NMR Metabolomics Methods

Chenomx Internal Standard solution (70 ul) and 230 ul D20 was added to each of the 36 urine sample (400 ul), vortexed for 30s, and centrifuged at 12000 rcf for 5min. Chenomx ISTD (Chenomx, Edmonton, Alberta, Canada) contains 5mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, Chemical Shift Indicator), 100 mM Imidazole (pH indicator), and 0.2% NaN3 (to inhibit bacterial growth) in D2O. 600 µl aliquot of the supernatant was transferred into 5mm NMR tubes (Bruker-Biospin, Germany). Phenotypic pooled urine samples were made by combining 150 µl aliquots from each of the study samples belonging to the same phenotype (cases and control). In addition, a combined phenotypic pooled sample was prepared by using 1000 µl aliquot from each of the phenotypic pooled sample. Pooled NMR samples were prepared as described above and used as quality check (QC) samples.

1H NMR spectra of urine samples were acquired on a Bruker Avance III 950 MHz NMR spectrometer (located at the David H. Murdock Research Institute at Kannapolis, NC, USA) using a 5 mm cryogenically cooled ATMA probe and ambient temperature of 25℃. A 1D NOESY presaturation pulse sequence (noesypr1d) was used for data acquisition. For each sample 64 transients were collected into 64k data points using a spectral width of 18.9 kHz (19.9 ppm), 2 s relaxation delay, and an acquisition time of 0.865 s per FID. Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS 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 processed using ACD NMR software (Advanced Chemistry Development, Toronto, ON, Canada). NMR bins (0.40-10.0 ppm) were made after excluding water (4.60-4.90 ppm), urea (5.55-6.00 ppm), and Imidazole (7.25-7.50 ppm) using Intelligent Bucketing Integration with a 0.04 ppm bucket width and a 50% looseness factor. Each of the NMR bins was normalized to total integral of each of the spectrum.