Identification of conserved gene expression changes across common glomerular diseases by spatial transcriptomics

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Article

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Abstract

Background: Glomerular diseases encompass a group of kidney diseases that may share common gene expression pathways. We aimed to analyze glomerular-specific gene expression profiles across various glomerular diseases.

Methods: We performed spatial transcriptomic profiling using formalin-fixed paraffin-embedded kidney biopsy specimens of controls and patients with five types of glomerular diseases using the GeoMx Digital Spatial Profiler. We identified common differentially expressed genes (DEGs) across glomerular diseases and performed Gene Ontology (GO) annotation by using the ToppGene suite.

Results: A total of 35 DEGs were consistently downregulated in glomeruli across the disease compared to the control, while none of the DEGs were consistently upregulated. Twelve of 35 downregulated DEGs, including the two hub genes FOS and JUN, were annotated with molecular function GO terms related to DNA-binding transcription factor activity. Other notable DEGs consistently downregulated and annotated in the pathway analysis included NR4A3, KLF9, EGR1, and ATF3. The annotated biological process GO terms included response to lipid-related (17/35 DEGs), response to steroid hormone (12/35 DEGs), or cell cycle regulation (10/35 DEGs).

Conclusions: Identifying common DEGs by spatial transcriptomic analysis provides insights into the underlying molecular mechanisms of glomerular diseases and may lead to novel assessment or therapeutic strategies.

Lay Summary

Glomerular diseases may share common gene expression pathways. In this spatial transcriptomic study, the authors investigated the commonly downregulated differentially expressed genes across five glomerular diseases, including IgA nephropathy, minimal change disease, membranous nephropathy, focal segmental glomerulosclerosis, and diabetic nephropathy. The authors identified that genes related to cell cycle regulation, transcription control, and lipid-related signals were commonly downregulated in these glomerular diseases. These findings provide insight into common pathogenetic mechanisms in various glomerular diseases.

Introduction

Glomerular diseases encompass a group of kidney diseases characterized by inflammation of the kidney glomeruli that contribute to the major socioeconomic burden related to chronic kidney disease. Despite differences in the underlying pathogenesis of various glomerular diseases, the resulting glomerular injury often manifests as urinary abnormalities, including proteinuria and hematuria, which
are common features of glomerular diseases. The complex network between podocyte slit diaphragm molecules, the actin skeleton, and cell adhesion molecules is essential in stabilizing the glomerular filtration barrier; however, the disruption of signaling in the filtration barrier induces these clinical features.\textsuperscript{4,5} Therefore, identifying shared molecular pathways in diseased glomeruli may offer insights into prognostic or therapeutic targets in glomerular diseases.

Spatial transcriptomics is a method for quantifying messenger RNA (mRNA) expression in multitudes of genes within localized areas in tissues that allows us to understand gene expression signatures and functional processes in a histological context.\textsuperscript{6} As the kidney is a highly differentiated organ with complex tissue substructures,\textsuperscript{7} spatial transcriptomic analysis of kidney tissue can be a valuable tool for investigating kidney microstructure-specific gene expression profiles. Particularly, as most pathogenetic processes of glomerular diseases originate from kidney glomeruli, the method can be used to identify glomerulus-specific gene expression. This advantage is important, as another advanced transcriptomic profiling technique, single-cell sequencing, can capture only a relatively low number of glomerular cells in human samples. Thus, spatial transcriptomic analysis has been introduced to investigate kidney glomerulus-specific gene expression in acute rejection after kidney transplantation\textsuperscript{8} and collapsing glomerulopathy.\textsuperscript{9}

In this study, we performed spatial transcriptomics analysis using GeoMx DSP of the glomeruli of 49 patients with diverse glomerular diseases and 11 healthy controls. We aimed to investigate the differences in gene expression levels between glomeruli in patients with glomerular diseases versus controls while also determining common pathways that contribute to the development of glomerular diseases. We hypothesized that there would be universally altered differentially expressed genes (DEGs) across patients with the five common glomerular diseases compared to healthy controls.

**Materials And Methods**

**Ethical considerations**

This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 2208-137-1353). Remnant formalin-fixed paraffin-embedded tissue (FFPE) samples from patients with five glomerular diseases were collected in the study hospital. Informed consent was obtained from most of the study participants before collecting any biospecimens or clinical information. For a few patients who were biopsied in the remote past periods, the use of the remnant tissue was approved and requirement for the informed consent was waived by the Institutional Review Board. The study was performed in accordance with the Declaration of Helsinki guidelines.

**Collection of kidney tissue specimens**

Kidney biopsies were performed for the participants with suspected glomerular diseases at the study hospital. Kidney biopsy specimens were acquired from the patients initially diagnosed with each of five
types of glomerular diseases (IgA nephropathy [IgAN], minimal change disease [MCD], membranous nephropathy [MN], focal segmental glomerulosclerosis [FSGS], and diabetic nephropathy [DN]) and embedded in FFPE blocks. The term “common glomerular diseases” refers to these five glomerular diseases, which were selected based on their prevalence among adults. For the control group, core biopsies were obtained from living-donor kidneys immediately after kidney implantation (e.g., zero-time biopsy). The inclusion criteria for spatial transcriptomic profiling were as follows: (1) ≥ 18 years of age, (2) a qualifying biopsy with > 10 glomeruli per section, (3) eGFR of ≥ 30 mL/min/1.73 m² at biopsy date, and (4) no pathological feature of diabetic kidney disease (except for DN). The control group comprised those with preserved kidney function (eGFR ≥ 80 mL/min/1.73 m²) with a similar distribution of sex and age to patients with glomerular diseases. The pathological investigation was also performed for the control biopsy specimens to ensure that the samples were free from any glomerular diseases.

Slide preparation for spatial profiling

Patients with IgAN, MCD, MN, FSGS, or DN and kidney donors with preserved kidney function (“normal” controls) were selected via database searches. For spatial transcriptomic profiling, slide preparation was conducted according to GeoMx spatial gene expression protocols (NanoString GeoMx DSP Manual Slide Preparation; MAN-10150-01). Briefly, 5-μm sections of FFPE kidney biopsy tissue were affixed to charged slides (Leica BOND Plus slides, cat# S21.2113.A) and baked at 60 °C for 30 minutes. After deparaffinization with CitriSolv and rehydration with ethanol and 1X phosphate-buffered saline (PBS) with DEPC (Diethyl pyrocarbonate), antigens were retrieved by incubating slides in 1X Tris-EDTA, pH 9, for 15 minutes and 1 μg/mL proteinase K in PBS for 15 minutes at 37 °C was followed to expose the targets for the probes. After washing in 1X PBS, human Whole Transcriptome Atlas (WTA, 18,694 target genes) probes were hybridized to the tissue section. Slides were incubated in the wet chamber overnight at 37 °C. The next day, these hybridized slides were stained with morphological markers for 1 hour at room temperature to demarcate the kidney tissue substructure and select glomerular regions of interests (ROIs). DNA-marker SYTO 13 (Nanostring, 121300303), a macrophage marker CD68 (Novus, NBP2-34587 AF532), epithelial cell marker Pan-CK (Novus, NBP2-33200 AF594), and glomerular marker alpha-smooth muscle actin (Abcam, ab202296) were strategically selected as morphological markers (Fig. 1).

GeoMx DSP profiling and data processing

Prepared slides were immediately loaded into the GeoMx DSP for the ROI selection. Configuration of glomerular ROIs were determined by a kidney pathologist. Oligonucleotide barcodes linked with target complimentary sequence were UV-cleaved and released from probes within the chosen ROIs and collected into the DSP collection plate. Then, libraries were prepared per the manufacturer’s protocol. Briefly, collected oligonucleotide barcodes were amplified by polymerase chain reaction (PCR) using primer pairs and i5/i7 dual-indexing sequences that preserved ROI identity. The PCR products were pooled into a single microtube and purified using AMPure XP beads (Cat # A63880) to generate sequencing libraries. Using a 4150 TapeStation System (Agilent), the concentration and purity were measured. Finally, libraries of 27 × 27 paired-end reads were sequenced on the Illumina Counting Platform, NovaSeq 6000.
FASTQ sequencing files were processed into digital count conversion files using GeoMx NGS Pipeline software (v2.0). We performed data analysis with the DSP Data Analysis Suite (v2.4) and data quality control. The limit of quantitation (LOQ) was set at the geometric mean multiplied by the standard deviations of the negative probes, and genes that were below the confidence threshold were excluded from the analysis. The genes that were expressed in less than half of the samples in the ROIs corresponding to each glomerular disease were also disregarded.

**DEG analysis and functional enrichment**

The identification of significant DEGs between the ROIs of samples of five glomerular diseases and control samples was performed by fitting a negative binomial generalized linear model using the R package “DESeq2”. To control for multiple comparisons, P values were adjusted for multiple testing using the Benjamini-Hochberg (B&H) procedure. DEGs commonly reaching the significance level of adjusted P values < 0.05 across the glomerular disease groups were identified, allowing a < 0.25 threshold in one of two groups with consistent directional changes. Functional gene enrichment analysis was performed using the ToppGene suite (https://toppgene.cchmc.org/). The DEGs were prioritized based on the FDR B&H q-value, and gene annotation according to molecular function, biological process, and cellular component pathways was implemented. Gene association network analysis and visualization were performed with the STRING platform (ver. 11.5, https://string-db.org/).

**Results**

**Characteristics of the study participants**

The study cohort included 60 participants who were diagnosed with IgAN (n = 8), MCD (n = 13), MN (n = 16), FSGS (n = 6), or DN (n = 6) and kidney donors (normal controls, n = 11). The clinical characteristics of the study population are summarized in Table 1. In patients with glomerular diseases, the mean age was 49.5 ± 12.2 years, and 30.6% (n = 15) were females, whereas the mean age of the kidney donors was 49.5 ± 9.8 years, and 45.5% (n = 5) were females. The median hemoglobin level was 12.6 (11.6–13.9) mg/dL in patients with glomerular diseases and 14.0 (14.1–15.4) mg/dL in the control group. In patients with MCD, the median serum albumin level was the lowest (2 [2-2.4] mg/dL), and the median total cholesterol level was the highest (360 [338–440] mg/dL) among patients with glomerular diseases. The median eGFR (interquartile range) of patients with glomerular diseases was 97 (60–109) mL/min/1.73 m², and the median baseline urine protein/creatinine ratio was 4.9 (2.2–8.6) g/g. The median eGFR of the normal control group was 88.8 (80.6–95.6) mL/min/1.73 m². For the eGFR category, 31 (51.7%), 16 (26.7%), 5 (8.3%), and 8 (13.3%) participants had an eGFR ≥ 90, 60–90, 45–60, and 30–45 ml/min/1.73 m², respectively. In 33 (67.3%) patients diagnosed with glomerular diseases, a urine protein/creatinine ratio ≥ 3.0 g/g was observed at the time of kidney biopsy. Patients had no pathological diagnosis of hypertensive or diabetic kidney disease in the biopsy samples, except for those with DN.
<table>
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<th>MCD</th>
<th>MN</th>
<th>FSGS</th>
<th>DN</th>
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<td>13</td>
<td>16</td>
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<td>36.6 ± 12.4</td>
<td>48 ± 12.4</td>
<td>52.1 ± 10.3</td>
<td>57.2 ± 5.5</td>
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<td>4 (30.8)</td>
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<td>albumin (mg/dL)</td>
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<td>8 (100)</td>
<td>1 (7.7)</td>
<td>3 (18.8)</td>
<td>4 (66.6)</td>
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<td>urine protein/creatinine</td>
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<td>9.3 (6.9–11.3)</td>
<td>7.4 (4.5–9.0)</td>
<td>4.5 (1.7–6.0)</td>
<td>2.7 (1.6–3.1)</td>
</tr>
</tbody>
</table>

IgAN = IgA nephropathy, MCD = minimal change disease, MN = membranous nephropathy, FSGS = focal segmental glomerulosclerosis, DN = diabetic nephropathy. *The urine protein/creatinine ratio was not calculated in 8 individuals in the control group.
### Spatial transcriptomics profiling results

We spatially profiled the expression of 18,694 genes using GeoMx WTA in samples from patients with five glomerular diseases and controls at the glomerular level. A total of 178 glomerular ROIs (3 ROIs per patient, except for those with MN with 1 ROI because of the low number of glomeruli available for profiling) were configured based on histological analysis following hematoxylin and eosin staining and immunostaining patterns for the morphological markers. Across a median surface area of 35326.7 (26193.3-52001.9) µm²/ROI, a median of 158 (124–227) cells/ROI were identified. Among a median of 1,253,995 raw reads/ROI, a median of 1,180,654 reads per ROI were aligned for RNA-seq and processed for quality control. RNA-seq saturation was 88.5 (82.5–95.7) %/ROI, and after excluding the genes that were expressed in less than half of the ROIs in each glomerular disease group, the remaining expression levels of 17,834 genes were studied in the downstream analysis.

We initially compared the gene expression profiles of each glomerular disease to the control and plotted the DEGs according to the respective glomerular diseases in volcano plots (Fig. 2).

### Universally downregulated DEGs across common glomerular diseases

To investigate the differences in the molecular pathways between glomerulus samples from patients with common glomerular diseases and those of normal controls at the transcriptional level, we identified universally downregulated DEGs in five common glomerular diseases with spatial transcriptomic profiling. A total of 35 DEGs were universally downregulated across samples from patients with the five glomerular diseases compared to those from controls (Supplemental Table 1), whereas none of the DEGs were universally upregulated. In particular, genes including Fos proto-oncogene (FOS), Jun proto-oncogene (JUN), early growth response 1 (EGR1), nuclear receptor 4A1 (NR4A1), KLF transcription factor 4 (KLF4), dual specificity protein phosphatase 1 (DUSP1), activating transcription factor 3 (ATF3), suppressor of cytokine signaling 3 (SOCS3), cyclin-dependent kinase inhibitor A1 (CDKN1A), and Serpin Family E Member 1 (SERPINE1) were significantly downregulated in samples from patients with glomerular diseases compared with those from controls, suggesting that immune-mediated and cell proliferation-related responses are closely linked with glomerular microenvironmental changes in glomerular diseases. For functional enrichment of universally downregulated DEGs in glomerular diseases, we annotated the spatial transcriptomic profiles with gene ontology (GO) terms, namely, molecular function, biological process, and cellular component terms, using the ToppGene suite. Twelve
of 35 downregulated DEGs, including the two hub genes FOS and JUN, which are components of transcription factor activator protein-1 (AP-1), were annotated with a molecular function GO term related to DNA-binding transcription factor activity (GO:0003700) or sequence-specific DNA binding (GO:0043565). Other notable DEGs consistently downregulated and annotated in the pathway analysis included NR4A1, NR4A3, KLF4, KLF9, EGR1, ATF3, and CDKN1A. The annotated biological process GO terms included response to lipid (17/35 DEGs), response to steroid hormone (12/35 DEGs), positive regulation of DNA-templated transcription (12/35 DEGs) or cell cycle regulation (10/35 DEGs). For cellular component GO terms, twelve of 35 DEGs were annotated with chromatin GO terms. Additionally, the gene–gene association network of universally downregulated DEGs in glomerular diseases was generated utilizing the STRING database (Fig. 3(a)), showing the close interaction between the identified DEGs consisting of a network with FOS/JUN-related genes.

**DEGs consistently identified in various glomerular diseases regardless of the directional changes**

A total of 126 genes were found to be differentially expressed (either up- or downregulated; FDR < 0.25; regardless of the direction of the fold changes; Supplemental Table 2) across the samples of the five glomerular diseases compared to the control samples. While most genes that were not commonly downregulated across the five GNs still demonstrated consistent direction in four types of glomerular diseases, namely, MCD, MN, FSGS, and DN, the direction of the difference in the gene expression was found to be opposite in IgAN to those of the others. The gene association network of DEGs consistently identified in glomerular diseases regardless of the direction, excluding commonly downregulated DEGs, was established via the STRING platform (Fig. 3(b)).

Among the DEGs, deubiquitination-related genes, including ubiquitin-specific peptidase 17 like (USP17L) family members 3, 11, 12, 15, and 17, were consistently downregulated in four glomerular diseases but upregulated in IgAN. USP17L family genes were annotated with a molecular function GO term related to deubiquitinase activity (GO:0101005) and a biological process GO term related to the regulation of protein processing, immune effector process, or protein localization to membrane. Along with USP17L family genes, THBS1 and CDH1 were also annotated to the above GO terms.

**Discussion**

In this study, we identified DEGs within the glomeruli of patients with common glomerular diseases compared to those of healthy controls by spatial transcriptomic analysis to characterize the consistently dysregulated molecular pathways in glomerular diseases. Using GeoMx DSP, we identified a set of 35 DEGs that were universally downregulated across five glomerular diseases. These conserved DEGs, including FOS, JUN, EGR1, NR4A1, NR4A3, KLF4, ATF3, SOCS3, DUSP1, and ZFP36, were assessed via functional enrichment analysis and GO term annotation to interpret the complex interactions and functions between genes and gene products. Seventeen of 35 DEGs, including the two hub genes JUN
and FOS, which were annotated to the AP-1 transcription factor complex,\textsuperscript{10} were annotated to lipid metabolism. Additionally, we observed significant upregulation of ubiquitin-specific peptidase genes in MN, MCD, FSGS, and DN samples compared to control samples, while their expression was downregulated in IgAN samples.

Spatial transcriptomic profiling using a microdissection-based method, including GeoMX DSP, has particular strength in the study of glomerular diseases, as the main focus of interest is usually on pathological changes in the glomerulus where most of the pathophysiological processes occur.\textsuperscript{6,9,11} Although there are other types of RNA-seq-based spatial transcriptomic methods (e.g., Visium), their current resolution is not sufficient to capture the glomerulus-specific transcriptome.\textsuperscript{12} On the other hand, single-cell RNA-seq is another useful transcriptomic analysis method for investigating cell-specific mRNA expression,\textsuperscript{13} yet most single-cell human data include a relatively low portion of glomerulus cells with low feature variance.\textsuperscript{14,15} Previously, we performed manual microdissection to investigate the glomerulus-specific transcriptome;\textsuperscript{16} however, laborious hand microdissection and sample availability limited the expansion of the data. In the current study, we used GeoMx DSP, which has been trialed in transplant rejection\textsuperscript{8} and collapsing glomerulopathy,\textsuperscript{9} to study the common transcriptomic alteration of the glomerulus in patients with various glomerular diseases. By implementing this advanced technique, we identified universal mRNA expression changes in diverse types of glomerular diseases, which may expand our knowledge of the common pathophysiological features of glomerular diseases. Another strength of the current study is the collection of kidney donor biopsy tissue during transplantation surgery, which closely approximates the physiological state of normal kidney tissue and facilitates the identification of DEGs in our study.

Nephrin, a podocyte-adhesive signaling molecule located in the slit diaphragm, is crucial for maintaining the structure and function of the glomerular filtration barrier. The slit diaphragm is a highly specialized lipid-raft structured cell junction that bridges adjacent podocyte foot processes and is commonly disrupted in nephrotic diseases.\textsuperscript{17,18} The AP-1 transcription factor is a dimeric molecule that plays a crucial role in cell proliferation, differentiation, and apoptosis by modulating the cell cycle.\textsuperscript{10,19} Nephrin is essential for activating the AP-1 signal transduction pathways\textsuperscript{20,21} along with Src family members\textsuperscript{22–24}. In nephrin-expressing NHP15 cells, higher c-Jun activity has been observed than in Finn-minor cells that lack human nephrin expression, indicating that nephrin is involved in activating the AP-1 signaling pathway through c-Jun activation.\textsuperscript{20} It has also been suggested that nephrin-triggered cellular signaling cascades may preserve the structural integrity of the podocyte slit diaphragm.\textsuperscript{21} In this study, we identified consistent downregulation of DEGs, including JUN, FOS, JUNB, and ATF3, which are components of the AP-1 transcription factor, in common glomerular diseases. Therefore, we suggest that the nephrin-induced AP-1 signaling pathway is universally downregulated in glomerular diseases accompanied by disruption of the slit diaphragm and dislocation of nephrin.

Furthermore, c-Fos is known to activate lipid synthesis, including phospholipids\textsuperscript{25} and glycolipids\textsuperscript{26}, and multiple DEGs, including JUN, FOS, KLF9, ATF3, and CDKN1A, were annotated to the ‘response to lipid’
biological GO term in this study. The slit diaphragm has been proposed as a lipid raft functioning as a signaling hub with the interaction of proteins including podocin and nephrin.\textsuperscript{17,27,28} Although the structures and metabolic mechanisms of the slit diaphragm are largely unknown, we infer that the downregulation of the above genes may interfere with lipid-protein interactions that are important in maintaining the function of the slit diaphragm.

Consistent with our findings, downregulation of FOS or JUN was detected in patients with IgAN, lupus nephritis, MCD, FSGS, MN, and DN in a study that compared transcriptomic profiles between patients with glomerular diseases and healthy controls.\textsuperscript{29} Additionally, there was a report that c-FOS expression was scant or c-FOS was not expressed in glomeruli but that c-FOS expression was detected in tubules, small vessels, or interstitial cells in crescentic IgAN patient samples.\textsuperscript{30} Despite several reports indicating AP-1 activation with an inflammatory response in kidney injury models or polycystic kidney diseases, the upregulation of AP-1 was mainly exhibited in tubules and interstitium, not in glomeruli.\textsuperscript{31–33} In most chronic glomerular diseases, the downregulation of JUN/FOS and AP-1 was predominant within the glomeruli consisting of a disrupted slit diaphragm and altered protein signaling.

We also identified that the glomerular mRNA expression of USP17L family genes in IgAN samples showed reverse directional changes when compared to the other types of glomerular diseases. Previous studies have reported the upregulation of the ubiquitin–proteasome pathway in IgAN, which demonstrated the overexpression of the proteasome and its switch to the immunoproteasome in peripheral blood mononuclear cells of IgAN patients.\textsuperscript{34} As the ubiquitin–proteasome system regulates the antigen processing and presentation of major histocompatibility complex (MHC) class I molecules,\textsuperscript{35} the enhanced immune response to certain MHC-1 antigens in IgAN has also been suggested.\textsuperscript{34} In addition, the association between genetic polymorphisms within the MHC regions and susceptibility to IgAN was identified in a previous genome-wide association study of IgAN.\textsuperscript{36} Our findings are in accordance with previous studies linking ubiquitin–proteasome system activation and the T-cell response in the pathogenesis of IgAN. Further investigation is needed to determine the significance of the difference in the direction of the ubiquitin–proteasome pathway in IgAN compared to other glomerular diseases.

There are some limitations in our study. First, the downregulated DEGs were not assessed by further experimental investigations. An additional mechanistic experiment is warranted to confirm the clinical implication of our findings, along with transcriptomic analysis focusing on disease-specific glomerular changes. Second, although challenging, identifying the cell specificity of the identified DEGs would enrich the meaning of our findings. With the advancement in platforms for spatial transcriptomic strategies, a cellular- or subcellular-level investigation should be widely available in the future, and our study data may provide background information for future studies. Third, the issue of generalizability should be considered, as the study participants were Asian.

In conclusion, we revealed universally downregulated DEGs annotated to AP-1 or lipid metabolism in the glomeruli of various glomerular diseases through spatial transcriptomic profiling. Our study elucidates
the potential for common molecular mechanisms underlying the pathogenesis of various glomerular diseases.

Declarations

Acknowledgments

None.

Authors’ contributions

JMC, MK, SP, HYS, YCK, SSH, KWJ, YSK, HL, HJK, and DKK contributed to conceptualization, data curation, and formal analysis. JMC, MK, SP, HYS, JHK, SMC, YK, SL, YCK, SSH, KWJ, YSK, HL, HJK, and DKK contributed to the investigation and methodology. JMC, MK, SP, HYS, YCK, SSH, KWJ, YSK, HL, HJK, and DKK contributed to the resources and software. JMC and SP contributed to visualization. SP, YCK, SSH, KWJ, YSK, HL, HJK, and DKK contributed to funding acquisition and project administration. SP, KWJ, HJK, and DKK contributed to supervision, writing-review & editing. All authors contributed to writing the original draft.

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Competing interests

None of the authors have disclosures to declare.

Data availability statement

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

References


Figures

Figure 1
Glomerular ROI scan for digital spatial profiling (membranous nephropathy). ROIs for spatial transcriptomic analysis were configured within the white circles based on histological staining with hematoxylin and eosin and immunostaining with the morphological markers PanCK (yellow, for epithelial cells), CD65 (green, for macrophages), alpha smooth muscle actin (red, for glomeruli), and SYTO-13 (blue, for nuclei staining). ROI = regions of interest

Figure 2

Volcano plots of DEGs between respective glomerular disease samples vs. control samples. IgA nephropathy, minimal change disease, membranous nephropathy, focal segmental glomerular sclerosis, and diabetic nephropathy (left to right). Each plot shows the changes in glomerular gene expression in the respective glomerular disease samples compared to control samples. The red dots indicate the upregulated DEGs with \( \log_2(FC) > 1.5 \), whereas the blue dots indicate the downregulated DEGs with \( \log_2(FC) < 1.5 \). DEG = differentially expressed gene
Figure 3

**Gene association network analysis of universally downregulated DEGs** Visualization of gene association network analysis of (a) universally downregulated DEGs and (b) DEGs in common glomerular diseases regardless of the direction of gene expression fold changes in samples from five glomerular diseases compared to control samples using the STRING database. DEG = differentially expressed gene

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- CommonGNSupplementalMaterial.pdf