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

An aliquot (180 µL) of each study sample was mixed with 60 µL 0.9% saline solution containing 0.2% NaN3 and 4 mM formate in D2O. A total pool was also created by combining 11 µL aliquots from each study sample, mixing, dividing into four 180 µL aliquots, and processing identically to the individual samples. Each sample was mixed using a multiple tube vortex, centrifuged, and transferred to NMR tubes.

1H NMR spectra of plasma samples were acquired with a 1D CPMG pulse sequence (cpmgpr1d) on a Bruker Avance III 700 MHz NMR spectrometer (located at the David H. Murdock Research Institute at Kannapolis, NC, USA) using a 3 mm cryogenically cooled CRYO QNP probe and ambient temperature of 25℃. For each sample 128 transients were collected into 64k data points using a spectral width of 8417.509 Hz (12.02 ppm), 2 s relaxation delay, 2 ms fixed echo time, loop for T2 filter (l4)=80, and an acquisition time of 3.89 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5 Hz. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the formate signal. The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. Spectra were assessed for missing data and underwent quality checks.

NMR spectra were pre-processed using Bruker TopSpin 3.2 and ACD 1D NMR Processor 12.0 (ACD Labs, Toronto, CA) for metabolomics analysis. NMR bins (0.0 – 8.35 ppm) were made after excluding water (4.55 – 5.15 ppm) and citrate (2.47-2.565 and 2.61-2.70) using intelligent binning with a width of 0.04 ppm and 50% looseness factor. Integrals of each of the bins were normalized to total integral of each of the spectrum. NMR signals were library matched to metabolites and the concentrations were determined relative to 1 mM formate using Chenomx NMR Suite 8.1 Professional (Chenomx, Edmonton, Alberta, Canada) software.