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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.

4530

## 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.

4277

## 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

https://doi.org/10.1017/cts.2020.299 Published online by Cambridge University Press

effects of a novel JIA risk variant at 1q24.3. METHODS/STUDY POPULATION: JIA patients meeting criteria for the two most common disease subtypes (oligoarticular and RF neg polyarthritis) were genotyped using the Immunochip, an Illumina array with dense coverage of the HLA region and 186 other loci previously reported in autoimmune diseases. Phase I association findings (Hinks, 2013) and Phase II analysis (unpublished) of an expanded cohort (4,271 JIA and 14,390 controls) identified new risk loci, including rs78037977 at 1q24.3. We prioritized rs78037977 and predicted possible impacted mechanisms based on Bayesian predictions of attributable risk, the surrounding chromatin landscape, and transcription factor binding data. A luciferase reporter assay was used to assess allele-dependent enhancer activity. RESULTS/ANTICIPATED RESULTS: rs78037977 is located between *FASLG* and *TNFSF18* at chromosome 1q24.3 is associated with JIA ($p = 6.3x10^{-09}$), and explains 94% of the posterior probability at this locus; no other SNPs in linkage disequilibrium ($r^2>0.6$). The chromatin landscape around rs78037977 contains H3K4Me1 and H3K27Ac marks, which are indicative of enhancer activity. Further, >160 transcription factors have chromatin immunoprecipitation followed by sequencing (ChIP-seq) peaks overlapping rs78037977 in various cellular contexts. In luciferase reporter assays, the region around rs78037977 containing the reference A allele had ~2-fold increased enhancer activity compared to the non-reference allele. DISCUSSION/SIGNIFICANCE OF IMPACT: This work provides *in vitro* evidence to support allele-dependent enhancer activity of a novel JIA-risk variant at 1q24.3. Our ongoing work investigates the effect of the DNA-containing region of rs78037977 on gene expression and differential transcription factor binding at rs78037977.

**4086**

## HIV-Associated Myocardial Diastolic Dysfunction and Soluble ST2 Concentration in Tanzanian Adults: A Cross-Sectional Study

Justin Kingery[1], Rahul Hosalli BA[1], Bernard Desdarius MD/MMED[2], Fredrick Kalokola MD/MMED[1], Edmund Damas MD[1], Karl Reis BA[1], Hyung Hee Lee PhD[1], Margaret McNairy MD[1], Daniel Fitzgerald MD[1], and Robert Peck MD/PhD[1]

[1]Weill Cornell Medicine; [2]Weill Bugando School of Medicine

OBJECTIVES/GOALS: To determine the prevalence of myocardial diastolic dysfunction (DD) and association of serum concentration of the cardiac biomarker serum soluble ST2 in HIV-infected as compared to uninfected Tanzanian adults at the time of HIV diagnosis. METHODS/STUDY POPULATION: In this cross-sectional study we consecutively enrolled HIV-infected participants and uninfected controls at a large, referral HIV clinic in Mwanza, Tanzania. Standardized history, physical examination, echocardiography and serum samples were obtained. The primary outcome was prevalence of myocardial diastolic dysfunction in HIV-infected as compared to uninfected adults. The secondary outcome was the association of baseline serum sST2 concentration with diastolic dysfunction prevalence. Regression models were used to quantify the associations. RESULTS/ANTICIPATED RESULTS: We enrolled 388 HIV-infected, ART naïve and 461 HIV-uninfected controls. Participants with HIV had a higher prevalence of DD (OR = 2.44, p = 0.001, controlled for age, sex, hypertension and BMI) and more severe dysfunction (66.7% vs 42.5%, p = 0.056) at an earlier age. Baseline serum sST2 concentration was significantly associated with DD in HIV-infected but not uninfected participants (p = 0.04 and 0.90, respectively). More HIV-infected adults with concurrent DD exceeded the threshold of 35ng/mL as compared to controls (15.7% vs 5.3%, p<0.0001). Additionally, a significant population level shift to higher sST2 concentration was observed in HIV-infected adults with dysfunction as compared to both HIV-infected without and HIV-uninfected adults with dysfunction (Kolmogrov-Smirnov test: p = 0.02 and 0.04). DISCUSSION/SIGNIFICANCE OF IMPACT: In a large population of HIV-infected adults in sub-Saharan Africa, HIV infection is associated with myocardial diastolic dysfunction. This dysfunction is associated with higher sST2 concentrations. Therefore, we conclude that the sST2 pathway may provide insight into the pathophysiologic mechanisms of dysfunction in HIV-infected adults.

**4078**

## Identification of distinct fibroblast populations with unique roles in pancreatic cancer progression and tumor immunity

Josephine Kebbeh Darpolor[1], Xiaofeng Zheng, Sujuan Yang, Hikaru Sugimoto, Julienne Carstens, Pedro Correa de Sampiao, Valerie LeBleu, and Raghu Kalluri

[1]The University of Texas Health Science Center at Houston

OBJECTIVES/GOALS: The desmoplastic reaction in PDAC involves a significant accumulation of immune cells and fibroblasts.The functional diversity of carcinoma associated fibroblasts (CAFs) remains largely unknown, and identification of immune regulating subsets would have a substantial impact in augmentation of immunotherapy efficacy. METHODS/STUDY POPULATION: Employing histology, FACs, multiplex immunohistochemistry, single cell RNA sequencing (sc-RNA-seq) and genetically engineered mouse models, we demonstrate that $aSMA^+$ cells are a dominant CAF population in PDAC with tumor restraining properties (TS-CAFs), as opposed those of the $FAP^+$ CAFs, which demonstrate tumor promoting activity (TP-CAFs). RESULTS/ANTICIPATED RESULTS: Analysis of bulk tumor depleted of either TS-CAFs or TP-CAFs showed that TS-CAFs predominantly modulate extracellular matrix (ECM) production, facilitate cell-ECM adhesion and regulate adaptive immunity, while TP-CAFs exhibit a lineage that is skewed towards a pro-inflammatory, chemokine secreting phenotype. Further, scRNA-Seq analyses demonstrate that CAFs share distinct gene expression profiles characteristic of lymphocytic and myeloid lineages. Together our data distinguish two populations of CAFs, one which is tumor suppressing with roles in ECM remodeling and another which is tumor promoting with roles in cytokine production, both with immune modulating capabilities. DISCUSSION/SIGNIFICANCE OF IMPACT: Our study identifies a complex network of functionally heterogeneous fibroblasts during PDAC progression with significant immunotherapeutic implication. The identification of distinct fibroblast subsets will allow us to discriminately target fibroblast populations to augment immunotherapy efficacy in pancreatic cancer.

https://doi.org/10.1017/cts.2020.299 Published online by Cambridge University Press