University of Washington

Center for Translational Muscle Research

4th Annual Symposium

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Poster Presentation Abstracts

Abstracts are listed in alphabetical order by presenting author's last name.

Indicates the presenter is also giving a lightning talk.

Poster # 1

Measuring myofilament specific calcium in HIPSC cardiomyocytes with improved optogenetic sensors.

Anthony Asencio[1], Justin D. Lee[2], Sarah Wait[2], Michael Regnier[3], Andre Berndt[1], Farid Moussavi-Harami[4]. [1]BioEngineering, University of Washington, Seattle, WA, USA, [2]Molecular Engineering, University of Washington, Seattle, WA, USA, [3]Dept BioEngg, Univ Washington, Seattle, WA, USA, [4]Cardiology, Univ Washington, Seattle, WA, USA.

Genetic cardiomyopathy can emerge from point mutations in sarcomeric proteins. To understand disease progression and develop targeted treatments, a better understanding of the affected molecular mechanisms regulating contraction is needed. New experimental tools have been developed that can assist in this regard. First, human cardiac cells and tissue derived from induced pluripotent stem cells (iPSCs) have become valuable models for studying the initiating events in genetic cardiomyopathies. Second, novel calcium indicators and localization strategies allow for time resolved compartmental measurements of ionic calcium. These strategies target cardiac troponin I or T (cTnT or cTnI). We aimed to improve upon the strategy of using fusion proteins to better quantitate measurements of the free calcium concentration in myofilaments using the green protein calcium sensor GCAMP6F fused to the n-terminus of cTnT. To estimate the local effect of decreased myofilament calcium buffering, we will lipofect our DNA construct into cells differentiated from three hiPSC lines that have different troponin C (TnC) calcium binding affinities. These cell lines have the WTC11 iPSC background and express: WT TnC, heterozygous cTnC I61Q (with reduced calcium binding), and homozygous cTnC D65A (that does not bind calcium in the N-terminal site II). Our preliminary results show that our probe tagged cTnT localizes the areas of the sarcomere filament with troponin as expected, the sensor brightness is improved over other fused protein probes, and the on/off rates are satisfactory for estimating myofilament calcium kinetics.

Poster # 2

Danicamtiv affected isometric force and cross-bridge kinetics similarly in skinned myocardial strips from male and female rats

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Myotropes are small molecule pharmaceuticals that have recently been developed or are under investigation for treatment of heart diseases. Myotropes have had varied success in clinical trials. Initial research into these myotropes have widely focused on animal models of cardiac dysfunction in comparison with normal animal cardiac physiology—primarily using males. In this study, we examined the effect of a myosin activator called Danicamtiv on contractile function in skinned myocardial strips from male and female Sprague-Dawley rats. We found that skinned muscle strips from both sexes showed an increased steady-state isometric force production at sub-maximal calcium levels (equivalent to an increase in Ca^{2+}-activated sensitivity to contraction) in the presence of 3 µM Danicamtiv at sarcomere length of 2.2 µm. While there was an increase in Ca^{2+}-sensitivity, there were no increases in maximal force for either sex. Danicamtiv also showed no sex differences in the crossbridge cycling, whereby Danicamtiv slowed down cross-bridge cycling kinetics via decreasing the cross-bridge detachment rate (as we had previously observed, Kooiker *et al.* 2023. Circ Res). Given what is known about Danicamtiv, these increases in contractility may follow from the myotrope shifting the population of cross-bridges from the OFF (super-relaxed state) to the ON (disorder relaxed state) to enhancing cross-bridge binding. The inclusion of both sexes in animal models during the formative stages of drug development could be beneficial for mitigating the efficacy, or in contrast the limitations, of therapeutic impacts on cardiac function.

Poster # 3

Developing novel epigenetic treatments for DMD using human stem cell-derived engineered muscle tissues

P. Barrett[1], K. Louie, C. Le Guiner, L. Maves, A. Smith, S. Luttrell, N. Geisse, J-B. Dupont and D. Mack.

Although Duchenne Muscular Dystrophy (DMD) is a progressive and degenerative disease there is evidence for embryonic and fetal stage defects during myogenesis, including transcriptional and epigenetic perturbations. Epigenetic small molecules are proving to be outstanding candidates as DMD therapies, they improve the phenotype in animal models, have a candidate in phase 3 clinical trials and they are already approved for use in cancers. However, many small molecule therapies for DMD have failed to show efficacy in clinical trials, which could be partly due to an inadequate preclinical evaluation or to targeting mechanisms too late in the disease. Human induced pluripotent stem cell (hiPSC) derived models recapitulate embryonic muscle development with high fidelity and enable characterization of contractile properties when cast into engineered muscle tissues (EMTs). Dystrophin-deficient EMTs were utilized to track the initiation and progression of pathology and then screened for functional recovery following treatment with novel epigenetic compounds identified in our high throughput DMD zebrafish screen. EMTs were repeatedly analysed using the Magetometric Analyzer for eNgineered Tissue ARRAY (MANTARRAY) platform. Dystrophin-null EMTs showed force deficits, relaxation delays, elevated resting calcium, blunted calcium transients and impaired mitochondrial function. The histone deacetylase inhibitor Trichostatin A reversed the contractile deficit and corrected other aspects of the pathology in a dose dependent manner. Combining developmental, structural and functional analyses at the engineered tissue level with the latest transcriptomic methods is improving our understanding the molecular drivers of DMD pathology and uncovering the mechanisms of new epigenetic therapeutics.

Poster # 4

The Association of Insulin Like Growth Factor Binding Protein-2 With Muscle Mass and Function in Prostate Cancer

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Elevated insulin-like growth factor binding protein (IGFBP)-2 levels are reported among various cancers, and recent findings indicate that it plays a role in muscle wasting in pancreatic cancer. In prostate cancer, the potential association of IGFBP2 with muscle mass and function has yet to be addressed. We hypothesize that plasma IGFBP2 will be negatively associated with muscle mass and function in prostate cancer patients.

IGFBP-2, physical function, and body composition were assessed in prostate cancer patients about to begin androgen deprivation therapy (n = 54). Body composition was assessed by dual x-ray absorptiometry (DEXA). Muscle strength by hand grip (HGS) and stair climb power (SCP), and muscle endurance through six minute walk test (6MWT) and VO2 Max. Muscle mitochondrial function was determined by obtaining the oxygen consumption rate under different stressors.

IGFBP2 presented negative correlations with BMI (r: -0.517, $p < 0.001$, n = 54), DEXA muscle mass (r: -0.360, p = 0.007, n = 54), HGS (r: -0.358, p = 0.008, n = 54), and SCP (r: -0.314, p = 0.023, n = 52). Additionally, it showed positive correlations with basal (r: -0.309, p = 0.037, n = 46), ADP-linked (r: -0.351, p = 0.017, n = 46), and maximal (r: -0.441, p = 0.002, n = 46) mitochondrial respiration.

IGFBP2 was associated negatively with body weight, muscle mass, and muscle strength, but positively with mitochondrial respiration. IGFBP2's relationship with muscle may be fiber-dependent having a negative impact on slow twitch fibers but a potential positive effect with fast twitch fibers.

Poster # 5

In Situ Transcriptomics Analysis of a Muscular Dystrophy Mouse Model for Identification of Cellular Therapeutic Targets

Chamberlain, Joel R., Zavaljevski, Maya, and Bisset, Darren R.

We are developing an adeno-associated viral vector (AAV) delivery of RNAi sequences targeting cellular mRNAs as a treatment for facioscapulohumeral muscular dystrophy (FSHD). To broaden candidate mRNA targets for AAV-RNAi therapy we are investigating FSHD disease mechanism, which is poorly understood. FSHD is caused by misexpression of the DUX4 gene in muscle, where it is normally repressed following early development. DUX4 is a transcription factor gene epigenetically repressed in D4Z4 macrosatellite repeats on chromosome 4q35, which are reduced in number or altered in DNA and chromatin methylation states in disease to relieve repression. Widespread cellular changes are initiated by DUX4 to cause muscle damage, although the mechanism of cell death is unclear. Most studies of DUX4 focus on myoblast expression, but contributions from myofibers, inflammatory infiltrates, and vascular changes also play a role in disease progression in FSHD muscle. To investigate the direct relationship between DUX4 and muscle changes in vivo we initiated a bioinformatics approach using a mouse model of FSHD based on AAV delivery of the DUX4 gene (AAV-DUX4) to wild-type muscle. We detailed the spatial transcriptomic profiles of 50 mm spots spanning transverse cryosections of AAV-DUX4 dosed or non-expressing AAV treated tibialis anterior muscles. Seurat cluster analysis showed gene enrichment for a dramatic decrease in mitochondrial function and indicated a myofiber functional decline. Positive changes indicate an increase in protein translation and immune cell stimulation. Further analyses of nearest neighbor regions adjacent to DUX4 expression and a mouse biomarker provided overlapping data with additional detail for immune cell activation. With information mined from the transcriptomics study we aim to identify muscle and non-muscle cell changes related to DUX4 spatiotemporal expression to identify new targets for development of candidate therapies for FSHD.

Poster # 6

Control Number: *I-11980*

Title:

USING 2'-DEOXY-ADP TO PROBE STABILITY OF THE MYOSIN INTERACTING HEADS MOTIF AT ATOMIC RESOLUTION

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Body:

In addition to active cycling states, myosins within the thick filament can access an 'inactive' conformation called the interacting heads motif (IHM) that has been associated with the energy-conserving super relaxed (SRX) state of muscle. Thus, accessing the IHM conformation is a means of thick filament-based regulation of muscle. Only a handful of cryo-EM structures of IHM myosin are available. The large size of the IHM has similarly challenged all-atom molecular simulations of the structure. have performed explicit solvent, all-atom molecular dynamics simulations of human cardiac β-myosin in the IHM confirmation on the microsecond timescale. In recent experimental studies, the small molecule ATP analogue, 2'-deoxy-ATP (dATP), has been shown to destabilize myosin heads from the IHM into disordered, more active states. To complement these experimental studies, we simulated β-myosin in the IHM conformation in which ADP.P_i was replaced by dADP.P_i. These simulations showed that dADP reduced the stability of the IHM by reducing the number of interactions between S1 heads as well as the net interaction energy between the heads. They also show that the tails of dADP.P_i -bound heads adopted conformations distinct from existing atomic models obtained with cryoEM. Thus, simulations suggest that dynamics in the RLC-binding region of the tail and at the head-head interface both contribute to IHM stability. Further, they suggest that departure from the IHM state involves coordinated motions in regions of myosin separated by over 100 Å. These novel simulations should also prompt further research into the contribution of tail dynamics into IHM stability as well as interest in mutations that influence tail dynamics. Ongoing coarse-grained simulations will probe the stability of the interacting heads over longer timescales.

Poster # 7

Comparing the Transcriptomic Trajectories of Differentiating iPSC-Spinal Motor Neurons with that of Developing Fetal Spinal Cord Ventral Horn

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Many muscle wasting diseases are triggered by dysfunction in innervating spinal motor neurons. To study the upstream of the neuromuscular pathology, iPSC-derived ventrospinal neurons have been used to model human spinal cord ventral horn that motor neurons reside. However, current differentiation protocols generate neurons with unknown subpopulation composition and transcriptomic relevance to their native counterpart. Moreover, we do not have enough understanding of whether the generated cells are capable of interacting with skeletal muscle to generate functional neuromuscular synapse (NMJ), which develops through exquisite crosstalk between those two cell types during the embryogenesis. To address this issue, we aimed to analyze the transcriptomic trajectories of developing ventrospinal neurons by using longitudinal single-cell RNAseq approach. The differentiating neurons are collected every 3-5 days from pluripotent state to the differentiation day 50 to generate 15 libraries to map their temporal change of transcriptome along the differentiation. Moreover, we sought to use the same approach with developing human spinal cord obtained from UW Birth Defect Research Laboratory at different gestational stages to align their transcriptomic profiles to that of iPSC-derived ventrospinal neurons. From the populational analysis of the *in vitro* part of the experiment, we found that the cells go under active neurogenic transition from day18 to day25, which turned out to be the critical temporal window that the majority of the unpatterned neural progenitors switch over to ventropsinal neurons. Since the more in-depth analysis is ongoing, we expect this comprehensive set of bioinformatics data will guide us to improve current *in vitro* differentiation protocols to closely recapitulate the native spinal cord development. Finally, we believe this study to provide an important insight to generate the first *bona-fide* human NMJ in-a-dish to model neuromuscular diseases as a whole.

Poster # 8

Role of AMPK in chemotherapy-induced muscle wasting

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Introduction: Muscle wasting is a common side effect of chemotherapy treatment and is associated with poor outcomes for cancer patients. 5'-adenosine monophosphate-activated protein kinase (AMPK) is an intracellular regulator of energy homeostasis, and plays an important role in muscle metabolism and function. Despite this, the function of AMPK in chemotherapy-induced muscle wasting remains unknown. To characterize the interaction between chemotherapy-induced wasting and AMPK and to decipher the underlying mechanisms, we used a genetic model to compare the effects of cisplatin on AMPK transgenic (TG) and wild-type (WT) mice.

Methods: Adult male AMPKα2i WT and TG mice were treated with vehicle or cisplatin (2.5 mL/kg/day) for 5 days and animals were euthanized after 4 days of the last cisplatin treatment. Body composition, grip strength, and treadmill endurance were assessed pre- and post-treatment. Skeletal muscle was collected following euthanasia. Statistical analysis was performed by two-way ANOVA or independent t-test (2-tailed), $p < 0.05$.

Results: At baseline, TG mice exhibited significantly lower treadmill-time-to-exhaustion and normalized grip strength, as well as greater fat mass and body weight. Treatment with cisplatin induced genotype-independent decreases in food intake, body weight, fat mass, and lean body mass. Declines in grip strength and treadmill running time were both significant and more prominent in TG mice compared to their WT counterparts. Additionally, TG mice tended towards a greater cisplatin-induced decline in hindlimb muscle mass than WT mice.

Conclusions: AMPK is necessary for the maintenance of muscle function during chemotherapy in mice.

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Poster # 9

ASCL1 pioneer factor activity switches from neurogenic to myogenic lineage following RTK modulation by synthetic proteins

Damien Detraux[1], Riya Keshri[1], Thomas Schlichthaerle[1,2], Jason Zhang[1,2], David Baker[1,2], Julie Mathieu[3]*, Hannele Ruohola-Baker[1]*

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The direct conversion of somatic cells into another terminally differentiated cell type, called transdifferentiation, is an attractive approach for the field of regenerative medicine. The control of cell fate conversion during transdifferentiation is so far done by the expression of specific transcription factors, called pioneer factors (PF), and a cocktail of small molecules. Our data suggest that while pioneer factors open the chromatin, novel AI-designed receptor tyrosine-kinase (RTKs) mini binders can further direct the fate. These proteins, designed to be small, specific and stable, could outperform and replace small molecules or endogenous growth factor. In the context of canonical neuron specific PFs such as ASCL1 and NGN2 expression, we screened a library of available minibinders and show that the activation of FGFR, TIE2, TRKA and EGFR signaling with concomitant TGFBR2 blockade surprisingly leads to a switch from the neurogenic to a myogenic Desmin positive lineage. We then demonstrate that this switch is ASCL1-dependent and results in the upregulation of MYOD, a skeletal muscle master regulator. We postulate that the cell fate switch following minibinder treatment results from a change in ASCL1 chromatin binding. Using this system, we aim to understand the mechanisms regulating cell fate decisions during direct conversion, especially focusing on the chromatin accessibility of PF after RTK modulation.

Poster # 10

A direct reprogramming of fibroblast into skeletal muscles targeting receptor signaling pathways with AI-designed synthetic ligands

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Abstract

A diminished repair capacity of skeletal muscles in aging, severe injuries, and disorders such as muscular dystrophies require advances in personalized regenerative medicine. Transdifferentiation/direct reprogramming is one of the safest and most desirable ways of therapeutics development. MyoD overexpression mediated myogenic transdifferentiation has widely been utilized where higher efficacy is yet to be achieved for clinical purposes. We utilize AI-designed synthetic minibinders and heterofusions to target extracellular receptors (RTKs) for ameliorating myogenic transdifferentiation. The synthetic minibinders (mb) show high receptor specificity, isoform specificity and better stability than natural ligands. The synthetic minibinders are antagonists of RTKs signaling and when scaffolded together acts as antagonists. While Heterofusions forcefully binds to two nonpairing receptors and bring them in close proximity to elicit rare signaling response. In human fibroblast we screened and identified C6_mbFGFR1/2c agonist, ALK1R_mb and TGFBRII_mb that increase the efficiency of myogenic transdifferentiation. We propose that these signaling pathways improve muscle transdifferentiation by altering myogenic regulators and by single cell sequencing we will unveil the molecular details further. Surprisingly, we identified two classes of heterofusions that improved the transdifferentiation. The first class of heterofusions are strong inactivators of the signaling pathways such as Lytac:mb-ALK1:mb and TGFbRII:mb-Her2:mb strongly inactivates ALK1R and TGFbR pathways. The second class of heterofusions are activators of unprecedented signaling responses such as, Her2:mb-FGFR:mb or TRKA:mb-BMPR2:mb. We are interested to know if presence of the two receptors is relevant for the activation of the unprecedented pathways or not. Additionally, we screened the library of heterofusions in another biological assay, the myogenic differentiation of hiPSCs overexpressing MyoD, and identified Alk1:mb-Lytac:mb, Her2:mb-TGFBRII:mb, Alk1R:mb-Gp130R:mb, IGF1R:mb-PDGFR:mb heterofusions. We report the rare and unique signaling response generated by heterofusions and their relevance in myogenic reprogramming and we are interested in understanding their molecular mechanism. Overall, These heterofusions and minibinders are extremely promising tools with enormous applications in future regenerative medicine development.

Poster # 11

A MOLECULAR SCALE INVESTIGATION OF THE MECHANISMS OF CONTRACTILE DYSFUNCTION FOR THE HYPERTROPHIC CARDIOMYOPATHY MYH7 G256E MUTATION

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Studying the molecular mechanisms of hypertrophic cardiomyopathy (HCM) in humans has several challenges, including availability of myectomy samples for specific mutations. To determine the effect of specific mutations on β-myosin heavy chain (MHC) function, we use gene-edited human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as a model system. Measurements from individual isolated contractile organelles (myofibrils) from hiPSC-CMs demonstrate that the specific force and rate of force generation for mutant G256E myofibrils is greater than isogenic control myofibrils. During relaxation, slow phase kinetics were decreased, indicating slower cross-bridge detachment rate. Furthermore, isolated G256E myofibrils were less sensitive to ADP product inhibition during relaxation, pointing to delayed ADP release as a mechanism of impaired relaxation. Using stopped-flow kinetic analysis, measures of myofibril ATP binding using mant-ATP, was slower for the G256E mutation. Together, these data identify impaired relaxation via delayed myosin detachment as a mechanism for the G256E hypercontractile phenotype. To understand the molecular-structural basis of these effects we used molecular dynamics computational simulations of wild type and mutant β-MHC. In the post-rigor (M.ATP) state structure, we observed reduced transducer region stability due to weakened hydrogen bonds between neighboring β-sheet strands as well as significant changes in local contacts. In post-powerstroke simulations (A.M.ADP), altered nucleotide pocket dynamics in G256E simulations suggest that structural communication leading to ADP release may be affected. Overall, our results suggest that the G256E mutation affects the myosin transducer region, leading to altered communication between the nucleotide binding pocket and the actin binding surface during the acto-myosin chemo-mechanical cycle.

Poster # 12

Title: Mitochondrial Electron Transport Chain Assays for Mouse Tissue Homogenates

Authors: Ernst-Bernhard Kayser[1] PhD, Mariya Sweetwyne[2] PhD, and Anna Scott[1] PhD

Affiliation: [1]Seattle Children's Hospital, Biochemical Genetics Lab; [2]UW Medicine Pathology

Abstract:

The mitochondrial electron transport chain (ETC) or respiratory chain maintains the electrochemical proton gradient across the inner mitochondrial membrane which is the driving force for mitochondrial ATP synthesis via oxidative phosphorylation. Dysfunction of electron transport, caused either by genetic lesions or environmental toxins, leads to a variety of primary neuro-muscular diseases (e.g. Leber's Hereditary Optic Neuropathy, Leigh syndrome) but is also implicated in nutrition and age-related chronic diseases (e.g. diabetes, Parkinson's disease). Since energy metabolism is affected, muscle weakness and exercise intolerance are frequently observable sequelae.

The Biochemical Genetics Lab of Seattle Children's Hospital has recently implemented a diagnostic service for functional testing of the ETC in homogenates from human muscle biopsies. Here, we report progress to extend the service to processing a variety of mouse tissues with high energy demand: skeletal muscle, heart, kidney, and liver.

The test panel comprises the ETC assays: Complex I (rotenone-sensitive NADH:ubiquinone reductase), Complex II (TTFA-sensitive succinate:ubiquinone reductase), Complex III (antimycin-sensitive ubiquinol:cytochrome c reductase), Complex IV (KCN-sensitive cytochrome c oxidase), Complex I-III (NADH:cytochrome c reductase), Complex II-III (antimycin A-sensitive succinate:cytochrome c reductase), succinate dehydrogenase (malonate-sensitive succinate:PES reductase to probe proximal Complex II). Two additional enzymes tested are not components of the ETC: citrate synthase serves to assess mitochondrial content of the homogenate; lactate dehydrogenase determines the capacity for ATP synthesis by glycolysis when mitochondrial reoxidation of NADH is impaired.

The widened scope of acceptable sample types may be useful for research collaborations. Please contact Anna Scott if you are interested.

Poster # 13

Novel promoters for GNEM gene therapy

Sherin Lucas[1], Violet Phan, Julie Crudele

[1]Undergraduate

GNE myopathy (**GNEM**) is caused by mutations in the *GNE* gene, which encodes the bifunctional enzyme **GNE** (UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine) in the sialic acid biosynthesis pathway. Missense mutations in *GNE* lead to hyposialylation of glycoproteins on the surface of muscle fibers and progressive muscle weakness and atrophy. We are developing an AAV gene therapy for GNEM. Due to recent dose-dependent adverse events and deaths in muscle-directed AAV clinical trials, we are developing a gene therapy that will be efficacious at lower, hopefully safer, doses. To that end we are designing novel tandem expression cassettes (**TECs**) using promoter and enhancer elements from genes with liver- and muscle-restricted expression. By using a TEC with dual expression in muscle and liver, we can 1) replace GNE in the muscle directly and 2) indirectly increase sialylation of muscle fibers by exploiting the sialic acid scavenging/recycling pathway utilizing glycoproteins secreted by the liver.

We have designed two iterations of TECs and tested them *in vitro* in C2C12 myoblasts, HepG2 hepatocytes, and HEK293s (negative control). 5 of 7 TECs retained at least 50% expression in C2C12s compared to a muscle-specific promoter, with 4 of the TECs actually showing enhanced expression. All of the TECs retained at least 70% expression in HepG2s compared to a liver-specific promoter, and none of them showed enhanced expression in HEK293s versus the muscle-specific promoter. While *in vivo* tests are pending, we have demonstrated that we can combine liver and muscle promoter elements for expression in both tissues without losing tissue specificity.

Poster # 14

Rat Model of Duchenne Muscular Dystrophy (DMD mdx)

Luo, S, Noffke, E., Tasfaout, H., Flint, G.V., Snyder, J.M., Chamberlain, J.S., Mack, D. L.

Duchenne muscular dystrophy (DMD) is a severe muscle wasting disease caused by mutations in the DMD gene encoding dystrophin. Much of the field's foundational understanding comes from mice studies; unfortunately, there are clear limitations due to their near-normal life span, mild to non-progressive clinical course, and robust muscle regeneration. The DMD mdx rat model mimics skeletal and cardiac functional abnormality and pathology observed in DMD patients (progressive muscle wasting, fibrosis, poor regeneration). We have accumulated a large data set characterizing clinically-relevant functional outcome measurements at 3-, 6-, 9- and 12-months in preparation for preclinical evaluation and have injected rats with AAVs ([AAV6-, AA9-, AA9Myo1]-CK9e-µDys5) to examine muscle-specific targeting, liver de-targeting, transduction efficiency, dosage, and explore delivery parameters to halt disease progression. DMD rats displayed muscle weakness compared to WT siblings with age (isometric torque-frequency relationship), and a trend towards weaker cardiac phenotype (shallow diaphragm deflections (amplitude; mm), elevated Myocardial Performance Index scores, reduced fractional shortening, and lower left ventricular mass) consistent with the cardiomyopathy observed in DMD boys. We have accumulated a large histopathology data set demonstrating morphological differences using Hematoxylin/eosin, trichrome stain, and picrosirius red (5 µm, formalin-fixed, paraffin-embedded, microtome-sectioned) to quantify necrosis, atrophy, fat accumulation, and connective tissue/fibrosis. The observed fibrosis (abundance in connective tissue), necrosis, inflammation in skeletal-, cardiac-, and diaphragmatic-muscles is consistent with the weaker cardiac and diaphragmatic phenotype and hindlimb muscle weakness. Post gene-therapy, we see a strong trend towards improved peak hindlimb force (leftward shift of the torque frequency), weight, cardiac and histopathology readouts.

Poster # 15

MYH3 mutations associated with distal arthrogryposis alter the contractility and myosin isoform switching of hiPSC derived skeletal muscle

Christian Mandrycky[1], Saffie Mohran[1], Matthew Childers[1], Shawn Luttrell[2], Elizabeth Choi[1], Kati Buckingham[3], Michael Bamshad[3], David L. Mack[1,2], Michael Regnier[1]

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Distal arthrogryposis (DA) is a skeletal muscle disorder characterized by joint contractures predominantly localized in the distal extremities. DA associated syndromes like Freeman-Sheldon Syndrome (FSS) are linked to mutations in the *MYH3* gene that encodes the embryonic skeletal muscle myosin. To study the mutation and its effect on developing muscle, we generated human induced pluripotent stem cell (hiPSC) lines bearing T178I or R672C *MYH3* mutations. hiPSC were differentiated into skeletal muscle and evaluated for differences in the maturation of the contractile unit and its functional performance. All lines efficiently differentiated into skeletal muscle and no differences in fusion efficiency or sarcomere length were observed between genotypes. R672C mutations in MYH3 were associated with alterations in myosin isoform content, with homozygous R672C having reduced MYH3 protein by day 7 of differentiation and near loss of MYH3 protein by day 9. This corresponded with elevated levels of MYH8 and MYH7 protein relative to isogenic control. Myofibril mechanics measurements showed no differences in specific force between mutant and normal myofibrils, but myofibrils from homozygous R672C cells had a faster rate of relaxation ($k_{rel,fast}$) and more complete relaxation. To investigate the functional effect of MYH3 mutations absent changes in myosin isoform content we have validated the siRNA induced knockdown of MYH8 and MYH7 in normal and mutant cells. Ongoing studies will further characterize the effect of these mutations on actin-myosin binding, crossbridge cycling kinetics, the maturation of skeletal myotubes over time, and myosin isoform switching dynamics.

Poster # 16

Title: Sex Differences in Frailty, Sarcopenia, and Mitochondrial Dysfunction.
Authors: Gavin Pharaoh, Nathan Kaiser, Anna Bakhtina, Jim Bruce, David Marcinek

Background: Frailty is a condition characterized by increased vulnerability to adverse health outcomes. Importantly, sex has a major impact on frailty development with females generally experiencing higher frailty. We hypothesized that disruptions in mitochondrial protein-protein interactions impair mitochondrial function and contribute to the development of sarcopenia and frailty. The goal of this study is to identify sex-specific contributions of the mitochondrial protein interactome to the development of frailty and sarcopenia.

Methods: We measured a clinical frailty index, hindlimb muscle mass, and in vivo force in 7- and 27-month-old C57Bl6/J mice from the NIA aging mouse colony (n=10/age and sex). At endpoint, we measured mitochondrial respiration, membrane potential, and the protein-protein interactome in isolated gastrocnemius muscle mitochondria.

Results: ADP sensitivity of mitochondrial respiration was reduced in aged muscle mitochondria. This was associated with significant changes in mitochondrial protein-protein interactions. Female mice had earlier onset and more severe frailty and a larger decline in muscle mass, while the loss of force was similar between sexes. Both sexes exhibited heterogeneity in the extent of frailty and sarcopenia with a correlation between the loss of muscle mass, force, or contraction speed with increased frailty. The severity of age-related changes in the mitochondrial proteome was correlated with the amount of frailty.

Conclusions: Age-related disruptions in the mitochondrial protein interactome contribute to the development of sarcopenia and frailty.

Poster # 17

The structural basis for human muscle PFK regulation

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Glycolysis is central to eukaryotic energy metabolism and must be finely tuned to respond to changing energetic and metabolic needs of the cell. The first step committing glucose to glycolysis is performed by the homotetrameric enzyme phosphofructokinase (PFK). In vertebrates, there are three isoforms of PFK: muscle (PFKM), liver (PFKL) and platelet (PFKP). All tissues express all three isoforms, except skeletal muscle in which only PFKM is expressed. PFK is an essential node controlling glucose catabolism, ATP production via glycolysis, and metabolite flux into anabolic pathways. Because of its central importance, PFK is tightly regulated, most significantly by ATP. The extent by which PFK activity is modulated by ATP varies by isoform. The unique energetic needs of skeletal muscle are reflected in the unique properties of PFKM regulation. While other isoforms of PFK are inhibited by high ATP concentrations, the muscle isoform retains activity at high ATP concentrations, except under acidic conditions. This pH dependent regulation by ATP is unique to PFKM and is important to understand energy production in the muscle during anaerobic exercise, when tissues become acidified by the buildup of lactic acid. The biophysical basis for this pH-dependent ATP regulation is not understood. Here, I determine the first high-resolution cryo-EM structures of human PFKM under active and inhibited conditions and define the structural basis for muscle-specific ATP- and pH-dependent allosteric regulation.

Poster # 18

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MYBPC3-c.772G>A mutation results in haploinsufficiency and altered myosin cycling kinetics in a patient induced stem cell derived cardiomyocyte model of Hypertrophic Cardiomyopathy.

Mutations in cardiac myosin binding protein-C (cMyBP-C; gene = *MYBPC3*) are the most common cause of hypertrophic cardiomyopathy (HCM). These mutations are either classified as missense mutations or truncation mutations. One mutation whose nature has been inconsistently reported in the literature is the *MYBPC3*-c.772G>A mutation. Using patient-derived induced pluripotent stem cells differentiated to cardiomyocytes (iPSC-CMs) we have performed a mechanistic study of the structure-function relationship for this *MYBPC3*-c.772G>A mutation versus a mutation corrected, isogenic cell line. Gene expression and liquid chromatography mass spectrometry results confirm that this mutation leads to exon skipping and mRNA truncation, resulting in 21.7% less cMyBP-C protein (i.e., haploinsufficiency), without any presence of a truncated peptide. This, in turn, results in increased myosin recruitment and accelerated myofibril cycling kinetics during both contraction and relaxation. Our mechanistic studies suggest that faster ADP release from myosin is a primary cause of accelerated myofibril cross-bridge cycling due to this mutation. Additionally, the reduction in force generating heads expected from faster ADP release during isometric contractions, is outweighed by a cMyBP-C phosphorylation mediated increase in myosin recruitment that leads to a net increase of myofibril force, primarily at sub-maximal calcium activations. These results match well with our recent report on contractile properties of myectomy samples of the patients from whom the iPSC-CMs were generated and demonstrates that these patient derived cell lines are a good model to study this pathological mutation and extends our understanding of the mechanisms of altered contractile properties of this HCM *MYBPC3*-c.772G>A mutation.

Poster # 19

Contractility and local stress patterns depend on directionality of fibrosis progression: Insights from microscale biomechanical simulations

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Abstract

Cardiac fibrosis is a common pathological process that leads to reduced myocardial contractility and increased myocardial stiffness. Fibrosis involves various cellular and subcellular events, hereunder myocyte necrosis and extracellular matrix (ECM) stiffening. We set out to explore the individual impact of these in a computational model, hypnotizing that tissue-scale effects are anisotropic as determined by the prevailing direction of fibrotic progression.

We used a mechanical modeling framework that allows for explicit geometrical representation of individual myocytes embedded in an ECM; independent adjustment of intra- and extracellular stiffness; and assignment of active contraction for each cell. We used a geometrical representation of 6×12 cells as a baseline, then explored different cellular configurations representing myocyte loss longitudinally (relative to the myofiber alignment direction), transversely, and randomly; removing 1/3rd of all cells. We considered the impact of original and increased ($\times10$) ECM stiffness for each configuration, tracking contractility and stress distributions as output variables.

We found myocyte loss perpendicular to the myofiber direction to be most consequential for loss in contractility (as much as 30% reduction; compared to up to 21% for longitudinal/random configurations). Longitudinal and random fibrosis progression on the other hand led to significant increases in both myocyte and matrix stresses. Increased matrix stiffness generally reduced contractility and enhanced stress magnitudes.

Our computational model predicts that tissue-scale scale changes are anisotropic with distinct impact on contractility and stress distributions. On an organ-scale level, these results might be correlated with respectively reduced cardiac function and risk of further fibrosis progression.

Poster # 20

Effects of regulatory light chain phosphorylation on porcine thick-filament structure and function

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Precise regulation of cardiac contraction is essential for normal cardiac function. The heart must generate sufficient force to pump blood throughout the body, but either inadequate or excessive force can lead to dysregulation and disease. Myosin regulatory light chain (RLC) is a thick-filament regulatory protein, which binds to the neck of the myosin heavy chain. Post-translational phosphorylation of RLC by myosin light chain kinase is thought to influence myosin head radial position relative to the thick-filament backbone, enabling more favorable binding with the actin thin-filament to facilitate force production. Decreases in RLC phosphorylation (RLC-P) are associated with cardiac dysfunction and disease. We aim to investigate the role of RLC-P on cardiac structure and function to better understand its regulatory mechanisms.

Small-angle x-ray diffraction revealed that RLC-P decreases myosin crown order (I_{M6}) and alters thick-filament backbone structure (I_{M6}). These modifications do not correlate with changes in force production and viscoelastic properties at low calcium levels (pCa 8.0), but rather translate into large phosphorylation-dependent increases at high calcium levels (pCa 4.8). Nucleotide handling rate measurements reflect that RLC-P decreases the rate of ADP dissociation ($-k_{ADP}$) and increases the rate of ATP association ($+k_{ATP}$), contributing to modifications in cross-bridge cycling kinetics in a sarcomere-length dependent manner. This may suggest that RLC-P has its largest effects on myosin head movement and force production during systole, when calcium levels are high. Our work further elucidates the effects of RLC-P on muscle function, thereby promoting a better understanding of thick-filament regulatory contributions to cardiac function in health and disease.