results will broaden scientific understanding of phage-bacterial interactions and determine the mechanisms by which phage impact virulence independent from toxin gene carriage. Identification of phage-encoded gene(s) enhancing CA-MRSA contagion will inform surveillance efforts and identify novel therapeutic targets.

4007

Medroxyprogesterone Upregulates the Glucocorticoid Receptor in Female Long Evans Rats

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OBJECTIVES/GOALS: Estrogen monotherapy in postmenopausal women can reduce kidney function, while dual therapy combining estrogen with a progestin improves renal health. Using the female Long Evans rat as a novel animal model of postmenopausal cardiovascular disease, we found similar results where estrogen worsens renal health while co-administration of medroxyprogesterone acetate (MPA) was protective. MPA cross-activates glucocorticoid receptors (GR), which are targeted clinically for their anti-inflammatory actions. Therefore, our goal was to determine if estrogen monotherapy induces renal damage by increasing inflammation, while dual therapy with MPA opposes inflammation by cross-activating GR. METHODS/STUDY POPULATION: Female Long Evans rats underwent OVX at 11 months of age and received a subcutaneous implant containing E2, E2+MPA or vehicle for 40 days. RESULTS/ANTICIPATED RESULTS: Coadministration of MPA prevented the E2-induced increase in proteinuria (Veh: 0.27±0.07; E2: 3.53±1.16; E2+MPA: 1.20±0.58 mg/mg creatinine; $P = 0.03$) and decline in glomerular filtration rate (Veh: 0.51±0.02; E2: 0.24 ±0.05; E2+MPA: 0.39±0.05 ml/min; $P<0.01$). Co-administration of MPA significantly increased renal GR transcript levels compared with E2 alone (Veh: 0.96±0.02; E2: 0.94±0.10; E2+MPA: 1.24±0.04 fold change; $P<0.01$). Inflammatory marker COX 2 renal transcript levels were significantly reduced by a similar degree in both mono and dual therapies compared with vehicle (Veh: 1.07±0.06; E2: 0.81±0.04; E2+MPA: 0.81±0.04 fold change; $P<0.01$). Neither TNF-alpha and IL-6 mRNA nor urinary beta-microglobulin levels (Veh: 1.71±0.31; E2: 2.88±0.78; E2+MPA: 3.07±1.15 mg/day; ns) were altered. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results show that the effect of E2 on renal pro-inflammatory markers was not altered by the addition of MPA despite the significant increase in renal GR levels. Therefore, the renoprotective effects of MPA in midlife hormone therapy may be independent of renal GR-mediated changes in the immune profile.

4006

Methionine Dependence in Cancer: From Metabolic Phenotype to Therapy

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OBJECTIVES/GOALS: Methionine dependence was described 45 years ago as an increased reliance on an exogenous supply of the essential amino acid methionine in most cancer cells compared to normal cells. Methionine depletion, using either synthetic diets or the enzyme methioninase, potentiates the effects of chemotherapy and radiotherapy in tumor-bearing animal models. Two main obstacles prevent methionine dependence from integrating the clinical treatment of cancer. The first is the weight loss associated with methionine depletion therapy, increasing the risk of cachexia in patients. The second is the stubborn absence of a mechanism to explain the inability of cancer cells to adapt to low methionine levels. METHODS/STUDY POPULATION: To address these two obstacles, we are using an immunocompetent murine model of metastatic melanoma to compare the effects of complete methionine deprivation with a moderate, 75-80% methionine restriction similar to the one used to increase lifespan in animal models. In an effort to identify a mechanism of action, we also performed a proteomic screen of two melanoma cell lines divergent for methionine dependence under methionine stress. RESULTS/ANTICIPATED RESULTS: We recently showed that methionine restriction is sufficient to provide gains in treating local and metastatic lesions in vivo, without weight loss. We observed few differences in pathway activation between the two cell lines in response to methionine stress, despite proliferation being cut by half in the methionine dependent cell line. We expect that subcellular translocation events may provide further information on the molecular bases of methionine dependence. DISCUSSION/SIGNIFICANCE OF IMPACT: A moderate restriction in methionine is sufficient to recapitulate the benefits of methionine depletion in cancer, without weight loss. The mechanism behind this effect remains unknown. This work contributes towards the integration of methionine dependence into clinical practice and the discovery of novel drug targets.

4196

MICROBIAL COMPOSITION DEFINES PELVIC PAIN PHENOTYPES IN REPRODUCTIVE-AGE WOMEN

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OBJECTIVES/GOALS: In young women, there is significant symptomatic overlap among lower urinary tract conditions, including bladder and pelvic pain, leading to misdiagnosis and delayed care. The epidemiology of pelvic pain suggests a microbial involvement, but previous studies have not definitively identified specific bacteria associated with pain diagnoses. METHODS/STUDY POPULATION: We examined urinary bacterial associations with specific symptom clusters, not diagnoses. Catheterized urinary samples were obtained from 78 pre-menopausal controls and cases with bladder and pelvic pain. 16S next-generation sequencing (NGS) characterized urinary microbial populations; validated questionnaires quantified symptom type and severity. *K* means unsupervised clustering analysis of NGS data assigned subjects to urotypes based on the urinary bacterial community state types. Quantitative PCR (qPCR) confirmed the NGS results and provided objective concentrations for critical taxa. Linear regression analysis confirmed the associations of bacterial concentrations and specific symptoms. RESULTS/ANTICIPATED RESULTS: In a pilot study of 35 reproductive-age women with a variety of complaints NGS revealed four urotypes that correlated with symptomatology. Isolated urgency incontinence was rare; the majority of subjects with symptoms complained of genitourinary pain. Bladder-specific pain (worse with filling, relieved by voiding) was associated with *Lactobacillus iners*. Asymptomatic patients almost universally had a non-iners, *Lactobacillus*-predominant microbiota. Vaginal and urethral pain unrelated to voiding correlated with increasing Enterobacteriaceae, primarily *Escherichia coli*. Detection of these species by qPCR in a validation population ($n = 43$) was highly

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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†

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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*

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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

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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

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