervation and increase the quality of life of ALS patients.
Title: Utilization of the collage-induced arthritis mouse model to evaluate molecular biomarkers of methotrexate efficacy

Author: Ryan S. Funk, Pharm.D., Ph.D.

Abstract: Methotrexate (MTX) is an anti-folate therapeutic and is the cornerstone of disease-modifying therapy in the treatment of autoimmune arthritis, including rheumatoid arthritis and juvenile idiopathic arthritis. Despite its established efficacy, a major barrier to its effective use is a highly variable and unpredictable response profile with approximately 1 in 3 patients failing to respond to initial therapy. Therefore, a critical need exists to identify clinical biomarkers of MTX efficacy to guide clinicians in the early optimization of drug therapy. This study utilizes the collagen-induced arthritis (CIA) mouse model as a model of autoimmune arthritis to evaluate molecular biomarkers of MTX response. Most notably, we demonstrate the dose-dependent efficacy of MTX in the CIA mouse model and find that mice fail to appreciably metabolize MTX into its biologically active intracellular form (i.e. polyglutamate metabolites). Further, we demonstrate that MTX therapy is associated with the depletion of intracellular folates and that this anti-folate effect is directly associated with various measures of drug efficacy. Together, this work demonstrates the dose-dependent efficacy of MTX in the CIA mouse model and directly links its efficacy with its pharmacological activity as an anti-folate.
Title: Ileus and dysautonomia can contribute to significant morbidity in Guillain-Barré syndrome independent of peripheral motor involvement.

Author: Aaron LacKamp, M.D.

Abstract: Guillain Barre Syndrome (GBS) is a humorally-mediated inflammatory polyradiculoneuropathy. The rate of respiratory failure can be predicted by the rapidity of onset of symptoms. At KU, endotracheal intubation has occurred in 9 out of 129 cases based upon a search of de-identified records using the HERON database.

Findings: Respiratory failure may occur in GBS as a result of diaphragmatic weakness and can be anticipated based upon bedside spirometry. We present a case of precipitous respiratory failure in spite of normal spirometry due to gut failure. The patient had pharyngeal weakness, failed a swallow evaluation, ileus, colonic gaseous distention, and did not have a bowel movement for 7 days prior to his hypoxic respiratory failure. There was cephalad displacement of the diaphragm and the patient had developed shunting in the left lung due to atelectasis in the left lower lobe. The patient required prolonged mechanical ventilation until resolution of the ileus and colonic distention.

In this case dysautonomia and branchiomotor weakness were disproportionate to the degree of peripheral motor weakness. There was no appreciable respiratory muscle weakness prior to precipitous hypoxic respiratory failure. Dysautonomia, ileus, and hyponatremia may suggest hypothalamic involvement of GBS. The identification of non-motor symptoms is associated with increasing severity of motor weakness, however in this patient non-motor symptoms were uncoupled in terms of severity from peripheral motor involvement.

Conclusion: Possible hypothalamic effects of Guillain-Barre Syndrome: ileus, and dysautonomia may independently contribute to significant morbidity and may be future areas of investigation.
Title: Applying Human Umbilical Cord Derived Mesenchymal Stem Cells for the Treatment of Amyotrophic Lateral Sclerosis

Author: Takashi Matsuda\textsuperscript{1}, Yomna Badawi\textsuperscript{1}, Kleiton Silva\textsuperscript{4}, Rupal Soder\textsuperscript{2}, Richard Barohn\textsuperscript{1}, Tadashi Yoshida\textsuperscript{4}, and Hiroshi Nishimune\textsuperscript{1}

\textsuperscript{1}Department of Anatomy and Cell Biology, \textsuperscript{2}Midwest Stem Cell Therapy Center, \textsuperscript{3}Department of Neurology, University of Kansas Medical Center, Kansas City, KS, USA, \textsuperscript{4}Department of Medicine and Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, MO, USA

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by a gradual loss of motor neurons. Studies indicate that neuromuscular junction (NMJ) denervation occurs in the early stages of the disease while neuronal cell bodies in the spinal cord remain intact. We found that the synapse organizer laminin $\beta_2$ decreases in NMJs of ALS model SOD1\textsuperscript{G93A} mice, and transgenic expression of laminin $\beta_2$ in skeletal muscles of SOD1\textsuperscript{G93A} mice ameliorates NMJ denervation. The objectives of this study are (1) to determine whether human umbilical cord-Wharton’s jelly derived mesenchymal stem cells (hMSCs) from multiple donors secrete laminin $\beta_2$ and neurotrophic factors at similar levels, and (2) to evaluate hMSCs derived from different donors in vivo in SOD1\textsuperscript{G93A} mice for NMJ maintenance and life-span extension. We discovered that hMSCs derived from multiple donors can increase the secretion of laminin $\beta_2$ and neurotrophic factors by stimulation in media supplemented with growth factors. Furthermore, we confirmed that unilateral injection of hMSCs increased NMJ innervation rates and average myofiber cross sectional area in MSC-injected muscles compared to non-injected contralateral muscles. Then, the hMSCs obtained from two donors were transplanted separately into SOD1\textsuperscript{G93A} mice by combined intrathecal and bilateral intramuscular injections. Importantly, injection of hMSCs improved neuromuscular function of injected SOD1\textsuperscript{G93A} mice, and prolonged the lifespan of SOD1\textsuperscript{G93A} mice compared to the vehicle injected SOD1\textsuperscript{G93A} mice. Thus, injection of hMSCs obtained from donors could be an efficient treatment method to improve the quality of life of ALS patients.
Title: Identification of the Prevalence and Patterns of Celiac Disease Associated Arthropathy and Coexistence of Celiac Disease with Rheumatic and/or Inflammatory Disorders in a Single Tertiary Medical Center

Author: Anita Moudgal¹, Pooja Bhadbhade², Ammar Haikal², Mehrdad Maz², ¹The University of Kansas School of Medicine, ²Division of Allergy, Clinical Immunology and Rheumatology, Department of Medicine; The University of Kansas Medical Center

Abstract: Background: Celiac disease (CD) is a gluten-sensitive enteropathy that develops in genetically predisposed individuals. Arthropathy has been reported as an extra-intestinal manifestation of CD. The pathogenesis of arthropathy is unclear; however, the immunologically mediated mucosal injury may lead to absorption of immune complexes or gut-derived antigens which may provoke antibody mediated autoimmune diseases including rheumatic diseases and arthropathy. We report a retrospective chart review of 35 confirmed cases of CD associated arthropathy and coexisting rheumatic or inflammatory disorders.

Methods: Heron database was used to search for patients seen by Gastroenterology and/or Rheumatology between January 2008 to January 2018 with suspected CD based on ICD-9 code for CD. These charts were retrospectively reviewed to determine who had confirmed CD, joint pain and pattern of involvement, dietary compliance, and other inflammatory or rheumatic disorders. The diagnosis of CD was based on serology specific for tissue transglutaminase (tTG) antibody, endoscopic and histological results, compliance with gluten free diet, and clinical judgement.

Results: A total of 95 patients with suspected CD were identified. 35/95 had confirmed CD. Of the 35 patients, 29 were female and 34 self-identified as Caucasian. The average age of patients was 49 years. Of the 35 patients, 22 patients did not have a co-existing rheumatic or inflammatory disorder. This cohort of 22 patients endorsed symptoms in the following distribution: 82% (18/22) had peripheral only, 0% (0/22) had axial only, and 18% (4/22) had peripheral and axial involvement. Of the 22 patients, 54% (14/22) endorsed arthropathy in small joints, 91% (20/22) in medium joints, and 82% (18/22) in large joints. Improvement in arthralgia after transitioning to a gluten-free diet was reported in 4/22 patients. A total of 13/35 with confirmed CD diagnosis had a rheumatic and/or inflammatory disorder. Rheumatoid Arthritis had the highest prevalence at 11% (4/35) followed by IBD (3), UCTD (2), Sjorgen’s Syndrome (1), Scleroderma (1), Symmetric Inflammatory Polyarthritis (1), and Psoriasis (1). Fibromyalgia, a non-inflammatory condition, was reported in 6% (2/35) of patients. 3 patients were not seen by rheumatology.

Conclusion: CD associated arthropathy and coexistence of CD with rheumatic and/or inflammatory disorders are under-recognized. Our data demonstrates that 63% of patients had CD associated arthropathy who appear to be a unique subset separate from those with coexisting CD and rheumatic/inflammatory disorders. Identifying the association and pattern of arthropathy in CD will aid in management of patients who either present to a gastroenterologist with extra-intestinal manifestations or to a rheumatologist with gastrointestinal manifestations. The awareness that CD can coexist in rheumatic diseases will aid rheumatologists in more effective recognition and management of patients with such presentations.
Title: Efficacy of mycophenolate mofetil in the treatment of rheumatoid arthritis associated interstitial lung disease

Author: Amos, J., Kendall, J., Moran, R., Krause, M., Schmidt, P., Hall, C., Hamblin, M., Maz, M. Division of Allergy, Clinical Immunology and Rheumatology, Division of Pulmonary and Critical Care, Department of Medicine; The University of Kansas Medical Center

Abstract: Background: Interstitial lung disease (ILD) as an extra-articular manifestation of rheumatoid arthritis (RA) can lead to significant morbidity and mortality. There is limited data on the efficacy of mycophenolate mofetil (MMF) in the treatment of RA associated ILD.

Methods: This retrospective chart review identified patients with a clinical diagnosis of RA and interstitial lung disease at a single tertiary academic medical center who were treated with MMF for at least 3 months between 1/01/2005 and 12/31/2018. Patients were identified by diagnosis codes, and then reviewed to confirm clinical diagnoses. Data regarding concurrent therapies including glucocorticoids, and pulmonary function tests, infections and hospitalizations were also collected.

Results: 26 patients were identified; 17 female (65.4%) and 23 Caucasian (88.5%) with a mean age of 57.8 at the time of diagnosis of RA. Rheumatoid factor (RF) was positive in 20 (76.9%), anti-cyclic citrullinated peptide antibody (ACPA) in 16 (61.5%) and 10 (38.5%) were seropositive for both, while 3 (11.5%) patients were seronegative. The mean time to diagnosis of ILD after diagnosis of RA was 40.6 months with a range of -50 to 504 months. Seven (26.9) patients had an ILD diagnosis prior to RA.

Fourteen (53.8%) patients had usual interstitial pneumonia (UIP), 5 (19.2%) had nonspecific interstitial pneumonia (NSIP), 3 (11.5%) had organizing pneumonia (OP), and 4 (15.4%) had other forms such as mixed UIP and NSIP. The average duration of MMF therapy was 24.9 months with an average maximum daily dose of 2163.5mg. 11 (42.3%) patients were concurrently on rituximab (RTX), 1 (3.85%) on methotrexate, 1 (3.85%) on certolizumab, and 1 (3.85%) on sulfasalazine. 15 (57.7%) patients were on concurrent prednisone ≥10mg daily and 6 (23.1%) were on prednisone <10mg daily for at least one month.

Average FVC for all patients was 62.4% (SD 21.9) predicted at the time of initiation of MMF, 61.9% (SD 23.0) predicted at 6 months, and 64.8% (SD 25.9) predicted at 12 months. In aggregate, 13 (50%) patients on MMF had stable or improved FVC over the 12-month period, of whom 6/13 (46.1%) were on concurrent RTX. Among patients on combination MMF and RTX; 6/11 (54.5%) had stable or improved FVC over the 12-month period.

There were 26 hospitalizations in 8 (30.8%) different patients; 17 (65.4%) for infections, 3 (11.5%) for respiratory failure, and the remainder were for cardiovascular events. There were 10 outpatient infections treated with antimicrobials. There were 9 (34.6%) deaths by the end of the study period.

Conclusion: In this cohort of 26 RA patients with ILD, treatment with MMF as mono or combination therapy with RTX was associated with stable FVC in 50% of patients at 12-month. About 55% of patients on combination MMF and RTX showed stability or improvement in FVC. Patients treated with MMF compared to combination MMF and RTX had similar FVC values at the end of the study period. Further studies are needed to better understand the efficacy and safety of MMF or combination therapy with RTX in RA associated ILD.
Title: Pattern of Arthropathy in Patients with Cystic Fibrosis

Author: Daniel Pham; Mehrdad Maz, MD; Michael Crosser, MD; Megan Krause, MD
Department of Internal Medicine, Division of Allergy, Clinical Immunology, & Rheumatology, The University of Kansas Medical Center

Abstract: Background: Arthropathy is a rare but debilitating manifestation of cystic fibrosis (CF) that has no formal definition. This study attempts to characterize the spectrum of joint pains in CF patients.

Methods: A retrospective chart review was conducted on 246 adult CF patients who were seen at a single tertiary care center between January 1, 2008 and December 31, 2017. Charts were individually reviewed for description of joint symptoms. Patients were excluded if they had an alternative explanation for joint symptoms. Association of joint symptoms with pulmonary exacerbation were abstracted and defined based on clinical diagnosis of the treating provider. Pattern of joint involvement, duration of symptoms (acute as defined by <6 weeks and chronic >6 weeks), and therapies utilized for joint pain were also abstracted.

Results: In the overall cohort of 246 adult CF patients, 43 (17%) had unexplained joint symptoms. In the overall cohort, 128 (52%) were female and 42 (97%) self-reported as Caucasian. In those with unexplained joint symptoms, 28 of 43 (65%) were female. Most commonly, the joint symptoms were not associated with pulmonary exacerbations (22, 51%). In 15 (35%) patients there was association between joint symptoms and pulmonary exacerbations while in 6 (14%) it was not specified. In 18 patients, the duration of symptoms were described and 14/18 had symptoms lasting less than 6 weeks. Knee (23), ankles (13), hips (10), and wrists (10) were the most affected joints. However, both small and large joints were affected. Both symmetric and asymmetric presentations were noted. No individuals had findings of sacroiliac joint involvement. There were no individuals with hypertrophic osteoarthopathy in this cohort. The most commonly used medications were NSAIDs. In the overall cohort of CF patients who had unexplained joint symptoms, therapies utilized were NSAIDs (25, 58%), acetaminophen (10, 23%), and prednisone (9, 21%). In terms of the use of DMARDs, hydroxychloroquine was the most frequently used (7, 16%) followed by sulfasalazine (1, 2.3%). No biologics were used in this cohort. There were no reports of fluoroquinolone associated tendinopathy or voriconazole induced periostitis.

Conclusion: This study characterizes the variety of joint symptoms in CF patients and expands on current knowledge. Females were most likely to experience joint symptoms and the knee was most commonly affected joint. Understanding the diverse spectrum of joint symptoms will result in greater recognition and improvement in quality of life for these patients.
Title: ASARM reverses hyperphosphatemia, prevents calciphylaxis-like lesions, corrects renal, bone brain and cardiovascular calcification in a rat model of chronic kidney disease.

Author: Peter S. N. Rowe, Jason R. Stubbs, Shiqin Zhang, Timothy Fields, Alan S. Yu and Ellen T. McCarthy.

Abstract: Background: Abnormalities in mineral metabolism, bone and vascular calcification occur in Chronic Kidney Disease (CKD-MBD). Cognitive function also declines as the disease progresses. Bone ASARM peptides are strong inhibitors of mineralization and induce hypophosphatemia by inhibiting phosphate uptake from the gut. We hypothesize treatment of CKD-MBD rats with ASARM peptides will reverse hyperphosphatemia, correct mineralization defects and improve mortality.

Methods: To test our hypothesis, we used a rat 5/6 Nephrectomy experimental model (NEPHREX) and sham operated rats (SHAM) as controls. Male rats (16 wk, 250 gm) were fed a high phosphate diet to worsen mineral metabolism defects (2% P, 2000 IU Vit D and 0.8% Ca; TEKLAD 170496). ASARM peptide was infused continuously for 4 weeks using subcutaneous implantation of Alzet osmotic pumps. Sera collections were taken at the beginning and end of the study.

Results: NEPHREX rats treated with ASARM-peptide showed major reductions in hyperphosphatemia, and improved renal, bone, brain and cardiovascular calcification compared to controls treated with vehicle. Also, the high phosphate diet NEPHREX rats developed sub-dermal medial blood vessel calcification and calciphylaxis like lesions. The subdermal blood vessel calcifications did not occur in 56-NEPHREX rats treated with ASARM-peptide.
Title: Nephrogenic systemic fibrosis is induced in high phosphate diet CKD rates exposed to Gd3+ binding contrast agents (GBCA); Role of ASARM peptides.

Author: Peter S. N. Rowe, Aditi Gupta, Timothy Fields, Travis Hagedorn, and Ellen T. McCarthy.

Abstract: Background: High contrast Magnetic Resonance Imaging (MRI) requires the use of Gadolinium Binding Contrast Agents (GBCAs). Subsets of chronic kidney disease (CKD) patients exposed to GBCAs develop Nephrogenic Systemic Fibrosis (NSF), a progressive disease that leads to acute morbidity and death. Our previous work showed circulating ASARM-peptides bind to GBCAs and induce release of toxic Gd. Bone-derived ASARM peptides induce hypophosphatemia and bone-mineralization abnormalities. We hypothesize increased levels of acidic ASARM-peptide exacerbates release of free Gd resulting in an NSF pathology with reduced ectopic mineralization defects. Methods: To test our hypothesis, we used a rat 5/6 Nephrectomy CKD disease model (NEPHREX). Male rats (16 wk, 250 gm) were fed a high phosphate diet (2% P, 200IU Vit D and 0.8% Ca; TEKLAD 170496). ASARM peptide was infused continuously for 4 weeks using subcutaneous implantation of osmotic pumps. As controls, co-implanted osmotic pumps were used to co-infuse SPR4 peptide - a peptide that neutralizes ASARM. Sera collections were taken at the beginning and end of the study. Three consecutive, daily bolus injections of Gd-containing contrast agent (Omniscan , gadodiamide) were given 3 days after pump implantation through surgically implanted jugular-vascular-catheters. Results: NEPHREX rats treated with Omniscan and ASARM developed severe skin pathology, behavioral abnormalities, and joint abnormalities that were consistent with NSF. Computed tomography (CT) showed renal, brain,heart dermal metastatic calcifications and bone defects in Omniscan treated Rats. ASARM peptide treatment corrected the Omniscan induced skin, bone and soft tissue mineral abnormalities and corrected the hyperphosphatemia. Conclusion: Our study shows CKD rats fed a high phosphate diet and treated with Omniscan develop severe NSF like pathology. ASARM infusion prevents Omniscan induced subdermal calcification, corrects mineral defects and hyperphosphatemia. In conclusion, ASARM peptides induce release of free Gd from GBCAs but reduce mineralization pathology. These findings have clinical importance for GBCA use in inherited or acquired renal bone-mineral loss disorders with increased circulating ASARM-peptides.
Title: Treatment of posttraumatic arthrofibrosis using high intensity ultrasound and laser in a novel rabbit model of knee contracture

Author: Authors: David Hazlewood, Yi Feng, Qinghua Lu, Xinmai Yang, Jinxi Wang
Presenter: Jinxi Wang

Abstract: Post-traumatic joint contracture induced by scar tissues can leave patients in a permanent state of pain and disability, which is difficult to resolve by current treatments. This study examined the therapeutic effect of pulsed high-intensity laser (PHIL) and pulsed high-intensity focused ultrasound (PHIFU) for posttraumatic joint contracture due to arthrofibrosis, with short pulses for prevention of tissue damage. Rabbit knee contracture was induced by surgical capsular damage. Twenty-one rabbits were divided into four groups: untreated control (n=5), PHIL (n = 5), PHIFU (n = 5), and PHIL + PHIFU (n = 6). Maximum knee extension of the surgically modified rabbit knee was compared to that of the contralateral control knee over the course of 16 weeks. The results revealed that the rabbits in the untreated control group maintained a consistent level of joint contracture, while rabbits in each of the treatment groups had improved range of motion, eventually leading to a restoration of normal joint extension. Average recovery time was 7.6 ± 1.5 weeks for the PHIL treatment group, 9.8 ± 3.7 weeks for the PHIFU group, and 7.6 ± 2.2 weeks for the combined group. Histopathology demonstrated reduced density and accelerated resorption of scar tissues in the treated knee joints. This study provides evidence that both PHIL and PHIFU are effective in treating posttraumatic arthrofibrosis in rabbits, and warrant further investigations into the underlying mechanisms and optimal parameters of PHIL and PHIFU therapies in a larger number of animals.
Mast cell deficiency amplifies inflammatory response in a mouse model of Kawasaki’s disease

Jason M Springer, Mingcai Zhang, Ryan Funk, Ossama Tawfik, Naohito Ohno, Noriko N Miura, Mehrdad Maz, Kottarappat N Dileepan
Division of Allergy, Clinical Immunology & Rheumatology, Department of Medicine, University of Kansas Medical Center, Kansas City, KS, USA

Abstract: Background: In Kawasaki’s disease (KD) higher serum IL-6 in the first week of the disease has been shown to be a risk factor for the development of coronary artery aneurysms. In this study, we used the Candida albicans water-soluble fraction (CAWS) mouse vasculitis model for KD, to determine the role of mast cells on IL-6 homeostasis.
Methods: Wild-type male C57Bl/6 (WT) or mast cell-deficient (MC) mice were intraperitoneally injected with Phosphate-buffered saline (PBS) or CAWS (2 mg/mouse) daily for 5 days. Mice were sacrificed at either 7 or 14 days after the last injection. Aortic root inflammatory score was blindly accessed.
Results: Seven MC mice injected with CAWS died unexpectedly within 24 hours of the first CAWS injection. MC-CAWS mice had higher systemic IL-6 compared to WT-CAWS at 7 days and 14 days. By 14 days mice in MC-CAWS had significantly higher serum INFγ compared to WT-CAWS mice. TNFα was higher in MC-CAWS compared to WT-CAWS at both 7 days and 14 days. The average AIS was higher in the MC-CAWS group compared to WT-CAWS at both 7 days (1.5 vs 1.3) and 14 days (3.7 vs 2.4).
Conclusion: By 14 days, mast cell deficient mice exposed to CAWS developed higher systemic levels of both IL-6 and INFγ, two important cytokines in pathogenesis of aortitis and coronary arteritis. By histology, mast cell deficient mice have higher inflammatory scores. This supports the novel concept that mast cells play a protective role in inhibiting the initial systemic inflammatory response in Kawasaki’s disease.
Accessory inspiratory muscle (i.e., pectoralis minor) activity is increased in a rodent model of respiratory motor neuron loss

Lauren F. Borkowski and Nicole L. Nichols, Department of Biomedical Sciences, University of Missouri, Columbia, MO

Patients with neuromuscular diseases experience loss of respiratory motor neurons (i.e., phrenic and intercostal) resulting in ventilatory failure, and ultimately death. There are currently no significant treatments to prolong or correct for these breathing deficits. Genetic rodent models of motor neuron loss develop global symptoms (e.g., dysphagia, limb paralysis, etc.), so we have developed an inducible model of only respiratory motor neuron death in order to study how motor neuron loss impacts respiration and to develop therapeutic interventions. Briefly, adult rats are intrapleurally injected with cholera toxin B conjugated to saporin (CTB-SAP) that is retrogradely transported to the phrenic and intercostal motor nuclei of the spinal cord, which results in selective elimination of phrenic and intercostal motor neurons. Despite deficits in maximal ventilatory capacity following CTB-SAP, eupneic ventilation is maintained. Our preliminary data suggest that one way eupnea may be maintained in CTB-SAP rats is via the recruitment of G-coupled protein receptor-dependent pathways to cause respiratory plasticity in the phrenic motor nucleus over the course of phrenic motor neuron death. However, our preliminary data also indicate that diaphragmatic amplitude is decreased at baseline in CTB-SAP rats vs. controls; thus, phrenic respiratory plasticity may only account for a portion of the maintenance of eupneic ventilation. We hypothesize that eupneic ventilation following respiratory motor neuron loss may also be maintained through the recruitment of accessory inspiratory muscles (e.g., the pectoralis minor muscles). The pectoralis minor muscles actively elevate the ribs upward and outward to move the chest wall following increased ventilatory demand. Pectoralis minor muscles are not normally utilized for eupnea, but have been shown to increase activity with disease or injury (e.g. ALS, spinal cord injury, and bilateral diaphragmatic paralysis). To begin to test this hypothesis, we are studying pectoralis minor output via electromyography in anesthetized, spontaneously breathing control and CTB-SAP rats. Our preliminary data suggest that indeed pectoralis minor activity is increased in CTB-SAP rats vs. controls, suggesting that the pectoralis minor may also be recruited to maintain eupneic ventilation. Future studies will evaluate the recruitment of other accessory inspiratory muscles (e.g., scalenes, sternocleidomastoid, etc.) over the course of respiratory motor neuron loss, and whether these muscles (including the pectoralis minor) are necessary for eupnea in CTB-SAP rats. This furthers our understanding of the potential contribution accessory inspiratory muscles, specifically the pectoralis minor, have on the maintenance of eupneic ventilation following respiratory motor neuron loss.
Positron emission tomography spinal imaging in a canine disease model of ALS

Joan R. Coates DVM MS Diplomate ACVIM (Neurology), Jeffrey N. Bryan DVM MS PhD Diplomate ACVIM (Oncology), Joni M. Lunceford, Kevin R. Lunceford, Stacy L. Wilder, Michael J. Schueller PhD, Richard A. Ferrieri PhD, Chih-Kai Chao, John M. Gerdes

1University of Missouri, Columbia, MO and 2University of Montana, Missoula, MT

Abstract: Canine degenerative myelopathy (DM) is a progressive adult-onset neurodegenerative disease that has similarities to amyotrophic lateral sclerosis (ALS). A hallmark of CNS tissues within ALS patients, DM dogs and SOD1 mutant rodents is the loss of the excitatory amino acid transporter (EAAT2) protein on CNS astrocytes, which causes regional excitotoxic injury. Recently, a new positron emission tomography (PET) imaging fluorine-18 radiolabeled tracer, $^{18}$F-FAA, which targets the CNS EAAT2 protein has been synthesized. The purpose was to detect EAAT2 changes in DM dogs relative to healthy controls (HC) by $^{18}$F-FAA spinal PET imaging.

Companion owned dogs were recruited as HC or DM affected. The cohorts included HC [age range: 5-11 years, 1 male and 6 females] and DM affected [age range: 9-14 years, 4 males and 2 females]. Multiple tracer doses were afforded per production run, with high molar activity (83-730 TBq/mmol) and purity (>95%). The $V_T$ estimates were derived with a two compartment (one tissue) kinetic. A significant statistical difference in $^{18}$F-FAA tracer $V_T$ was found for T5-T12 thoracic spine, where the DM cohort had a lower $V_T$ value than the HC cohort (ANOVA p=0.0307). A significant $V_T$ change in cervical spine (HC vs. DM) was not found. We conclude that the HC vs. DM cohort findings demonstrate that $^{18}$F-FAA PET imaging is sensitive for the detection of thoracic EAAT2 protein target changes. Thus, longitudinal PET imaging in DM dogs is advancing in order to assess progressive EAAT2 changes in spine regions and brain.
Title: Voluntary Wheel Running Partially Compensates for the Effects of Estrogen Receptor-α Knockout on Cortical Bone in Male Mice

Author: Rebecca K. Dirkes, Nathan C. Winn, Thomas J. Jurrissen, Dennis B. Lubahn, Victoria J. Vieira-Potter, Jaume Padilla, Pamela S. Hinton, University of Missouri- Columbia, MO

Abstract:  

Introduction: Loss of estrogen activity negatively impacts bone mass and strength, whereas exercise is osteogenic. Here, we determined whether exercise could compensate for the negative effects of estrogen receptor-α (ERα) loss.

Methods: At 12 weeks of age, male ER-α knockout (KO) and wild-type (WT) mice were given a high-fat diet and randomized to exercise (EX) treatment or sedentary (SED) control, resulting in four groups: WT-SED, WT-EX, KO-SED, and KO-EX (n=8-10 per group). After 10 weeks animals were sacrificed, and tibiae collected. Cortical geometry and trabecular microarchitecture were assessed via micro-CT. Biomechanical properties were assessed via three-point bending. Two-way ANCOVA with body mass was used to test the effects of KO and EX on cortical geometry and biomechanical properties; two-way ANOVA was used to test the effects of KO and EX on other outcomes.

Results: EX decreased body mass and body fat percentage, regardless of genotype. KO had lower total bone area, marrow area, and cortical thickness of the tibia mid-diaphysis versus WT. There was a significant genotype-by-exercise interaction for cortical area; EX increased cortical area in the KO animals, such that KO-EX was not different from WT animals. EX increased cortical thickness regardless of genotype. There were no differences in biomechanical properties among groups. KO improved measures of trabecular microarchitecture compared to WT, with no effect of EX.

Conclusion: Loss of ERα negatively impacts cortical bone geometry in young, sedentary, male mice, but exercise started after skeletal maturity can partially compensate for the loss of ERα activity.
Multi-modal Pain Assessment of Client-owned Dogs with Primary Bone Tumors

Flesner, Brian; Torres, Bryan; Hutcheson, David; Maitz, Charles; Tate, Debbie; Donnelly, Lindsay; McCleary-Wheeler, Angela; Rindt, Hans; Lunceford, Joni; Bryan, Jeffrey
University of Missouri; Presenter Brian K. Flesner, University of Missouri, Columbia, MO

Abstract: Current evidence for pain/gait outcomes in canine osteosarcoma is described by relatively few studies. Subjective owner questionnaires, force plate analysis, and serum biomarkers have been utilized to assess response. We aimed to use multiple diagnostics to objectively evaluate pain relief after therapeutic intervention in dogs with primary bone cancer. We hypothesized that intervention would cause objective evidence of pain control.

Methods: Evaluations of 8 dogs with primary bone cancer included $^{18}$F-FDG PET/CT scans, motion analysis, validated owner questionnaires (CBPI), and serum N-telopeptide (NTx) concentration. Dogs were staged and had $^{18}$F-FDG PET prior to treatment, and day 0, 7, 14, and 28-day CBPI, serum banking for NTx, orthopedic exam, and gait analysis. Dogs treated with radiation underwent day 28 $^{18}$F-FDG PET.

Results: Four dogs were amputated; four received neo-adjuvant zoledronate and hypofractionated radiation therapy. CBPI revealed significant improvements in pain severity and pain interference scores compared to baseline. Positive changes in peak vertical force (16.7%) and vertical impulse (29.1%) were noted at day 28. Dogs receiving zoledronate and RT had a significant (at least 30%) reduction in serum NTx from baseline compared to amputated dogs (p=0.029). Max$_{SUV}$ and Intensity values from PET scans decreased while Tumor Uniformity significantly increased in irradiated tumors; Gross Tumor Volume did not change.

Conclusions: Owner questionnaires, gait analyses, serum NTx, and maximum SUV on $^{18}$F-FDG PET/CT scans showed improved pain relief in dogs receiving zoledronate and radiation therapy. Larger, prospective studies are warranted to identify the best objective indicator of pain relief.
Title: Developing Swallow-Evoked Potentials (SwEPs) to Identify Pathological Neural Generator Sources for Targeted Dysphagia Treatment

Author: Ashley Kloepper, Joseph Arnold, M2, Brian Kinealy, M4, Chandler Haxton, Nicole Nichols, PhD, Kazutaka Takahashi, PhD, (Ilker Ozden, PhD) Department of Biomedical, Biological and Chemical Engineering, (Teresa Lever, PhD) Department of Otolaryngology-Head and Neck Surgery

Abstract: Objectives: Dysphagia is a poorly understood complication of many neurological diseases, often leading to fatal aspiration pneumonia. To address this clinical gap, we are adapting the fundamentals of auditory brainstem response testing to investigate dysphagia in rodents. Here, we share our progress toward developing a swallow evoked potential (SwEP) protocol in healthy mice and rats, in preparation for future studies with rodent models of neurogenic dysphagia.

Methods: Twenty C57BL6/SJL mice (4-8 months) and 20 Sprague Dawley rats (3-4 months) of either sex were used for SwEP protocol development. While lightly anesthetized and immobilized in ear bars, needle electrodes were inserted at multiple sites on the skull (subcutaneous) and ventral neck (intramuscular) for recording of EEG and swallow-related EMG activity, respectively. A chemical stimulus (2.7% citric acid) was applied to the oropharynx to evoke swallowing, confirmed by EMG bursts in synchrony with endoscopic pharyngeal constriction. SwEP responses were extracted from EEG signals time-locked to the onset of EMG swallowing activity during a 10-minute period post-stimulus application.

Results: Swallows were more frequent and consistent in rats versus mice. The averaged SwEP waveform consisted of 8 peaks within 10 ms for rats and 5 peaks within 6 ms for mice, immediately preceding the onset of EMG swallowing activity.

Conclusions: We developed a minimally invasive evoked potential protocol to investigate swallow-related neuropathology in rodents. Methodological optimization is underway, along with optogenetic experiments to unmask the corresponding neuroanatomical source(s) for each SwEP peak. Ultimately, we envision this work may accelerate the discovery of targeted dysphagia therapeutics.
Preload-induced ventricular dysfunction in isolated hearts from Duchenne Muscular Dystrophy (DMD<sup>mdx-4CV</sup>) mice.

Zahra Nourian, Laurin M. Hanft, Maike Krenz, Christopher P. Baines, Dongsheng Duan, Gang Yao, Kerry S. McDonald, and Timothy L. Domeier

Departments of Medical Pharmacology and Physiology, MU School of Medicine; Biomedical Sciences MU College of Veterinary Medicine; Molecular Microbiology and Immunology MU School of Medicine, Bioengineering, MU College of Engineering, and the Dalton Cardiovascular Research Center

Abstract: DMD is an inherited muscle wasting disease caused by absence of dystrophin. Clinically, DMD-associated muscle weakness presents early in life, progresses rapidly, and causes premature death. Subclinical signs of cardiac disease present early and usually progress to dilated cardiomyopathy in late stage DMD patients. Currently, it is unknown how dystrophin deficiency causes dystrophic cardiomyopathy and heart failure. Our group has undertaken a project to (i) examine underlying molecular mechanisms of dystrophic cardiomyopathy, (ii) identify novel diagnostic and prognostic biomarkers of disease progression, and (iii) identify novel gene therapies to ameliorate ventricular dysfunction. In this study we examined the tolerance of young male Dmd<sup>mdx-4CV</sup> dystrophic mice to sustained elevation in ventricular preload. Hearts were isolated, cannulated via both the aorta and left atrium, and perfused with physiological saline solution in Langendorff mode (i.e., no ventricular preload). In the absence of ventricular preload hearts of wildtype mice and Dmd<sup>mdx-4CV</sup> mice exhibited similar pressure development (60±6 mmHg wildtype versus 64±8 mmHg Dmd<sup>mdx-4CV</sup>). However, following pre-load challenge (20 mmHg in working heart mode for 30 minutes), hearts from Dmd<sup>mdx-4CV</sup> mice exhibited impaired pressure development (43±9 mmHg) versus wildtype mice (76±7 mmHg, P<0.05). The Dmd<sup>mdx-4CV</sup> hearts also had visible signs of damage in response to higher preloads. Taken together, the studies support susceptibility to stretch-induced ventricular damage and dysfunction in male Dmd<sup>mdx-4CV</sup> hearts. Future work will address sub-cellular mechanisms of stretch-induced ventricular dysfunction by systematic assessment of regulated cell death pathways, altered Ca<sup>2+</sup> handling, and varied myofilament structure/function in Dmd<sup>mdx-4CV</sup> mice.
Skeletal muscle mitochondrial dysfunction and whole-body metabolic alterations in a mouse model of osteogenesis imperfecta

Victoria Gremminger a, Emily Harrelson a, Laura C. Schulz b, R. Scott Rector c, Charlotte L. Phillips a

Department of Biochemistry, a Department of Obstetrics, Gynecology, and Women’s Health, b Department of Child Health, University of Missouri, Columbia, MO 65211. c Departments of Nutrition and Exercise Physiology and Medicine-GI, University of Missouri; Harry S Truman Memorial VA Hospital, Columbia, MO

Abstract: Osteogenesis imperfecta (OI) is a heritable connective tissue disorder with 85% of patients having type I collagen gene defects. OI occurs in approximately 1:15,000 livebirths and can be subdivided into four types based on clinical severity from mild with few fractures to perinatal lethal. Although bone fragility is the most common manifestation, intrinsic muscle weakness affects roughly 80% of OI patients. Homozygous osteogenesis imperfecta murine (oim/oim) mouse modeling moderately severe human OI type III have inherent muscle weakness and exhibit severe mitochondrial dysfunction. Oim/oim mitochondrial dysfunction was evidenced by significant reductions in gastrocnemius mitochondrial respiration rates, exhibiting only 35-48% of wildtype [Wt] mitochondrial respiration rates and citrate synthase activity. Mitochondria play essential roles in the metabolism and bioenergetics of the cell. To investigate parameters associated with metabolic health we measured glucose tolerance, energy expenditure, VO2 consumption, VCO2 production, and evaluation of the respiratory quotient (RQ) in Wt and oim/oim mice. RQ ratios (CO2 expelled:O2 consumed) can predict the primary fuel sources being utilized. Although glucose tolerance was not altered in male oim/oim mice, energy expenditure and VO2 consumption were increased and RQ reduced relative to WT mice; suggesting a change in metabolic fuel preference. Male oim/oim mice also exhibited increased lean mass and reduced fat mass. While further evaluation of these parameters is still required, preliminary data suggests that oim/oim mice may exhibit a metabolic phenotype with potential changes in metabolic fuel utilization, which may be associated with the previously observed mitochondrial dysfunction and compromised skeletal muscle force.
Title: Neurostimulation of Bladder Efferents After Spinal Cord Injury to Preserve Autonomic Ganglion Function

Author: David J. Schulz, Division of Biological Sciences, University of Missouri-Columbia

Abstract: It is now apparent that spinal cord injury (SCI) results in substantial changes in neurons and neural networks below the site of the injury as a result of loss of input, even though the cells of these networks are not directly injured. For example, in motor systems these changes in excitability below the injury manifest as spasm due to hyperexcitability in motor neuron populations. While these ideas have been investigated more in motor and sensory systems, there is a paucity of work in the autonomic nervous system in this regard, and virtually none of these concepts or approaches have been applied to efferent control of bladder and bowel function. Functional recovery or improvement will remain elusive if we do not understand these changes, as they are critical for the success of regenerated inputs, artificial interfaces, or pharmacological interventions. We use a rodent model of SCI to demonstrate how loss of descending inputs alters the gene expression, cellular properties, and activity of spinal motor and peripheral bladder innervating neurons below the site of injury. We have demonstrated that these injuries substantially reconfigure the gene expression profiles of these target tissues, as well as change the excitability and synaptic integration of peripheral neurons that directly innervate target organs such as the bladder. We are currently working towards designing and deploying implantable stimulation devices to determine whether acute stimulation shortly after injury can prevent or ameliorate these changes, with the hope that by preserving the underlying neural architecture in uninjured tissue below the site of injury, prognosis for functional recovery or efficacy of therapeutic intervention will be greatly enhanced.
Biomechanical Role and Strength of the Anterolateral Ligament in the Rotational Control of the Knee

Amy Whitaker, Matthew Daggett, Barth Wright, Kyle Barner, Catherine Mayer, Loretta Laughrey, Jayda Jones, Anthony Pitter and Thiagarajan Ganesh

University of Missouri-Kansas City, Department of Civil and Mechanical Engineering, 350K Robert H. Flarsheim Hall, 5110 Rockhill Road, Kansas City, MO 64110, USA
Kansas City University of Medicine and Biosciences, Kansas City, MO

Abstract: The anterolateral ligament (ALL) is a ligament located between the femur and tibia. The ligament’s importance and role has recently been discovered. It has been hypothesized by researchers that the ALL controls the rotational stability of the tibia/femur. In Anterior Cruciate Ligament (ACL) construction surgery, the proposed connection between ALL and ACL is often disregarded. Understanding the biomechanics of the knee joint and the role that the anterolateral ligament plays in rotational stability is crucial to making good surgical decisions. To study the contribution of the ALL to the rotational control of the knee, we designed and fabricated a testing apparatus capable of applying torsional and axial loads simultaneously to the knee using the BOSE® testing machine. Then a testing protocol was designed and developed to evaluate the contributions of the anterior and posterior bands of the ALL to torsional stability of unembalmed (fresh frozen) cadaveric knees when positioned at various degrees of flexion. The results of the biomechanical analysis of the ALL from the mechanical testing and strain analysis using the non-contact strain measurement technique called Digital Image Correlation (DIC) analysis of strains are presented in this poster.
Title: Multi-scale finite element analysis of 3D multiplexed images to examine bone mechanotransduction and heterogeneous activation of β-catenin signaling in osteocytes.

Author: Loretta E. Laughrey, Nuria N. Lara-Castillo, LeAnn M. Tiede-Lewis, Sarah L. Dallas, Mark L. Johnson, Thiagarajan Ganesh, University of Missouri - Kansas City: School of Computing and Engineering, School of Dentistry

Abstract: Wnt/β-catenin signaling in osteocytes is known to be necessary for bone formation. We have observed that cyclic compression loading of the mouse forearm results in heterogeneous activation of Wnt/β-catenin signaling in osteocytes at the mid-shaft of the ulna. This is in contrast to results from previous bone finite element (FE) models, which predict a homogeneous osteocyte response.

To develop a more detailed understanding of mechanotransduction between bone loading and Wnt/β-catenin activation, we have developed realistic, predictive computer FE models that incorporate tissue imaging data to compute bone strains in the lacunar walls in response to macroscopic bone loading and to correlate those strains with β-catenin activation in osteocytes.

In preliminary studies, Micro-CT scans and confocal fluorescence images of murine bones were collected. The images were converted into finite element models using the Materialise Innovation Suite®, and strain analysis was done using the FEBio Software Suite. β-catenin activation was assessed using fluorescence intensity values from a LacZ reporter. Pearson correlation was used to identify relationships between strain and β-catenin activation for individual osteocytes within the same bone sample.

Using this experimental process with bone from mice in three age cohorts, we will look for patterns in bone strain that may suggest new ways to mechanically induce better bone stimulation for fracture and osteoporotic patients.
**Title:** Jaw Morphogenesis: a budding role of cranial neural crest in bone mineral density and microarchitecture

**Author:** Hammad Mumtaz, Kathleen Nguyen, Brianne Schmiegelow, LeAnn Tiede-Lewis, Maria Gonzalez, and Erin Bumann

**Abstract:** Mandibular bone reconstruction is still the only option for craniofacial jaw defects, trauma, and cancer. An in-depth study of jaw bone development is crucial to develop non-surgical options. We can learn a lot about jaw bone formation and morphogenesis by studying vertebrates. Our lab uses two commercially-available avian with differences in jaw size and shape, quail and duck. We have shown previously that osteoblast lineage, cells involved in bone deposition, derived from cranial neural crest control bone mineral density (BMD) during development. The objective of this study was to determine species-specific differences in BMD and microarchitecture. Fertilized eggs of quail and duck were incubated, mandibles collected at stages of early bone deposition (HH36) or remodeling (HH39), and analyzed by osteomeasure or microcomputed tomography. Duck had an average BMD of about 140mg calcium hydroxyapatite (CaHA)/cm$^3$, which was significantly higher than the quail 115mg CaHA/cm$^3$ at HH39 ($p<0.0005$). Quail had significantly more BMD volume from 90-120mg CaHA/cm$^3$, while duck had significantly more BMD volume from 180-270mg CaHA/cm$^3$ at HH39 ($p<0.01$). From HH36 to HH39 significant differences were seen in bone surface to bone volume (BS/BV) and trabecular thickness in both quail and duck ($p<0.05$). Trabecular width was significantly higher in duck compared to quail at HH39 ($p<0.01$). No significant differences were found in BS/BV, trabecular number, or width between species of the same stage. Species-specific differences were evaluated but further studies are needed to determine the precise mechanisms by which these properties are controlled.
Regulation of myofilament force and loaded shortening by skeletal myosin binding protein-c

Joel Robinett, Department of Medical Pharmacology and Physiology, University of Missouri

Abstract: Skeletal Myosin Binding Protein-C (sMyBP-C) is a 125-140 kDa protein located on each half-thick filament in a region known as the C-zone. In this study we investigated mechanisms by which sMyBP-C regulates myofilament function using rat permeabilized skeletal muscle fibers. Slow-twitch skeletal muscle fibers were mounted between a force transducer and motor and Ca^{2+} activated to produce a range of forces. Contractile properties were measured including stretch-induced transient force overshoot, force development rates, and loaded sarcomere shortening. In slow-twitch fibers, protein kinase A (PKA) treatment (i) augmented phosphorylation of sMyBP-C, (ii) doubled the magnitude of the relative transient force overshoot at low Ca^{2+} activation levels, and (iii) increased force development rates at all Ca^{2+} activation levels. We also investigated the role that sMyBP-C phosphorylation state plays in loaded sarcomere shortening. We tested the hypothesis that MyBP-C acts as a brake to filament sliding within the myofilament lattice by measuring sarcomere shortening as thin filaments traversed into the C-zone during lightly loaded slow-twitch fiber contractions. Before PKA, shortening velocity decelerated as sarcomeres traversed from ~3.10 to ~3.00 µm. After PKA, sarcomeres shortened a greater distance and exhibited less deceleration during similar force clamps. Following sMyBP-C dephosphorylation, sarcomere length traces displayed a brief recoil (i.e., “bump”) that initiated at ~3.06 µm during loaded shortening. Our results suggest sMyBP-C and its phosphorylation state regulate sarcomere contraction by a combination of cross-bridge recruitment, modification of cross-bridge cycling kinetics, and alteration of drag forces that originate in the C-zone.
Title: Overlapping functions of matrix vesicles and extracellular vesicles in bone

Author: Kun Wang, LeAnn M. Tiede-Lewis, Lora A. Shiflett, Donggao Zhao, Jennifer L. Rosser, Andrew Keightley, Lynda F. Bonewald, Sarah L. Dallas, University of Missouri Kansas City, Kansas City, MO.

Abstract: A recent paradigm in cell-cell communication involves cell shedding of extracellular vesicles (EV) (exosomes and microvesicles), which deliver their protein, mRNA and miRNA cargo to target cells to alter their function. Using Dmp1-mGFP mice expressing a membrane-GFP in osteocytes, we have shown that osteocytes shed EV, which can signal to osteoblasts, be released into the circulation or be deposited in bone ECM. Matrix vesicles (MV) are another type of ECM-bound vesicle that initiate mineralization in calcified tissues. Because EV and MV have similar characteristics it was proposed that MV are a specialized type of anchored exosome. To examine this, we compared the properties and function of MV and EV from IDG-SW3 cells, a model of osteoblast/osteocyte transition.

In Dmp1-mGFP mice, GFP+ve vesicles were observed in the ECM of bone and dentin. Confocal and electron microscopy showed that osteocyte-enriched SW3 cells release similar vesicles and confirmed ECM-bound MV containing mineral crystals. EV were isolated from SW3 cell culture supernatants and MV were isolated from the cell layer. Both EV and MV were enriched in mineralization-related proteins, alkaline phosphatase, annexin A5 and PHOSPHO1, with comparable levels of mRNAs for osteocyte-expressed genes Dmp1, E11, Rankl, and HIF1α. Treatment of undifferentiated SW3 cells with EV or MV from mature SW3 cells increased Dmp1-GFP and induced comparable changes in osteocyte gene expression (upregulation of Dmp1 and RankL; no change in E11/gp38; downregulation of Phex, Colla1 and TNAP). Both EV and MV induced equivalent mineralization in SW3 cells. These data show that EV and MV from SW3 cells overlap in composition and function, supporting the concept that MV are a specialized exosome that nucleates mineralization and functions in cell-cell signaling in bone.