APPLICATION OF OMICS- AND FUNCTIONAL NETWORK ANALYSIS FOR PAEDIATRIC PATIENTS DIAGNOSED WITH IDIOPATHIC NEPHROTIC SYNDROME

Lee A. Gethings1, Johannes P.C. Vissers1, John Shockcor1, Stephen McDonald1, Mathias Hofmann2, Marc Kipping2, Sandra Kraljević Pavlic3, Mirela Sedlić4, Maja Lemac4, Danica Batinić4, Mirela Sedlić4, Maja Lemac4, Danica Batinić4, Pavelić3, Mirela Sedic3, Maja Lemac4, Danica Batinić4, Pavelić3.1Waters Corporation, Manchester, UK; 2Waters GmbH, Eschborn, DE; 3University of Rijeka, Croatia; 4University of Zagreb, Croatia; 5University of Liverpool, UK.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

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 prove 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 responses 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

Proteomic and metabolite samples intended for peptide analysis were prepared for LC-MS/MS as previously described [1]. Samples were treated with 1% 1,9-diazabicyclo[5.4.0]undec-7-ene (DBU) at 60 °C under a nitrogen atmosphere for 30 minutes. The resulting supernatant was digested using trypsin overnight. The digest was then purified using Oasis HLB reversed phase C18 column (20 mg, 96 mm OD, Waters) followed by a second Oasis HLB reversed phase C18 column (20 mg, 96 mm OD, Waters). A series of washes using 10% acetonitrile (ACN) at 5 µL/min using an ACQUITY UPLC system (Waters) was used to elute peptides.

Label-free LC-MS was used for qualitative and quantitative analysis. Data were acquired in data independent analysis (DIA) that enables acquisition of LC-MS or LC-MS/MS spectra from the ion source. An important advantage of DIA over data dependent acquisition schemes, illustrated in Figure 3.

Bioinformatics

The LC-MS peptide data were processed and searched with ProteinLynx Global Server. Normalized label-free quantification was achieved using TransOmics LC-MS software. The resulting metabolomic data was also processed using TransOmics with additional statistical analysis conducted. Ingenuity IPA was used for network and pathway analysis.

RESULTS

Small amounts of the purified urine were analysed to identify, quantify and investigate the proteomics and metabolomics variance between control and disease pre-treated subjects. PCA was used to identify significant changes between control and disease pre-treated subjects. The resulting metabolomic data was also processed using TransOmics and confirmed by Ingenuity IPA as shown in Figure 4. Similar clustering patterns are observed for both the protein and metabolite data. Proteomic data were aligned, normalised and quantified. A large proportion of the identified proteins were discovered and over 80% of the total variance is explained by disease condition. The identified proteins indicate relationship to HDL-trafficking and are shaded in blue. Pathways of related categories.

CONCLUSIONS

- 80% of the proteins identified were expressed, with 31% of proteins having a maximum fold change ≥ 2 and ≥0.8. The majority of identified proteins are glycoproteins of which many of which also show changes in relative abundance.

- PCA analysis shows both protein and metabolite data to be complimentary.

- A label-free multi-omic approach has been applied for the analysis of the urine of INS patients by combining proteomic and metabolomic analysis. metabolomics and protein profiling are complimentary. The metabolomics workflow results are summarized in Figure 5. Using the metabolite contrasting loadings plot, significantly affected metabolites that are shared in-tand are shown in Figure 6. The identified proteins indicate relationship to HDL-trafficking and complementarity to disease pre-treated subjects. Functions associated with the metabolic changes identified are also represented in Figure 7. Significant over-represented metabolites and proteins were used to provide reconstructed connectivity pathway analysis.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

REFERENCES


