A MULTI-OMICS APPROACH TO QUANTITATIVELY CHARACTERISE URINE FROM CHILDREN DIAGNOSED WITH IDIOPATHIC NEPHROTIC SYNDROME USING MOBILITY-ASSISTED DATA-INDEPENDENT LC-MS

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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.

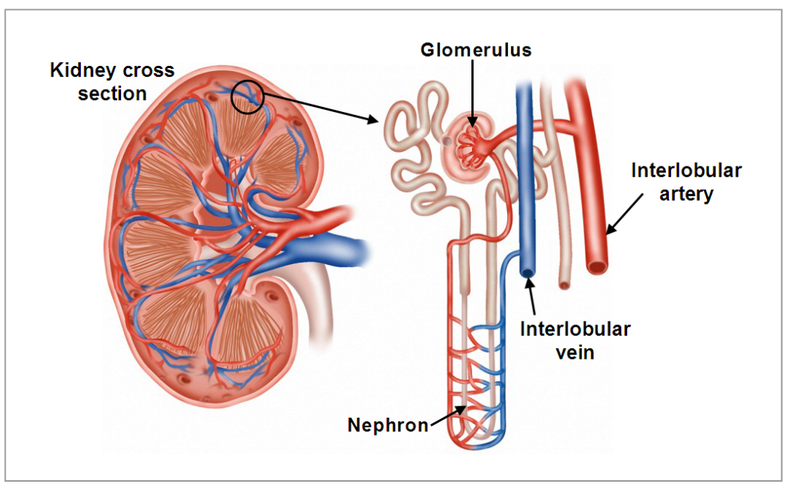

Figure 1. Kidney section highlighting a single nephron. A malfunctioning glomerulus is were INS occurs.
(Courtesy of Wellcome Images)

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-HSA resin and centrifuged using Vivaspin 5,000 MWCO filters. A series of washes using water were implemented to ensure adequate recovery. The resulting supernatant was digested using trypsin overnight as shown in Figure 2.

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.

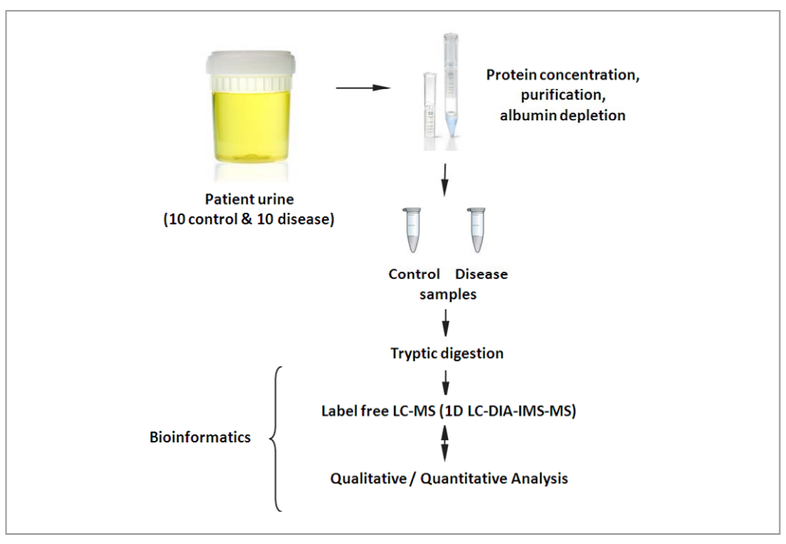

Figure 2. Experimental design study for urinary proteins

LC-MS conditions

Label-free LC-MS was used for qualitative and quantitative peptide analyses. Experiments were conducted using a 90 min gradient from 5 to 40% acetonitrile (0.1% formic acid) at 300 nL/min using a nanoACQUITY system and a BEH 1.7 µm C18 reversed phase 75 µm x 20 cm nanoscale LC column.

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 2.1 x 10 cm LC column was used.

Data were acquired in data independent analysis (DIA) that utilized a nanoscale LC nanoACQUITY or ACQUITY system directly interfaced to a hybrid IMS-oaToF Synapt G2 mass spectrometer. Ion mobility (IMS) was used in conjunction with both acquisition schemes, illustrated in Figure 3.

Figure 3. Retention and drift time principle ion mobility enabled data-independent analysis (DIA-IMS-MS).

Bioinformatics

The LC-MS peptide data were processed and searched with ProteinLynx GlobalSERVER. Normalized label-free quantification was achieved using Progenesis LC-MS software. The resulting metabolomic data was processed using MetaboLynx software and statistical analysis conducted with EZ Info.

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.

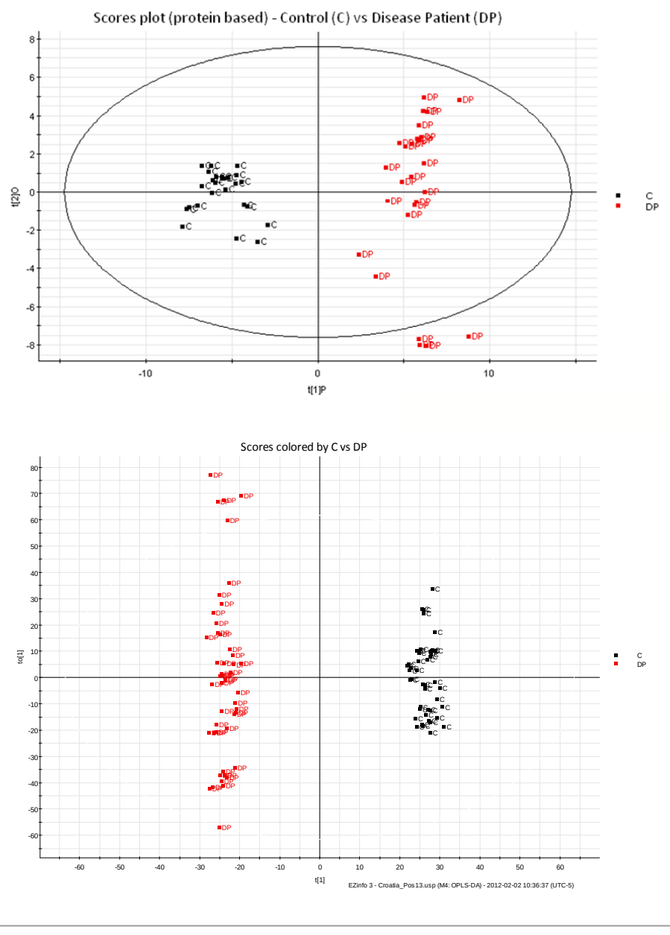

Figure 4. Scores plot from OPLS-DA analysis of disease pre-treated (red) vs. control (black).

Proteomic data were aligned, normalized and quantified. A large proportion of the identified proteins were glycosylated and over 80% of the total number of proteins identified, exhibited a significant expression fold change. Figure 5 highlights the proteins which have greater than a 2-fold change between sample sets.

The fold change at the peptide level can be visually displayed using 3D montage images. A charge state feature of one of the peptides of interest is shown in Figure 5. The metabolomics workflow 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 indent.

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. Postsynaptic Ca^{2+} is thought to increase through the NMDA receptors, which activate several signal transduction pathways including Erk/MAP kinase and cAMP regulatory pathways.

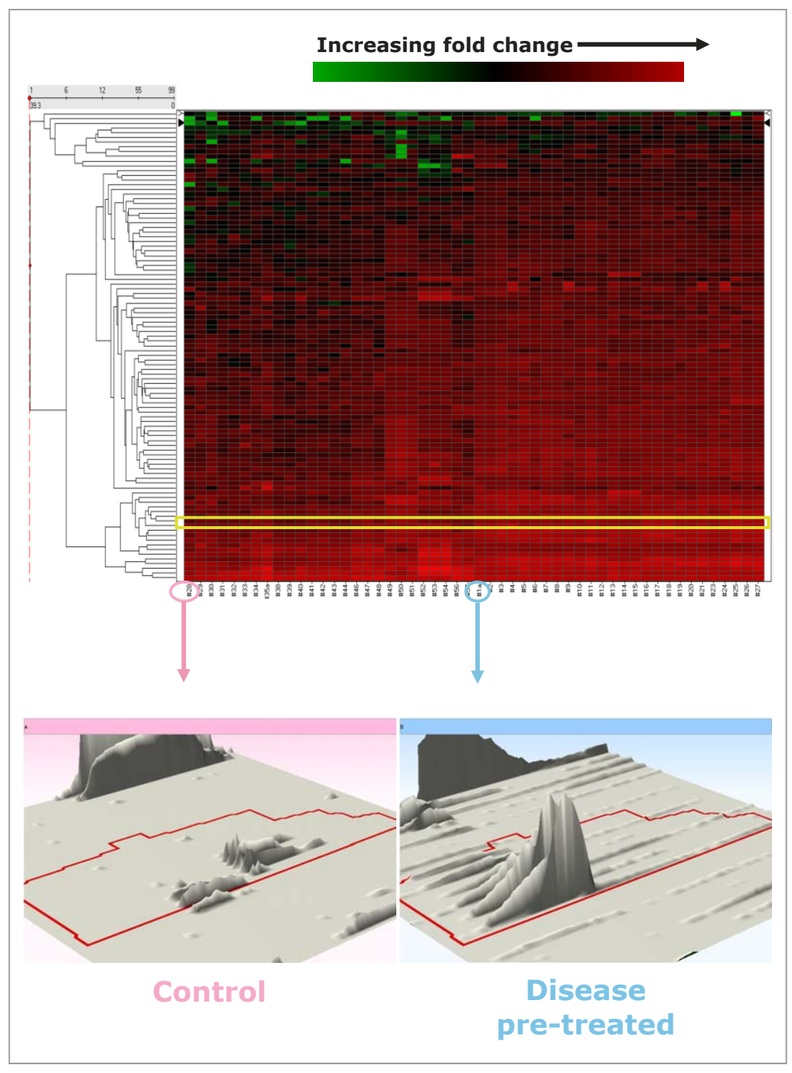

Figure 5. Hierarchal cluster analysis regulated proteins with a minimum of 3 identified peptides and a fold change greater than The highlighted region represents prostaglandin with associated 3D montage images for TMLLQPAGSLGSYSYR.

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 complimentary.**
- **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

1. One-step sample concentration, purification and albumin depletion method for urinary proteomics. Vaezzadeh AR, Briscoe AC, Steen H, Lee RS. J Proteome Res. 2010;9(11):6082-89.
2. Database searching and accounting of multiplexed precursor and product ion spectra from the data independent analysis of simple and complex peptide mixtures. Li GZ, Vissers JP, Silva JC, Golick D, Gorenstein MV, Geromanos SJ. Proteomics. 2009 Mar;9(6):1696-719.
3. A multiplexed quantitative strategy for membrane proteomics: opportunities for mining therapeutic targets for autosomal dominant polycystic kidney disease. Han CL, Chien CW, Chen WC, Chen YR, Wu CP, Li H, Chen YJ. Mol Cell Proteomics. 2008 Oct;7(10):1983-97.
4. Src kinases: a hub for NMDA receptor regulation. Salter MW, Kalia, LV. Nature Reviews Neuroscience, 2004 April; 5: 317-328.

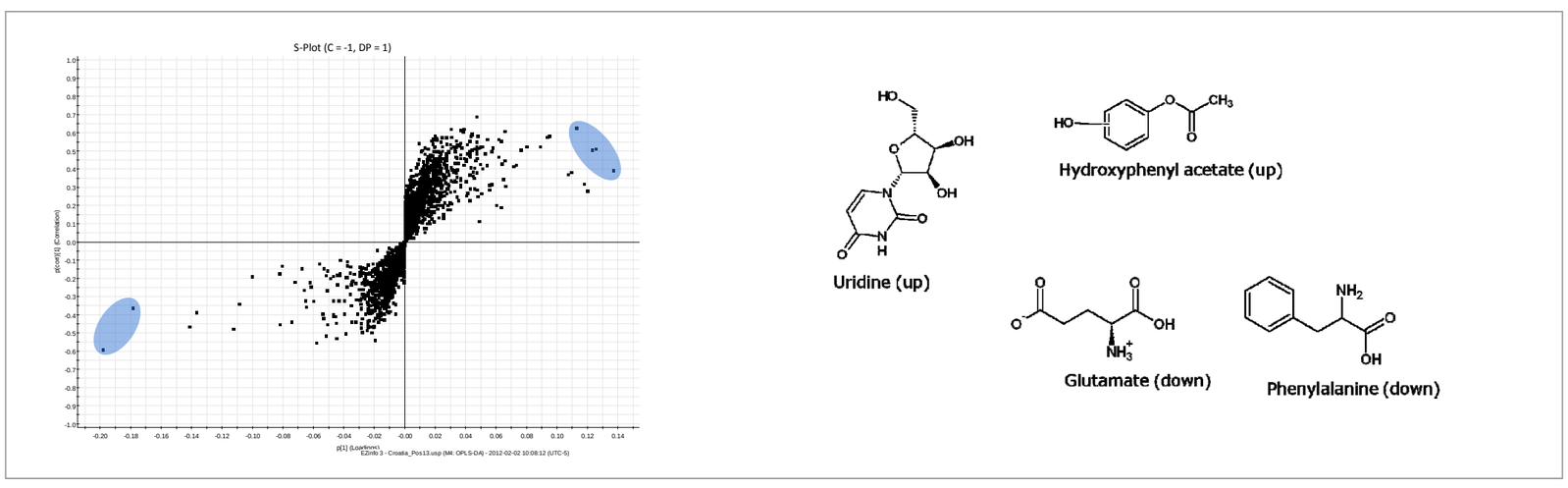

Figure 6. Metabolite loadings plot from OPLS-DA analysis of disease pre-treated vs. control subjects based in positive ion mode. Metabolites contributing the greatest variance are represented within the blue shaded areas with examples provided.

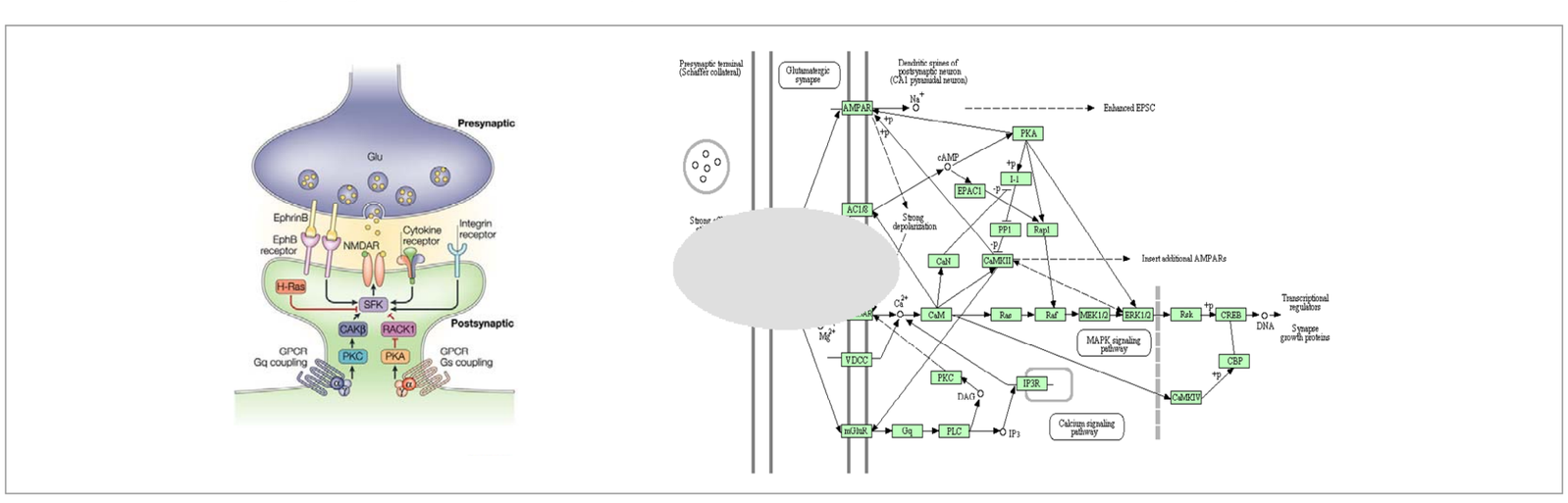

Figure 7. Neuronal system pathway, specifically highlighting the role of the Glutamate [NMDA] receptor subunit and glutamate for downstream transmission in the postsynaptic cell.

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