implantation, potentially leading to a new diagnostic or treatment target in early pregnancy failure. OBJECTIVES/GOALS: Studies suggest interferon signaling regulation is tightly balanced between physiologic and pathophysiologic growth in early pregnancy. We propose to determine the impact of interferon-mediated inflammation on embryo implantation and early pregnancy failure in normal conditions and chronic inflammatory diseases in a novel mixed-mouse model. METHODS/STUDY POPULATION: To probe the role of type-I interferons (IFNs) in implantation, we will utilize a mouse model and non-surgically transfer both Ifnar1-/- and Ifnar1-/+ embryos into an immune-competent pseudopregnant wild-type female recipient. This will allow analysis of a litter with distinct genotypes within the same, immune-competent, uterine environment. Type-I IFN stimulation will be systemically induced with Poly-(I:C) at various time points around implantation. A similar approach will be used in mouse models of chronic inflammatory disease states associated with early pregnancy loss (e.g. systemic lupous erythematous). With this model, we will be able to control for deficiencies in maternal immune response to specifically determine the embryonic response to inflammation during implantation and development. RESULTS/ANTICIPATED RESULTS: We anticipate the Ifnar1-/+ embryos - those able to respond to Type-I IFN - and their surrounding implantation sites will exhibit more maternal-fetal barrier dysfunction in the form of impaired trophoblast fusion, improper formation of the microvascular architecture, and increased permeability of the maternal-fetal barrier, compared to embryos unable to respond to IFN. We will also conduct similar analyses in mouse models of chronic inflammatory diseases. We hypothesize these mice to have baseline endometrial inflammation that stimulated the IFN-pathway in IFN-capable embryos, producing breakdown of the maternal-fetal barrier. In these mice, we predict Ifnar1-/- embryos will show improved molecular outcomes when compared to Ifnar1-/+ embryos, and thus improved associated pregnancy outcomes. DISCUSSION/SIGNIFICANCE OF FINDINGS: This work can insight into the immunological mechanisms that govern embryo implantation and early placentation. This could provide more pointed means for management and intervention of early pregnancy failure and/or disease states that are commonly associated with poor reproductive outcomes.

38766

Massively Parallel Reporter Assay Reveals Functional Impact of 3'-UTR SNPs Associated with Neurological and Psychiatric Disorders*

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ABSTRACT IMPACT: Screening the effect of thousands of non-coding genetic variants will help identify variants important in the etiology of diseases OBJECTIVES/GOALS: Massively parallel reporter assays (MPRAs) can experimentally evaluate the impact of genetic variants on gene expression. In this study, our objective was to systematically evaluate the functional activity of 3'-UTR SNPs associated with neurological disorders and use those results to help understand their contributions to disease etiology. METHODS/STUDY POPULATION: To choose variants to evaluate with the MPRA, we first gathered SNPs from the GWAS Catalog that were associated with any neurological disorder trait with p-value < 10-5. For each SNP, we identified the region that was in linkage disequilibrium ($r2 > 0.8$) and retrieved all the common 3'-UTR SNPs (allele-frequency > 0.05) within that region. We used an MPRA to measure the impact of these 3'-UTR variants in SH-SY5Y neuroblastoma cells and a microglial cell line. These results were then used to train a deep-learning model to predict the impact of variants and identify features that contribute to the predictions. RESULTS/ANTICIPATED RESULTS: Of the 13,515 3'-UTR SNPs tested, 400 and 657 significantly impacted gene expression in SH-SY5Y and microglia, respectively. Of the 84 SNPs significantly impacted in both cells, the direction of impact was the same in 81. The direction of eQTL in GTEx tissues agreed with the assay SNP effect in SH-SY5Y cells but not microglial cells. The deep-learning model predicted sequence activity level correlated with the experimental activity level (Spearman's corr = 0.45). The deep-learning model identified several predictive motifs similar to motifs of RNA-binding proteins. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study demonstrates that MPRAs can be used to evaluate the effect of non-coding variants, and the results can be used to train a machine learning model and interpret its predictions. Together, these can help identify causal variants and further understand the etiology of diseases.

49193

The Association Between Specialized Pro-resolving Lipid Mediators and Markers of Neuroinflammation and Disease Severity in Acute Traumatic Brain Injury: A Prospective, Observational Cohort Study

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ABSTRACT IMPACT: Characterizing specialized pro-resolving lipid mediators (SPMs) of inflammation in a cohort of adult patients with traumatic brain injury (TBI) will potentially identify novel biomarkers of disease and would generate hypotheses regarding the role of SPMs in the pathophysiology and recovery after brain injury OBJECTIVES/GOALS: Primary aim: evaluate the association between plasma and cerebrospinal fluid (CSF) SPM levels with inflammatory markers known to mediate blood brain barrier (BBB) disruption. Secondary aim: evaluate the association between SPM levels and disease severity, 14-day Glasgow Coma Scale score (GCS) and survival. METHODS/STUDY POPULATION: This is a single-center, prospective, observational cohort study (target N=50). Adult participants with moderate-to-severe non-penetrating TBI will have blood and CSF sampled serially for laboratory measures of SPMs (resolvins, protectins, lipoxins, maresins) and inflammatory peptides (TNF-α, IL-1β, IL-6, MMP-9, TIMP-1) by liquid chromatography‟ mass spectrometry and RT-PCR & ELISA, respectively. Baseline patient characteristics, admission GCS, and 14-day GCS and mortality will be prospectively collected. To evaluate the association between SPMs and other continuous variables, Pearson's and Spearman's correlation will be used. Cox regression will be used to evaluate the association between SPM lipidomes and 14-day survival. RESULTS/ANTICIPATED RESULTS: As the primary outcome measure, the association between SPM levels with assayed inflammatory markers will be determined at 24 and 72 hours post-TBI. We hypothesize that SPMs will be negatively correlated with TNF-α, IL-1β, IL-6, MMP-9 and positively correlated with

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TIMP-1. As secondary outcome measures, the association between SPM levels with disease severity by admission GCS will be determined at 24 hours post-TBI, and the association between SPM levels and GCS and mortality will be determined at 14-days. We hypothesize that SPM levels will be positively associated with admission and 14-day GCS and will be independently associated with 14-day survival. DISCUSSION/SIGNIFICANCE OF FINDINGS: Using the SPM lipidome as a biomarker of disease is a novel tool for future translational research. It will inform a foundational mechanistic framework for TBI pathophysiology and attenuation of neuroinflammation post-TBI, providing rationale for pre-clinical and clinical research focused on novel therapeutics

77085

Impact of MYH6 Variants on Development and Clinical Course of Hypoplastic Left Heart Syndrome

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ABSTRACT IMPACT: This work represents a novel way in which genetic information can be used to improve clinical decision making as it pertains to both treatment and management of congenital heart disease. OBJECTIVES/GOALS: Our lab found that MYH6 variants are both enriched in hypoplastic left heart syndrome (HLHS) and associated with decreased cardiac transplant-free survival. To elucidate the mechanisms of MYH6 variant pathogenicity, we are assessing their impact on atrial function during HLHS development and progression. METHODS/STUDY POPULATION: We are using 2D speckle-based tracking to retrospectively evaluate echocardiograms (echos) from 51 HLHS patients, 17 with MYH6 variants and 34 matched controls. Atrial function will be assessed by myocardial strain and strain rate at seven time points, beginning at the time of the patients' earliest prenatal echo, and ending with their last available echo before death or cardiac transplant. Early left atrial function will examine the role of MYH6 variants in the development of HLHS in vivo, while longitudinal right atrial function will be assessed in order to look for differences that could be contributing to the decreased transplant-free survival seen in MYH6 variant carriers. RESULTS/ANTICIPATED RESULTS: We hypothesize that MYH6 variants cause HLHS by impairing early left atrial (LA) contractility, resulting in altered left ventricular hemodynamics and consequent hypoplasia. We therefore expect to find diminished prenatal LA function in HLHS patients with MYH6 variants. We also hypothesize that MYH6 variants continue to impair right atrial (RA) function in surgically-reconstructed HLHS hearts, necessitating earlier transplantation. Accordingly, we expect variant carriers to exhibit lower RA function at birth versus controls. We expect differences between groups to persist over time, and possibly increase in magnitude. In HLHS patients with MYH6 variants, we anticipate declining RA function will precede right ventricular function and therefore be an early indicator of transplant need. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study represents a novel way in which genetic information can inform clinical decision-making. Identifying MYH6 variants as an early cause of HLHS offers chances for intervention. Understanding long-term effects of MYH6 on right atrial function in HLHS may aid in cardiac transplant risk stratification, thus improving patient outcomes.

88462

Fluconazole distribution in CNS and gynecological tissues in HIV-related cryptococcal meningitis decedents

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ABSTRACT IMPACT: Plasma and CSF are not reliable estimates of drug exposure in tissue compartments relevant for treatment and prevention of infectious diseases. OBJECTIVES/GOALS: Globally, high dose fluconazole is widely used in the management of cryptococcal meningitis. While it is known to readily penetrate into cerebrospinal (CSF), less is known about drug concentrations in brain parenchymal tissues. Similarly, distribution of fluconazole into gynecological tissues has not been robustly characterized. METHODS/STUDY POPULATION: With informed consent from next-of-kin, we conducted autopsies within 24h of death for hospitalized Ugandans receiving fluconazole for treatment or secondary prophylaxis of cryptococcal meningitis. Dosing history was abstracted from medical chart and caregiver interviews. Fluconazole concentrations were determined using high-performance liquid chromatography- tandem mass spectrometry (LC-MS/MS) in plasma, CSF, 10 brain compartments (frontal, parietal, and occipital lobes, corpus callosum, globus pallidus, hippocampus, midbrain, medulla oblongata, spinal cord, and choroid plexus) and 4 female genital compartments (cervix, vagina, ovary, and uterus), depending on tissue availability. Descriptive statistics of tissue to plasma ratios were used to describe concentrations relative to plasma. RESULTS/ANTICIPATED RESULTS: Fluconazole concentrations were measured in available tissues of 21 individuals with detectable fluconazole in plasma. Daily doses of fluconazole were 200 mg (n=4), 400 mg (n=1), 800 mg (n=4), 1200 mg (n=9) or unknown (n=3). CSF concentrations (n=10) ranged from 93-1380% (median 100%) of plasma while brain concentrations (n=3) across all 10 compartments ranged from 45% to 89% (median 69%) of plasma. In the female genital tract, cervical concentrations (n=10) were 9-78% (median 65%) of plasma and in the 2 individuals with available tissue, concentrations in vaginal, ovarian, and uterine tissues were similar to cervix, ranging from 63-105% of plasma. DISCUSSION/SIGNIFICANCE OF FINDINGS: Measuring drug concentrations directly in tissues, the presumed site of action, improves estimates of drug efficacy. While fluconazole concentrations in CSF were similar to plasma, actual brain tissues were consistently lower. Concentrations were similar between upper and lower female genital tracts, but were consistently lower than plasma.

Regulatory Science/Team Science

10122

Development of an In Vitro in Vivo Correlation of Itraconazole Spray-Dried Dispersion Tablets

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ABSTRACT IMPACT: As the number of poorly water-soluble drugs in development increases, our research will expand on the science behind improving drug solubility and absorption and ensuring that promising poorly-water solubility drugs do not fail drug

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