**Predicting and Defining Steroid Resistance in Pediatric Nephrotic Syndrome using Plasma Metabolomics**

Metabolomics Analysis: NIH ERCMRC

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**Abstract:**

Nephrotic syndrome (NS) is a very common kidney disease in children. Glucocorticoids (GC) are the primary therapy, but are ineffective in ~20% of children and ~50% of adult cases. Patients with steroid resistant NS (SRNS) fail to enter remission after prolonged oral GC treatment, and are at high risk for GC-induced side effects and progression to end-stage kidney disease. This study aimed to discover markers of steroid resistance that could be potentially used to predict SRNS at presentation, and develop an improved mechanistic definition of pediatric SRNS. Plasma samples were collected from 30 steroid sensitive NS (SSNS) and 15 SRNS patients, and paired samples analyzed which were collected both at disease presentation, prior to any steroid therapy, and after ~7 weeks of daily GC treatment. Broad spectrum 1HNMR data were acquired, binned, and concentration fit. Multivariate analyses and hypothesis testing were used to determine the metabolites that best differentiated the four phenotypic groups. Treatment effects on metabolomics profiles were observed between paired Pre- and Post- treatment SSNS groups, and between Post SSNS and SRNS groups. Metabolites most perturbed by GC treatment included lipoproteins, adipate, pyruvate, alanine, creatine, glucose, tyrosine, valine, and glutamine. Logistic regression using a stepwise variable selection method was used on Pre- samples to model the odds at clinical presentation of SRNS. After controlling for age, the step-wise logistic regression model selected increased glutamine (OR= 1.01; 0.99-1.02 95% CI) as a marker of SRNS. A similar model with children age >3 only, indicated that children with reduced levels of malonate (OR=0.94; 0.89-1.00 95% CI) had increased odds of SRNS. Thus, malonate concentration may be a potential plasma biomarker for identifying SRNS at initial clinical presentation.

**Sample Description**

Citrate plasma samples were collected from 30 steroid sensitive NS (SSNS) and 15 SRNS patients, and paired samples analyzed which were collected both at disease presentation, prior to any steroid therapy, and after ~7 weeks of daily GC treatment.

The data required for the NMR metabolomics analysis can be found in the accompanying files and folders:

Procedures: 1. Steroid Resistant Nephrotic Syndrome Procedure.docx

Study Design Table: 2. Steroid Resistant Nephrotic Syndrome Study Design Table.xlsx

Metadata: 3. Steroid Resistant Nephrotic Syndrome METADATA.xlsx

Processed Data: 4a. Steroid Resistant Nephrotic Syndrome Normalized Binned Data.xlsx

4b. Steroid Resistant Nephrotic Syndrome Concentration Data.xlsx

Raw Data (folder): 5. Steroid Resistant Nephrotic Syndrome Raw Data.zip

**Notes:**

Each of the bin integrals were normalized to the total integral of each of the NMR spectrum (for more details, see accompanying Procedures file, **1. Steroid Resistant Nephrotic Syndrome Metabolomics Procedure.docx**

Descriptions of abbreviations for factors are available in the Variable Dictionary in the accompanying Study Design Table files, organized by sub-study i.e. **2. Steroid Resistant Nephrotic Syndrome Study Design Table.xlsx.**

The normalized binned NMR data and concentrations of select metabolites are available in the accompanying Processed Data (i.e. **4a. Steroid Resistant Nephrotic Syndrome Normalized Binned Data.xlsx** and **4b. Steroid Resistant Nephrotic Syndrome Concentration Data.xlsx**).

Sample ID serves as the unique identifier of the individual samples and is used as the NMR folder name in the raw NMR data file.