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Dysregulation of Skeletal Muscle Mitochondrial Function following Critical Illness: a Translational Approach

Luther Gill[1], Liz Simon[2], and Patricia Molina[2]
[1]LSUHSC School of Medicine- LA CaTS; [2]LSU Health Science Center

OBJECTIVES/GOALS: The objective of the study was to determine whether CLP altered genes associated with mitochondrial function in the diaphragm. METHODS/STUDY POPULATION: A rodent cecal-ligation and puncture (CLP) model used to mimic sepsis-induced critical illness. The CLP model involved ligation of 50% of the cecum below the ileocecal valve in adult C57BL6 mice, followed by needle puncture of the cecum resulting in mid-grade sepsis. Mice survived for 48 hours or more, following injury. Diaphragm and limb muscles were harvested 24 hours following CLP (N = 6) and following a sham CLP procedure (N = 6). RESULTS/ANTICIPATED RESULTS: Gene expression of mitochondrial related genes (mef2c, myh1, pgc1-α), were significantly decreased in the diaphragm of CLP injured animals when compared to controls. In addition, ubiquitin ligases, genes associated with skeletal muscle atrophy murf1 and atrogin were increased in the diaphragm 24 hours after injury ($p < 0.01$). DISCUSSION/SIGNIFICANCE OF IMPACT: Our results indicate that sepsis-induced critical illness significantly impacts the expression of genes implicated in mitochondrial homeostasis and atrophy. Ongoing studies will identify whether CLP injury decreases skeletal muscle mitochondrial function.

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Elucidating the Influence of Chemotherapy (melphalan) and /or C. difficile toxin B Exposure on Beta-catenin Protein Expression in Caco-2 Monolayers

Jailene Canales, Alison Weiss, and Senu Apewokin[1]
[1]UC Physicians

OBJECTIVES/GOALS: We previously reported that genetic polymorphisms in the beta-catenin gene (CTNNB) are associated with the development of *Clostridiodes difficile* colitis during autologous stem cell transplantation (https://www-ncbi-nlm-nih-gov.proxy.libraries.uc.edu/pubmed/29594489). To biological validate these findings, we sought to evaluate the development of chemotherapy-associated *Clostridiodes difficile* infections by assessing the effect of C.difficile toxin B (TcdB) and of using melphalan in beta-catenin protein expression in Caco2 cells. METHODS/STUDY POPULATION: To determine the effect of melphalan and/or C.difficile toxin B on expression of *Beta-catenin* from human gut epithelial cells:

- Adenocarcinoma cells (Caco-2) cells were seeded and allowed to grow into monolayers
- Monolayers were treated with PBS, TcdB, melphalan and/or TcdB + melphalan for 24 hours and then washed with PBS
- Immunofluorescence was measured on the monolayers to visualize three markers -DAPI-Nuclear Stain (blue),Actin-ccytoskeletal stain (red), B-Catenin (green)
- Analysis of images with ImageJ (NIH). Statistical analysis of the effect of TcdB and/or melphalan on β-catenin protein levels was determined by One-way ANOVA

Cells stained with a primary anti-β catenin antibody and an Alexa-488 secondary antibody were evaluated by flow cytometry to quantify the effect of melphalan and/or C. difficile toxin B on Caco2 cells. RESULTS/ANTICIPATED RESULTS: Immunofluorescent intensity was higher in the control (PSS exposed) cells when compared to melphalan, TcdB and mephalan+TcdB exposed cells ($p = 0.026$, 0.004 and 0.049 respectively) DISCUSSION/SIGNIFICANCE OF IMPACT: A significant difference was seen in β catenin expression in Caco-2 monolayers exposed to TcdB and/or melphalan. These data support the a role of β-catenin in the pathophysiology of CDI during chemotherapy and support GWAS findings reporting a difference in CDI susceptibility based on β-catenin genotype.

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Evaluation of Neurotransmitters in Channelopathy-Related Epilepsy

Sunita N Misra[1], Theresa M. Czech, and Jennifer A. Kearney
[1]Northwestern University

OBJECTIVES/GOALS: Variants in voltage-gated sodium channels (VGSC) are a common cause of severe early onset epilepsy. Changes in CSF neurotransmitters (NT) were identified in 2 cases of VGSC-related epilepsy. Here we investigate NT changes in patients and a novel mouse model of VGSC-related epilepsy. METHODS/STUDY POPULATION: We conducted a single site IRB approved retrospective chart review of patients with VGSC-related epilepsy who underwent CSF NT testing for diagnostic purposes. In parallel, we examined NT levels from the brains of wild-type (WT) and a novel VGSC-related epilepsy mouse model after obtaining IACUC approval. We rapidly isolated forebrain, cortex, striatum, and brainstem from 5-6 animals per sex and genotype. A combination of HPLC with electrochemical detection and mass spectrometry were used to quantify NT levels from brain samples. RESULTS/ANTICIPATED RESULTS: We identified 10 patients with VGSC-related epilepsy who received CSF NT testing. Two of these patients had abnormal NT results including changes to dopamine (DA) or serotonin (5-HT) metabolites. We analyzed NT levels from four brain regions from male and female WT and VGSC-related epilepsy mice. We anticipate that most of the NT levels will be similar to WT, however subtle changes in the DA or 5-HT metabolites may be seen in VGSC-related epilepsy. DISCUSSION/SIGNIFICANCE OF IMPACT: Patients with VGSC-related epilepsy often have autism spectrum disorder, sleep, and movement disorders. Understanding the role of aberrant NT levels in VGSC-related epilepsy may provide additional therapeutic targets that address common neuropsychological comorbidities as well as seizures.

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Functional consequences of the juvenile idiopathic arthritis risk variant at 1q24.3

Halima Moncrieffe[1], Katelyn Dunn[1], Yongbo Huang[1], Xiaoting Chen[1], Carl D. Langefeld[2], Matthew T. Weirauch[1], John B. Harley[1], Leah C. Kottyan[1], Susan D. Thompson[1], and Juvenile Arthritis Consortium for the Immunochip
[1]Cincinnati Children's Hospital; [2]Wake Forest School of Medicine

OBJECTIVES/GOALS: Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatologic disease childhood and a cause of pain and potential disability. JIA has a strong genetic component and no known cure. The goal of this study is to evaluate allele-dependent

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