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

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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 *murfl* and *atrogen* 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

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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-cytoskeletal 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 melphalan+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 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

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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

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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