

367

Designing an Untargeted Metabolomics Assay to Detect Biomarkers for Inborn Errors of Metabolism in the Clinical Laboratory*

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OBJECTIVES/GOALS: To develop an untargeted metabolomics assay that can holistically characterize the small molecule signatures of different inborn errors of metabolism (IEM) for biomarker discovery and identification of novel IEMs, with the goal of implementing the assay into the clinical laboratory to improve testing efficiency. **METHODS/STUDY POPULATION:** A hydrophilic interaction liquid chromatography (HILIC) column and reverse phase (RP) column were assembled in tandem on a SCIEX X500B quadrupole time-of-flight (QTOF) system to create a dual liquid chromatography (LC), tandem mass spectrometry method. The X500B was operated in data-independent acquisition mode with both positive and negative ionization. A mixture of 165 reference standards from eleven compound classes common to IEMs were used to evaluate the capability of the assay to resolve small molecules. Chromatographic resolution for each standard was determined qualitatively by comparison to a reference spectral database. External validation of the assay will be performed by analyzing a commercial library of reference metabolites. **RESULTS/ANTICIPATED RESULTS:** A total of 88% (146/165) of the standards were detected by the assay. The RP column alone resolved 71% (117/165) of the standards, the HILIC column resolved 33% (55/165), while 17% (29/165) of the standards were resolved by both columns. The HILIC column resolved standards that were more polar, while the RP column resolved more non-polar compounds. To evaluate matrix effects, the reference standard mixture was spiked into pooled plasma. In the presence of plasma 6/146 (4%) of the standards were suppressed to levels below the limit of detection. We expect external validation with the commercial metabolite library will corroborate these results, and that the high-quality spectral data attained from this reference library can be used to improve identification of unknown metabolites in patient samples. **DISCUSSION/SIGNIFICANCE:** We have shown our untargeted metabolomics assay can detect known biomarkers for IEMs. Clinical implementation of this method could streamline diagnosis of IEMs while simultaneously improving patient outcomes by leveraging the metabolome for biomarker discovery, and improved understanding of IEM mechanisms to inform novel treatment strategies.

368

Development of Interactome Network to Understand Adverse Drug Reactions for Antidepressant Medications

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OBJECTIVES/GOALS: Patient adherence with antidepressant therapy is a critical aspect of clinical management. Drug molecules often interact with other off-target proteins resulting in adverse events. We utilized an interactome model for predicting the likelihood of adverse drug reactions in order to accurately prescribe antidepressant medications to a patient. **METHODS/STUDY POPULATION:** We utilized an interactome model to study physical interactions between proteins and biological functions disrupted by

major depression to understand how changes in genes alter patient-specific drug efficacy and cause adverse drug-reactions. To study how drugs and diseases propagate through the proteins and biological functions, we harnessed diffusion profiles to represent nodes in the graphical model and used a biased random walks model to illustrate how signals propagate in a heterogeneous biological network. The edge weights were defined for the drug, disease, protein, and biological function node types. The interactions studied included drug-protein, disease-protein, protein-protein, protein-biological function, biological function-biological function. **RESULTS/ANTICIPATED RESULTS:** Based on previous studies, we anticipate that the genetic variants modulating antidepressant response include 5-HTT, STin2, HTR1A, HTR2A, TPH1 and BDNF. Additionally, genetic variants such as SLC6A4 as well as the HPA pathway may play an important role in antidepressant therapeutic response. With regards to medication drug response, the genes, SLC6A4 and HTR2A, have been known for encoding proteins that affect the synaptic cleft of serotonergic neurons. Also, the gene SLC6A4 has been shown to encode for the serotonin reuptake transporter, which is the main pharmacological target of SSRIs. In female patients with major depression, the polymorphism associated with MAOA gene may be involved in the pharmacological response. **DISCUSSION/SIGNIFICANCE:** The key to successful depression treatment is early adherence but nearly 60% of patients discontinue antidepressants within three months. By developing a strong understanding of the genes that alter treatment efficacy, we can provide patients with more awareness regarding the most effective treatment plan to minimize adverse events.

369

Differentiating Dementia with Lewy Bodies and Alzheimer's Disease Using Extracellular Vesicles

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OBJECTIVES/GOALS: Extracellular vesicles (EVs) in biofluids can reflect pathology in the brain (like alpha-synuclein protein linked to Dementia with Lewy Bodies) making them attractive disease biomarkers to distinguish disease. Here, we will investigate their potential as non-invasive biomarkers to differentiate Dementia with Lewy Bodies & Alzheimer's Disease. **METHODS/STUDY POPULATION:** We will leverage the collection of antemortem plasma from autopsy confirmed Dementia with Lewy Bodies (DLB), Alzheimer's Disease (AD) & healthy controls (HC) through the Mayo Clinic Brain Bank. We will characterize antemortem brain-derived plasma EVs (bpEVs) & post-mortem brain-derived EVs (bEVs) of the same patient using nanoparticle tracking, electron microscopy, & western blotting for EV-associated proteins. We will then measure levels of total & phosphorylated alpha-synuclein (asyn) using AlphaLisa. We will assess EVs' ability to initiate asyn aggregation using a real-time quaking-induced conversion (RT-QuIC) assay & a cell-based Fluorescence Resonance Energy Transfer (FRET) assay. Once characterized, this will allow differentiation of bpEVs in DLB, AD, or healthy controls. **RESULTS/ANTICIPATED RESULTS:** We hypothesize that asyn levels and seeding capacity will discriminate DLB from AD. With AlphaLisa assay, we expect higher total and phosphorylated asyn levels in DLB bpEVs and their corresponding post-mortem bEVs. Using

RT-QuIC and FRET assay, we expect EVs isolated from DLB patient samples to support seeded aggregation, whereas EVs from AD and HC will not. **DISCUSSION/SIGNIFICANCE:** To date, no diagnostic or less invasive biomarkers can distinguish DLB from AD. The successful completion of the aims outlined in this proposal will identify characteristics of bpEVs that differentiate DLB from AD or HC and support the development of bpEVs as a non-invasive, early biomarker to diagnose patients presenting with dementia from DLB.

370

Epicardial adipose tissue and cardiometabolic health in youth-onset type 2 diabetes undergoing vertical sleeve gastrectomy

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OBJECTIVES/GOALS: The goal of this study is to investigate the potential independent relationship between epicardial adipose tissue (EAT) and cardiometabolic health in youth-onset type 2 diabetes (T2D) and explore changes in EAT as a potential mediator of changes in cardiometabolic health in response to vertical sleeve gastrectomy (VSG). **METHODS/STUDY POPULATION:** We will assess glycemic control, insulin sensitivity and secretion in youth with T2D before and 3 months after VSG. Fasting labs, anthropometrics, and a 4-hour, frequently sampled liquid mixed meal tolerance test (45g carbohydrates, 14g fat, and 14g protein) were performed. Calculations included glucose, insulin, and GLP-1 area under the curve (AUC), Matsuda Index, HOMA-IR, and oral disposition index (DI). These cardiometabolic outcomes will then be assessed for associations between total EAT volume, measured from cardiac MRI. **RESULTS/ANTICIPATED RESULTS:** Previous studies have shown that individuals with obesity have higher EAT than lean controls, and adults with T2D have even higher EAT than obese controls. Therefore, we anticipate that our participants will have higher volume of EAT than what has been reported in the literature and that they will have worsening cardiometabolic outcomes without MBS. Our anticipated results will include: Weight and BMI, hemoglobin A1c, diabetes medications, Matsuda Index, HOMA-IR, DI, and glucose and insulin AUC during an MMTT. Cardiac MRI's are being analyzed and will give total EAT volume and will be analyzed for correlations with the cardiometabolic outcomes of body composition, aortic stiffness, blood pressure, cardiac structure and function, as well as lipid panel and insulin sensitivity. **DISCUSSION/SIGNIFICANCE:** This study is the first to specifically assess EAT in adolescents with T2D. The assessment of EAT will be done with gold-standard MRI and correlated with cardiometabolic health assessed by gold-standard methods. Together, the results will give insight into EAT as a potential independent cardiometabolic risk factor in adolescents undergoing VSG.

371

Fractalkine isoforms using gene therapy differentially regulate microglia activation and vascular damage in the diabetic retina

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OBJECTIVES/GOALS: Retinal inflammation caused by the activation of resident macrophages (microglia) during diabetes exacerbates glial cell dysfunction, resulting in neuronal loss. The goal is to use rAAV gene therapy to deliver neuronal-derived fractalkine (FKN), minimizing inflammation and vascular damage in the diabetic retina. **METHODS/STUDY POPULATION:** The human microglial receptor (CX3CR1) binds to FKN, a protein that is expressed on neuronal membranes (mFKN), and undergoes constitutive cleavage to release a soluble domain (sFKN). Deficiencies in CX3CR1 or FKN showed increased microglial activation and elevated retinal pathology. To understand the mechanism by which mFKN and sFKN regulate microglia function, recombinant adeno-associated viruses (rAAVs) expressing mFKN or sFKN were delivered to intact retinas during diabetes. Markers of neuronal loss, vascular damage, and inflammation were analyzed. We hypothesize that the administration of rAAV-sFKN but not rAAV-mFKN will prevent vascular and neuronal damage, and improve visual function. **RESULTS/ANTICIPATED RESULTS:** rAAV-sFKN minimized microglial activation, blood vessel rupture, fibrinogen deposition, and prevented neuronal loss, compared to mice treated with rAAV-mFKN in a mouse model of diabetic retinopathy (DR). rAAV-sFKN treated mice showed improved visual acuity using a two-choice discrimination task through learning-based behavior. rAAV-sFKN treatment correlated with the success rate of the mice finding the reward based on their ability to distinguish visual cues. Future studies will test the effects of rAAV-sFKN and rAAV-mFKN on microglia inflammatory cytokine release, optic nerve damage and synaptic neurotransmission, peripheral immune responses, and transcriptomic changes in microglia during diabetes. **DISCUSSION/SIGNIFICANCE:** Current therapies for DR are ineffective in restoring vision. rAAVs-sFKN delivery appears to act as a neuroprotective approach in the diabetic retina. sFKN serves as an alternative pathway to implement translational and therapeutic approaches, minimizing pathology and improving visual function.

372

HMGB1 Localization as a Driver of Carcinogenesis in RDEB-Associated Squamous Cell Carcinoma

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OBJECTIVES/GOALS: This study investigates whether localization of high mobility group box 1 (HMGB1) controls inflammatory signaling and DNA damage response in human keratinocytes, the cell of origin for squamous cell carcinoma (SCC). SCC is especially