predictive of each phenotype (P<0.00001). Pathologic bacteria were associated with the severity of specific pain symptoms. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results implicate a microbial role in genitourinary pain. We describe clinically-useful bacterial biomarkers for specific pelvic and bladder pain phenotypes. This objective, rapid, and inexpensive testing to classify bladder and pelvic pain would allow more accurate diagnosis and improve treatment. CONFLICT OF INTEREST DESCRIPTION: Dr. Anger is an expert witness for Boston Scientific. Dr. Eilber is an investigator and expert witness for Boston Scientific, an investigator for Aquinox, and a consultant for Boston Scientific and Allergan. Dr. Ackerman is an expert witness for Cynosure.

4582

NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD) DEPLETION MUST BE SEVERE TO INDUCE CARDIAC DYSFUNCTION AND EVENTUAL FAILURE†

Timothy Luongo[1], Lin Wang, Karthikeyani Chellappa, Melanie R. McReynolds, Daniel P. Kelly, Joshua D. Rabinowitz, and Joseph A. Baur
[1]University of Pennsylvania

OBJECTIVES/GOALS: Nicotinamide adenine dinucleotide (NAD) plays essential roles in energy metabolism and cell signaling pathways. NAD functions as a coenzyme by accepting electrons during glycolysis and the TCA cycle and subsequently donates them to complex I of the electron transport chain providing the driving force for ATP production. NAD also acts as a co-substrate for several classes of enzymes, including sirtuin deacetylases. Both NAD and the enzyme that is rate limiting for synthesis, Nicotinamide phosphoribosyltransferase (Nampt), are depleted in the failing heart, concurrent with hyperacetylation and mitochondrial dysfunction. Moreover, treatment with NAD precursors reduced cardiac injury in several heart failure models. However, NAD precursors may have systemic effects, and it remains unproven whether depletion of myocardial NAD is causative or merely correlative for the onset and progression of heart failure. METHODS/STUDY POPULATION: To test this, we generated a cardiac-specific tamoxifen-inducible (αMHC-MerCreMer) model for deletion of Nampt (Nampt cKO) in cardiomyocytes. Adult mice were administered tamoxifen for 5 days leading to deletion of *Nampt*, resulting in a 72% reduction in myocardial NAD after two-weeks. RESULTS/ANTICIPATED RESULTS: Echocardiography revealed that Nampt cKO mice displayed a significant reduction in left ventricular (LV) contractility as well as cardiac hypertrophy. Despite the further loss of NAD, the majority of animals survived to 8 weeks of age before experiencing sudden deaths resulting in significant mortality over the next several weeks. Remarkably, we observed only a slight increase in acetylation of mitochondrial proteins, and cardiac mitochondria isolated from Nampt-null mice even at 8 weeks displayed a normal or higher oxygen consumption rate. We found that mitochondrial NAD levels were preferentially maintained and depleted at a slower rate compared to those in bulk tissue. DISCUSSION/SIGNIFICANCE OF IMPACT: While mild depletion of cardiac NAD has been reported in heart failure, our data indicate that the heart can adapt to much more severe loss of NAD prior to the loss of viability.

4478

Not just GLUT1: genome sequencing reveals genetic heterogeneity in Doose syndrome*

Jeffrey Dennis Calhoun[1], Jonathan Gunti, Katie Angione, Elizabeth Geiger, Krista Eschbach, Garnett Smith, Charuta Joshi, Tamim Shaikh, Scott Demarest, and Gemma Carvill
[1]Northwestern University

OBJECTIVES/GOALS: Epilepsy with myoclonic-atonic seizures (EMAS) is a childhood onset epilepsy disorder characterized by seizures with sudden loss of posture, or drop seizures. Our objective was to use short-read genome sequencing in 40 EMAS trios to better understand variants contributing to the development of EMAS. METHODS/STUDY POPULATION: Eligibility for the cohort included a potential diagnosis of EMAS by child neurology faculty at Children's Hospital Colorado. Exclusion criteria included lack of drop seizures upon chart review or structural abnormality on MRI. Some individuals had prior genetic testing and priority for genome sequencing was given to individuals without clear genetic diagnosis based on previous testing. We analyzed single nucleotide variants (SNVs), small insertions and deletions (INDELs), and larger structural variants (SVs) from trio genomes and determined those that were likely contributory based on standardized American College of Medical Genetics (ACMG) criteria. RESULTS/ANTICIPATED RESULTS: Our initial analysis focused on variants in coding regions of known epilepsy-associated genes. We identified pathogenic or likely pathogenic variants in 6 different individuals involving 6 unique genes. Of these, 5 are *de novo* SNVs or INDELs and 1 is a *de novo* SV. One of these involve a *de novo* heterozygous variant in an X-linked gene (*ARHGEF9*) in a female individual. We hypothesize the skewed X-inactivation may result in primarily expression of the pathogenic variant. We anticipate identifying additional candidate variants in coding regions of genes previously not associated with EMAS or pediatric epilepsies as well as in noncoding regions of the genome. DISCUSSION/SIGNIFICANCE OF IMPACT: Despite the genetic heterogeneity of EMAS, our initial analysis identified *de novo* pathogenic or likely pathogenic variants in 15% (6/40) of our cohort. As the cost continues to decline, short read genome sequencing represents a promising diagnostic tool for EMAS and other pediatric onset epilepsy syndromes. CONFLICT OF INTEREST DESCRIPTION: The authors have no conflicts of interest to disclose. SD has consulted for Upsher-Smith, Biomarin and Neurogene on an unrelated subject matter. GLC holds a research collaborative grant with Stoke therapeutics on unrelated subject matter.

4418

Optimization and Validation of a Silk Scaffold-Based Neural Tissue Construct

Ben Jiahe Gu[1], Dennis Jgamadze, Guoming (Tony) Man, and Han-Chiao Isaac Chen
[1]University of Pennsylvania

OBJECTIVES/GOALS: Our goal is to develop a silk fibroin scaffold-based neural tissue construct and characterize it in a rat model of cortical injury. We aim to optimize the construct for transplantation, test pharmacologic interventions that may enhance its survival, and

https://doi.org/10.1017/cts.2020.83 Published online by Cambridge University Press