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# Proteomic patterns in glomerular research, a laser capture microdissection and liquid chromatography-tandem mass spectrometry approach

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## ABSTRACT

**Introduction**: Molecular techniques have the potential to shed light on glomerular diseases that conventional renal pathology may be unable to reveal. The aim of this study was to investigate whether proteomic patterns of glomeruli obtained from kidney biopsies can differentiate between minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS) and control groups (CTR).

**Methods**: 18 formalin-fixed, paraffin-embedded (FFPE) renal biopsies comprising three groups of samples (CTR=3, MCD=6, FSGS=9) were subjected to label-free quantitative mass spectrometry. Glomeruli were excised from FFPE renal biopsies by laser capture microdissection (LCM) and, to increase both yield and protein identifications, single-pot solid-phase-enhanced sample preparation (SP3) digest method was applied. The samples were analyzed by mass spectrometry based shotgun proteomics.

**Results**: The proteome profiling resulted in the identification of a total of 723 proteins. Multivariate analysis provided several proteins important in the separation of the three groups. Pattern Hunter analysis revealed moderate and high correlation of proteins against CTR-FSGS-MCD or CTR-MCD-FSGS patterns. The most significant pathways involved were associated with nephrin family and cytoskeleton interactions, as well as laminin/extracellular matrix related proteins. Univariate analysis revealed 58 significant different proteins among the three groups. Signaling pathways of these proteins were also associated with nephrin family interactions and cytoskeleton organization.

**Conclusions**: This study demonstrates that mass spectrometry-based shotgun proteomic analysis of LCM glomeruli yields reproducible and quantitative data capable of discriminating between different disease conditions. Differentially expressed proteins provide insights into pathogenesis of glomerular disease.

**Keywords**: proteomics, minimal change disease, focal segmental glomerulosclerosis, laser capture microdissection, tandem mass spectrometry

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## INTRODUCTION

Regardless of their rarity, primary glomerular diseases place a significant burden on individuals, healthcare systems and the society [1,2]. Histologic injury patterns still guide diagnosis, prognosis, and treatment [3]. Biopsies only give a "snapshot" of the disease and do not reflect the complex pathogenesis-driven disease activity and course. Biopsies frequently fail to distinguish primary from secondary disease or to predict prognosis or treatment response [4]. Additionally, biopsies are invasive. Unfortunately, many trigger agents cause a similar glomerular injury pattern. Thus, diseases with the same histology but different pathogenesis and prognosis are often treated similarly. Even successful treatments improve prognosis in only about 50% of patients, and identifying those who will benefit remains a clinical challenge [3].

On the other hand, while certain glomerular diseases can be recognized by a serologic signature or clinical

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picture this is not the case for minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS). Both are characterized by a clinical presentation with nephrotic syndrome, mostly inactive sediment, and have a histological feature of podocyte foot process effacement (FPE) as marker of extensive podocyte injury but different prognosis under therapy. The majority of MCD patients respond to corticosteroid treatment, whereas FSGS patients are typically resistant to steroid therapy and experience a progressive decline in glomerular filtration rate [5,6].

In the context of kidney biopsies, where available material is limited laser capture microdissection (LCM) can be a useful option to precisely isolate structure of interest, glomeruli, for further molecular analysis. The advantage of this technique is that it offers an inframilimetric dissection precision that allows isolation of glomeruli under direct visual observation by a pathologist. Isolated structures, both from fresh tissue and formalin-fixed, paraffin-embedded (FFPE), can be used for downstream applications such as RNA or DNA sequencing, proteomics analysis, or gene expression analysis [7–9].

Proteomics analyses offer the possibility of screening and quantifying altered expression levels of proteins present in the tissue sample isolated using LCM. This can provide information about changes in protein expression and post-translational modifications, as well as protein-protein interactions and signaling pathways that are involved in kidney disease. Proteomics analyses of kidney biopsies can be performed using various techniques, including mass spectrometry, 2D gel electrophoresis, and protein microarrays. These methods can provide high-throughput analysis of protein expression and can be used to compare protein profiles between healthy and diseased kidney tissue [10].

Using this proteomic approach on LCM glomeruli, important advances have been made in the field of pathogenesis of several glomerular disorders. Our study aims to determine feasibility of such an approach in our center and whether proteomic patterns of glomeruli from kidney biopsies can discriminate between MCD, FSGS and controls and whether it may shed light on the pathogenesis of disease.

## METHODS

### Samples collection and storage

We conducted an observational, retrospective and cross-sectional study using MCD and FSGS FFPE historic biopsies from the Emergency Hospital of Cluj-Napoca and the control kidney tissue from the Institute of Urology and Transplantation of Cluj-Napoca. Normal control kidney tissue was obtained from patients who underwent nephrectomies for tumors, collected at distance from the tumor and that was certified by a pathologist to normal tissue on light microscopy. We included patients with historic biopsies in the last 3 years for nephrotic syndrome, who had a diagnosis of MCD and FSGS, validated by an experienced pathologist, who provided consent for the use of remaining biopsy tissue. Only patients with sufficient tissue for proteomic analysis after LCM were included. We collected clinical and paraclinical data available at the time of biopsy including age, gender, renal function, serum albumin, and proteinuria/24 h (or proteinuria/creatinine ratio).

### Laser-capture microdissection sample preparation

LCM was performed at the Centre of Research in Functional Genomics, Biomedicine and Translational Medicine of Iuliu Haţieganu University of Medicine and Pharmacy of Cluj-Napoca. Each case was sectioned at 10 µm. Multiple sections per case, depending on tissue availability, were affixed onto MMI RNAse/DNAse-free membrane slides and subjected to staining via Mayer's hematoxylin staining protocol. The LCM System MMI CellCut Plus was used to perform the isolation of glomerular sections. In the LCM protocol, the tissue image is displayed on an LCD monitor and subsequently, an experienced pathologist identifies and demarcates each glomerulus. After identifying the glomeruli, the tissue sections are isolated in a contamination free environment. This is achieved by placing the tissue sections between a clean glass slide and the MMI slide membrane. Sections of tissue were collected using adherent MMI Collection Caps, which were designed to prevent contamination during the collection process. A number between 27-100 glomeruli were collected for each case and subsequently subjected to proteomics analysis.

### Sample preparation for proteome profiling

Sample preparation, proteomic analysis and data processing were performed at the Medfuture Research Center for Advanced Medicine of Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca. LCMs were resuspended in Rapigest® 0.1% (prepared in 100mM ammonium bicarbonate) and transferred into protein low retention tubes. Protein extraction was facilitated by sonication (3x3'', A:80%) and two heat incubation steps (95°C, 15' and 80°C, 60'). Sample preparation was further conducted by means of Single-pot solid-phase-enhanced sample preparation (SP3) as described by Hughes et. Al [11]. Beads were combined in an 1:1 (v/v, in water) ratio, the final concentration used being 20µq/µL. Supernatant was reduced and alkylated (Dithiothreitol - DTT final concentration 5mM for 30' at 60C and

10mM Iodoacetamide -IAA, dark, room temperature, 30', respectively). Briefly, 10 µL SP3 bead stock (20 µg/µL) were added to each sample, binding of proteins to magnetic beads being induced by addition of 100 % acetonitrile- ACN. After 15 min sample incubation at room temperature, beads were immobilized using a magnetic rack and supernatant was discarded. The beads were washed with 100 % ethanol and 100% ACN being afterwards redissolved in Rapigest® 0.1% buffer (Waters Corporation). Trypsin was used as digestion agent at an enzyme-to-protein ratio of 1:25 (w/w). After overnight digestion (37°C, 18h, 1000rpm), ACN 100% was used for peptide binding to the magnetic beads. The washing step was performed as stated above. Finally, Beads were pelleted on the magnetic rack for 2 minutes; the supernatant was removed, and beads were dried for 5 min at RT. Peptides were eluted using dimethylsulfoxide-DMSO 2% (v/v in water). The purified peptides obtained (supernatant) were transferred into an injection vial and reconstituted in 1% formic acid- FA. Before LC-MS analysis, a digested standard of yeast alcohol dehydrogenase (ADH1) prepared in 5% ACN, 0.1% FA was added to each sample as used as an internal standard protein (the final concentration of ADH was 25 fmol/µL).

### Liquid chromatography - tandem mass spectrometry analysis (LC-MS/MS)

LC–MS/MS analyses were performed using a NanoAQUITY UPLC system (Waters Corporation, Milford, MA) coupled to Synapt G2-S high-definition mass spectrometer (Waters Corporation) via a NanoLockSpray dual electrospray ion source (Waters Corporation). Peptides were trapped on ACQUITY UPLC M-Class Trap column Symmetry C18, 5µm particles, 180 µm×20mm, (Waters Corporation), for 2 min at 5 µL/min in 0.5% solvent B (0.1% (v/v) formic acid in acetonitrile. ACQUITY UPLC M-Class reverse phase C18 column HSS T3, 1.8 µm, 75µm×250mm (Waters Corporation) was used for peptide separation. The method used for peptide separation was adapted from Ute Distler et al [12]. Briefly, peptides were separated by running a gradient of ACN+0.1%FA (mobile phase B) from 5 to 85% (v/v) at a flow rate of 300 nL/ min over 45 min gradient on the analytical column heated to 55 °C. MS analysis of eluting peptides was performed by ion-mobility separation (IMS) enhanced data-independent acquisition (DIA) in UDMSE mode [12]. Lockmass compound Glu-1-fibronopeptide B (GluFib) (100fmol/µL) was delivered by auxiliary pump at a flow rate of 0.3uL/min, spectra of the doubly charged species (m/z 785.8426) being recorded every 45sec.

### Data Processing and Label-Free Quantification

Raw MS data was processed by ProgenesisQIP version 4.2 (Nonlinear dynamics, Waters corporation) where data was post-acquisition lockmass corrected using the doubly charged monoiotopic ion of GluFib and aligned to the most suitable reference run identified automatically by the software. Normalization to all proteins was used. Data was searched against a target-decoy UniPortKB/Swiss-Prot Human database containing 20361 proteins (downloaded Jan 2022) to which yeast alcohol dehydrogenase (P000330) was added, using the following parameters: trypsin as digestion agent, maximum missed cleavages: one; fixed modification: carbamidomethylation and methionine oxidation; hydroxylation of asparagine, methylation at aspartic acid, proline, lysine; lysine methylation and lysine formylation at N-term as variable modifications; false discovery rate (FDR) was set to <1%. Ion matching requirements implemented were: (i) minimum one fragment ion matches per peptide ion; (ii) 3 fragment ions matched per protein identification and (iii) at least one peptide match per protein identification. Peptides with mass error of more than 20 ppm and length <5 amino acids were removed. Protein quantitation was performed using ADH1 (at concentration 25fmol/uL). The reviewed list of proteins was exported as .csv file for subsequent data analysis. Metaboanalyst 5.0 was used for subsequent post-processing protein analysis. Differential expression analysis and data visualization (PCA plots, heatmaps) were obtained using Metaboanalyst 5.0.

Reactome Pathway and gene ontology (GO) enrichment analysis was visualized using ClueGO, a Cytoscape plug-in that visualizes the non-redundant biological terms for large clusters of genes in a functionally grouped network [13]. The tissue specificity and distribution of the selected proteins was evaluated based using data from Human Protein Atlas proteinatlas.org version 22 [14,15]. Standard gene names were used for protein annotation.

### Declarations

The study was approved by the ethics boards of Iuliu Hatieganu University of Medicine and Pharmacy (No.142/2021) and the Emergency County Hospital Cluj-Napoca (No. 3/2021) and the Institute of Urology and Renal Transplantation (No. 3/2021) in accordance with the ethical standards of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

## RESULTS

Clinical characteristics and biochemical data of the study subjects

The study included a total of fifteen biopsies obtained from patients diagnosed with MCD and FSGS, as well as three control normal tissue samples. Demographic and

paraclinical data of patients are presented in Table 1. The values are represented as the average and standard error of the mean (average ± SEM).

## Multivariate Analysis

Tissue proteomics profile was acquired from a total of 18 FFPE renal tissue samples divided into three groups: control (CTR, n=3), FSGS (n=9) and MCD (n=6). First, multivariate exploratory data analysis methods were applied as they simultaneously take all variables into consideration and are generally considered more suitable for high-dimensional omics data analysis. Accordingly, principal component analysis (PCA), an unsupervised clustering or dimensionality-reduction method was utilized for data overview and pattern discovery (Fig. 1a, b). PCA chemometrics analysis performed using all 628 proteins identified (Fig. 1a, b) was able to explain 74.3% protein variation via five principal components (PCs), PC1 embracing 43.2% of the variation. The group separation was provided mainly by PC1 and PC2. On the PC1 direction (along the Y-axis, on the first row), there was a relatively good separation between all three groups. On the PC2 direction there was a tendency of separation between the CTR (red) and the two pathologic conditions MCD (blue) and FSGS (green) groups (Fig. 1a).

Partial least square-discriminant analysis (PLS-DA) was performed to sharpen the separation between the groups. The first 5 components captured 71.6% of variation (Fig. 2). As expected, the separation capacity of the PLS-DA was superior to PCA (Fig. 2a). Good separation between all three groups was observed along the Y-axis on component 1 direction comprising 42.3% of the observed variation. Along with the second component Y-axis, separation was obtained mostly between CTR (red) and FSGS (green) groups. On the component 3 direction, there was separation between the MCD (blue) and FSGS (green) groups. Figure 2b shows the 2D score plots of the first three PLS-DA components. Top 25 proteins ranked based on the variable importance in projection (VIP score) on the first component explaining most of the separation are shown in Fig. 2c (Online Resource 1). One important issue associated with PLSDA is overfitting, consequently, model validation is compulsory. The model had modest prediction accuracy (Fig. 2d) while the quality of prediction and accuracy ($R^2$=0.6; $Q^2$=negative value) was low; this concluding that the model was either not predictive or it was overfitted.

**Table 1. A summary of clinical demographics and laboratory readings of patients**

| | MCD (n=6) | FSGS (n=9) |
|---|---|---|
| Age at biopsy (years) | 39±9.01 | 42 ±5.26 |
| Sex (M:F) | 4:2 | 5:4 |
| Serum creatinine (mg/dl) | 0.90±0.11 | 1.13±0.14 |
| Serum albumin (mg/dl) | 2.16±0.40 | 2.77±0.29 |
| Proteinuria (g/24h) | 12.88±3.48 | 10.70±2.98 |

M- male, F-female

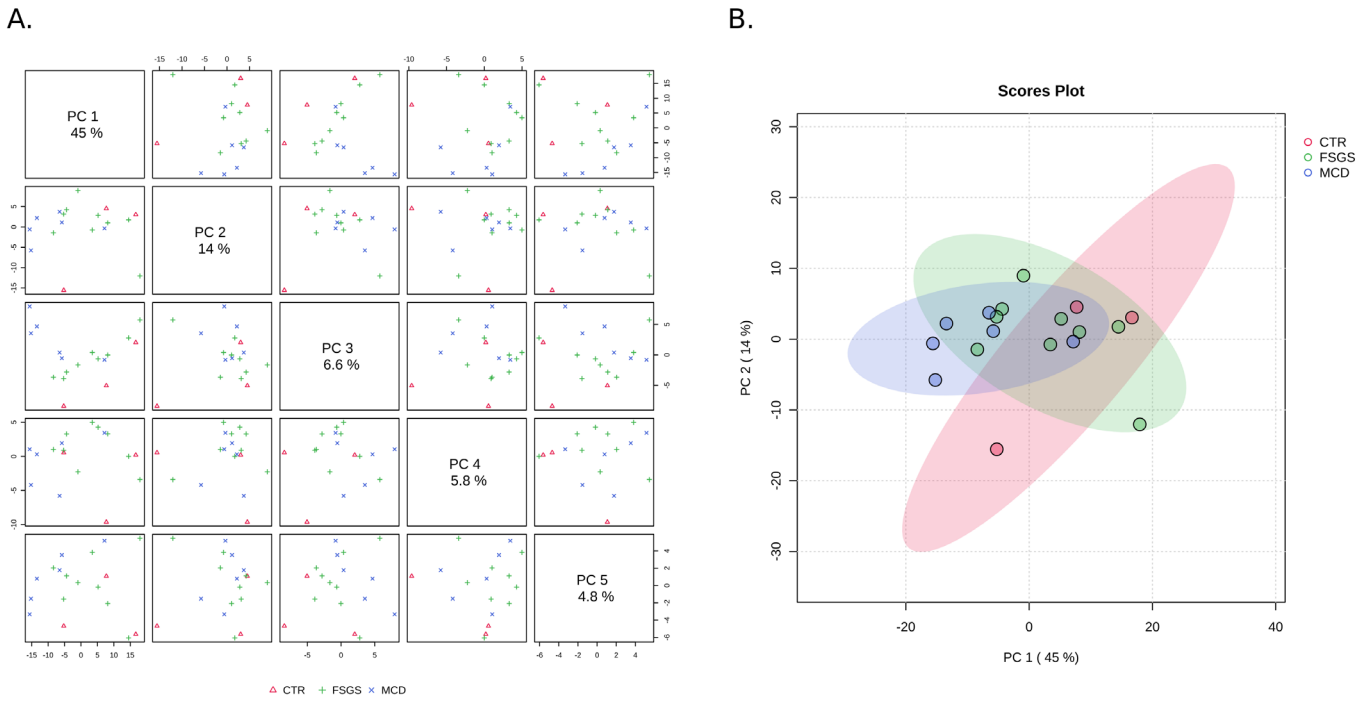



**Fig.1 Principal Component Analysis (PCA). a. Pairwise score plots between the selected PCs. The explained variance of each PC is shown in the corresponding diagonal cell; b. Scores plot between the selected PCs. The explained variances are shown in brackets. control (CTR), minimal change disease (MCD) , focal segmental glomerulosclerosis (FSGS)**

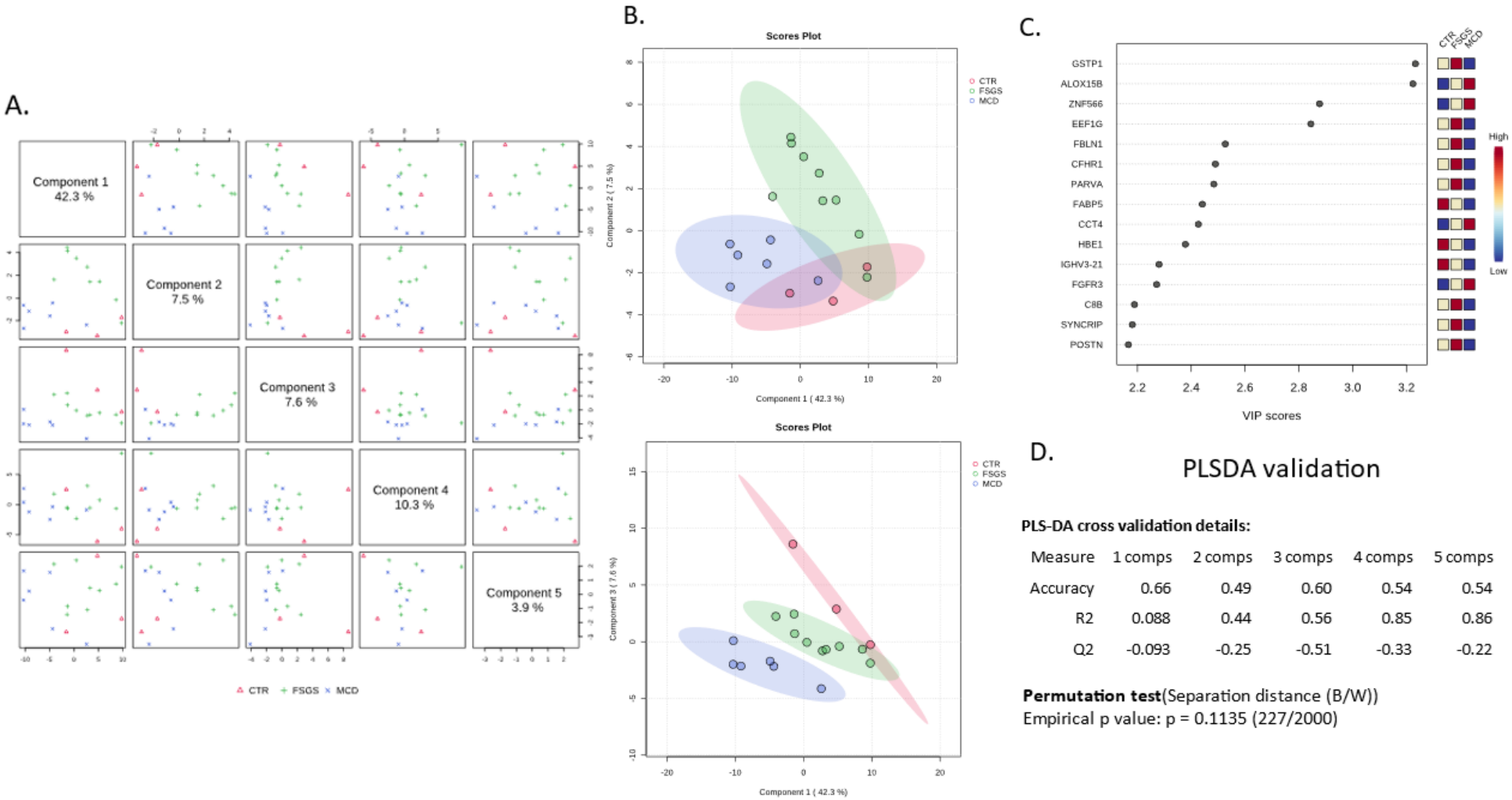



Fig.2 Partial Least Squares - Discriminant Analysis (PLS-DA). a. PLS-DA overview; b. Scores plot between the selected PCs; c. Important features identified by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding protein in each group under study; d. PLS-DA model validation

### Identification of variables showing a particular pattern of change

Pattern Hunter analysis was used to identify patterns across the groups of samples applying Spearman's rank correlation coefficient distance measurement to rank proteins in concentration order. Several proteins showed high ($|r| \geq 0.70$, $p \leq 0.05$; n=23) or medium ($0.50 \geq |r| \leq 0.70$, $p \leq 0.05$, n=174) correlation against CTR-FSGS-MCD (Fig. 3a, b, Online Resource 1) pattern. Moreover, the high correlating proteins were found associated with Signaling by Interleukins, vesicle-mediated transport, and metabolism of proteins ReactomeDB pathways (ClueGO analysis). Then again, the medium correlating proteins were involved in cell-cell communications, glucose metabolism and keratinization pathways. Only medium correlations were found against CTR-MCD-FSGS pattern (Fig. 3c, d), the list of variables being enriched in proteins associated mostly with glucose metabolism and signaling by PDGF pathways.

### Identification of variables significantly different among experimental conditions

Further, one-way ANOVA (p-value FDR cutoff: 0.2) with Tuckey post-hoc test ($p \leq 0.05$) identified 58 proteins significant different between groups (Online Resource 2). The proteome profile across groups of ANOVA-significant proteins is represented in Fig. 4a. Several patterns of protein concentration that change across different groups are distinct: there is a group of proteins that show an increasing concentration trend from CTR group to MCD (Fig. 4a, blue arrow), one group of proteins showing highest concentrations in MCD group with no different trend in CTR and FSGS groups (Fig. 4a, black arrow) and one group showing lowest concentration in MCD (Fig. 4a, orange arrow) including one small protein group with increasing MCD-FSGS-CTR tendency. ANOVA post-hoc test (Fig. 4b) revealed 2 common proteins with significantly altered abundance in all comparisons, 25 proteins mutual for MCD vs FSGS and MCD vs CTR and 6 proteins shared in MCD vs CTR and FSGS vs CTR. While no proteins were found exclusively in FSGS vs CTR, 10 and 15 proteins were found exclusively in MCD vs CTR and MCD vs FSGS, respectively. The two proteins that could discriminate between the three groups were Chaperonin containing TCP1 subunit 4 (CCT4) and G protein subunit alpha transducin 2 (GNAT2), both showing lowest level in CTR and highest in MCD. ClueGo Gene Ontology and Pathway enrichment functional analysis of the ANOVA-significant proteins (Fig. 4c) revealed Signaling by interleukins, Glucose metabolism and Nephrin family interactions pathways as significantly enriched in the dataset.

## DISCUSSION

This study provides evidence that glomerular proteome from clinical kidney biopsies can be successfully characterized using LCM followed by mass spectrometry based shotgun proteomics. To date, the majority of proteomic analyses have been conducted with urine and plasma, biological fluids that can be easily collected [8]. However, tissue investigations demonstrate the enormous potential of proteomic analysis for enhancing our comprehension of glomerular disorders [16].

To our knowledge, there are few studies that evaluate glomerular proteomics in MCD vs FSGS patients. To date, we only found one report, published in abstract form comparing 9 patients with MCD and 7 patients with FSGS. Compared to MCD, primary FSGS glomeruli had higher levels of proteins that regulate cell-cell and cell-matrix adhesion and differentiation [17].

Both FSGS and MCD are primary podocyte-damaging diseases and represent an interesting target for proteomic studies. In both these conditions, little is known about the molecular composition of the glomerulus and how it alters as the disease progresses; pathogenesis of these two glomerular diseases might be very different. Podocyte FPE the most prevalent pathological feature in podocyte disorders, can be caused by either a dysregulated podocyte cytoskeleton or the loss of integrity of the slit diaphragm (SD) [18,19]: while FPE in MCD could be directly triggered by alterations in cytoskeleton and/or loss of integrity of the SD, in FSGS FPE might be an adaptation, with podocyte rearrangements reactive to focal podocyte injury in an effort to minimize exposed areas, as podocytes are terminally differentiated cells that do not divide. Within MCD and FSGS itself various pathways of disease might be involved in different patients.

For such proteomic kidney tissue studies, LCM was used to selectively separate the glomeruli, from frozen or FFPE tissue [8]; Finne et al. found no difference between the number of proteins detected in these two types of tissues [9,20,21]. Prior research required a minimum of 4 to 100 glomeruli to be dissected from 10-µm-thick tissue sections [9,22,23].

Our study proves that proteomic analysis of isolated glomeruli from kidney biopsy tissue offers a unique window into the pathogenesis of these podocytes disorders and enables the molecular profiling of a patient's biopsy. The high degree of clustering between the groups suggests the capability of the method to discern between different types of podocyte injury. The complexity of the FSGS cohort, which included both primary and secondary FSGS as well as varying treatment responses, may account for the intra-group variability.

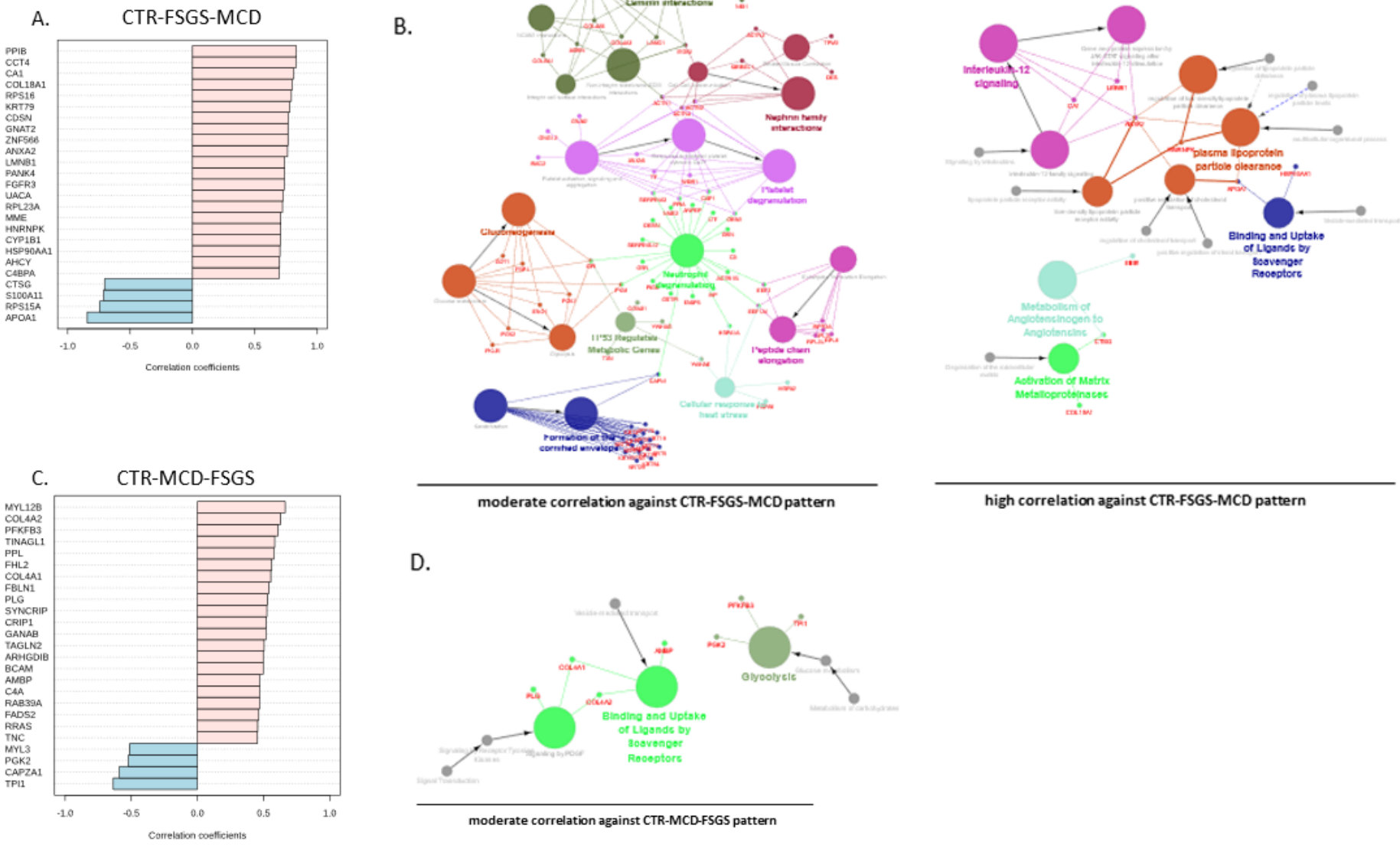



**Fig. 3** Pattern analysis- proteins with concentration changes that follow a specific pattern; **a.** Top 25 proteins correlating against CTR-FSGS-MCD pattern; **b.** ClueGO functional enrichment analysis of the significantly (B-H corrected p≤0.05) associated Reactome pathways with proteins correlated against CTR-FSGS-MCD pattern; **c.** Top 25 proteins correlating against CTR-MCD-FSGS pattern; **d.** ClueGO functional enrichment analysis of the significantly (Benjamini-Hochberg corrected p≤0.05) associated GO terms and Reactome pathways with proteins correlated against CTR-MCD-FSGS pattern

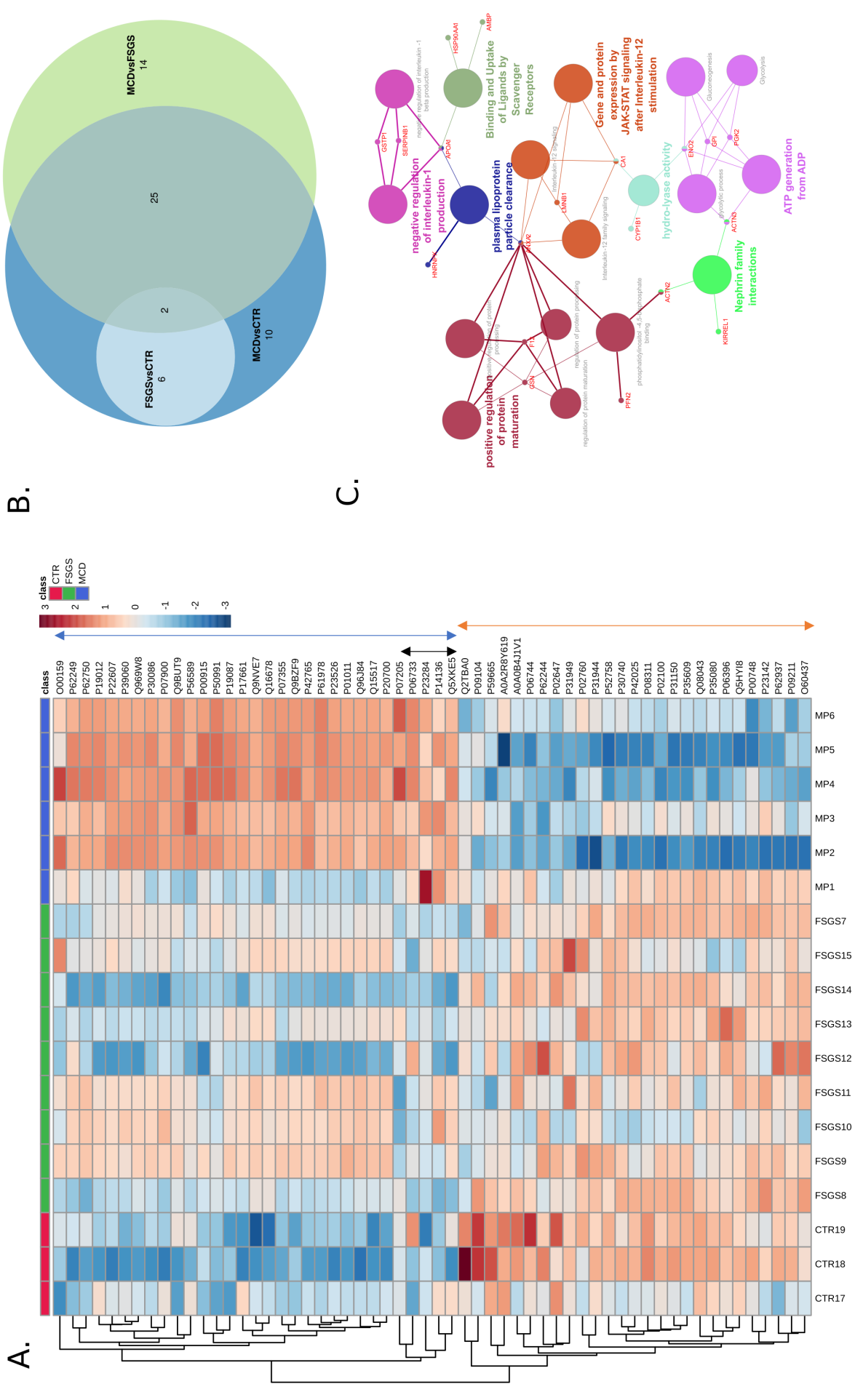



**Fig. 4 a.** Heatmap of hierarchical cluster analysis results based on ANOVA significant different proteins (distance measure using Euclidean, and clustering algorithm using average; **b.** Euler diagram of Anova and Tuckey Honestly Significant Difference post-hoc analyses; **c.** ClueGO functional enrichment analysis of the significantly associated GO molecular functions, biological processes, immune system process and Reactome pathways with ANOVA significant different proteins (corrected BH p-value:0.05, min. 3 genes/pathway)

First, multivariate analysis (PCA and PLS-DA) was able to discriminate between groups based on proteomic pattern (Fig.1, Fig.2). Proteins linked to extracellular matrix organization retained their discriminatory capabilities when analyzed in multivariate analysis. Examples include proteins involved in cell adhesion, migration and ECM (extracellular matrix) organization such as FBLN1 [24], chaperonins involved in folding of cytoskeletal proteins (CCT4) [25].

Second, pattern Hunter analysis identifies moderate or high correlations across the groups (Fig.3). Proteins linked to patterns of glucose metabolism, peptide chain elongation, cellular response to heat stress, interleukin-12 signaling, vesicle-mediated transport were all related to CTR-FSGS-MCD pattern, but the most interesting patterns from a pathogenic point of view remained nephrin family interaction and laminin signaling.

Lastly, in univariate analysis, Reactome points up two particular pathways structurally and functionally specific to podocyte function, namely nephrin family interactions and proteins involved in cytoskeleton organization. These were upregulated in MCD. Whereas ECM/laminin interactions proteins characterize FSGS [26].

Nephrin family proteins are important components of the SD, a highly specialized protein structure which is located between tertiary foot processes of mature podocytes. The structural and functional integrity of the SD is crucial for the selectivity of glomerular filtration (GF) [27]. As an example, Neph 1 (Kirrel 1) a member of the nephrin-like proteins and a major component of the SD was found to be more abundantly expressed in MCD versus FSGS or controls and able to differentiate between these conditions. Malfunctioning or mutation of this protein has been involved in subtypes of nephrotic syndrome in humans and is associated to FPE [28]. Increased expression of Neph1 might be a response to podocyte injury or the cause of SD dysregulation, FPE and proteinuria per se. Besides its role in GF selectivity, SD functions as a major signaling hub for cytoskeleton dynamics. Noteworthy, cytoskeleton related pathway was also relevant for discrimination between groups. Cytoskeleton proteins were upregulated in univariate and multivariate analysis, especially in MCD.

Renal biopsy, an invasive procedure, may not be possible in certain high-risk patients, and serial renal biopsies are typically avoided [8]. Also, due to sampling error and the focal nature of the lesions in FSGS the latter might sometimes be initially misdiagnosed as MCD with important implications in therapy [28,29]. Finding 'useful' biomarkers for glomerular disease and having them validated is important not just for scientific advancement and patient stratification in clinical trials, but for the future of precision medicine in the treatment of glomerulopathies. To facilitate such work, discovery studies and replication cohorts are being assembled globally [1,3].

The main limitation of the study is the limited number of samples included in the study. In the field of glomerular research, it is typical for studies to employ a small number of patients as samples [9]. Also, the immunostaining of the potential biomarkers detected at a higher level in FSGS and MCD than in controls would be necessary to validate our findings.

Our study is in line with this novel approach for glomerular diseases among research groups in Romania; further research is necessary to confirm our findings, and we intent to prospectively correlate proteomics from glomeruli with plasma and urine proteomic studies to identify biomarkers for MCD and FSGS from more accessible in a noninvasive collected biofluids.

## CONCLUSIONS

Glomerulopathies are rare diseases and access to kidney tissue is limited; the need of cutting-edge techniques that were used in our study (LCM and mass spectrometry-based shotgun proteomics) limits this approach to a few locations around the world. Proteomic analysis using mass spectrometry-based shotgun proteomics on LCM glomeruli from historic biopsies can discriminate between podocytopathies such as MCD and FSGS and to separate them from controls, providing insights into the pathogenesis of these glomerular conditions.

## ABREVIATIONS

ACN- acetonitrile
ADH1- alcohol dehydrogenase
CTR- control
DIA- data-independent acquisition
DMSO- dimethylsulfoxide
DTT- dithiothreitol
FDR- false discovery rate
FFPE- formalin-fixed, paraffin-embedded
FA- formic acid
FPE- foot process effacement
FSGS- focal segmental glomerulosclerosis
GF- glomerular filtration
GN- glomerulonephritis
GluFib- glu-1-fibronopeptide B
IAA- iodoacetamide
IMS- ion-mobility separation
LCM- laser capture microdissection

MCD- minimal change disease

PLS-DA- partial least square-discriminant analysis

PCA- principal component analysis

PCs- principal components

SP3 - single-pot solid-phase-enhanced sample preparation

SD- slit diaphragm

VIP score- variable importance in projection

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## AUTHORS' CONTRIBUTION

AAB – formal analysis and investigation, original draft preparation, review and editing, funding acquisition, resources

AN – methodology, resources

ARP - funding acquisition, resources, validation, review and editing

CAI – conceptualization, methodology, formal analysis and investigation, original draft preparation, review and editing, resources, supervision, validation

IBN – conceptualization, resources, supervision, review and editing, methodology

IMK – conceptualization, original draft preparation, review and editing, resources, supervision, visualization, project administration, validation

IEP – methodology, software, formal analysis and investigation, original draft preparation, review and editing, resources, validation

RP – methodology, resources, review and editing

YM - formal analysis and investigation, funding acquisition, resources

Approval of the final manuscript: all authors.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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