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

Rachel Wurth, Coleman Turgeon, Zinandra Stander, Dimitar Gavrilov, Patricia Hall, Dietrich Matern, Matthew Schultz, Silvia Tortorelli, Devin Oglesbee
Mayo Clinic

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.

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

Beenish Patel, Yu-Ping Wang
Tulane University

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.

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

Maria Bregendahl¹, Ayman Farooq², Zhengrong Zhang³, Yang You³, Tsuneya Ikezu², Suelen Boschen Lucio De Souza², Pamela McLean²
¹Mayo Clinic Graduate School of Biomedical Sciences ²(Mayo Clinic Department of Neuroscience, Mayo Clinic Graduate School of Biomedical Sciences, Jacksonville, FL, USA) ³(Mayo Clinic Department of Neuroscience, Jacksonville, FL, USA)

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