INTRODUCTION

Idiopathic nephrotic syndrome (INS) results from the malfunction of the glomerular filter and is the most prevalent glomerular disease in children. In spite of some progress, its pathogenesis is still unknown and the therapy options are confined to gross immune modulation. A variety of methods for diagnostic and treatment purposes are available for the patients; however, the lack of understanding regarding the pathogenic mechanisms underlying INS can lead to poor therapeutic response and adverse side-effects. Here, we describe a multi-omic approach to reveal new molecular factors involved in pathogenesis of INS with potential diagnostic and therapeutic significance.

METHODS

Sample preparation
Pediatric urine samples intended for peptide analysis were prepared for LC-MS analysis as previously described [1]. Samples were treated with 1% Rapigest SF prior to reduction and alkylation. Aliquots were incubated with anti-HSMA resin and centrifuged using Vivascience 5,000 MWCO filters. A series of washing steps were implemented to ensure adequate recovery. The resulting supernatant was digested using trypsin overnight at 55°C as illustrated in Figure 1.

Metabolite analysis samples were purified using Oasis HLB extraction cartridges. Water/methanol (90/10) washes were performed, followed by analyte elution using methanol. The resulting residue was reconstituted in 200 µL mobile phase and vortexed prior to LC-MS.

LC-MS conditions
Label-free LC-MS was used for qualitative and quantitative peptide analysis. Experiments were conducted using a 90 min gradient from 100 to 50% acetonitrile (0.1% formic acid) at 5 µL/min using a ACQUITY UPLC system. Here, a BEH 1.7 µm C18 column was used.

For metabolite identification, the LC-MS experiments consisted of a 10 min gradient from 100 to 50% acetonitrile (0.1% formic acid) at 5 µL/min using a ACQUITY UPLC system. Here, a BEH 1.7 µm C18 reversed phase 75 µm x 20 cm nescale LC column.

Data were acquired in data-independent analysis (DIA) that utilized a nanoscale LC nanoACQUITY or ACQUITY system directly interfaced to a hybrid PDA-TOF Synapt G2 mass spectrometer. Ion mobility (IMS) was used in conjunction with both acquisition schemes, illustrated in Figure 2.

RESULTS

Small amounts of the purified urine were analyzed to identify, quantify and investigate the proteomic and metabolomic variance between control and disease pre-treated subjects. Principal component analysis was used to identify and highlight significant changes between control and disease pre-treated samples, of which an example is shown in Figure 4. Similar clustering patterns are observed for both the protein and metabolite datasets.

The fold change at the peptide level can be visually displayed using 3D montage images. A change state feature of one of the peptides of interest is shown in Figure 5. The metabolomics workforce results are summarized in Figure 6. Using the metabolite contrasting loadings plot, significant metabolite identifications can be found at the extremes and are shaded in blue. Example compounds which are found to contribute most significantly to the variance are shown in red.

A common pathway is shown in Figure 7, illustrating Glutamate (NMDA) receptor subunit is one such example. NMDA belongs to the glutamate-gated ion channel family of proteins and is used in neuronal system pathways. Glutamate can also be located within the same pathway. Posttranslational CA2+ is thought to increase through the NMDA receptors, which activate several signal transduction pathways including ERK/MAP kinase and cAMP regulatory pathways.

CONCLUSIONS

- 80% of the proteins identified were expressed, with 31% of proteins having a maximum fold change ≥ 2 and ANOVA (p) value ≤ 0.05
- The majority of identified proteins are glycosylated of which many of which also show changes in relative abundance.
- PCA analysis shows both protein and metabolite data to be complementary.
- Variety of compounds are identified as contributing towards the metabolite variance.
- Complementary information obtained from metabolite and protein analysis has been shown through the use of glutamate and NMDA within the neuronal system pathway.
- A label-free multi-omics approach has been applied for the analysis of the urine of INS patients by implementing DIA-IMS-MS, provides both qualitative and quantitative information in a single experiment.

REFERENCES


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