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14th Biennial International Podocyte Conference

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Abstracts

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Conflict of Interest Statement

The laboratory of Katalin Susztak receives research support from Gilead, GSK, Novo Nordisk, Bayer, Regeneron, Calico, and Novartis. Katalin Susztak is on the advisory board of Jnana Therapeutics, has received consulting fees from Pfizer and Jnana, and holds a provisional patent for Jag1- and Notch-based targeting of chronic kidney disease (patent WO2009035522A1).

Larry Holzman does not have any conflicts of interest to declare.

The abstracts included in this supplement were reviewed and selected by members of the 14th Biennial International Podocyte Conference Organizing Committee as indicated above. The committee has no conflicts of interest in connection with the Conference or the selection of abstracts.

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Dear Attendee:

On behalf of the Scientific Organizing Committee, welcome to the *14th Biennial International Podocyte Conference*!

The Podocyte Conference series began informally in 1999 when a small group of six "podocytologists" met in Heidelberg, Germany. This was a time when we recognized the clinical burden of glomerular disease yet had simple understanding and had what today we would consider rudimentary experimental tools to advance our knowledge. The meetings that followed became an annual and then a biannual series that helped foster a strong, collaborative international scientific community focused on understanding the mechanisms of glomerular disease with the hope of identifying targets for clinical interventions. Led by workers working from multiple perspectives and employing a variety of experimental approaches, our science has evolved dramatically, from that narrowly focused on the biology of the podocyte to understanding the podocyte in the context of its larger niche. Reflecting today's broader scientific views, the 14th International Podocyte Conference has also widened its scope, recognizing advances in mechanisms of glomerular diseases, developments using modern discovery methods, and reflecting recent excitement and investment in drug development for glomerular diseases.

Founded in 1740 by Benjamin Franklin, the University of Pennsylvania has a rich history and is recognized as one of the top research institutions in the world. The university's innovative spirit and commitment to advancing knowledge make it the perfect setting for our conference, where we strive to deepen our understanding of glomerular diseases and identify potential therapeutic targets.

This *Podocyte Conference* includes more than 300 attendees from twenty-five countries discussing their research through oral presentation or presentation of over 120 abstracts. We are especially pleased to welcome more than eighty early career investigators, who will participate in a full day *Early Career Investigator Premeeting* on Tuesday, May 23, and then engage in the proceedings of the primary meeting. We are thrilled that you are bringing your energy, intelligence, and innovative ideas to the glomerular disease field, and we are certain that your interactions during this conference–both formal and informal—will enhance your understanding and create new opportunity and will move our field forward.

The *International Podocyte Conference* remains a unique venue for addressing the broad scientific complexities of the many diseases that comprise kidney glomerulopathies. It has thrived for more than two decades because of your enthusiastic participation and by the support of our generous sponsors. Again, welcome to Philadelphia and to the *14th Biennial International Podocyte Conference*! We wish you a productive meeting.

Sincerely,

Katalin Susztak and Larry Holzman for the Organizing Committee

Podocyte cell biology (e.g., cytoskeleton, intercellular junction/slit diaphragm, intracellular signaling)

1 ADIPOKINE UPD2 AND JAK/STAT PATHWAY MEDIATE HIGH-FAT DIET-INDUCED NEPHROCYTE DYSFUNCTION

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Background/Aims: The consumption of superfluous macronutrients disrupts systemic metabolism, which can lead to pathogenesis.
Methods: In this study, we investigated the effect of high-fat diet (HFD) on kidney function using Drosophila melanogaster. We showed that HFD decreased the absorption function of nephrocytes, the fly's functional equivalent of human podocytes. Transmission electron microscopes (TEM) analysis of the subcellular structure reveals morphological changes of the lacuna channels and the vacuoles caused by HFD.
Results: We found that HFD increases the expression of adipokine Upd2, a ligand for the Jak/Stat pathway, and increased its secretion from the fat body. Overexpression of Upd2 in the fat body compromised nephrocyte function. We further showed that HFD increases Jak/Stat pathway activity in the nephrocytes. Consistently, genetic activation of the Jak/Stat pathway in nephrocytes reduces nephrocyte function, whereas nephrocyte function impaired by HFD can be restored by inhibiting the Jak/Stat pathway.
Conclusion: Our study revealed an adipose tissue and nephrocyte axis for HFD-induced kidney pathogenesis, and identified Upd2 and Jak/Stat pathway as potential therapeutic targets for diabetic kidney dysfunction.

2 AN IN VITRO APPROACH TO UNDERSTAND CONTRIBUTION OF KIDNEY CELLS TO HUMAN URINARY EXTRACELLULAR VESICLES

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Background/Aims: Extracellular vesicles (EV), membranous particles secreted by all cell types, carry distinct sets of RNA, proteins, lipids and metabolites that accurately reflect the physiologic status of their parental cells. As EV are present in biofluids such as urine, they represent a promising source of biomarkers for diagnostics, prognostics and identification of pharmacological interventions. However, the knowledge of the contribution of specific cell types to biofluid EV mixtures is limited, as well as methodologies to study it. Thus, the aim of this study was to understand the contribution of each glomerular cell type to the final urinary EV (uEV) repertoire and their changes in diabetic kidney disease (DKD).

Methods: EV from cell culture media derived from immortalized glomerular cells (podocyte, glomerular endothelial, mesangial) were isolated by hydrostatic filtration dialysis. EV RNA was isolated and subjected to miRNA and RNA sequencing and proteins profiled by tandem mass tag proteomics. To study cell-type-enriched EV we defined lists of features and compared them to available kidney bulk and single cell datasets as well as reported urinary in vivo EV datasets. To study differences in the secretion of EV in cells grown in diabetogenic media we compared cells grown in basal media or diabetogenic, insulin-resistance inducing media (supplemented with pro-inflammatory cytokines, high glucose, and high insulin).

Results: Interestingly, we identified characteristic sets of cell-type-enriched EV features, many of which were found in human kidney and, most importantly, in human urinary uEV datasets. Comparing highly insulin sensitive and insulin resistant cell models, we found changes in EV cargo derived distinctly from podocytes. The dysregulated “diabetic” miRNAs and mRNAs could be linked into relevant networks of regulatory pathways.

Conclusion: These results demonstrate the value of studying extracellular vesicles from different glomerular cell types in vitro and in disease models. This information could be utilized as an easy method to identify novel molecular and cellular pathways, biomarkers and targets involved in glomerular disease.

3 DEEP LEARNING IDENTIFIES SUBPHENOTYPES OF DIABETIC KIDNEY DISEASE DRIVEN BY GENETIC VARIATIONS IN THE RHO PATHWAY

Nanditha Anandakrishnan, Ishan Paranjpe, Xuan Wang, Jonathan Haydak, Tielman Van Vleck, Stefanie Defronzo, Zhengzhe Li, Anthony Mendoza, Ruijie Liu, Jia Fu, Iain Forrest, Weibin Zhou, Kim Lee, Ross O'Hagan, Sergio Dellepiane, Kartikeya Menon, Faris Gulamali, Samir Kamat, Luca Gusella, Alexander Charney, Ira Hofer, Judy Cho, Ron Do, Benjamin Glicksberg, John He, Girish Nadkarni, Evren Azeloglu

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Background/Aims: Diabetic kidney disease (DKD) is a leading cause of end-stage kidney disease (ESKD) however disease heterogeneity has limited therapies for targeting disease causing pathways. Here, we use deep learning to identify a novel genetic variant of ARHGEF18 associated with significantly higher risk of DKD and ESKD. We further employ quantitative microscopy techniques and biochemical assays to elucidate the mechanistic role of ARHGEF18 and its variant in podocytes.
Methods: DKD patients from the Mount Sinai BioMe Biobank were used in this study. We performed unsupervised clustering and accounted for population structure in a deep learning framework. We then performed a genome wide association study (GWAS) of the patients compared with healthy controls. For mechanistic studies of the novel variant, cytoplasmic, focal adhesion as well as live-cell motility, Rho GTPase activity, and protein degradation experiments were performed using quantitative total internal reflection fluorescence (TIRF) microscopy and biochemical assays using immortalized human podocytes expressing ARHGEF18 wild-type (WT) and mutant transcripts.
Results: We employed autoencoders and unsupervised clustering of 1,372 DKD patients to establish two cohorts with differential prevalence of proteinuria and ESKD. GWAS analysis on these patients identified a novel variant of ARHGEF18, a Rho guanine exchange factor highly enriched in podocytes. Nephroseq database showed increased ARHGEF18 expression in chronic kidney disease kidney biopsy samples compared to healthy controls. Overexpression of ARHGEF18 transcripts in podocytes led to impaired cell adhesion, focal adhesion architecture, and cell motility. Live TIRF imaging showed preferential subcellular localization of GEF18 mutant to the periphery of migrating podocytes whereas GEF18 WT localized at the perinuclear/cytoplasmic region. GEF18 mutant cells displayed increased RhoA activation. In vitro protein degradation assays using WT and mutant exogenous GEF18 showed that the mutant protein has increased stability and half-life. Our assays also showed that mutant protein has higher resistance to ubiquitin mediated degradation leading to pathologically increased protein levels.
Conclusion: In summary, we report a novel variant of ARHGEF18 that drives podocyte dysfunction through impaired protein localization and degradation. Targeting this pathway could help regulate RhoA activity and cytoskeletal rearrangement, preventing foot process effacement in DKD.

4 DELAYED TREATMENT OF COQ10 PRECURSORS FAILS TO RESCUE COQ10-DEFICIENT GLOMERULOPATHY

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Background/Aims: Four genes (COQ2, COQ6, ADCK4 and PDSS2) involved in Coenzyme Q10 (CoQ10) biosynthesis cause monogenic steroid resistant nephrotic syndrome. CoQ10 is an antioxidant and electron carrier in the respiratory chain. CoQ10 precursors have been shown to prevent renal failure. It remained if these CoQ10 precursors are still effective when renal function begins to decline.
Methods: To evaluate the therapeutic effect of CoQ10 precursors, podocyte specific Adck4 knockout (Adck4ΔPod) mouse was used as a CoQ10-deficient model. We delayed CoQ10 precursors (2,4-diHB or probucol) treatment of Adck4ΔPod mice from 3-months (early treatment) to 5-months (delayed treatment). We then analyzed renal histology by light microscopy and immunofluorescence and performed proteinuria assessment
Results: Histologic analysis of kidneys from mice treated at 3-months showed normal histology. Adck4ΔPod mice treated at 3-months showed glomerular sclerosis and a progressive increase in plasma creatinine, plasma BUN and urinary albumin levels. Compared with early treated mice, delayed-treated mice developed significantly decreased expression of the podocyte markers, nephrin, and increased expression of αSMA, a marker of glomerular fibrosis.
Conclusion: We showed that whereas CoQ10 precursors treatment of Adck4ΔPod mice at 3-months is therapeutic, the effect was lost if treatment delayed to 5-months when chronic renal failure was already present. These results imply that to properly medicate after diagnosis, it is important to validate correct timing point of drug administration.

5 DEVELOPING NOVEL ALLOSTERIC AGONISTS OF INTEGRIN ALPHA 3 BETA 1

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Background/Aims: Podocyte damage and loss is a key determinant of proteinuria and glomerular injury. Therefore, maintaining podocyte viability and preventing their urinary loss is a validated therapeutic strategy against glomerular diseases. Podocytes selectively utilize integrin a3b1 to adhere to the glomerular basement membrane (GBM) and such attachment is essential for maintaining their viability. Using a novel high-content imaging-based assay, we previously identified small molecule integrin b1 agonists as podocyte-protective. This suggests that reinforcement of podocyte adhesion, via increased integrin-mediated cell adhesion and strengthened cytoskeleton, is a protective strategy in various kidney diseases.
Methods: A novel assay using recombinant integrin a3b1 was used to identify novel integrin agonist antibodies via phage display. K562 cells stably expressing a3b1 and differentiated podocytes (mouse and human) were used in in vitro assays. K562 cells were used in flow-cytometry based assays to characterize novel integrin agonists. Podocytes were utilized in cell adhesion based functional assays. Furthermore, podocyte damage was induced using puromycin aminonucleoside (PAN) and treatment in the absence or presence of various agonists, for up to 48 hours, was used to determine efficacy of integrin agonists. Podocytes were fixed and stained for detection using the confocal imaging based High-Content Screening (HCS) assays. Imaging software was used to quantify morphology properties such as roundness, as well as the overall F-actin and focal adhesion signal. Currently, the agonists are being tested for efficacy in in vivo assays.
Results: PAN damage resulted in quantitative reduction in F-actin fiber numbers and intensity, and increased roundness in podocytes. It also reduced binding of active b1 integrin antibody 9EG7. Newly discovered integrin integrin agonists increased a3b1-dependent cell adhesion and ameliorated PAN-mediated podocyte damage. They also reduced migration of a3b1-expressing cells.
Conclusion: We have previously shown that integrin agonists are a novel therapeutic strategy for targeting integrins in various diseases. Activation of integrin a3b1 in podocytes shows that it increases cellular adhesion to matrix proteins and protects cells from damage. Ongoing in vivo studies will demonstrate efficacy of this approach and will highlight integrin activation as a novel therapeutic strategy against various glomerulopathies.

6 EFFICACY OF VOCLOSPORIN IN MAINTAINING PODOCYTE FUNCTION AND VIABILITY IN A RAT STREPTOZOTOCIN MODEL OF DIABETIC NEPHROPATHY

James Tumlin, Janet Klein, John Viel

Clinical Research, NephroNet Clinical Trials; Emory University; Aurinia Pharmaceuticals

Background/Aims: Diabetic nephropathy is a leading cause of end-stage renal disease. Diabetes stimulates the activity and expression of calcineurin; a serine-threonine phosphatase that is a critical regulator of podocyte function and stability. Because calcineurin has been linked to podocyte apoptosis, synaptopodin degradation and slit pore function we investigated the effects of Voclosporin, a third generation calcineurin inhibitor (CNI), on the progression of diabetic nephropathy.
Methods: Voclosporin (5.0 mg/kg) was administered to 75 streptozotocin (60 mg/kg) treated diabetic male Sprague-Dawley rats by 2X per day via gavage feeding. Animals were divided into three 25 subject cohorts including normal animals, diabetic controls and diabetics treated with oral Voclosporin (5.0 mg/kg). Animals were euthanized at 6, 9, and 12 weeks and renal cortex was harvested for histologic analysis of calcineurin, WT-1 (Wilms tumor-1) and CTGF (connective tissue growth factor) expression.
Results: Streptozotocin treated animals developed hyperglycemia (BG-500-600 mg/dl) within 30 mg/dl) within 30 days and proteinuria by Day 84. After 6 weeks, renal cortical expression of calcineurin in diabetic controls increased by 3.5X fold compared to normal controls while treatment with voclosporin reduced the increase by 50%. Similarly, voclosporin blocked the rise in glomerular and renal tubular expression of connective tissue growth factor (CTGF). Sirius Red staining renal cortex demonstrated that in contrast to diabetic controls, the addition of voclosporin blocked a rise in glomerular and interstitial fibrosis. Lastly diabetic animals treated with voclosporin maintained podocyte density as evidenced by WT-1 positive cells per unit glomerular area.
Conclusion: This study demonstrates that in rat models of uncontrolled diabetes calcineurin expression is upregulated in the renal cortex via a calcineurin dependent mechanism and can be blocked by voclosporin. Treatment of diabetic animals with oral voclosporin stabilized synaptopodin levels and reduced depletion of WT-1 positive cells in the glomerulus and blocked a diabetes induced rise in CTGF expression. The reduced level of interstitial fibrosis in the voclosporin treated animals suggest possible use of 3rd generation CNI in diabetic nephropathy

7 FINE-TUNED REGULATION OF PODOCYTE AUTOPHAGY AND NEPHRONECTIN BY MICRORNAS UPREGULATED IN MEMBRANOUS GLOMERULONEPHRITIS

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Background/Aims: Autophagy has been identified as a crucial maintenance program in podocytes and its dysregulation has been linked to glomerular diseases, such as membranous glomerulonephritis (MGN). As dysregulated micro-RNAs (miRs) are found in glomerular injury and can alter autophagy, our aim was to investigate the relationship between MGN associated miR-378a, its target nephronectin (NPNT) and autophagy.
Methods: To unravel the influence of miR-378a overexpression or NPNT knockdown on autophagy, immortalized human podocytes were transfected with miR-378a-3p mimic or NPNT siRNA. Analysis of autophagy flux was carried out after Bafilomycin A treatment. Expression levels of autophagy related genes (ATGs) were determined in cell lysates, as well as on renal tissue from patients with MGN.
Results: Although miR-378a has several potential binding sites on ATGs, we did not observe differences in their mRNA expression in podocytes after miR-378a transfection, while NPNT siRNA treatment decreased mRNA expression of ATGs 2, 6 and 7. However, increased miR-378a and knockdown of NPNT both increased autophagy flux. We detected reduced p-mTOR/mTOR ratios after miR-378a transfection as a mechanism of increased autophagy flux. LC3 and ATG staining were increased in podocytes of patients with MGN.
Conclusion: We suggest that the previously published increase in miR-378a in MGN might not only alter the extracellular protein NPNT, but also increase autophagy flux. Furthermore, decreased NPNT itself seems to impact autophagy in podocytes, indicating a fine-tuned regulatory system between miRs, extracellular matrix proteins and autophagy, which might open new avenues for therapeutic options.

8 GIT2 REGULATES RAC1 ACTIVITY AND IS CRITICAL FOR CYTOSKELETAL DYNAMICS IN PODOCYTES

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Background/Aims: Rho-family of small GTPases (Rho GTPases), including Rac1, play important roles in actin cytoskeletal remodeling required for cell adhesion and migration. We previously showed that Rac1 activation in podocytes causes foot process effacement and proteinuria. Rac1 activity is tightly regulated by guanine nucleotide exchange factors (GEF) and GTPase-activating proteins (GAP). Using proximity-dependent biotin identification, we identified that Rac1 in immortalized human podocytes (HP) interacts with GIT ArfGAP 2 (GIT2) protein, which is not GEF/GAP.
Methods: GIT2 localization in HP was examined by immunocytochemistry. HP with GIT2 knockdown (KD) and control cells (CTRL) were established using shRNAs. Rac1 activity was assessed by pull-down assay. Cell areas were quantified in fixed cells stained by phalloidin. Cell migration was studied by wound healing assay. Mice with heterozygous GIT2 deletion were established using CRISPR/Cas9, and systemic GIT2 knockout (KO) mice were generated by crossing them. Urine albumin to creatinine ratio (ACR) and foot process (FP) width were quantified up to 48hrs after intraperitoneal lipopolysaccharide (LPS) injection. Data are shown as the mean±SE.
Results: GIT2 was localized with paxillin in focal adhesions. GTP-bound (active) Rac1 levels were higher in GIT2 KD HP than in CTRL. GIT2 KD elicited cell spreading with marked lamellipodial protrusions, which was significantly attenuated by the Rac1 inhibitor NSC23766 (CTRL 5427±309 μm2, KD 8958±806 μm2, KD+NSC 4337±363 μm2). GIT2 KD HP had smaller focal adhesions than CTRL and accelerated wound-induced motility. ACR and FP width were significantly increased in GIT2 KO mice than in CTRLs at 48 hours following LPS treatment (ACR: CTRL 77.6±11.2 mg/gCr vs. KO 129.9±26.8 mg/gCr, FP width: CTRL 441.1±9.7 nm vs KO 500.5±14.1 nm).
Conclusion: Our findings demonstrate that GIT2 is localized in the focal adhesion in podocytes and plays a critical role in podocyte cytoskeletal dynamics and glomerular barrier function. Rac1 regulation by GIT2 has distinct mechanisms from GAP/GEF and may offer a new therapeutic target.

9 IDENTIFICATION OF ALTERNATIVE SPLICING EVENTS IN MECHANICALLY STRETCHED PODOCYTES AS A MODEL FOR GLOMERULAR HYPERTENSION

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Background/Aims: Alterations in pre-mRNA splicing play an important role in disease pathophysiology. However, the role of alternative splicing (AS) in hypertensive nephropathy (HN) has not been investigated. The purpose of the Sys_CARE (Systems Medicine Investigation of AS in Cardiac and Renal Diseases) project is to identify AS events that play a role in the development and progression of HN.
Methods: Cultured mouse podocytes were exposed to mechanical stretch for 3 days under low and high stretch conditions. Subsequently, mRNAs and proteins were analyzed by RNA-Seq and LC-MS/MS. Enrichment analysis (GSEA) identified proteins which were classified according to biological processes and molecular function. Splicing and transcript expressions were studied with bioinformatical tools (e.g. rMATS).
Results: Proteomic analysis identified a total of 135 and 424 significantly regulated proteins under low and high stretch conditions, respectively, compared to unstretched conditions. Gene Ontology analysis revealed that a higher number of actin-binding proteins were down- and mRNA processing proteins were up-regulated. RNA_Seq identified over 1000 different splicing events including all types of AS events. Here we show an isoform switch of Myl6 and Shroom3 by RNA-Seq after mechanical stretch.
Conclusion: Mechanically stretched podocytes can be used as a model of hypertensive nephropathy. In-depth RNA-Seq analysis identified AS changes that could be associated with the development of HN.

10 IDENTIFICATION OF RNASE1 AS A NOVEL MODULATOR OF PODOCYTE CYTOSKELETON ARCHITECTURE

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Background/Aims: Central to the health and function of the podocytes is a dynamic cytoskeleton that not only provides structural support, but also facilitates the interaction with the basement membrane and functions as an important signaling center. In this project, we used a unique human blood peptide library to identify native bioactive peptides with the capacity to modify the podocyte cytoskeleton.
Methods: PKCε knockout mouse podocytes were used as a model for cytoskeleton dysfunction to systematically test the fractions derived from the peptide library. The candidate peptides (CP) were identified by mass spectrometry. Morphological changes due to exposure to the CP were monitored by immunofluorescence, and the mechanistic targets of the CP were analyzed by western blot and transcriptomics.
Results: We identified RNASE1 as a candidate for cytoskeleton remodeling. Exposure to recombinant RNASE1 increased cell area and the number of focal adhesions, resulting in improved attachment, in models of podocyte injury. Analysis of the possible mechanisms involved in cytoskeleton rearrangement suggest that there may be differential tyrosine kinase signaling after exposure to RNASE1. Additionally, RNA sequencing identified 11 down- and 7 upregulated genes as candidates for further analysis.
Conclusion: Through our systematic screening approach, we could successfully identify a novel candidate protein that can alter the podocyte cytoskeleton. By rescuing the structural changes that result from injury, RNASE1 could provide new insights into the molecular mechanisms that result in podocyte dysfunction and proteinuria as well as open new avenues for treatment.

11 INHIBITION OF HEPARANASE ATTENUATES PODOCYTE INJURY INDUCED BY PUROMYCIN AMINONUCLEOSIDE

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Background/Aims: Podocytes adhere to glomerular basement membrane, which is a dense network of extracellular matrix composed of type IV collagen, laminin, nidogen, and heparan sulfate proteoglycans. Literature suggests that heparanase is involved in the development of various proteinuric diseases; it is unclear whether heparanase plays a role in podocyte injury.
Methods: In this study, a conditional immortalized mouse podocyte line was used to induce differentiation in vitro. The formation of focal adhesions (FA) , stress fibers, and interdigitating cellular junctions were observed in differentiated podocytes. Treatment of podocytes with puromycin aminonucleoside (PAN), a podocyte injury reagent, reduced the formation of FAs and stress fibers.
Results: Notedly, interdigitating cellular junctions shortened into punctate structures and accumulated with F-actin. We found that co-treatment of heparanase inhibitor prevented the PAN-induced cellular abnormalities. Furthermore, we used BSA filtration assay to measure the podocyte monolayer permeability and found that the inhibition of heparanase activity attenuated the PAN-induced BSA leakage.
Conclusion: Taken together, our results suggest that heparanase may be a therapeutic target to prevent podocyte injury.

12 INVESTIGATING PODOCYTE-PARIETAL EPITHELIAL CELL COMMUNICATION THROUGH INTERCELLULAR BRIDGES

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Background/Aims: Podocytes are terminally differentiated visceral epithelial cells that are critical for maintenance of the glomerular filtration barrier. Podocyte loss is characteristic of multiple kidney diseases, including FSGS and RPGN which have a high risk of progression to ESKD, requiring dialysis. In these diseases, podocyte loss triggers the activation and proliferation of neighboring parietal epithelial cells (PECs) which reside along the bowman's capsule. Aberrant PEC proliferation leads to crescent or pseudo-crescent formation in the bowman's space and eventual glomerular injury.

Recent evidence suggests that in the setting of injury podocytes may physically interact with neighboring cells by forming intercellular bridges. However, the functional role of these bridges between podocytes and PECs has not been characterized.

Methods: We investigated intercellular bridges in three models of podocyte injury: podocyte-specific loss of Krüppel-like factor 4 (Klf4ΔPod) nephrotoxic serum nephritis (NTS) and diabetic kidney disease (db/db). Immunohistochemistry, immunofluorescence staining, and electron microscopy were used to identify intercellular bridges in glomeruli. Single nucleus RNA-seq was performed on each injury model. Immortalized mouse podocytes were differentiated and co cultured with mPEC257 cells prior to immunostaining for potential markers of intercellular bridges.

Results: We observed de novo bridges in glomeruli of Klf4ΔPod mice and in mice treated with NTS. We noticed colocalization of endogenous podocyte markers (synaptopodin, nestin) and markers for activated PECs (CD44) in both models, suggesting that intercellular bridges form between injured podocytes and activated PECs. The number of glomeruli with intercellular bridges correlates with the severity of injury, as few intercellular bridges were observed in db/db compared to proteinuric models with rapid podocyte loss. Enrichment analysis of differentially expressed genes between Klf4ΔPod mice and NTS treated mice showed common upregulated pathways including focal adhesion, axon guidance, and actin cytoskeleton regulation in the podocyte and PEC clusters. Immunofluorescence staining of a coculture of mouse podocytes and PECs reveals that intercellular bridges contain actin, CRIM1, AHNAK, BIRC3, and microtubule markers (RAB8A, RAB11A), suggesting potential protein trafficking. Bright-field microscopy also showed vesicle-like structures within intercellular bridges.

Conclusion: To date, this is the first study to demonstrate the potential role for intercellular bridges between injured podocytes and activated PECs in proliferative glomerulopathies.

13 LIVE CELL IMAGING-BASED ANALYSES TO CHARACTERIZE THE PATHOGENIC POTENTIAL OF CRUMBS2 VARIANTS

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Background/Aims: Mutants of the slit diaphragm protein CRB2 are associated with nephrotic syndrome. However, less is known about the pathogenic potential of novel identified CRB2 variants or about enzymes that influence CRB2 processing. Therefore, we aimed to characterize several CRB2 variants in a cell-culture based system and tested how the knockout of PDIA3 affects the transport of CRB2 to the plasma membrane.
Methods: We used HEK293T cells stably expressing BFP-CAAX-signal at the PM and transiently expressed GFP-tagged CRB2 wildtype and various CRB2 variants with putative pathogenic potential. Using live cell imaging, we analyzed intracellular CRB2 localization after 48h of expression and quantified the degree of PM localization of CRB2-GFP signal. In addition, we tested the impact of PDIA3 on CRB2 transport.
Results: CRB2 WT is efficiently transported towards the PM (in over 85% of the cells). In contrast, most disease-associated missense variants like CRB2 C620S or N800K fail to reach the cell surface. Only 10-15% of the cells show a CRB2 signal at the PM. Strikingly, we also found differences between the CRB2 variants in their degree of PM localization, which might indicate differences in pathologic severity. In addition, we show that PDIA3 function is essential for correct PM transport of CRB2 WT.
Conclusion: Our in vitro cell culture system based on HEK293T BFP-CAAX cells is well suited to characterize surface localization of CRB2-GFP variants and thereby allows a first evaluation of their pathologic potential. Proper folding in the ER, posttranslational modifications and ER stress might be so far underestimated injury factors in podocytopathies of SD proteins.

14 OSBPL7 IN CHRONIC KIDNEY DISEASE

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Background/Aims: OSBPs are implicated in various processes such as cholesterol transfer from the endoplasmic reticulum to the Golgi, cholesterol efflux, and regulation of ABCA1 expression. Previous studies have shown that OSBPL7 deficiency leads to decreased autophagy in other cell types, which may impact lipid trafficking and podocyte function. This study aims to investigate the role of OSBPL7 deficiency in CKD.
Methods: The study utilized immortalized mouse podocytes and a Tg[l-fabp:DBP:eGFP] zebrafish model to investigate the effects of OSBPL7 deficiency on the development of renal dysfunction in podocyte disease and measure the integrity of glomerular filtration. OSBPL7 deficient podocytes and zebrafish were generated and levels of ER stress, autophagy, lipid droplets, and proteinuria were determined.
Results: OSBPL7 protein levels were reduced in the renal cortex of metabolic and non-metabolic mouse models of CKD. OSBPL7 deficiency leads to an increase in apoptosis levels and ER stress markers in podocytes and a decrease in autophagic flux. Knocking down OSBPL7 expression in Tg[l-fabp:DBP:eGFP] zebrafish leads to increased proteinuria and an edema phenotype. The findings suggest that OSBPL7 plays a critical role in the regulation of autophagy and ER stress in podocytes contributing to CKD.
Conclusion: This study sheds light on the important role of OSBPL7 in the regulation of autophagy and ER stress in podocytes and its potential as a therapeutic target in CKD. The results show that OSBPL7 deficiency may lead to lipid accumulation inside the podocyte and contribute to the proteinuria observed in CKD. Further studies are needed to understand the impact of dysregulated lipid trafficking in CKD.

15 PODOCYTE-SPECIFIC REGULATION OF PROTEIN PHOSPHATASE 2A WORSENS DKD PROGRESSION

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Background/Aims: Diabetic kidney disease (DKD) remains a leading cause of chronic kidney disease with limited treatment options. We previously showed that the main active agent in Fructus Arctii confers renoprotection in DKD through increased activation of protein phosphatase 2A (PP2A) in podocytes. Notably, while PP2A is expressed in many cell types, a specific isoform encoding a regulatory subunit of PP2A, PPP2R2B, is expressed specifically in the podocytes in the kidney, and its missense variant (T202M) is associated with worsened renal outcome in diabetic nephropathy patients. Therefore, we examined the consequence of PPP2R2B missense variant in DKD pathogenesis.
Methods: We generated knock-in (KI) mice with analogous ppp2r2b missense variant (ppp2r2b T288M), and diabetes was induced with streptozotocin. Vehicle-injected KI mice and STZ-injected wildtype (WT) mice served as controls. Renal function was assessed every two weeks, and all mice were euthanized after 24 weeks post diabetes induction for examination.
Results: Although no differences were observed in renal function in non-diabetic WT or KI mice, diabetic KI mice developed significantly higher levels of albuminuria in comparison to diabetic WT mice. Diabetic KI mice also showed significantly worsened podocyte injury and loss, and mesangial matrix expansion. As PP2A regulates the dephosphorylation of p65 NF-κB, we evaluated the phosphorylation level of p65 NF-κB in the mice glomeruli as a secondary readout of PP2A activity in control and diabetic mice. Compared to wildtype mice, KI mice had a higher level of glomerular phosphorylated p65 level, regardless of the disease condition, indicating that ppp2r2b T202M variant resulted in reduced PP2A activity in glomeruli.
Conclusion: The missense ppp2r2b variant related to clinical worse renal outcome in humans resulted in worsened diabetic glomerulopathy in KI mice. Thus, pharmacological enhancing the function of PPP2R2B, a podocyte-specific PP2A regulatory subunit, may open up a potential therapeutic avenue of podocyte-specific renoprotection in DKD.

16 PODOCYTES EXHIBIT A DISTINCT ALTERNATIVELY SPLICED LANDSCAPE OF PPARΓ MRNA COMPARED TO ADIPOSE TISSUE

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Background/Aims: Podocytes are terminally differentiated epithelial cells, and their structural and functional integrity is compromised in glomerular disease, characterized by proteinuria. Traditional agonists (e.g., pioglitazone) and selective modulators (e.g., GQ-16) of peroxisome-proliferator-activated-receptor-γ (PPARγ) reduce proteinuria in glomerular disease and protect podocytes from injury via PPARγ activation. While the transcriptional role of PPARγ in activating adipogenic genes is well-established in adipose tissue, liver and muscle, understanding of podocyte PPARγ signaling remains limited.
Methods: We performed a comprehensive analysis of PPARγ mRNA variants due to alternative splicing, in human podocytes and compared with adipose tissue.
Results: We found that podocytes express the ubiquitous PPARγ Var 1 (encoding γ1) and not Var2 (encoding γ2), which is mostly restricted to adipose tissue and liver. Additionally, we detected expression at very low levels of Var4, and barely detectable levels of other variants, Var3, Var11, VartORF4 and Var9, in podocytes. Furthermore, a distinct podocyte vs. adipocyte PPAR-promoter-response-element containing gene expression, enrichment and pathway signature was observed, suggesting differential regulation by podocyte specific PPARγ1 variant, distinct from the adipocyte-specific γ2 variant.
Conclusion: In summary, podocytes and glomeruli express several PPARγ variants, including Var1 (γ1) and excluding adipocyte-specific Var2 (γ2), which may have implications in podocyte specific signaling and pathophysiology. This suggests that new selective PPARγ modulators can be potentially developed that will be able to distinguish between the two forms, γ1 and γ2, thus forming a basis of novel targeted therapeutic avenues.

17 RAPGEF1 (C3G) IS ESSENTIAL FOR SLIT-DIAPHRAGM INTEGRITY IN MICE

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Background/Aims: Podocytes play a central role in the pathogenesis of glomerular diseases. The slit-diaphragm protein nephrin is necessary for the integrity of the filtration barrier. Nephrin signals to the actin cytoskeleton and to integrin β at focal adhesions via the small GTPase Rap1 and its activating factor C3G. The goal of this project is to understand the role of C3G in glomerular podocytes in vivo.
Methods: C57BL/6 C3G flox mice were crossed to C57BL/6 Six2-Cre transgenic mice to generate a C3G knockout in the metanephric mesenchyme (C3G-KO). Mice kidneys were analyzed histologically and ultra-structurally. Mice were evaluated for proteinuria. Bulk RNA-sequencing was performed with C3G-KO mice glomeruli.
Results: Ultrastructural analysis of C3G-KO mice revealed foot process effacement concomitant with proteinuria starting 4 weeks after birth progressing to focal segmental glomerulosclerosis. C3G-KO animals exhibited significant podocyte loss. Bulk RNA-sequencing of C3G-KO mice glomeruli revealed significantly enriched gene regulation in cellular processes such as cell adhesion and signal transduction.
Conclusion: C3G is essential for an intact glomerular filtration barrier. C3G may play a role in anchoring podocytes to the basement membrane as C3G deficiency in the nephron results in podocyte loss. Moreover, RNA-sequencing analysis indicates that C3G is involved in cell adhesion and signaling processes.

18 RAPID AND DIRECT ISOLATION OF MITOCHONDRIA FROM KIDNEY PODOCYTES SHOWS HIGH OXIDATIVE PHOSPHORYLATION CAPACITY THAT IS DIMINISHED IN PRIMARY PODOCYTE CULTURES

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Background/Aims: Whether and how podocytes depend on mitochondria (mito) across their long post-mitotic lifespan is yet unclear. Isolation of podocyte mitochondria typically requires first isolating podocytes themselves. Subsequent disassociation of podocytes from their basement membrane, however, recapitulates an injured state that stresses mitochondria and may not be representative of in vivo mito-function.
Methods: To address this, we made novel mouse lines by crossing floxed HA-mitochondria tagged (MITO-Tag) mice with those expressing Cre in either podocytes (NPHS2) or tubules (CDH16), thus allowing for rapid, kidney cell-specific, isolation of mitochondria via immunoprecipitation.
From kidneys of ~6-month-old mice, we isolated mitochondria from either podocytes or tubules (n each mouse line=4 male, 2 female) using HA-tag MACS columns. We also isolated total mitochondria from the same kidneys using an indiscriminate TOMM22 MACS pull-down. Mito-function was measured in fresh isolates (<2 hours) by Oroboros Fluo-O2k.
Results: Surprisingly, when normalized to mito-content by citrate synthase (CS) levels, mitochondria isolated from podocytes had twice the respiratory capacity of tubule mitochondria in Complex I+II (podocyte vs. tubule = 109 vs. 42 pmol O2*s-1*Unit CS-1, p=0.011) and Complex IV-driven respiratory states (podocyte vs. tubule = 371 vs. 153 pmol O2*s-1*Unit CS-1, p=ns). This was not an anomaly of the mouse strain because mitochondria isolated by TOMM22 from total MITO-Tag/NPHS2Cre cortex, comprised largely of tubule cells, respired similarly to those isolated from CDH16 tubules (p=0.91-0.99).
To test if cultured podocyte mitochondria retain the same respiratory capacity as those isolated from intact kidney, we performed a paired experiment of podocyte-mitos isolated from kidney vs. from cultured glomerular outgrowths. (MITO-Tag/NPHS2Cre, 7.5-month-old females, n=4). Mitochondria from day 7 cultured podocytes maintained the same level of CS (p= 0.68) but had significantly reduced Complex I+II (culture podo-mito =39% of kidney podo-mito, p=0.04) and Complex IV (culture podo-mito = 55% of kidney podo-mito, p=0.04) respiratory capacity in paired comparisons to day 0 mito-isolates from the same kidneys.
Conclusion: Thus, podocytes maintain mitochondria capable of robust respiration capacity that outstrips that of tubules, when normalized to mitochondrial content, however, they are sensitive to culture conditions. Mitochondrial capacity, efficiency and dependance may be underestimated by standard primary podocyte culture experiments.

19 THE DISTINCTIVE FUNCTIONS OF ENDOCYTIC MECHANISMS WITHIN THE MAINTENANCE OF SLIT DIAPHRAGMS IN DROSOPHILA NEPHROCYTES

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Background/Aims: Evidence from in vitro studies and murine knockout models support a role of endocytosis for the glomerular filtration barrier. Furthermore, two endosomal regulators, GAPVD1 and TBC1D8B, were discovered as monogenic causes of nephrotic syndrome. However, the mechanistic role of endocytosis remains unclear. The nephrocytes of Drosophila provide an accessible, molecularly conserved podocyte model.
Methods: Endosomal regulators and their impact on the trafficking of slit diaphragm proteins were manipulated by acutely induced gain- and loss-of-function and the resultant phenotypes analyzed by immunofluorescence, functional assays, and live cell imaging. Endocytic turnover of fly nephrin was investigated by live antibody labeling.
Results: Sns, the Drosophila nephrin, had a protein half-life of ~2 days, but live antibody labeling showed turnover after 2 h. Silencing of Rab5 caused ectopic slits and diminished Sns turnover. Rab5-RNAi reduced permeability of the filter. We blocked dynamin-dependent endocytosis acutely, which caused ectopic slit diaphragms while the Sns turnover remained unchanged. Conversely, inhibition of raft-mediated endocytosis by flotillin-RNAi or Cyclodextrin significantly reduced the turnover of fly nephrin.
Conclusion: While dynamin-dependent endocytosis prevents lateral diffusion and formation of ectopic slit diaphragms in nephrocytes, flotillin-dependent endocytosis regulates turnover of nephrin within the slit diaphragm. Selective endocytosis maintains a stable yet dynamic filtration barrier.

20 THE IMPACT OF PHASE-SEPARATED CONDENSATES FORMED BY YAP AND TAZ IN PODOCYTES

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Background/Aims: Podocytes are highly dynamic cells and continuously have to adjust to physiological filtration pressure and considerable mechanical strains. Therefore, balanced intra- and intercellular signaling are pivotal for their sustained cellular metabolism. One central signaling cascade is the Hippo signaling pathway with YAP and TAZ as the transcriptional effectors. In podocytes, YAP/TAZ localize to the nucleus, and while podocyte-specific YAP deletion in vivo results in FSGS, TAZ-knockout mice show a milder phenotype. Recent reports provided evidence that YAP and TAZ can form condensates that generate liquid-liquid phase separation (LLPS) to regulate downstream transcriptional programs. The impact of this YAP/TAZ property on podocyte homeostasis remains unclear.
Methods: We made use of a podocyte in vitro cell model exogenously expressing GFP-tagged YAP or TAZ. We performed live imaging to analyze the basal and induced formation of LLPS. Different conditions were tested to drive or interfere with the formation of LLPS. We further analyzed the effects of LLPS formation on the composition of YAP/TAZ protein complexes by label-free mass spectrometry and co-immunofluorescence. The effect of LLPS in YAP and TAZ-dependent transcriptional regulation was analysed by measuring the level of target gene expression.
Results: Here we demonstrate that YAP and TAZ form LLPS in podocytes. While most exogenous YAP-LLPS fusions require induction, TAZ-LLPS occur constitutively and are primarily observed in the cytoplasm and plasma membrane of podocytes. Hyperosmotic stress promotes nuclear YAP and TAZ shuttling and the dynamics of LLPS fusion. Increase of calcium signaling, production of reactive oxygen species and cytoskeleton disorganization failed to affect YAP/TAZ-LLPS. Exogenous YAP/TAZ condensates compartmentalized the transcription factor TEAD1 and YAP/TAZ. Notably, this did not subsequently induce YAP/TAZ-TEAD1 interaction and transcription of specific target genes in podocytes. Nevertheless, disruption of the phase-separated condensates decreased levels of YAP/TAZ target genes.
Conclusion: This study identifies LLPS modulation of YAP and TAZ as a novel level of regulation balancing hippo signaling in podocytes. We postulate that differences in LLPS formation might be a mechanism underlying the distinct functions of the two highly similar transcriptional regulators YAP and TAZ.

21 THE INSULIN / IGF AXIS IS CRITICALLY IMPORTANT IN THE KIDNEY PODOCYTE AND CONTROLS GENE TRANSCRIPTION AND SPLICEOSOME FUNCTION

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Background/Aims: Insulin signalling to the podocyte via the insulin receptor (IR) is crucial for kidney function and insulin-like growth factor 1 (IGF1) signalling through the structurally related insulin-like growth factor 1 receptor (IGF1R) is also known to directly affect the podocyte. Since the IR and IGF1R may act redundantly in some contexts, this study sought to elucidate the role of the insulin/IGF1 axis in podocyte function using mouse and cell culture models deficient in both receptors.
Methods: To examine the effects of combined receptor loss in vivo, a transgenic mouse model with conditional inactivation of podocyte IR and IGF1R was generated. In vitro, conditionally immortalised genetic IR/IGF1R dual knockout podocytes were characterised using global proteomic and transcriptomic analysis.
Results: Podocyte specific IR/IGF1R knockout mice developed significant albuminuria and a severe renal phenotype with global sclerosis, renal failure and death occurring between 4 and 24 weeks. >90% loss of IR/IGF1R in cultured mouse podocytes was also detrimental resulting in >50% cell death 7 days after gene knockdown. Enrichment analysis of total proteomic data revealed a striking downregulation of gene ontology terms associated with DNA repair, splicing and RNA processing activity in IR/IGF1R knockdown cells. Long-read RNA sequencing was performed to further explore the effect of dual receptor knockdown on spliceosome function. Analysis of this data revealed higher levels of intron retention and an increase in the proportion of transcripts containing premature termination codons in IR/IGF1R knockdown podocytes.
Conclusion: This work underlines the critical importance of podocyte insulin/IGF signalling and reveals a novel role for this signalling axis in the maintenance of genomic integrity and in RNA processing by regulating spliceosome activity.

22 THE PODOCYTE-SPECIFIC ANGULIN ILDR2 AND ITS ROLE IN TRICELLULAR TIGHT JUNCTIONS AND GLOMERULAR LOCAL IMMUNE TOLERANCE

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Background/Aims: Tricellular tight junctions (tTJ) regulate the paracellular flux where three cells meet. In tTJs, tricellulin (MARVELD2) and one angulin (ILDR1, ILDR2, LSR) recruit claudins (CLDN). ILDR2 is mainly expressed in immunologically privileged tissue inducing local immune tolerance. Besides the fact of having tricellular junctions, little is known about the function of tTJ in podocytes.
Methods: tTJ component expression was investigated in scRNA-seq datasets, RT-qPCR, and western blot and super-resolution microscopy (3D-SIM) in human and mouse kidneys. Co-immunoprecipitation (co-IP) of ILDR2 and binding partners was performed in HEK293 cells. Glomeruli from C57BL/6J ILDR2 knockout or wild-type mice were analyzed with tandem mass spectrometry.
Results: Podocytes expressed the tTJ components MARVELD2 and ILDR2 colocalized with CLDN5 and ZO-1. In co-IPs, ILDR2 formed a multiprotein complex with CLDN5, MARVELD2, and ZO-1. Upon podocyte injury, ILDR2 was recruited from tri- to bicellular junctions. Aged ILDR2 KO mice showed foot process effacement, glomerular hypertrophy, and decreased podocyte density but without proteinuria. Proteome analysis of ILDR2 KO glomeruli revealed upregulated pro-fibrotic matrix proteins and decreased podocyte proteins.
Conclusion: Podocytes have tTJs with ILDR2 as the cell-specific angulin. ILDR2 is bound to TJ components of the slit diaphragm complex and is recruited to bicellular tight junctions during effacement. ILDR2-deficient mice presented glomerular changes without albuminuria. NTS-N induction in ILDR2-deficient mice will be performed to further investigate the role of ILDR2 in podocytes.

23 THE PODOCYTE: GLOMERULAR SENTINEL AT THE CROSSROADS OF INNATE AND ADAPTIVE IMMUNITY

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Background/Aims: Focal segmental glomerulosclerosis (FSGS) is a common glomerular disorder that manifests clinically with the nephrotic syndrome and has a propensity to recur following kidney transplantation. The pathophysiology and therapies available to treat FSGS currently remain elusive. Since the podocyte appears to be the target of apparent circulating factor(s) that lead to recurrence of proteinuria following kidney transplantation, this abstract is focused on the podocyte.
Methods: In the context of kidney transplantation, the performance of pre- and post-reperfusion biopsies, and the establishment of in vitro podocyte liquid biopsies/assays allow for the development of clinically relevant studies of podocyte biology. This has given insight into new pathways, involving novel targets in innate and adaptive immunity, such as SMPDL3b, cGAS-STING, and B7-1.
Results: Our studies demonstrate that the successful clinical use of rituximab and abatacept, two immunomodulating agents, in our case series, may be due to direct effects on the podocyte, in addition to, or perhaps distinct from their immunosuppressive functions. Our recent work identifies the cGAS-STING signaling pathway in podocytes as a significant contributor to the pathogenesis of glomerular disease.
Conclusion: Tissue biomarker-directed therapy may provide a rational approach to validate the mechanism of disease and allow for the development of new therapeutics for FSGS. Our work highlights recent progress in the field and emphasizes the importance of kidney transplantation and recurrent FSGS (rFSGS) as a platform for the study of primary FSGS.

24 TRANSCRIPTION-BLOCKING DNA LESIONS IN PODOCYTES CAUSE FOCAL SEGMENTAL GLOMERULOSCLEROSIS AND INTERSTITIAL INFLAMMATION IN THE KIDNEY THAT CAN BE AMELIORATED BY CALORIE RESTRICTION

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Background/Aims: Terminally differentiated podocytes need a strong cell stress response to maintain their integrity. Transcription stress by deleting the endonuclease cofactor Ercc1 in podocytes results in the development of FSGS. DNA damage and aging in podocytes are not well described, yet highly important phenomena in an aging population. This study addresses the podocytes' response to transcription stress.
Methods: We analyzed immune cells in Ercc1 pko mice by flow cytometry and immunohistochemistry. Podocyte repair capacity, involvement of NER-components, antioxidant defense, and actin cytoskeleton reorganization in response to DNA damage were characterized in vitro. A calorie restriction intervention was conducted in Ercc1 pko mice and the relevance of the mTORC1 pathway was assessed in vivo.
Results: DNA damage in podocytes causes interstitial inflammation driven by Th1 cells inducing macrophages at later disease stages. The post-mitotic podocyte displays sufficient repair mechanisms and reacts to induced DNA damage by reorganization of the actin cytoskeleton and an oxidative stress response. Calorie restriction ameliorates the FSGS-like phenotype and reduces T cell infiltration. Genetically reducing mTORC1 activity by deleting one allele of Raptor does not modulate the Ercc1 pko phenotype.
Conclusion: Ercc1 deficiency causes a progeroid phenotype. Podocyte-specific deletion of Ercc1 leads to glomerulosclerosis and early death, accompanied by mTORC1 activation and interstitial inflammation. Calorie restriction inhibits mTORC1, reduces inflammation, and oxidative stress - hallmarks of aging in the Ercc1 pko mice, dramatically ameliorating the renal phenotype in our model.

Glomerular basement membrane biology and disease

1 A DROSOPHILA MODEL OF ALPORT SYNDROME AS A PLATFORM FOR VALIDATING PATHOGENICITY OF COL4A5 GENETIC VARIANTS

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Background/Aims: Alport syndrome is a progressive kidney disease affecting 3% of children with chronic kidney disease. A common mutation that patients carry is in the X-linked COL4A5 gene, which encodes one of the three proteins that form the trimeric type IV Collagen α3α4α5. The COL4A5 protein is essential for the glomerular basement membrane which forms the kidney filtration barrier.
Methods: Here, we show that Drosophila Col4a1 is the functional homolog of human COL4A5 in the fly nephrocyte (equivalent of human podocytes that produce COL4A5). We then used this platform to screen seven COL4A5 variants from patients with uncertain functional significance.
Results: Silencing Col4a1 in nephrocytes led to irregular and thickened basement membrane, as well as significantly reduced nephrocyte filtration function. This deficiency phenotype could be restored by expressing human reference (wildtype) COL4A5, but not by COL4A5 carrying any of three established pathogenic COL4A5 variants. We provide evidence to support pathogenicity for four variants and found the other three to be benign.
Conclusion: Thus, we demonstrate the feasibility of using an efficient Drosophila platform to provide the much needed functional validation for many COL4A5 variants identified from patients. This approach could be readily expanded to accommodate other candidate kidney disease genes for their variant pathogenicity validation.

2 DEEP-INTRONIC VARIANTS IN THE X-LINKED ALPORT SYNDROME: FROM DETECTION TO THERAPEUTIC HOPES

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Background/Aims: X-linked Alport syndrome is a monogenic inherited nephropathy caused exclusively by pathogenic variants in the COL4A5 gene. This gene encodes collagen IV alpha 5 chain which is a crucial structural component of the glomerular basement membrane. In 20% of cases, no molecular cause is identified in COL4A5 exons or flanking regions, suggesting deep intronic variants involved in the disease.
Methods: We developed a transcriptomic approach to detect missing splicing events in the COL4A5 gene. We applied targeted-RNAseq to patient-derived fibroblasts to detect pathogenic deep intronic variants. Using a bioinformatic score, we excluded the false events. We then established an antisense oligo (ASO) therapy to correct mis-splicing in our models.
Results: We identified a pathogenic splicing event in 17/19 patients with XLAS without variant identified in coding / flanking regions, including 14 pseudo-exon retention. A rare deep-intronic variant involved in splicing was systematically identified in each patient. We successfully restored physiological splicing by using specific ASO in 8/10 patients, leading to the rescue of WT protein in the extracellular matrix.
Conclusion: The therapeutic potential of ASOs compels us to develop a bioinformatics method for precise diagnosis of splicing variants. We developed a reliable method for identifying aberrant transcripts in patients without variation in coding region. These findings illustrate the importance of discovering deep-intronic variations and the applicability of ASO treatment in patients with XLAS.

3 THE LONG NON-CODING RNA MEG3 MAY AFFECT THE EXPRESSION OF BOTH APICAL AND BASEMENT MEMBRANE COMPONENTS OF GLOMERULAR EPITHELIAL CELLS IN DIABETIC KIDNEY DISEASE

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Background/Aims: Diabetic kidney disease is a complication of diabetes that takes years, sometimes decades, to develop. The loss of epithelial phenotype in an in vitro model of diabetic glomerular epithelial cells (HGEC), as indicated by downregulation of podocalyxin and nephrin expression, is gradual and irreversible. The long non-coding RNA Maternally Expressed 3 (MEG3) is upregulated in the same cell line when cultured at high glucose levels.
Methods: We performed deep sequencing experiments and computational analysis to discover the genomic targets of MEG3, a DNA:RNA triplex forming lncRNA.
Results: We found that MEG3 may play a role in the observed changes by binding to the genomic loci of genes that are differentially expressed following chronic high glucose treatment. Loci that are predicted to form triplexes include genes that are associated (among other processes) with the extracellular matrix and the cell shape. FN1 and PODXL, the genes that encode fibronectin and podocalyxin, respectively, are also among the predicted MEG3 target genes.
Conclusion: MEG3 may be a potential regulator of glomerular epithelial phenotype by regulating the expression of glomerular basement membrane components.

Glomerular cell paracrine signaling/cross talk

1 INJURY INDUCED PARACRINE EFFECTS ON THE PODOCYTE'S TRANSCRIPTOME

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Background/Aims: Knowledge gaps in glomerular diseases include the crosstalk between injured and healthy podocytes, and how podocytes respond to different forms of injury. The goal of this study was to establish an in vitro microfluidic coculture model to determine what mediators from directly injured podocytes cause paracrine damage to neighboring podocytes, and to distinguish the responses to different injuries.
Methods: We engineered an open microfluidic coculture device consisting of two separate but interconnectable chambers. Following seeding with either mouse or human podocytes and induction of injury in one chamber by cytotoxic sheep anti-podocyte IgG (IgG), puromycin (PAN), or Adriamycin (ADR), chambers were connected to study the paracrine effects between injured and naïve podocytes. Imaging, cell viability and RNA-sequencing were temporally measured in podocytes of both chambers.
Results: Injured mouse and human podocytes displayed foot process effacement, cell body shrinkage, and decreased cell viability. Gene set enrichment analysis showed several common pathways for injury by IgG, PAN, and ADR, including p53, apoptosis, TGFß and inflammation (TNF, IFN, IL2). IgG and ADR but not PAN increased ROS. The naïve podocytes cocultured with injured ones showed similar enrichments. Among the enriched ligand-receptor pairs mediating paracrine effects were VEGF-NRP1, CSF1-CSF1R, DLL1-NOTCH2, IGF1-IGF1R, TNF-DAG1, TGFß3-TGFR.
Conclusion: The in vitro open microfluidic coculture device represents a new model for studying autocrine and paracrine signaling between healthy and injured podocytes. The paracrine injury response is similar to the direct injury response. Mechanistic studies are currently ongoing to identify which ligands from the injured podocytes and which receptors on naïve podocytes mediate these paracrine effects.

2 PODOCYTE DERIVED ENDOTHELIN-1 AND CROSSTALK WITH ENDOTHELIAL CELLS THROUGH EDNRA IS ESSENTIAL FOR GLOMERULAR INJURY

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Background/Aims: Crosstalk between activated podocytes and glomerular endothelial cells (GECs) has been demonstrated in mouse models of focal segmental glomerulosclerosis (FSGS). We have previously demonstrated that podocytes activation can result in GEC stress and dysfunction and concomitant albuminuria via increased endothelin-1 and endothelin receptor A (Edn1/Ednra) signaling. However, the mechanistic link between podocyte activation and Edn1 production, GEC injury, and reciprocal crosstalk that results in podocyte loss and albuminuria remains unclear.
Methods: We examined the effects of TGFβ on podocyte activation. We measured Edn1 expression and Edn1 release into the conditioned media of podocytes in culture treated with TGFβ. We generated a mouse model through targeted gene deletion of Edn1 in podocytes using the Cre-loxP system (pET1fl/fl). We also generated a quadruple transgenic mice line with podocyte specific Edn1 knockout, and inducible, podocytes specific TGFβR1 podocyte-specific ET1fl/fl;TbrIAADTg/Tg mice . Mice were treated with adriamycin or with doxycycline (Dox) to induce nephropathy.
Results: TGFβ signaling resulted in a significant increase in Edn1 expression and Edn1 release by podocytes in culture. This effect was prevented by a TGFβR1/Smad inhibitor or with Smad2 or Smad2/3 knockout, suggesting canonical TGFβ signaling dependent pathway. Compared to adriamycin treated control pET1+l+, adriamycin treated pET1fl/fl mice had reduced glomerular injury, albuminuria and podocyte depletion. Next, we compared Npsh1-rtTATg/Tg;TbrIAADTg/Tg with ET1fl/fl;TbrIAADTg/Tg mice treated with Dox. Mice with podocyte specific Edn1 ko has no glomerular injury, albuminuria and podocyte depletion was completely prevented and no increase in GEC Ednra
Conclusion: Our studies provide evidence for the essential role of podocyte derived Edn1 upon activation and crosstalk with GECs via Ednra that results in GEC injury, podocyte depletion and albuminuria in experimental FSGS.

3 SHIGA TOXIN TARGETS THE PODOCYTE CAUSING HAEMOLYTIC URAEMIC SYNDROME THROUGH ENDOTHELIAL COMPLEMENT ACTIVATION

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Background/Aims: Shiga toxin (Stx) associated Haemolytic uraemic syndrome (HUS) is the leading cause of acute kidney injury (AKI) in children with a mortality of 5%. It is associated with devastating complications including End-stage renal disease, neurological damage and death. Exactly why the glomerular microvasculature is so susceptible to injury following systemic Stx infection remains unclear. Additionally terminal complement inhibition has proven therapeutically beneficial in atypical (complement driven) HUS but its role in the common form of Stx HUS is unclear.
Methods: Using:
1. Transgenic mouse in which we expressed the STX receptor [GB3] exclusively in the podocyte by engineering podocyte specific GB3 receptor mice on a GB3 null background.
2. Human podocyte and glomerular endothelial cell (GEnC) co-culture experiments.
3. Human STX-HUS kidney biopsy specimens.
Results: Specific activation of the podocyte Stx receptor (Gb3) triggers endothelial cell injury through podocyte to GEnC crosstalk. This is mediated by a reduction in podocyte VEGF-A which leads to loss of GEnC glycocalyx, a reduction in GEnC complement factor H binding and local activation of the terminal complement pathway. Therapeutic inhibition of the terminal complement pathway with a C5 inhibitor rescued this podocyte driven Stx-induced HUS phenotype.
Conclusion: Our work potentially explains why systemic Stx exposure targets the glomerulus and supports the use of terminal complement pathway inhibition in this devastating disease.

Glomerular cell lipid biology/intracellular signaling

1 APOLIPOPROTEIN M TREATMENT RESTORES KIDNEY FUNCTION IN ALPORT SYNDROME

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Background/Aims: We previously reported decreased glomerular Apolipoprotein M in glomerular diseases.ApoM is a protein linking cholesterol and sphingolipid metabolism, both important players in the development of glomerular injury in Alport Syndrome among other glomerular disorders. Here we test the hypothesis that systemic replacement of ApoM in Alport Syndrome protects from the development of kidney failure.
Methods: To investigate if rhAPOM improves renal function, we injected Col4a3 KO mice with rhAPOM by I.P. weekly, at 4 weeks of age until sacrifice (8 weeks of age). Cortical APOM levels were measured by Western blot. Serum and urine samples were used to determine BUN, creatinine, and urinary ACR. PAS, Picro Sirius Red and Oil Red O staining were performed to quantify fibrosis and parenchymal lipids.
Results: APOM mRNA and protein expression were markedly reduced in glomeruli and podocytes from Col4a3 KO mice compared to WT mice. Treatment of Col4a3 KO mice with rh-APOM normalized renal APOM levels and prevented the development of renal failure (serum BUN and creatinine), proteinuria (urinary albumin/creatinine ratio), and protected from the development of glomerulosclerosis, tubular atrophy and dilation.
Conclusion: Our results suggest that restoring renal APOM levels in an experimental model of Alport Syndrome protect from renal failure and that treatment with rh-APOM may represent a novel therapeutic strategy for the treatment of glomerular diseases.

2 GENERATING A COMPREHENSIVE LIPOTOXICITY PROFILE FOR PODOCYTES

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Background/Aims: Lipids such as free fatty acids (FFAs) have been shown to mediate diverse biological effects, ranging from cellular injury to cytoprotection. More specifically, lipotoxicity refers to a dysfunctional cellular state resulting from maladaptive cellular metabolism of excess circulating lipids. Podocyte lipotoxicity has been implicated in both systemic diseases with glomerular involvement such as diabetic kidney disease as well as in primary podocytopathies such as certain forms of focal segmental glomerulosclerosis. However, to date, only a limited number of FFAs have been studied in podocyte models. Using several biological readouts to advance our understanding of podocyte lipid biology, we investigated the effects of podocyte exposure to a comprehensive library of FFAs developed in our prior work (FALCON, Fatty Acid Library for Comprehensive ONtologies; Wieder, Coraor-Fried et al., 2023).
Methods: We performed time course and dose-response experiments of the well-studied palmitic and oleic acids using cell viability and cytoskeletal structure as readouts in order to define the treatment windows and FFA concentrations to be used for a comprehensive FFA screen. Based on these data, a selection of FFAs was probed for their effects on cell viability, production of reactive oxygen species, and cell morphology before these biological readouts were investigated through the entire FALCON platform. Experiments were performed in immortalized mouse podocytes and results were compared to a similarly acquired lipotoxicity profile from a human immortalized tubular epithelial cell (TEC) model.
Results: While exposure to palmitic acid resulted in cell death in a time- and concentration-dependent manner, oleic acid was protective for podocytes. Screening a wide spectrum of structurally diverse FFAs revealed distinct FFA clusters defined through differential effects on podocyte viability, production of reactive oxygen species, and cell morphology. Comparison with the corresponding lipotoxicity profile of TECs showed cell type-specific effects and susceptibilities, likely reflecting diverging functional demands upon podocytes and TECs in their native environments.
Conclusion: By applying the FALCON platform to podocytes, we generated a rich dataset resulting in a comprehensive lipotoxicity profile for podocytes which will inform our understanding of metabolic challenges to podocytes in health and lipotoxicity-driven kidney diseases.

3 ROLE OF SMPDL3B IN REGULATION OF ALBUMINURIA IN EXPERIMENTAL ALPORT SYNDROME

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Background/Aims: Alport Syndrome (AS) is characterized by progressive loss of kidney function with very limited cure options. Sphingomyelin phosphodiesterase acid like 3b (SMPDL3b) plays an important role in podocyte survival and regulates the availability of sphingosine-1-phosphate (S1P). We hypothesized that podocyte SMPDL3b overexpression affects the generation of S1P and contributes to the renal failure in AS.
Methods: In vitro, immortalized murine podocytes were used for illumina sequencing, qRT-PCR and Western blot analyses. In vivo, AS podocyte-specific Smpdl3b deficient mice (DKO) were generated and in-depth kidney phenotypical analysis was performed, including lipidomic (LC-MS and MALDI-MSI) analysis. Two-tailed t-test or One-Way ANOVA were used to detect statistical changes.
Results: SMPDL3b expression is significantly higher in AS podocytes and kidney cortices, which was associated with significantly increased S1P levels in kidney cortices and urine from AS mice. Podocyte-specific Smpdl3b deletion resulted in decrease of ACR levels and increased number of foot processes, which was associated with decreased cortical and urinal S1P levels. S1P injections in AS and DKO mice resulted in worsening albuminuria and glomeruli morphology.
Conclusion: Our data indicate that SMPDL3b expression may affect availability of S1P and regulate proteinuria levels in experimental AS. Thus, targeting SMPDL3b expression levels in the kidney may represent a novel approach to improve renal outcome in patients with AS.

Glomerular metabolism

1 NICOTINE INDUCES PODOCYTE INJURY THROUGH NOS UNCOUPLING AND PEROXYNITRITE PRODUCTION

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Background/Aims: Although the harmful effects of smoking on health are established, the precise cellular mechanisms of nicotine-related renal damage are understudied. Smoking-Related Glomerulopathy (SRG) is a renal disease phenotype associated with vaping/smoking. This condition histologically mimics diabetic nephropathy, however, SRG patients present with proteinuria, glomerular pathology, and renal insufficiency without hyperglycemia. Here we investigated nicotine-induced oxidative and nitrosative stress in glomerular podocytes. We hypothesized that nicotine triggers rapid peroxynitrite production (ONOO-), NOS uncoupling, and impairs mitochondrial function.
Methods: To test the hypothesis, we performed live confocal imaging on conditionally immortalized human podocyte line to detect the production of ONOO- (HPF), Ca2+ (Fluo-8), and nitric oxide NO (DAF-FM) in response to nicotine. The presence of nicotinic acetylcholine receptors' (nAChR) subunits in podocytes was confirmed with immunocytochemistry and functional tests. NO bioavailability was probed in response to Ang II in presence of specific NOS subunits' blockers. Seahorse assay was used to detect changes in mitochondrial health.
Results: Immunostaining indicated that human and rat glomerular podocytes express nAChR7. Acute application of nicotine promoted an intracellular Ca2+ transient and ONOO- production. Nicotine-mediated ONOO- response was efficiently blocked in the presence of superoxide dismutase, indicating that ONOO- production requires superoxide. The application of specific nAChR agonists elicited Ca2+ transients but did not produce the ONOO- response, suggesting that nitrosative processes may occur independently from Ca2+ influx or nAChR activation. Chronic exposure to nicotine (12-hrs) resulted in a significant decline in podocytes' NO bioavailability. While imaging data shows that under normal conditions in podocytes, NO is produced primarily by NOS1 (70%), chronic nicotine exposure led to pathological changes, where NOS2 becomes a primary isoform (75%). Finally, chronic exposure to nicotine significantly decreased mitochondrial maximum respiration in podocytes.
Conclusion: In podocytes, nicotine promotes cascade or oxidative-nitrosative reactions leading to peroxynitrite formation, activation of NOS2, decline in NO bioavailability, and mitochondrial dysfunction. The pathological shift in redox balance is directly associated with nicotine, the key component of tobacco and vaping products. It may be the primary mechanism responsible for podocyte damage and renal function decline in smokers.

Glomerular cell mechanisms of injury and aging

1 ANTIPROTEINURIC AND PODOCYTOPROTECTIVE EFFECTS OF THROMBIN INHIBITION THERAPY IN GLOMERULAR DISEASE

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Background/Aims: Thrombotic disease is a life-threatening co-morbidity of proteinuric glomerular disease. Previous research suggests that thrombin exacerbates in vitro podocyte injury and that pharmacologic thrombin inhibition decreases proteinuria in rat nephrotic syndrome models. Our aim was to manipulate circulating thrombin activity to investigate thrombin-mediated podocyte injury in situ using two mechanistically distinct models of glomerular proteinuria. We hypothesized that thrombin inhibition would alleviate podocytopathy, podocytopenia, and glomerular disease progression.
Methods: We first sought to directly examine the effects of thrombin on podocyte health by manipulating its zymogen precursor, prothrombin (PT). Using male Wistar rats, puromycin aminonucleoside-induced proteinuria was treated with PT antisense oligonucleotide to induce hypoprothrombinemia (LoPT), or serial prothrombin infusions to sustain hyperprothrombinemia (HiPT). Next we examined effects of pharmacologic thrombin inhibition using direct oral anticoagulants. In female transgenic rats expressing podocyte specific human diphtheria toxin receptor, diphtheria toxin-induced proteinuria was subsequently treated with Dabigatran (direct thrombin inhibitor) or Rivaroxaban (direct FXa inhibitor). Both models were compared to appropriate Sham and healthy Control groups (n=6-12/group). Morning spot urine was collected at days 0 and 10 (peak proteinuria). Glomeruli were isolated, dissociated into single-cell suspensions, and analyzed by flow cytometry after immunofluorescent synaptopodin antibody and TUNEL staining.
Results: Prothrombin knockdown reduced podocyte exposure to prothrombin, podocytopathy (TUNEL positive podocyte fraction), and proteinuria ($p<0.05$). In contrast, hyperprothrombinemia increased podocyte injury and proteinuria ($p<0.05$). Furthermore, pharmacologic anticoagulant treatment also improved in situ podocyte preservation. Both Dabigatran and Rivaroxaban decreased terminal podocyte injury, enhanced podocyte survival, and alleviated overall disease severity as measured by proteinuria and albuminemia ($p<0.05$).
Conclusion: These data demonstrate that thrombin inhibition directly enhances in situ podocyte function, health, and survival during acute glomerular proteinuria, while elevated thrombin activity exacerbates proteinuria and podocyte injury. Currently available anticoagulants may be repurposed as a novel approach to slow or halt proteinuric glomerular disease progression, thus enabling simultaneous thromboprophylaxis and podocytoprotective therapy.

2 FOXC2 AND WT1 REGULATE TRANSCRIPTIONAL REPROGRAMMING DURING THE PODOCYTE RESPONSE TO INJURY IN THE CONTEXT OF HUMAN DISEASE, THE MECHANISMS WHEREBY TRANSCRIPTION FACTORS REPROGRAM GENE EXPRESSION IN REPARATIVE RESPONSES TO INJURY ARE NOT WELL UNDERSTOOD. WE HAVE STUDIED THE MECHANISMS OF TRANSCRIPTION

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Background/Aims: In the context of human disease, the mechanisms whereby transcription factors reprogram gene expression in reparative responses to injury are not well understood. We have studied the mechanisms of transcriptional reprogramming in disease using murine kidney podocytes as a model for tissue injury. Podocytes are a crucial component of glomeruli, the filtration units of each nephron. Podocyte injury is the initial event in many processes that lead to End Stage Kidney Disease. FOXC2 is a transcription factor known to regulate gene expression in podocytes. FOXC2 and WT1 are both required for podocyte differentiation. We hypothesized that WT1 and FOXC2 act coordinately to mediate a repair process after podocyte injury.
Methods: Using murine models and human kidney organoids, we used ChIP-seq and transgenic approaches to investigate FOXC2 and WT1 -mediated transcriptional reprogramming during the course of podocyte injury. These included conditional knockout of Wt1 and conditional knockdown of Foxc2 in podocytes of adult mice. We also over-expressed Wt1 and FoxC2 in podocytes of adult mice, prior to Adriamycin mediated injury.
Results: FOXC2 and WT1 co-bind many genes important in maintaining the glomerular filtration barrier. Peak Intensity and peak number of both transcription factors increase after injury. Knockout of Wt1 or knockdown of Foxc2 in podocytes of adult mice attenuates the transcriptional response to injury. Over-expression of FoxC2 or Wt1 confers protection during podocyte injury.
Conclusion: Transcriptional Reprogramming plays an important role in the podocyte response to injury. WT1 and FOXC2 mediate what appears to be a reparative response, aimed at restoring the glomerular filtration barrier.

3 PODOCYTE INJURY LEADS TO A PREMATURE AGING PHENOTYPE

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Background/Aims: Healthy aging leads to a decrease in kidney function and the development of glomerulosclerosis. The risk, incidence, and prevalence of chronic kidney diseases increases with progressive aging, and the outcomes of glomerular diseases are typically worse in the elderly. The following studies were undertaken to test the hypothesis that injury to podocytes causes and accelerates an aging phenotype.
Methods: Experimental focal segmental glomerulosclerosis (FSGS) was induced in young (4-month-old) C57BL/6J mice with a cytopathic sheep anti-podocyte antibody (FSGS group). 4m old control mice received the isotype control IgG2A antibody (Control group). Animals were sacrificed at adulthood - 12m control (n=9) and 12m FSGS (n=9) and at middle-age - 18m control (n=8) and 18m FSGS (n=11). Spot urines were collected to measure albumin/creatinine ratios, and kidney tissue was used for immunostaining.
Results: Compared to young and control adult mice, middle-aged control mice showed several features of aging including glomerular hypertrophy, decreased podocyte density, podocyte hypertrophy, and a reduction in the following health-span characteristics: decreased Nephrin, and VEGF-A expression, increased Desmin and Collagen IV expression. Interestingly, 12m old mice that had FSGS at 4m of age displayed similar characteristics of podocyte aging as control middle-aged 18m old mice. In addition, podocyte senescence and aging were significantly higher in 18m old mice that had FSGS at 4m of age compared to 18m old control middle-aged mice.
Conclusion: Podocyte injury induced by FSGS in young mice causes a premature senescent and aging phenotype characterized by the expression of senescent markers, accompanied by a decrease in podocyte lifespan and health-span.

4 STING ACTIVATION BY MTDNA TRIGGERS PODOCYTE INJURY IN DKD

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Background/Aims: Diabetic kidney disease (DKD) remains the most common cause of end stage kidney disease in the USA, and is characterized by mitochondrial dysfunction, increased circulating serum mtDNA levels, and activation of STING, a DNA-sensing innate immune adaptor, expressed in podocytes. This study aims to investigate mechanisms of cytosolic mtDNA escape in DKD and their role in glomerular injury.
Methods: 16-week-old control (db/+) and diabetic (db/db) mice were used. Glomeruli were isolated to determine the expression of OXPHOS complex subunits, TFAM, PINK-1, BAK/BAX, and VDAC. Mitochondrial morphology was evaluated by TEM. Plasma and urine mtDNA were measured. Control and STING KO mice were treated with nDNA and mtDNA followed by analysis of pSTING/STING expression, body weight, and ACR.
Results: We found TFAM and PINK1 expression to be altered in db/db mice, with no changes in BAK/BAX expression. Mitochondria appeared circular on TEM analysis, suggesting mitochondrial dysfunction, which was confirmed by increased expression of OXPHOS complex II (SDHA). Db/db mice were also found to have increased mtDNA in plasma and urine. Treatment with mtDNA, but not nDNA were associated with increases in pSTING expression and the development of proteinuria.
Conclusion: Our data suggest that TFAM deficiency may contribute to PINK1 accumulation, altered mitophagy and mtDNA presence in the cytosol of podocytes. This, in turn, may lead to STING activation, unresolved inflammation and cell death. Targeting of the relevant pathways of mtDNA escape in podocytes may lead to new treatment options for DKD patients.

5 TRPC6-MEDIATED CALPAIN ACTIVATION IMPAIRS PODOCYTE AUTOPHAGY AND CAUSES PODOCYTE INJURY IN DIABETIC KIDNEY DISEASE

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Background/Aims: Diabetic kidney disease is associated with impaired podocyte autophagy and subsequent podocyte injury. The molecular mechanisms underlying the impaired autophagy in podocytes in diabetic kidney disease remain largely elusive. This study investigated how the calcium channel TRPC6 and the cysteine protease calpains deleteriously command podocyte autophagy in diabetic kidney disease.
Methods: Conditionally immortalized mouse podocytes (MPC-5) were transfected with a TRPC6 shRNA construct to knock-down TRPC6.
Diabetic kidney disease was induced in vivo in mice by using the streptozotocin-induced diabetes model upon unilateral nephrectomy and the BTBRob/ob model.
Human kidney biopsies from diabetic patients with diabetic kidney disease and control biopsies were stained.
Results: TRPC6 knockdown in podocytes increased the autophagic flux due to decreased calpains activity. In mice, diabetes resulted in increased TRPC6 expression in podocytes together with a decreased podocyte autophagic flux. Transgenic overexpression of the endogenous calpain inhibitor calpastatin as well as pharmacological inhibition of calpain activity normalized podocyte autophagic flux, reduced nephrin loss and prevented the development of albuminuria.
Conclusion: We discovered a new mechanism that connects TRPC6 and calpain activity to impaired podocyte autophagy, increased podocyte injury, and the development of proteinuria in the context of diabetic kidney disease. Therefore, targeting TRPC6 and/or calpain might be a promising therapeutic strategy treating diabetic kidney disease by restoring podocyte autophagy.

6 URINARY PLASMINOGEN AS A MARKER OF DISEASE PROGRESSION IN HUMAN GLOMERULAR DISEASE

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Background/Aims: Glomerular disorders have a highly variable clinical course and biomarkers that reflect molecular mechanisms underlying progression are needed. Serine proteases such as plasminogen and plasmin (active form) are elevated in the urine of patients with proteinuric disease due to the disruption of the glomerular filtration barrier. Our previous work identified plasminogen as a direct cause of podocyte injury via CD36 and oxidative stress mechanisms and established a correlation between urinary plasminogen levels and renal dysfunction at the time of kidney biopsy. We hypothesized that excessive trans-glomerular plasminogen may serve as a “second hit” in glomerular disease progression independent of the initial insult. This study evaluates the association between urinary plasmin(ogen) and longitudinal kidney outcomes in patients with biopsy-proven glomerular disease.
Methods: The study included 1010 patients enrolled in the CureGN Cohort with biopsy confirmed diagnosis of FSGS, MN, or IgAN. A de novo electrochemiluminescent immunoassay (ECLIA) was developed to measure urine plasminogen levels. Cox regression was used to examine the association between plasmin(ogen)uria and time to end stage kidney disease (ESKD).
Results: Log2uPlasminogen/Cr was significantly associated with ESKD (HR per doubling uPlasminogen/Cr 1.23, 95% CI 1.11-1.34, $P < 0.001$) adjusted for age, sex, race, eGFR, proteinuria and diagnosis. When analyzed via quintiles, the 4th vs. 1st (HR 4.89, 95% CI 2.03-11.80) and 5th vs. 1st (HR 12.22, 95% CI 5.28-28.29) quintiles of uPlasminogen/Cr) were associated with ESKD. The cut-point for the concentration of uPlasminogen/Cr that maximized the product of sensitivity and specificity to predict the 36-month cumulative incidence of progression to ESKD was 1183.1 µg/g with a sensitivity of 81% and specificity of 80%. ROC curves based on predicted probability of 36-month cumulative incidence of ESKD comparing three models (Clinical variables, Clinical variables + Log2UPCR and Clinical variables + Log2UPCR + Log2uPlasminogen/Cr) showed that the AUC of the model with plasmin(ogen)uria was significantly higher than the model with clinical variables + proteinuria: AUC (0.85 (0.81-0.89) vs. 0.89 (0.85-0.92), $P = 0.033$).
Conclusion: Our study identifies urinary plasminogen as a potentially useful noninvasive biomarker that can assist in prognosticating glomerular disease progression. In combination with our previous experimental findings, plasmin(ogen) could be a target for the development of podocyte specific therapies in patients with proteinuria and glomerular disease.

7 YAP-DEPENDENT EPITHELIAL: STROMAL INTERACTIONS IN THE KIDNEY GLOMERULUS ARE DEPENDENT ON INJURY DRIVEN CHANGES IN THE INTEGRIN REPERTOIRE

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Background/Aims: The kidney glomerulus is a unique microenvironment in which capillary loop architecture is maintained by epithelial-stromal interactions between podocytes and mesangial cells. A glomerular filtration barrier allows blood to be filtered, retaining proteins and cells in the circulation. Loss of normal architecture and failure of this barrier results in life-threatening kidney disease. Podocytes are a terminally differentiated cell within the glomerulus that express YAP and TAZ, the transcriptional effectors of HIPPO signaling. We hypothesized that changes in cell adhesion affect the roles of YAP and TAZ as transcription factors that mediate transcriptional reprogramming during the response to injury.
Methods: We performed podocyte specific inducible knockouts of Yap and Tax (Wwtr1) in adult mice. Immunostaining was used to localize YAP and TAZ during the response to injury. Loss of a3b1 Integrin in podocytes of adult mice was used to study how adhesion affects nuclear localization of YAP.
Results: Loss of both YAP and TAZ in podocytes of adult mice rapidly leads to loss of the filtration barrier and collapse of capillary loop architecture. Nuclear localization of YAP increases in response to podocyte injury whereas inactivation of Yap in podocytes of adult mice renders them more sensitive to injury. . YAP and TAZ are required to maintain the glomerular filtration barrier. Loss of YAP in podocytes confers increased sensitivity to injury. a3b1 and avb5 are two prominent integrins on murine podocytes that have distinct localization patterns. avb5 integrin increases in abundance along the capillary loops after injury. Loss of a3b1 integrin in podocytes results in increased abundance of avb5 integrin along capillary loops and increased nuclear localization of YAP. Using immortalized podocytes, blockade of avb5 integrin or focal adhesion kinase reduced nuclear localization of YAP.
Conclusion: YAP and TAZ are required to maintain the glomerular filtration barrier and play a role in the podocyte response to injury. Integrin localization on podocytes changes during the response to injury and appears to have a role in driving nuclear localization of YAP.

Glomerular physiology

1 MECHANICAL FORCES AT THE KIDNEY FILTRATION BARRIER GOVERN SPATIAL ORIENTATION OF PODOCYTE PROCESSES ON CAPILLARIES

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Background/Aims: We have previously described that the main role of podocyte foot processes is to counteract capillary wall distension, thereby compressing the glomerular basement membrane to optimize sieving properties. Here, we expand on these studies by investigating how the spatial orientation of podocyte processes might play a role in the biophysics of kidney filtration.
Methods: New imaging protocols developed by our group allow for 3D optical imaging at a resolution and throughput sufficient to resolve several thousands of primary and secondary podocyte processes on glomerular capillaries. This opens up for the first ever quantitative analysis of whether or not podocyte processes display a preferred spatial orientation on capillaries, a question which has been debated among kidney researchers for decades.
Results: We found a significant preference for secondary foot processes to be oriented in parallel with the capillary orientation and for primary processes to be oriented perpendicularly to the capillary orientation. The orientation preference was neither observed in newborn mice nor in two different glomerular disease models of different origin. Further, the orientation preference was more significant in straight capillary segments, which to a larger extent resemble a cylindrical geometry as compared to curved capillary segments.
Conclusion: For any pressurized object which resembles a cylinder in shape, such as a capillary, the wall stress will be highest along the circumferential axis. Based on this, we conclude that the non-random orientation of podocyte processes likely originates from the difference in wall stress between the circumferential and longitudinal axes. According to our previous models, a high density of foot processes is needed to provide buttress forces in order to counteract wall stress. The observed orientation would maximize the density of foot processes along the circumferential axis, where the need for counteracting wall stress is highest. In addition, the massive flow of fluid over the filtration slit will result in forces acting to narrow the slit diaphragm. The observed orientation of foot processes positions the slit diaphragm such that the higher circumferential wall stress is utilized to counteract such forces.

2 SHEAR STRESS ON PODOCYTE FOOT PROCESSES ARISING FROM FLOW IN FILTRATION SLITS STUDIED BY NUMERICAL FLOW SIMULATIONS

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Background/Aims: The glomerular filtration barrier is exposed to flow dynamic forces arising from filtration pressure (tensile stress) and filtrate flow (shear stress). Filtrate flow acts on podocyte cell bodies in Bowman's space and on foot processes (FPs) lining the filtration slit. Besides a previous estimate (Endlich & Endlich, Semin. Nephrol. 2012), the magnitude of shear stress to FPs remains unknown.
Methods: We used numerical flow simulations to study forces arising from glomerular filtration. Simulations were run with a realistic model of a filtration unit and the corresponding filtration parameters of the rat kidney. The filtration unit consists of fenestrated endothelium, the GBM, and two opposing halves of FPs bridged by the slit diaphragm (SD). The GBM and SD were regarded as porous media.
Results: Modeling the GBM and SD as one homogenous porous medium, a peak wall shear stress of 65.2 Pa acting on FPs in the filtration slit was found; pressure dropped by 2.5 mmHg across the SD. Increasing filtration slit width from 30 to 40 nm reduced peak wall shear stress by only 9% to 59.4 Pa. Modeling the GBM and SD as two separate homogenous porous media with an increased viscous resistance of the SD further increased the pressure drop across the SD, but also wall shear stress on FPs.
Conclusion: Our data demonstrate that FPs are likely to experience high levels of wall shear stress that exceed levels of endothelial wall shear stress. Shear stress on FPs represents the only flow dynamic force that directly tends to disconnect viable podocytes from the GBM–a hallmark of podocyte loss in many glomerular diseases.

Glomerulus: novel disease models or imaging methods

1 A NOVEL IN VIVO HIGH-CONTENT DRUG SCREENING ASSAY IDENTIFIES A PAN-HDAC INHIBITOR AS PODOCYTE-PROTECTIVE IN A FSGS-LIKE ZEBRAFISH MODEL

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Background/Aims: Focal segmental glomerulosclerosis is a rare disease caused by damage and depletion of podocytes. Drugs that protect podocytes are missing until today. Novel and effective screening assays are needed to identify potential drugs to treat patients. The aim of this work was to develop an imaging-based in vivo high-content screening assay in a FSGS-like podocyte injury model in zebrafish larvae.
Methods: Treatment of a screening strain with 80 µM metronidazole (MTZ) for 24 hours results in a FSGS-like phenotype. Proteinuria and podocyte depletion is detected with a screening microscope and measured with custom codes. A 138 drug / compound library was screened for protective effects and substances that prevented proteinuria or podocyte depletion were considered as hits and were further validated.
Results: Screening of a 138 epigenetic drug / compound library revealed 14 podocyte-protective candidates. One candidate which belongs to the family of pan-HDAC inhibitors was validated in a larger zebrafish larvae group. Drug 171 could be confirmed to ameliorate proteinuria and podocyte depletion significantly and prevent subsequent edema formation in a dose-dependent manner. Structurally similar drugs / compounds could recapitulate the protective effect in this specific podocyte injury model.
Conclusion: A semi-automated in vivo high-content screening system that automatically detects proteinuria and podocyte depletion on a large scale was successfully established. This novel drug screening assay is able to identify podocyte-protective drugs / compounds. The pan-HDAC inhibitor Drug 171 and similar drugs / compounds were confirmed as podocyte-protective agents in a FSGS-like zebrafish model.

2 A NOVEL VASCULARIZED HUMAN KIDNEY ORGANOID TO STUDY PODOCYTE AND ENDOTHELIAL HEALTH AND DISEASE

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Background/Aims: Increasing the vascular density in human kidney organoids is critical for mechanistic studies in kidney health and disease. Particularly in the glomerulus, where endothelial cells and podocytes interact in a highly regulated way, which, when dysregulated, drives various pathophysiological outcomes. Many methods for vascularizing in vitro kidney models have been taken, however, few have been robust, reliable, high-throughput, and can be used to study podocyte/endothelial morphology and morphological changes in injury settings.
Methods: Using a combinatorial approach, a doxycycline inducible ETV2 iPSC line was combined with a naïve iPSC line to generate highly vascularized human kidney organoids.
Results: Here, we show these organoids generate a robust endothelial cell network that invaginates into podocyte cell clusters, and on electron microscopy form glomerular basement membrane between interdigitated foot processes as well as highly organized fenestrations on endothelial cells. With snRNAseq we demonstrate that vascularized podocytes exhibit enhanced VEGF signaling, basement membrane formation, glomerular development signatures, and endothelial differentiation and migration markers. Lastly, we demonstrate that vascularization of human kidney organoids enables the formation of a functional interstitium with a drug responsive set of renin cells. These renin cells were confirmed to exist within podocyte clusters of vascularized kidney organoids only. Finally, in previous work, our group has identified HDAC8 as a potential target for the regulation of renal health and disease. In our current studies, we demonstrate the HDAC8 pathway is a potential targeted pathway to regulate mesenchymal transition of endothelial cells and podocytes. Here, we demonstrate the upregulation of mesenchymal transition markers in patients with glomerular disease, and show in our kidney organoid injury model that HDAC8 inhibition results in the amelioration of this disease process.
Conclusion: Overall, this work demonstrates the generation and validation of a vascularized human kidney organoid model that can be used for mechanistic studies of podocyte and endothelial health and disease.

3 HIGH-RESOLUTION IMAGING AND DEEP LEARNING-BASED SEGMENTATION OF GLOMERULAR FILTRATION BARRIER PATHOLOGIES

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Background/Aims: Current routine examination of kidney pathology involves many different imaging protocols and most assessments of pathological alterations are carried out in a manual and qualitative manner. We present a combination of imaging and analysis tools which allows for an automated and quantitative three-dimensional assessment of pathologies in the glomerular filtration barrier.
Methods: We applied imaging protocols, previously published by us, for three-dimensional high-resolution imaging of the glomerular filtration barrier. In those protocols, podocyte foot processes and the glomerular basement membrane are selectively visualized using fluorescent probes. An automated quantitative assessment of a wide range of foot processes and GBM pathologies was then made using image analysis based on deep learning networks.
Results: We here show that a wide range of quantitative parameters which in detail describe glomerular filtration barrier pathologies can be extracted using our approach. Recent results show that, in addition to podocyte foot processes, also the glomerular basement membrane can be automatically segmented in three dimensions. This is important in order to improve the characterization of morphological alterations, increase data throughput and to remove sectioning artifacts that can occur in two-dimensional methods (such as electron microscopy) which results in more accurate measurements of volumetric parameters, such as thickness.
Conclusion: High-resolution three-dimensional imaging and image analysis provides a novel and unique tool for quantitatively assessing filtration barrier pathologies. These methods can possibly be used in future clinical pathology for more accurate diagnostics and to stratify patients for different treatments.

4 INVESTIGATING PODOCYTE INJURY IN ACUTE MURINE KIDNEY SLICES BY COMBINING MULTIPHOTON WITH SUPER-RESOLUTION MICROSCOPY

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Background/Aims: Dysregulation of podocyte calcium homeostasis is causing kidney disease. The direct effects of persistently increased podocyte calcium on the slit diaphragm remain elusive. Therefore, we established a co-imaging workflow combining multiphoton and STED microscopy in acute murine kidney slices. Our approach allows correlating functional measurements with structural information in an ex vivo model.
Methods: Healthy control or diseased mice with podocyte-specific expression of GCaMP3 were sacrificed. Acute kidney slices were cut using a vibratome. The slices were imaged with multiphoton microscopy to measure GCaMP3 fluorescence intensity. For subsequent identification of glomeruli, reference points were created by laser damage in the tissue. The slice was then fixed and prepared for STED microscopy.
Results: Using both multiphoton and STED microscopy consecutively revealed no correlation between slightly elevated intracellular calcium levels in podocytes due to nephrotoxic serum nephritis and slit diaphragm morphology within single glomeruli. However, robustly elevated calcium levels were accompanied by destruction of the slit diaphragm.
Conclusion: This new workflow allows imaging of the same glomerulus with two microscopy techniques to combine their advantages. We matched functional, intracellular calcium measurements with structural impairments of the slit diaphragm, visible only with super-resolution microscopy. We are convinced that our co-imaging method using acute kidney slices is applicable to a variety of podocyte research questions.

5 PATIENT IPSC-BASED PHENOTYPIC AND TRANSCRIPTOMIC SCREENING OPTION TO UNRAVEL NEW TARGETS AND PATHWAYS IN CONGENITAL NEPHROTIC SYNDROME

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Background/Aims: Prof. Nishinakamura's group previously generated pluripotent stem cell (iPSC)-derived kidney organoids from a patient suffering from a congenital nephrotic syndrome caused by the mutations (c.G1379A, c.2515delC) in the nephrin (NPHS1) gene. We further developed this system into a robust, reproducible, and high-throughput organoid assay useful for the progression of drug discovery programs.
Methods: To enable readouts in 384w, we scaled up the production of iPSC-podocytes and iPSC-kidney organoids. We also developed and applied a 3D whole mount immunostaining readout to quantify the phenotypic signature of iPSC-derived podocytes and kidney organoids. Bulk ScreenSeq™ and single-nucleus sequencing was applied to characterize the transcriptomic profiles of both systems.
Results: A specific lack of nephrin phosphorylation was confirmed via automated western blotting. Besides, our kidney organoids completely lacked nephrin signals in synaptopodin-positive regions of glomerular structures. We further quantified the general nephrin deficit with a new script-based whole mount imaging analysis. Finally, by comparing transcriptomic disease signatures, we showed the translational relevance of these organoids for drug discovery in the field of podocytopathies.
Conclusion: We successfully developed and characterized a patient-derived kidney organoid assay and made it available in a high-throughput format. This translational system will prove useful in the identification and validation of candidate targets and for the progression of new kidney therapeutics.

6 TYPE 2 DIABETIC NEPHROPATHY (T2DN) RAT AS A MODEL TO STUDY PODOCYTES IN THE SETTING OF DIABETIC NEPHROPATHY

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Background/Aims: Diabetic kidney disease (DKD) is the leading cause of chronic renal pathology. Understanding the molecular underpinnings of DKD is critical to designing tailored therapeutic approaches. We have demonstrated previously that type 2 diabetic nephropathy (T2DN) rats develop renal and physiological abnormalities similar to clinical observations in humans with DKD (Palygin et al., 2019). Furthermore, our recent studies revealed sexual dimorphism in this model, indicating that while both male and female T2DN rats develop a non-obese DKD phenotype, females have significant protection from the development of severe forms of glomerular and tubular damage (Spires et al., 2021).
Methods: Here we describe the use of this model to study glomerular diseases and podocyte function. Special emphasis was paid to sex differences and the contribution of aging to the progression of diabetic nephropathy (DN). To explore glomeruli and podocyte function in the development of DN, we utilized young (12-week-old) and aged (>48 weeks-old) T2DN rats. Male and female T2DN rats were used to evaluate hyperglycemia, renal injury, and podocyte function during the progression of DN. To delineate transcriptional changes, RNA-Seq analysis was performed in the kidney cortex of T2DN rats of both sexes at a younger and older age.
Results: The development of albuminuria and glomerular damage was significantly different between sexes. These changes were accompanied by a significant increase in urinary nephrin shedding in males, suggesting pathological changes at the glomerular filtration barrier from loss of podocyte foot processes. Scanning Ion Conductance Microscopy (SICM) was further applied to study morphological changes in cultured podocytes and freshly isolated glomeruli. Using 3D visualization and topographical quantification of the length and irregular shape of the podocyte foot processes, we showed an absence of interdigitated foot processes on a selected region of the glomerular capillary loop in isolated T2DN glomeruli.
Conclusion: Our study revealed that T2DN rats develop renal and physiological abnormalities similar to clinical observations in human patients with DN, including progressive glomerular damage, indicating these rats are an excellent model for studying the glomeruli and podocyte function during the progression of DN.

Immunobiology of glomerular disease (including complement cascade biology)

1 ANTI-NEPHRIN ANTIBODIES IN MINIMAL CHANGE DISEASE

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Background/Aims: The pathogenesis underlying Minimal Change Disease (MCD) remains incompletely understood. Recently, antibodies against nephrin, a key protein of the podocyte slit diaphragm, were identified in 29% of patients with MCD. So far, this finding has not been reproduced independently. Our study aims to validate and further examine the presence and pathophysiologic role of anti-nephrin antibodies in MCD.
Methods: We performed a blinded screening of serum samples from 73 patients with MCD, 67 patients with FSGS, 30 patients with IgA nephropathy (IgAN) and 30 patients with membranous nephropathy (MN) from the Hamburg Glomerulonephritis Registry, in comparison to 67 healthy controls. Anti-nephrin antibodies were determined by immunoprecipitation using recombinant nephrin and subsequent western blotting.
Results: We detected anti-nephrin antibodies in 29/73 (39.7%) sera from patients with MCD, 1/67 (1.5%) sera from patients with FSGS, and 1/30 (3.3%) sera from patients with MN. Sera from 30 patients with IgAN as well as from 67 healthy controls were negative. MCD patients positive for anti-nephrin antibodies had significantly lower serum albumin, higher proteinuria, and more severe dyslipidemia compared to anti-nephrin antibody negative MCD patients.
Conclusion: We could independently reproduce the presence of anti-nephrin antibodies in sera from patients with MCD. Anti-nephrin positive patients had more severe disease than anti-nephrin negative patients. Further studies are necessary to better understand the pathomechanisms underlying MCD and to assess the value of antibody detection for diagnosis, prognosis, and monitoring of therapy.

2 BTLA STIMULATION ALLEVIATES EXPERIMENTAL CRESCENTIC GLOMERULONEPHRITIS

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Background/Aims: Treatment of acute, crescentic GN consists of unspecific, potentially toxic immunosuppression. T cells are central in the pathogenesis of GN, and various checkpoint molecules control their activation. Modulating T lymphocytes through manipulation of these checkpoint molecules, thus, may represent a promising targeted therapeutic option for GN . The immune checkpoint molecule B and T lymphocyte attenuator (BTLA) has shown its potential to restrain inflammation in other T cell-mediated disease models. Its role in GN, however, has not been investigated. We hypothesised that BTLA activation may lead to a downregulation of nephritogenic T cells and dampens glomerular inflammation.
Methods: Nephrotoxic nephritis (NTN) was induced in Btla-deficient (BtlaKO) mice and littermate controls (WT), and disease severity was assessed using functional as well as histological parameters at different time points after disease induction. Immunological changes were comprehensively evaluated by flow cytometry, single cell RNA sequencing and in vitro assays for dendritic cell and T cell function. Transfer experiments into Rag1KO mice confirmed the observed in vitro findings. Lastly, the potential of an agonistic anti-BTLA-antibody to treat experimental GN was evaluated in vivo.
Results: BtlaKO mice develop aggravated NTN, driven by an increase of infiltrating renal Th1 cells. Single cell RNA sequencing showes increased renal T cell activation and positive regulation of immune response. While BTLA-deficient Tregs exhibit preserved suppressive function in vitro and in vivo, BtlaKO T effector cells evade Treg suppression. Administration of an agonistic anti-BTLA antibody robustly attenuates NTN by suppressing nephritogenic T effector cells and promoting Treg expansion.
Conclusion: BTLA signaling effectively restrains nephritogenic Th1 cells and promotes regulatory T cells in experimental crescentic glomerulonephritis. Suppression of T cell- mediated inflammation by BTLA stimulation may prove relevant for a broad range of acute GN.

3 DECIPHERING THE CONTRIBUTION OF INFLAMMATORY MACROPHAGES TO FOCAL SEGMENTAL GLOMERULOSCLEROSIS

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Background/Aims: Focal segmental glomerulosclerosis (FSGS) refers to a histologic pattern of glomerular injury that results from different disease entities, which in turn respond differently to therapeutic regimens. Causes for FSGS include immunological diseases that respond to immunosuppressive treatments. Modern biotechnological tools allow us to characterize diseases resulting in an FSGS pattern in detail with the prospect of providing pathophysiology-based precision medicine for the patients.
Methods: Here, we use the genetic model of heterozygous compound mutations in Nphs2 that features an FSGS phenotype and develops strong infiltration of immune cells into the glomerulus. We employed bulk and single nucleus RNA sequencing of glomerular isolates, flow cytometry, and immunohistochemistry to profile these immune cells in depth.
Results: We identified mononuclear phagocytes to be the most abundant immune cells in diseased glomeruli. A macrophage subpopulation with an inflammatory and tissue-remodeling transcriptomic signature significantly expanded over time. We confirmed this intraglomerular infiltration of macrophages in immunohistochemistry staining and saw signs of translocation to the tubulointerstitium at a later stage of the disease. Transcriptomic ligand-receptor-target gene network analysis indicated progressively altered signaling between glomerular cells resulting in a pro-fibrotic inflammatory milieu characterized by strong signaling activity of the TGFb-superfamily and upregulation of CC-chemokines and matrix metalloproteinases. Intriguingly, fractalkine (CX3CL1), a macrophage recruiting chemokine, was strongly upregulated at an early stage of the disease. Immuno-staining localized fractalkine to glomerular endothelial cells while flow cytometry showed significant upregulation of its receptor CX3CR1 on macrophages.
Conclusion: In this model, mutations in a slit-diaphragm protein result in FSGS with prominent glomerular inflammation and strong macrophage infiltration that may be linked to fractalkine signaling. Based on these results, defining macrophage function in FSGS might provide the rationale for new therapeutic approaches.

4 KIDNEY C5AR IS EXPRESSED IN RESIDENT MACROPHAGES, TUBULAR EPITHELIAL CELLS, AND IN ASSOCIATION WITH FIBROSIS

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Background/Aims: Identifying the cellular targets of avacopan, a potent antagonist of C5a receptor (C5aR), is critical for understanding the mechanism of action in kidney disease; however, published reports on C5aR expression in the kidney vary widely (Abe et al., 2001; Yuan et al., 2012). To address this issue, we investigated the mRNA and protein expression of C5aR in human and mouse kidneys.
Methods: We performed in situ hybridization (ISH) on formalin-fixed, paraffin embedded tissue blocks using mRNA probes for C5aR and C5L2 and immunohistochemistry (IHC) with validated C5aR antibodies. Macrophage (CD68) and tubule subpopulation (CALB1, SLC13A3) antibodies were validated for IHC and dual IHC-ISH. Kidney tissues were from normal human subjects and lupus nephritis (LN) patients, or from mice.
Results: Using both ISH and IHC we observed expression of C5aR in interstitial and tubular cells. The cells expressing C5aR were macrophages and a discrete subpopulation of tubule epithelial cells. C5L2 was observed in the same kidney cell types, but with lower expression. The human C5aR knock-in mouse kidney had a similar expression pattern to human. C5aR expressing cells localized to areas of fibrosis in kidney disease models and LN biopsies.
Conclusion: In both mouse and human kidneys, we observed C5aR expression on macrophages, on a subpopulation of tubular epithelial cells, and in association with fibrosis. C5aR expression in the kidney is consistent with its role in inflammation and tissue remodeling, and suggests the hypothesis that treatment with avacopan may reduce inflammation-driven fibrosis in disease.

Membranous nephropathy: pathobiological mechanism, genetics

1 ROLE OF NON-ANTI-PLA2R AUTOANTIBODIES IN MEMBRANOUS NEPHROPATHY

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Background/Aims: In primary membranous nephropathy an increasing number of antigens and pathogenic antibodies have been described, most notably antibodies directed against podocyte antigen PLA2R. However the course of the disease can be extremely variable with approximately 30% remitting spontaneously, 30% with stable CKD and proteinuria and 30% inexorably progressing to renal failure. While titers of the PLA2R autoantibody are correlated with prognosis, other secondary autoantibodies could play a role in enhancing pathogenicity or of compounding complement mediated podocyte injury. Anticardiolipin antibodies are a class of antiphospholipid antibody that are found elevated in a variety of proteinuric kidney diseases and renal allografts and have been demonstrated to have prognostic significance. We hypothesized that sera from membranous patients with elevated titers of ACLA would bind to an immortalized podocyte lineage, which does not expression the PLA2R antigen. This secondary autoantibody would cause compounded injury to the podocyte via complement fixation and would correlate with clinical outcomes.

Methods: We obtained serum samples from a cohort of patients diagnosed with biopsy proven membranous nephropathy and preformed ELISAs against anti-cardiolipin IgG and IgM antibodies in serum. We then incubated the sera for 1 hour at 37C on an immortalized podocyte lineage and washed with 1% BSA in PBS as a blocking agent. As controlled we incubated the cells with fetal bovine serum, podocyte media with no serum and control human serum obtained from presumably healthy cohort. We then lifted the cells off the plate with Accutase and incubated the cells with a mouse anti-human IgG or IgM attached to a fluorophore for 1 hour at 4C. We then preformed flow cytometry for anti-human IgG and IgM.

Results: Sera of patients with membranous nephropathy showed elevated levels of IgG and IgM anticardiolipin antibodies compared to healthy controls. Additionally there appears to be a cohort with membranous who have markedly elevated levels of anticardiolipin. Additionally, these levels were elevated when compared to serum from patients with CKD, FSGS and minimal change disease. By flow cytometry some of the membranous nephropathy samples showed significantly increased IgG binding compared to controls of human sera, FBS or no sera. This IgG binding was of variable amount and not found in every membranous sample. No differences in IgM binding were found. Interestingly, the ELISA data showed that while both anticardiolipin IgG and IgM were elevated in membranous samples, the difference for IgM was relatively minor compared to IgG.

Conclusion: Here we show that a subset of membranous patients have elevated levels of IgG, and to a lesser extent IgM, anticardiolipin antibodies in their sera compared to healthy controls, as well as patients with CKD and other proteinuric diseases. Additionally we have shown that an IgG autoantibody, but not IgM, in the sera from membranous patients strongly binds immortalized podocytes that do not express PLA2R. These findings together suggest that a secondary autoantibody either generated from the initial insult or already present in the patient binds strongly to podocytes.

To further elucidate the antigen and antibody we plan to preform immunofluorescence using sera that was found to have IgG binding via flow to see if there is clustering of the autoantibody on the surface of the podocyte. We also plan to preform flow cytometry to evaluate what IgG class this antibody belongs to. Finally we will obtain clinical data to see if any correlation can be made between samples which had antibody binding and clinical outcome, response to therapy, remission rates and PLA2R levels.

2 A MOUSE MODEL OF SERINE PROTEASE HTRA1 - ASSOCIATED MEMBRANOUS NEPHROPATHY

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Background/Aims: Prototypical podocyte autoantigens in membranous nephropathy (MN) are surface-expressed transmembrane proteins like PLA2R or THSD7A. More recent antigens may be secreted or not even normally expressed by the podocyte. We sought to investigate the ability of the secreted podocyte protein serine protease HTRA1 to contribute to in situ immune deposits in an animal model of MN.
Methods: WT and global HTRA1 knockout (KO) mice were actively immunized with human HTRA1. Glomerular immune deposits were analyzed via immunofluorescence and confocal microscopy, and podocyte HTRA1 expression was analyzed via RNAscope microscopy. The presence and specificity of circulating anti-HTRA1 was assessed.
Results: HTRA1 and mouse (m) IgG co-localized in a pattern typical of MN in the immunized WT, but not KO, mice, despite the presence of circulating anti-HTRA1 in both animals. Immunized WT mice developed proteinuria. RNAscope suggested increased podocyte expression of HTRA1 mRNA in the WT disease model vs unimmunized WT mice.
Conclusion: The presence of HTRA1-mIgG deposits in actively immunized WT but not KO mice, along with increased podocyte expression of HTRA1 by podocytes, argues against deposition of circulating immune complexes and instead argues for a podocyte origin of this secreted autoantigen and formation of in situ immune deposits. We will further confirm this hypothesis using a podocyte-specific HTRA1 KO.

FSGS: pathobiological mechanism, genetics

1 ALTERNATIVE MRNA SPLICING AND POLYADENYLATION PROVIDE A NOVEL MECHANISM OF REGULATION OF THE GLOMERULAR FILTRATION BARRIER

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Background/Aims: Glomerular disease, often characterized by podocyte injury and proteinuria, can lead to chronic kidney disease and end stage kidney disease. We hypothesized that the glomerular pathophysiology can be regulated by alternative mRNA processing of critical podocyte, slit diaphragm and matrix genes associated with the glomerular filtration barrier by alternative splicing (AS) and polyadenylation (APA) events.
Methods: Glomerular damage, accompanied with proteinuria, hypoalbuminemia and hypercholesterolemia was induced by puromycin aminonucleoside (PAN) or adriamycin (ADR) to mimic human minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS), respectively. Glomerular RNA-seq analyses was performed followed by JunctionSeq and APATrap bioinformatics analyses to detect APA and AS glomerular events. Novel splice sites, differences in alternative splice forms, and alternative isoform regulation were queried in JunctionSeq and APATrap pipelines after high-quality transcript annotations.
Results: Altered glomerular mRNA processing by AS/APA was identified in 136/71 and 1875/746 genes in MCD and FSGS models, respectively. Transcript annotation and prioritization of significant AS and APA identified key events in genes known to cause disruption in the glomerular filtration barrier. These events localized in the regions of slit diaphragm (NPHS1, NPHS2), glomerular basement membrane (COL4A3/4/5, LAMB2, LAMA5), podocyte mitochondria (COQ2/6), podocyte membrane (PODXL) and endothelial cells (INF2). Concomitantly, numerous polyadenylation and splicing factors were differentially expressed in these two models of glomerular nephropathy.
Conclusion: Findings from our studies shed light on a new landscape of research in glomerular pathophysiology, i.e., AS and APA of glomerular genes, many of which are crucial determinants of podocyte structure and function, slit diaphragm complex, and glomerular basement membrane. These findings present new implications for improved mechanistic understanding of glomerular disease and development of novel therapeutic intervention strategies.

2 AN INTEGRATED ORGANOID OMICS MAP EXTENDS MODELING POTENTIAL OF KIDNEY DISEASE

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Background/Aims: Kidney organoids are a promising model to study kidney disease, but their use is constrained by limited knowledge of their functional protein expression profile. We aimed to define the organoid proteome and transcriptome trajectories over culture duration and upon exposure to TNFα, a cytokine stressor.
Methods: We used proteomics to compare organoids with existing model systems such as native glomeruli and cultured podocytes. We characterized the trajectory of organoid differentiation and delineated innate immune responses in organoids to expand their scope as a model system in nephrology. We also compared our proteomics with bulk and single cell transcriptomics data.
Results: Older organoids increased deposition of extracellular matrix but decreased expression of glomerular proteins. Single cell transcriptome integration revealed that most proteome changes localized to podocytes, tubular and stromal cells. TNFα-treatment of organoids affected 320 differentially expressed proteins, including cytokines and complement components. Transcript expression of these 320 proteins was significantly higher in individuals with poorer clinical outcomes in proteinuric kidney disease. Key TNFα-associated protein (C3 and VCAM1) expression was increased in both human tubular and organoid kidney cell populations, highlighting the potential for organoids to advance biomarker development.
Conclusion: By integrating kidney organoid omics layers, incorporating a disease-relevant cytokine stressor and comparing to human data, we provide crucial evidence of functional relevance of the kidney organoid model to human kidney disease.

3 ATF4 MEDIATES PODOCYTE INJURY RELATED TO ENDOPLASMIC RETICULUM STRESS

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Background/Aims: Mutations in the gene OSGEP, which is part of a protein complex required for posttranslational modifications of tRNA (transfer RNA), cause an inherited renal-neurological disease that manifests which an FSGS-phenotype. This project's aim was to elucidate the molecular pathogenic mechanisms of podocyte injury in this disease and to test endoplasmic reticulum (ER) stress as a therapeutic principle.
Methods: We used nephrocytes of Drosophila melanogaster to assess how loss of function of Tcs3 (OSGEP's fly orthologue) affects the nephrocyte's slit diaphragm and to test pharmacological interventions and genetic interaction. Further, we performed RNASeq in OSGEP-depleted human podocytes and validated the fly findings in this model.
Results: Knockdown of Tcs3 caused slit diaphragm defects in nephrocytes. ATF4, an ER-stress mediating transcription factor was unregulated in both nephrocytes and human podocytes, upon knockdown of OSGEP/Tcs3 and transcriptome analysis revealed an upregulation of genes related to ER stress. Genetic and pharmacological inhibition of ATF4-signaling mitigated apoptosis and slit diaphragm defects of Tcs3 knockdown nephrocytes and improved the survival of human podocytes exposed to ER stress.
Conclusion: Our data underline the role of ER stress in podocyte injury and suggest ATF4-related signaling as a promising therapeutic target for certain glomerular diseases.

4 FVB/N-NOS1APEX3-/EX3- MICE DEVELOP SEVERE GLOMERULAR KIDNEY DISEASE, WHICH IS AMELIORATED BY ANTIPROTEINURIC TREATMENT

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Background/Aims: Monogenic nephrotic syndrome (NS) is a leading cause of pediatric chronic kidney disease. We previously discovered recessive NOS1AP variants as a novel cause of early onset NS in children and observed moderate glomerular disease in Nos1apEx3-/Ex3- C57BL/6 mice (Sci. Adv. 7:1386, 2021). We next hypothesized that the Nos1apEx3-/Ex3- phenotype depends on the genetic background of the studied mice.
Methods: To test, if severity of the observed glomerular disease in Nos1apEx3-/Ex3- mice depends on the genetic background, we crossed the allele onto FVB/N and 129/sv backgrounds. Albumin-to-creatinine ratios (ACR), blood parameters of kidney failure (BUN, albumin), and histology by light microscopy were assessed. We further evaluated the effects of anti-proteinuric treatment (lisinopril and Bis-T-23).
Results: FVB/N-Nos1apEx3-/Ex3- mice developed increased albuminuria, beginning at weaning age with levels one order of magnitude higher than C57BL/6 and 129/sv mice at 3-6 months. Furthermore, FVB/N-Nos1apEx3-/Ex3- mice showed renal dysfunction, and increased mortality. Lisinopril treatment (100 or 200 mg/L lisinopril in drinking water) reduced albuminuria and prevented premature death in FVB/N-Nos1apEx3-/Ex3-. However, injections with dynamin regulator Bis-T-23 did not lead to any therapeutic effects.
Conclusion: FVB/N-Nos1apEx3-/Ex3- mice develop severe proteinuric kidney disease with increased serum parameters, accompanying histologic findings and premature death – all consistent with early onset NS in patients with NOS1AP variants. Disease progression, including premature death, in Nos1apEx3-/Ex3- mice were halted by ACE inhibition. We, thereby, strengthen the case of NOS1AP as a novel NS disease gene.

5 MITOCHONDRIAL BIOGENESIS AND THERAPEUTIC INTERVENTIONS IN PODOCYTOPATHIES USING NOVEL APPROACHES

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Background/Aims: Focal segmental glomerulosclerosis is a heterogeneous disease that can lead to kidney failure. Mitochondria, sensors of cellular stress, play a key role in podocyte homeostasis, and their dysfunction can lead to podocyte injury and death. Inducing mitochondrial biogenesis in podocytes by Formoterol, β2-adrenergic receptor agonist, is promising to treat podocyte injury using stem cell and mouse models, and Nano-delivery approach. This work investigates the role of mitochondria in podocytopathies (FSGS and MCD) using patient-derived induced pluripotent stem cells (piPSC) kidney organoids system and assess the efficacy of Formoterol to podocyte injury.
Methods: We obtain piPSC from patients with genetic-related podocytopathies (COQ8B and NPHS2 [podocin] pathogenic variants causing FSGS), and nongenetic primary podocytopathies (primary FSGS, MCD) compared to PAN-induced podocyte injury and healthy cohorts. Then, we screen Formoterol, to induce mitochondrial biogenesis, using the kidney organoid system of these podocytopathies models.
Results: Our single cell data from FSGS model using kidney organoids showed that mitochondria-related pathways are significantly downregulated in primary FSGS compared to control. Delineation of how the cellular stress and triggers cause podocyte injury can improve our understanding of the mechanism of FSGS progression. We used Formoterol to increase mitochondrial biogenesis (MB) and to restore mitochondrial functions.
Conclusion: Inducing mitochondrial biogenesis to treat podocyte injury is promising.
Understanding the metabolic needs of podocytes and defining the signaling molecules and pathways during podocyte injury will be crucial for discovery of other potential therapies to target specific pathways based on molecular analysis in these models. Using a patient-derived kidney organoid system as a drug screening model and targeting mitochondria in podocyte injury are novel models for drug screening and development.

6 PODOCYTE PROTEASE ACTIVATED RECEPTOR 1 ACTIVATION IN MICE CAUSES FOCAL SEGMENTAL GLOMERULOSCLEROSIS, AND MIRRORS HUMAN DISEASE SIGNALLING EVENTS

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Background/Aims: Idiopathic nephrotic syndrome (INS) is thought to be caused by a circulating factor. This effector molecule can cause recurrence of nephrotic syndrome in the graft in minutes. Previous work under the supervision of Professor Moin Saleem, has shown that the circulating factor could be a serine protease that signals via Par-1 (Harris et al, 2013). We also looked at the role of TRPC6.
Methods: Podocytes were used to explore the signalling responses to PAR-1 stimulation. We then developed a transgenic mouse that expresses an overactive form of the PAR-1 receptor. This mimics constant stimulation of the receptor by an exogenous effector molecule. We expressed this transgene under the control of both a developmental and inducible podocyte-specific promoter system.
Results: PAR-1 agonist, thrombin and relapse plasma treatment of human podocytes in vitro all significantly stimulated pVASP, pJNK and p38 MAPK. The same signalling was seen in the glomeruli of the NPHS2 Cre Par-1 active+/- mice. Interestingly we saw the same signalling in the glomeruli of human kidney biopsies from patients with INS. TRPC6 inhibition significantly reduced this signalling. The NPHS2 Cre Par-1 active+/- mice died of renal failure at 42 days. Knock out of TRPC6 increased lifespan by 50%.
Conclusion: Human relapse plasma and PAR-1 agonist treatments elicit the same signalling response suggesting that there is an effector molecule in the relapse plasma that can act on the PAR-1 receptor. Persistent signalling through the PAR-1 receptor leads to a nephropathy that histologically resembles human FSGS. We have identified the PAR-1 receptor and TRPC6 as important therapeutic targets in INS.

7 PRIMARY FIBROBLASTS AS A NOVEL ASSAY FOR THE FUNCTIONAL TESTING OF NEW α-ACTININ 4 VARIANTS IN GLOMERULAR DISEASE

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Background/Aims: Podocytes are highly specialized, terminally differentiated cells. Mutations in a variety of genes can cause disruption of normal podocyte biology and lead to glomerular diseases. α-actinin 4 (ACTN4) is a ubiquitously expressed protein that plays a major role in the organization of the cytoskeleton. In podocytes, ACTN4 is mainly localized in the foot processes and is substantial for the maintenance of the filtration barrier. Mutations in ACTN4 were discovered as monogenetic causes of glomerular diseases. Currently, functional testing heavily relies on the generation of stable cell lines harboring the ACTN4 variant or the transient transfection into cells.
Methods: ACTN4 variants were detected by Whole Exome Sequencing using an Illumina HiSeq 2500, and confirmed by Sanger sequencing. Primary fibroblasts were generated from sterile skin punctures. The full-length human ACTN4 cDNA sequence was cloned in pcDNA3.1, and mutations were generated using site-directed mutagenesis. Transfection in HEK293 cells was performed using Lipofectamine. Antibodies against ACTN4 were used in combination with phalloidin for immunofluorescence. For immunoblot, antibodies against GAPDH and ACTN4 were used. Migration assays were performed using removable cell culture inserts.
Results: We identified two novel ACTN4 variants (c.450C>G and c.459C>G) and tested these variants in vitro using primary fibroblasts. Five different platforms predicted impaired protein stability of both ACTN4 variants. Primary fibroblasts showed actin/ACTN4 agglomerates and fewer stress fibers compared to wildtype control fibroblasts. Using actin fractionation, actin/ACTN4 agglomerates could be confirmed using transfected HEK293 cells. Additionally, cell migration was reduced in patient fibroblasts.
Conclusion: We identified and characterized two novel disease-causing ACTN4 variants. We showed that primary fibroblasts are a useful in vitro model to evaluate the pathogenicity of ACTN4 variants in glomerular kidney diseases, although no specific skin phenotype is present in these patients.

8 REVEALING THE MOLECULAR NUANCES OF TRPC6 IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN AN IN VITRO HUMAN PODOCYTE MODEL

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Background/Aims: We aim to unravel the mechanism behind podocyte loss in FSGS, proposed to be caused by the gain of function (GOF) or loss of function (LOF) mutation in TRPC6, expressed in podocytes, and supplement the lost podocytes by producing them in vitro. We report a novel heterozygous TRPC6 mutation in a large kindred showing no signs of FSGS-related pathophysiology, encoding for a truncated TRPC6 protein.
Methods: The molecular effects of TRPC6 mutants were studied using the tridimensional cryo-EM structure of the tetrameric TRPC6 protein, calcium influx, and patch-clamp experiments.
Results: An inducible FSGS model system in hiPSCs was established by targeting the dual genomic safe harbor locus. The Tet-ON system allowed the controlled expression of the GOF/LOF TRPC6 proteins. These lines were induced to differentiate into podocytes and showed variable responses of Calcium influx. Furthermore, we assessed the impact of a novel TRPC6 mutant that encodes a non-functional, fully inactivated TRCP6 channel, which is consistent with a complete loss-of-function (LOF) phenotype.
Conclusion: Our novel systemic glomerulus platform offers a deeper understanding of the TRPC6-associated mechanism of podocyte loss by allowing the comparison between healthy and diseased podocytes in their morphology and behavior. This humanized and personalized system can provide helpful insights for future therapeutic interventions that can slow down or even abolish the progress of FSGS and other CKDs.

ApoL1: genetics, biology, disease

1 A SNARE PROTECTIVE POOL ANTAGONIZES APOL1 RENAL TOXICITY IN DROSOPHILA NEPHROCYTES

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Background/Aims: People of sub-Saharan African ancestry are at higher risk of developing chronic kidney disease (CKD), attributed to the APOL1 gene risk alleles (RA) G1 and G2. The underlying mechanisms by which the APOL1-RA precipitate CKD remain elusive.
Methods: We developed and performed a Drosophila genetic modifier screen to identify genes that altered APOL1-induced toxicity in nephrocytes, which are equivalent of mammalian podocytes. The top hits were then investigated in-depth for their roles in nephrocytes, with or without APOL1-induced toxicity. We also examined their cellular co-localization with APOL1 in COS-7 cells, and their APOL1 binding affinity in human kidney HEK 293T cells.
Results: We found that SNARE proteins Syx7, Ykt6 and Syb have a significant impact on APOL1-induced toxicity in nephrocytes. Reducing the expression of these SNARE proteins dramatically increased APOL1 cytotoxicity, whereas overexpression of Syx7, Ykt6 or Syb attenuated the toxicity of APOL1-G1 in nephrocytes. We also found that these SNARE proteins co-localized with APOL1 in a subset of kidney cell vesicular structures and showed higher binding affinity for APOL1-G0 than for APOL1-G1/G2.
Conclusion: Based on these findings, we propose a new model for the “protective” roles for certain SNARE proteins in the pathogenesis of APOL1 nephropathy and identified novel targets to develop drugs against these diseases.

2 COVID-19 VACCINATION IS NOT ASSOCIATED WITH INCREASED INCIDENCE OF PROTEINURIC KIDNEY DISEASE IN AFRICAN AMERICANS WITH HIGH-RISK APOL1 GENOTYPE

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Background/Aims: Cytokines induced by COVID-19 drive pathogenic APOL1 expression which can lead to podocytopathy and collapsing glomerulopathy in persons with apolipoprotein L1 high-risk genotype (APOL1 HRG). SARS-CoV-2 vaccination also increases systemic cytokines and has been associated with new and relapsing glomerular disease. The impact of APOL1 HRG on the risk of SARS-CoV-2 vaccine-associated glomerular disease is unknown.
Methods: To investigate the risk of new-onset glomerular disease following SARS-CoV-2 vaccination in persons with APOL1 HRG, we enrolled self-identified African American/Black with APOL1 HRG, age 50 years or older, with no prior kidney disease from the MURDOCK registry. Pre- and post-vaccine serum creatinine and urine protein-to-creatinine ratio (UPCR) were compared using paired two-tailed t-test.
Results: Of the 12,526 participants in the registry, 1507 self-identified as African American/Black and 174 met eligibility criteria. 26 of the 174 had APOL1 HRG. Final analysis included 21 of the 26 individuals whose vaccination history and post-vaccine laboratory studies could be obtained. All participants received a minimum of two doses of vaccine. We found no significant difference between the pre- and post-COVID-19 vaccination serum creatinine and UPCR. Less toxic APOL1 haplotypes do not explain the lack of new-onset kidney disease.
Conclusion: In this community-based cohort of individuals with APOL1 HRG, SARS-CoV-2 vaccination was not associated with new-onset glomerular disease. This result supports the renal safety of SARS-CoV-2 vaccine in individuals with APOL1 HRG.

3 ENDOTHELIAL CELL-SPECIFIC G2APOL1 EXPRESSION INDUCES HYPERTENSION VIA STING AND NLRP3 PATHWAYS

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Background/Aims: Genetic studies showed an association between ApolipoproteinL1 (APOL1) high risk (G1/G2) genotype and hypertension (HTN) and hypertensive kidney disease (H-CKD). The causal role of APOL1 in the development of HTN and H-CKD is not fully understood.
Methods: In the human kidney, APOL1 is highly expressed in endothelial cells and podocytes. We generated endothelial and podocyte-specific inducible G0 and G2 APOL1 transgenic mice by crossing the TRE-G2APOL1 and TRE-G0-APOL1 mice with the endothelial-specific Cdh5tTA and podocyte-specific (NPHS1rtTA) transgenic mice. We induced APOL1 expression by doxycycline diet. In the Cdh5tTA/TRE-G2APOL1 and control mice, we performed uni-nephrectomy (UNX) at five weeks age and kept mice on a 4% salt diet for 12 weeks. Blood pressure was monitored by the tail-cuff method. To understand the role of the inflammasome (NLRP3 and GSDMD) and the cytosolic nucleotide sensor (STING) in disease development, we crossed the STING, NLRP3, and GSDMD knock-out mice with the Cdh5tTA/TRE-G2APOL1 animals.
Results: Podocyte-specific G2APOL1 transgenic mice developed severe HTN only after proteinuria and renal damage was observed, likely representing a secondary HTN. Systolic, diastolic, and mean arterial blood pressure was mildly but significantly higher in Cdh5tTA/TRE-G2APOL1 mice compared to control mice. Cdh5tTA/TRE-G2APOL1 mice also developed a slight increase in urinary albumin/creatinine ratio and renal fibrosis after the animals became hypertensive. Hypertension, renal fibrosis, and proteinuria were ameliorated in STING, NLRP3, and GSDMD knock-out Cdh5tTA/TRE-G2APOL1 mice compared to Cdh5tTA/TRE-G2APOL1 mice. Our preliminary data indicate an increased cytosolic leakage of mitochondrial DNA in Cdh5tTA/TRE-G2APOL1 mice, which is likely responsible for the STING, NLRP3, and GSDMD activation in the Cdh5tTA/TRE-G2APOL1 mice.
Conclusion: EC-specific G2APOL1 transgenic mice developed mild salt-sensitive hypertension and renal damage, indicating the causal role of G2APOL1 in hypertension and hypertensive kidney disease, while podocyte-specific G2APOL1 was associated with secondary hypertension.

4 HIV-1 PROTEIN NEF ACTS IN SYNERGY WITH APOL1-G1 TO IMPAIR NEPHROCYTE FUNCTION BY INHIBITING AUTOPHAGY AND ENDOCYTOSIS PATHWAY

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Background/Aims: Individuals carrying APOL1 risk alleles G1 and G2 have higher chances of developing HIV-associated nephropathy (HIVAN). The role of APOL1 in HIVAN is mostly derived from studies done in cultured renal cells or clinical genetic studies and lacks in vivo evidence. The basic mechanisms responsible for the synergistic renal pathological effects of APOL1 risk alleles and HIV-1 are still unclear.
Methods: Structure, function, and molecular pathways are highly conserved between mammalian podocytes and Drosophila nephrocytes. Thus we generated transgenic flies selectively expressing APOL1 nephropathy risk Alleles G1 and HIV-1 protein Nef in Drosophila nephrocytes. We then investigated the structural and functional effects on the Drosophila nephrocytes.
Results: We found nephrocyte function declined, cell size increased and slit diaphragm structure defected in aged APOL1-G1 transgenic flies. APOL1-G1 expression impaired the nephrocyte function by inhibiting the endocytosis pathway to affect the acidification of organelles. Nef expression facilitated these nephrocyte impairments by inducing the accumulation of autophagosomes and inhibiting the autophagy pathway, which facilitated the impairments of the acidification of organelles and nephrocyte function
Conclusion: These results suggested that Nef and APOL1-G1 lead to the increased risk of renal failure through their synergistic regulation of the autophagy and endocytosis pathway, which will provide new therapeutic targets to prevent HIV-associated nephropathy.

5 ROLE OF CELL-FREE HEMOGLOBIN IN APOLIPOPROTEIN L1-MEDIATED SICKLE CELL NEPHROPATHY

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Background/Aims: Apolipoprotein L1 (APOL1) risk variants (G1 and G2) have been implicated in various non-diabetic kidney diseases, including sickle cell nephropathy (SCN), the kidney complication of sickle cell disease (SCD). In SCN, APOL1 risk variants are associated with hemoglobinuria, albuminuria and hyperfiltration. However, the underlying mechanism remains unknown. We hypothesized that cell-free hemoglobin (Hb) released during hemolysis in patients with SCD is a second-hit factor for APOL1-mediated SCN.
Methods: Conditionally immortalized human podocytes (ciPodocytes) cell lines expressing different APOL1 genotypes (G0/G0 and G2/G2) and CRISPR/Cas 9 edited ciPodocytes with C-terminal helix truncation (APOL1Δ) or APOL1 deletion (APOL1KO) were stimulated with sickle hemoglobin (Hb S) (0–500 µg/mL, corresponding to 0–30 µM of heme molar equivalents) or heme (0–30 µM) for different time periods. To model the pro-inflammatory environment seen at steady state in patients with SCD, cells were incubated with 200 µg/mL of HbS in the presence of 0.1 – 1 ng/mL of recombinant cytokines (IL-1β, TNF-α and IFN-γ).
Results: Exposure of ciPodocytes to at least 100 µg/mL of cell-free HbS or 12 µM of heme for 24 hours causes a significant decrease in cell viability. This decrease was independent on the APOL1 genotype of the podocytes. Upon incubation with HbS, APOL1 G0/G0 ciPodocyte cell line showed a time and concentration dependent upregulation of APOL1 mRNA. In addition, more pronounced APOL1 upregulation was observed when the pro-inflammatory state seen in patients with SCD was mimicked in vitro.
Conclusion: This study provides evidence that cell-free HbS or heme at concentrations seen in vivo in patients with SCD can be toxic to the podocytes and can play a crucial role in upregulating APOL1 expression in SCN.

6 SMALL MOLECULAR APOL1 PORE BLOCKER AMELIORATES RENAL INJURY IN PODOCYTE-SPECIFIC G2APOL1 TRANSGENIC MICE

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Background/Aims: African Americans have a 3-5 times higher incidence of chronic kidney disease (CKD). Most of this excess renal disease risk can be explained by coding variants in the Apolipoprotein L1 gene (variants called G1 and G2). Mice with transgenic expression of risk variant APOL1 (RV) (in the NEFTA-rtTA/TRE-G2APOL1mice) in podocytes recapitulate features of kidney disease observed in patients. Previous studies reported that the APOL1 RV acts as a membrane permeability of the cation channel leading to cytolysis of podocytes. Here we tested the effect of a small molecular APOL1 channel blocker (ACB) on kidney disease development in NEFTA-rtTA/TRE-G2APOL1 mice.
Methods: We generated mice with podocyte-specific inducible expression of APOL1 (NEFTA-rtTA/TRE-G2APOL1) by crossing the TRE-G2APOL1 mice with the NEFTA-rtTA mice. NEFTA-rtTA/TRE-G0APOL1 (reference allele). APOL1 expression in the NEFTA-rtTA/TRE-G2APOL1 (RV) was induced by a doxycycline diet (200mg/kg) at 6 weeks of age. Mice were treated with vehicle or ACB compound (20mg/kg BW/day, i.p.,) for 14 days.
Results: NEFTA-rtTA/TRE-G2APOL1 developed severe proteinuria (ACR=5002µg/mg), elevated creatinine (0.7mg/dl), glomerulosclerosis and tubulointerstitial fibrosis. Mice treated with ACB had lower proteinuria (ACR=979 µg/mg), plasma creatinine (0.24mg/dl), and improved kidney function. We observed less severe glomerulosclerosis and fibrosis on histological analysis. Administration of ACB did not impact kidney function and renal histology in reference allele mice. We did not observe significant toxicity in reference allele animals. Renal injury was associated with increased expression of genes for renal fibrosis, NLRP3-Inflammatory (pyroptosis), and STING-inflammatory pathways, which was significantly reduced by the treatment of ACB in NEFTA-rtTA/TRE-G2APOL1 mice.
Conclusion: ACB treatment markedly improved kidney function and suppressed the NLRP3 and STING pathway activation, which in turn reduces renal inflammation and fibrosis leading to improve kidney function and histology. ACB may be considered a potential therapeutic target for APOL1 RV-associated renal disease.

Glomerular biology/pathobiology/disease: Other

1 A DESIGN-OF-EXPERIMENT APPROACH TOWARDS DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO PODOCYTES

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Background/Aims: The kidney is a vital organ required for waste excretion as well as water and solute reabsorption. Its functionality relies on a range of highly specialized cells such as podocytes. While we understand many of the properties of the different renal cell types, we lack effective methods to generate large quantities of terminally differentiated human-derived kidney epithelial cells.
Methods: We utilize Quality-by-Design-based methods, in particular the Design-of-Experiment (DoE) theory, to provide a systematic, data driven approach towards renal cell differentiation. In a single experiment we simultaneously test the effect of up to 12 morphogen inputs on more than 50 target genes. Experimental design and statistical methods provide an in-depth understanding of the input parameters.
Results: Here we report our progress for the generation of podocytes. Starting from established protocols we differentiate human stem cells into nephron progenitor cells (NPCs). Using DoE, we identified a complex combination of signaling agonists and/or antagonists that differentiate NPCs along the podocyte lineage in a stepwise protocol. These cells express transcription factors as well as structural proteins characteristic for podocytes, but also exhibit their structural and physiological properties.
Conclusion: The stem cell-derived podocytes will not only provide a valuable tool to understand the signaling inputs required for podocyte development/homeostasis but will also provide a critical tool towards developing treatments for human kidney diseases characterized by podocyte dysfunction.

2 ALTERED BONE MARROW MYELOPOIESIS CONTRIBUTES TO GLOMERULAR DYSFUNCTION

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Background/Aims: Myeloid-biased hematopoiesis is commonly observed in various disease conditions associated with CKD, including infection, chronic inflammation, diabetes, cardiovascular disease, and aging. Despite identifying immature myeloid cells in the bone marrow (BM) as a significant contributor to glomerular dysfunction in mice, the link between BM and kidney function in humans is not yet determined. Here, we tested if inflammatory signals alter BM myelopoiesis leading to podocyte injury.
Methods: Multicolor flow cytometry was performed to evaluate the immunophenotyping of BM cells from CKD patients and healthy individuals, as well as human myeloid cells differentiated in vitro. Bioenergetic and epigenetic changes in monocytes were assessed by Seahorse and ATAC-seq, respectively. Secretome analyses were performed using ELISA and multiplex cytokine assays. We assessed the impact of functionally altered myeloid cells and their soluble factors to podocyte structure and filtration function by conducting a high-throughput immunofluorescence assay on cultured podocytes and two different animal models.
Results: CKD patients exhibited markedly increased levels of TNFα and suPAR in their BM and peripheral blood and a myeloid-biased hematopoiesis, with a robust increase in inflammatory monocytes expressing uPAR. Further in vitro assays revealed that TNFα can alter myelopoiesis by skewing hematopoietic stem cell (HSC) differentiation towards monocytic lineage cells, leading to functional changes in the resultant monocytes. These TNFα-driven monocyte subsets exhibited increased uPAR expression and suPAR secretion, as well as increased metabolic activity and production of proinflammatory cytokines. Additionally, TNFα treatment increased chromatin accessibility to genes for proinflammatory cytokines, metabolic activity, and those involved in inflammation signaling pathways, as evidenced by ATAC-seq. The soluble factors secreted by TNFα-driven myeloid cells caused cytoskeletal rearrangement in cultured podocytes and led to filtration dysfunction in a transgenic zebrafish model. Moreover, mice injected with TNFα and IFNγ (essential for myelopoiesis) exhibited suPAR-associated glomerular dysfunction, demonstrated by albuminuria, and a significant increase in BUN levels, suPAR levels, and uPAR-expressing BM myeloid cells.
Conclusion: This study reveals, for the first time, that TNFα contributes to podocyte injury by altering BM myelopoiesis and promoting the development of metabolically and epigenetically reprogrammed monocyte subsets that secrete proinflammatory cytokines and soluble permeability factors.

3 ALTERED MITOCHONDRIAL METABOLISM AND INCREASED OXIDATIVE STRESS CAUSE PODOCYTES DYSFUNCTION IN CYSTINOSIS

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Background/Aims: Cystinosis is a rare, incurable disease caused by mutations in the CTNS-/- gene and leading to lysosomal cystine accumulation in all cells. It affects podocytes and presents with increased podocyte losses into urine and early stage glomerular proteinuria. Cysteamine decreases cystine accumulation but does not reverse podocyte injury. Thus, other pathogenic mechanisms are involved in cystinosis.
Methods: Immortalized cystinosis patient-derived (PD; n=2), healthy control (n=1) and CTNS-/- knockdown (KD; n=2) podocytes were used.The results were validated in our in-house developed fluorescent ctns-/- zebrafish larvae model (l-fabp:DBP-eGFP;CTNS).
Results: PD and KD cystinosis podocytes present decreased cell adhesion and increased static and dynamic permeability, caused by accumulation of mitochondrial reactive oxygen species. Moreover, they show fragmented mitochondrial network with impaired energy and TCA cycle and decreased expression of the scavenging enzyme Superoxide Dismutase 2. Treatment with the mitochondrial superoxide scavenger MitoTEMPO in combination with cysteamine can rescue cell adhesion and permeability in vitro and in vivo.
Conclusion: Impaired mitochondrial function and increased oxidative stress are critical features in cystinosis podocytes, leading to loss of cell adhesion and increased permeability. Importantly, these characteristics are the main cause of glomerular dysfunction in cystinosis patients. We found that targeted mitochondrial antioxidant therapy can revert these phenotypes when combined with cysteamine treatment.

4 ASSESSING THE SPECTRUM AND PATHOGENICITY OF BIALLELIC VARIANTS IN NPHS2 IN 216 INDIVIDUALS WITH STEROID-RESISTANT NEPHROTIC SYNDROME

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Background/Aims: We tested the hypotheses that there is a NPHS2-related genotype-phenotype correlation between onset of steroid-resistant nephrotic syndrome (SRNS) and (i) novel in silico deleteriousness prediction scores (REVEL, EVE) for homozygous missense variants, (ii) predicted truncation for homozygous frameshift and stop gain variants, and (iii) pathogenic alleles trans-associated with R229Q.
Methods: We reevaluated exome sequencing data of 2,403 individuals with SRNS for biallelic variants in NPHS2, including phase and co-segregation analysis for uncertain and likely pathogenic alleles. We performed podocin structure modeling, and correlated REVEL and EVE scores, and specific alleles (homozygous truncating and alles trans-associated to R229Q) with SRNS onset in 216 individuals.
Results: We analyzed 80 NPHS2 alleles (53 unreported in ClinVar) in 216/2,403 (9%) individuals with SRNS, including one novel allele (p.E281K) trans-associated with R229Q. (i) REVEL and EVE scores for homozygous NPHS2 missense variants slightly correlate with SRNS onset in 94 individuals. (ii) SRNS onset due to homozygous truncating variants ranges from 0 – 9 years. (iii) Compound heterozygous alleles trans-associated to R229Q cause late and early onset SRNS in our cohort.
Conclusion: REVEL and EVE scores for homozygous NPHS2 missense variants cannot predict SRNS onset to a clinically relevant extent. We identified one novel missense allele trans-associated with R229Q. We could not reproduce that compound heterozygous alleles trans-associated with R229Q tend to cause a late (>8 years) age of SRNS onset.

5 DECREASE OF THE FILTRATION SLIT DENSITY IS AN EARLY INDICATOR OF DISRUPTION OF THE FILTRATION BARRIER INTEGRITY

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Background/Aims: Intact podocyte foot processes (FP) are essential for renal function. Changes in FP morphology are thought to be the first event leading to proteinuria, but this has yet to be proven. Since FP are below the optical resolution of a light microscope, changes are studied on ultrathin sections by EM. Here, we analyze the relationship between morphology and proteinuria using a 3D-SIM-based procedure.
Methods: Mice were treated with NTS and sacrificed 24, 48, and 72 h after injection. 3D-SIM was done on podocin and integrin co-stained standard formalin-fixed and paraffin-embedded kidney sections (3 µm). The filtration slit density (FSD) was measured by podocyte exact morphology measuring procedure (PEMP) (Siegerist, 2017). Albumin to creatine ratio and electron micrographs were also analyzed.
Results: After NTS injection, mice developed moderate proteinuria after 48 h. Using PEMP, we found that the FSD was already significantly reduced in NTS-treated mice at 24 h compared to control-injected mice. Transmission electron microscopic data confirmed this structural alteration.
Conclusion: PEMP analysis of standard histological sections from NTS-injected and control mice demonstrated that the first event in the functional loss of the size selectivity of the filtration barrier is the effacement of FP morphology, followed by detectable proteinuria. Therefore, measuring FSD provides a novel and rapid approach to evaluate the early loss of filtration barrier integrity.

6 ESTABLISHING QUANTITATIVE AND REPRODUCIBLE PHENOTYPING OF AN NPHS1 KNOCKOUT MOUSE MODEL

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Background/Aims: Steroid-resistant nephrotic syndrome is the second most frequent cause of chronic kidney disease before the age of 25 years. Nephrin, encoded by NPHS1, localizes to the slit diaphragm of glomerular podocytes and plays a crucial role in forming the glomerular filtration barrier. Biallelic loss-of-function variants in NPHS1 can cause congenital nephrotic syndrome of the Finnish type for which, to date, no causative therapy is available. Recently, adeno-associated virus vectors targeting the glomerular podocyte, have been assessed as means for gene replacement therapy. Here, we established quantitative and reproducible phenotyping of conditional Nphs1 knockout mouse model.
Methods: A conditional-ready transgenic Nphs1 mouse model (Nphs1<tm1.1Pgarg>/J, Verma et al. PLoS ONE, 2018) and mice expressing podocyte-specific Cre-recombinase (Moeller, et al. Genesis 2003) were acquired. I) We characterized median survival of Nphs1fl/fl Nphs2-Cre+ mice compared to Nphs1+/+, Nphs1fl/+ or Nphs1fl/fl Nphs2-Cre- controls. II) Furthermore, we characterized its renal phenotype by analyzing the presence of proximal tubular microcysts through light microscopy, and III) by quantifying podocyte foot process density using transmission electron microscopy. To that end, we counted the number of foot process (FP) cross-sections per length of glomerular basement membrane (GBM). IV) Finally, we performed proteinuria, serum albumin and blood urea nitrogen (BUN) measurements.
Results: I) The median survival rate of the nephrin-deficient mice after birth was 20 days (range from 9-43 days, n=15). II) Nphs1fl/fl Nphs2-Cre+ mice clearly separated for a high number of proximal tubular microcysts. III) The average FP density per GBM length was 1.0 fp/µm in the Nphs1fl/fl Nphs2-Cre+ mice compared to 2.0 FP/µm in controls at day 9, 11, 14 and 21. IV) Finally, Nphs1fl/fl Nphs2-Cre+ mice developed proteinuria within a few days of birth as evidenced by urine albumin-to-creatinine ratios of 4.8-54. Blood serum testing of Nphs1fl/fl Nphs2-Cre+ mice revealed significantly reduced levels of serum albumin (median 2.1 vs. 3.8 g/dL, n=15) and total protein, as well as elevated BUN levels.
Conclusion: We quantitatively characterized the phenotype of the Nphs1fl/fl Nphs2-Cre+ mice. Our findings provide a thorough and reproducible way to phenotype a conditional Nphs1 knockout mouse model, which can facilitate future gene replacement therapy projects.

7 KETOGENIC DIET IN EXPERIMENTAL GLOMERULONEPHRITIS

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Background/Aims: Ketogenic diets (KD) have aroused interest in medicine due to their potential health benefits and therapeutic applicability in diseases. While it is known that ketogenic diets can have a major regulatory impact on inflammatory processes, it is unknown if a ketogenic diet is also beneficial in glomerulonephritis (GN). Here we studied the effects of a ketogenic diet as a treatment in an experimental mouse model of immune-complex-mediated GN.
Methods: Male C57BL/6 mice were switched to a KD three days after induction of nephrotoxic serum nephritis (NTS) and sacrificed on day 21. Renal phenotype evaluation was performed on PAS and Sirius Red stained kidney sections. To evaluate the long-term outcome, mice were switched back from KD to SC on day 21 and remained in the experiment for additional 5 weeks. Further analysis included kidney function estimation using a transdermal GFR measurement device, immunophenotyping of blood and lymphatic tissues using flow cytometry, and NMR spectroscopy of the renal metabolome.
Results: Animals fed a KD presented an ameliorated glomerular phenotype and showed significantly reduced levels of albuminuria on days 14 and 21, reduced levels of kidney fibrosis, and a preserved kidney function at the end of the long-term experiment. Animals showed major immunological and metabolic adaptions including reduced blood leucocyte numbers and an increase in the concentration of ketone bodies in the kidney. Collectively, metabolic adaptions due to a ketogenic diet counteracted metabolic differences observed between healthy and nephritic mice, indicating a favorable metabolic environment due to the dietary treatment.
Conclusion: A therapeutic ketogenic diet for 18 days initiated after disease induction was protective in an experimental mouse model of GN. Mice fed a ketogenic diet showed preserved kidney function and less renal fibrosis compared to controls even 5 weeks after completion of the dietary treatment. These observations were associated with major immunological and metabolic renal adaptions in animals fed a KD. We now plan further detailed investigations to better understand the protective mechanisms of a ketogenic diet and its translational potential for human GN patients.

8 LIMITED ROLE OF ANTITHROMBIN DEFICIENCY IN NEPHROTIC SYNDROME–ASSOCIATED HYPERCOAGULOPATHY

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Background/Aims: Nephrotic syndrome is associated with an acquired hypercoagulopathy thought to drive its predisposition for venous thromboembolism. Previous studies have suggested that urinary antithrombin loss leading to acquired antithrombin deficiency is the primary mechanism underlying this hypercoagulopathy, but this hypothesis has not been directly tested. The objectives of this study were to test the influence of antithrombin levels on hypercoagulopathy in nephrotic syndrome patient samples and perform meta-analyses to evaluate the likelihood of antithrombin deficiency in nephrotic syndrome patients.
Methods: Samples from 3 independent nephrotic syndrome cohorts were analyzed. Antithrombin antigen and activity assays were performed using ELISA and amidolytic assays, respectively. Plasma thrombin generation, albumin, and urine protein-to-creatinine ratios were determined using established methods. Meta-analyses were performed by combining these new data with previously published data.
Results: Antithrombin levels were not consistently related to either plasma albumin or proteinuria. Antithrombin was quantitatively related to hypercoagulopathy in adult nephrotic syndrome. Whereas antithrombin activity was inconsistently associated with hypercoagulopathy in childhood nephrotic syndrome. Notably, hypercoagulopathy did not differ between patients with normal antithrombin levels and those with levels below the threshold used to define clinical antithrombin deficiency (<70%). Moreover, ex vivo antithrombin supplementation did not significantly alter hypercoagulopathy in antithrombin deficient plasma samples. The meta-analyses demonstrated that antithrombin deficiency was not a uniform feature of nephrotic syndrome and was more common in children than adults.
Conclusion: These data suggest that antithrombin deficiency plays only a limited role in the mechanisms underlying the acquired hypercoagulopathy of nephrotic syndrome. Moreover, antithrombin deficiency was not present in all nephrotic syndrome patients and was more likely in children than adults despite the higher risk for venous thromboembolism in adults than children. Future studies should focus on elucidating antithrombin-independent mechanisms behind the pathophysiology of nephrotic syndrome hypercoagulopathy, to increase the accuracy of predicting thromboembolic risk and effective prophylactic anticoagulation therapies.

9 MATHEMATICAL MODELING OF PODOCYTE ADHESION HIGHLIGHTS THE ROLE OF CELL CONTRACTILITY AND FLUID SHEAR STRESS ON KIDNEY FUNCTION

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Background/Aims: Diseases of the kidney's glomerular filtration barrier can lead to podocyte detachment that compromises filtration and eventually leads to end-stage kidney disease. Although the podocyte cytoskeleton is known to regulate podocyte shape and attachment to the underlying glomerular basement membrane (GBM), the mechanisms by which structure, force, and cytoskeletal dynamics enable this regulation are not known.
Methods: We developed a mathematical model of the actin cytoskeleton in podocyte foot processes and their connections to the GBM, and tested it by simulating our experimental observations of the effects of changing mechanical forces on podocyte adhesion.
Our model suggests that tensions on the integrin-based bonds of podocytes to the GBM are key to their attachment. Considering that integrin bonds require a minimum tension for activation, but that excessive tension leads to bond breakage, our model predicted that homeostasis requires a balance between extrinsic stresses, associated with fluid shear in blood filtration, and intrinsic stresses, associated with actomyosin contractility.
Results: To test our model in vivo, we manipulated both actomyosin contractility and fluid shear stress in a mouse model through myosin inhibition, AAV-renin injection, or anti-hypertension drugs. As predicted, reducing both actomyosin contractility and fluid shear stress increased proteinuria. Additionally, our model predicted stress-dependent spatial distributions of integrins along podocyte foot processes that were observed by immunostaining and super-resolution microscopy.
Consistent with our predictions, synaptopodin -/- mice, which show increased susceptibility to podocyte injury and detachment, exhibited increased sensitivity to high blood pressure, as evidenced by severe proteinuria.
Conclusion: Taken together, mathematical modeling and animal experiments suggest that controllable mechanobiological factors determine the pathological progression of glomerular disease, and suggest new directions for therapeutic intervention targeting the balance of cell contractility and blood filtration shear stress.

10 MC1R AGONIST PL8177 PROTECTS AGAINST PODOCYTE LOSS IN A STREPTOZOTOCIN-INDUCED RAT MODEL OF DIABETIC NEPHROPATHY

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Background/Aims: A growing body of evidence suggests that melanocortins have a protective role in many kidney diseases. Melanocortin-1 receptors (MC1Rs) are expressed in several kidney cell types, including podocytes, glomerular endothelial cells, mesangial cells, and tubular epithelial cells. PL8177 is a potent MC1R agonist that demonstrates binding characteristics similar to those of α-melanocyte–stimulating hormone (α-MSH), and it shows promise as a therapy that promotes the resolution of inflammation and tissue repair. PL8177 was investigated in a rat model of diabetic nephropathy to determine effects on kidney cell populations and on glomerular pathology.
Methods: Diabetes was induced in Norwegian brown rats by a single 50-mg/kg intraperitoneal injection of streptozotocin. Diabetic rats were treated twice daily on days 4–113 with subcutaneously administered PL8177 (1.0 mg/kg; n=6) or vehicle (n=6). Kidneys were harvested on day 114, and protein expression and transcriptomics were investigated by Western blotting of homogenized kidney tissue and single nuclei RNA sequencing, respectively. Kidney samples were also fixed, immunostained, and analyzed for WT1 expression and glomerular size. Fifty glomeruli per animal were annotated manually, and automated image analysis was performed.
Results: WT1 protein, a marker for podocytes, was reduced in diabetic kidneys compared to healthy controls. WT1 expression was significantly increased after PL8177 treatment compared to vehicle treated. Transcriptomics analysis showed that PL8177-treated rats had increased numbers of podocytes and proximal tubule cells compared to vehicle treated. Mean podocyte density (WT1+ cells by immunostaining) in PL8177-treated rats was 42 cells per glomerular area vs 27 cells per glomerular area for vehicle and was similar to that seen in healthy rats (40 cells per glomerular area). Glomerular area and total number of cells were both trending lower in PL8177-treated rats compared to vehicle treated, suggesting less glomerular hypertrophy with PL8177 treatment.
Conclusion: MC1R agonist (PL8177) treatment of streptozotocin-induced diabetic rats increased the expression of WT1 protein and increased the number of podocytes and proximal tubule cells compared to vehicle-treated controls. Glomerular hypertrophy, a characteristic of kidney injury, trended toward reduction in PL8177-treated kidneys. These results suggest that melanocortin agonists may represent a new therapeutic avenue to treat diabetic nephropathy.

11 NEPHRIN AUTOANTIBODIES IDENTIFY TWO THIRDS OF STEROID-NAÏVE CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

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Background/Aims: We recently identified nephrin autoantibodies in 29% of adults and children with biopsy proven minimal change disease (MCD) (Watts, Keller et al, JASN). The majority of these patients had received corticosteroid (CS) therapy prior to sampling which likely underestimated the true prevalence. We therefore sought to evaluate nephrin autoantibodies in an exclusively treatment-naïve pediatric population with idiopathic nephrotic syndrome (INS). We hypothesized that a larger subset would be serologically positive for nephrin autoantibodies.
Methods: This was a prospective multicenter study of children with a clinical diagnosis of INS from North America (via the Pediatric Nephrology Research Consortium (PNRC)). Steroid responsiveness was determined by the referring board-certified pediatric nephrologist. Nephrin autoantibodies were evaluated on paired serum samples obtained at presentation and 7-10 weeks post-treatment. The previously published indirect ELISA protocol for anti-nephrin antibodies was used. The Wilcoxon test was used to compare anti-nephrin antibody titers at presentation and follow-up.
Results: Two thirds, 66.6% (n=14/21), of steroid-naïve INS patients, encompassing both steroid sensitive (SSNS) and steroid resistant (SRNS) patients, were positive for nephrin autoantibodies prior to therapy. Of those, 78.6% (n=11/14) became negative following CS therapy. Differentiating SSNS from SRNS at initial presentation, we found that 73.3% (n=11/15) of patients with SSNS were serologically positive for nephrin autoantibodies vs. 50% (n=3/6) with SRNS, with median nephrin autoantibody titers 2.2-fold higher among SSNS vs. SRNS patients (277 vs. 124 units/mL, respectively; P=NS). Among SSNS patients, CS treatment resulted in a statistically significant reduction in nephrin autoantibody titers (73%; P=0.0004 vs. pre-treatment), concordant with clinical remission. Among SRNS patients, however, CS treatment neither reduced nephrin autoantibody titers (44%; P=NS vs. pre-treatment) nor induced clinical remission.
Conclusion: In this exclusively steroid-naïve pediatric population with INS, we found that two thirds of patients were serologically positive for nephrin autoantibodies at initial clinical presentation, supporting their potential pathophysiological role in INS. This prevalence of nephrin autoantibody positivity among INS patients is more than double that reported in our previous study. The higher prevalence in SSNS vs. SRNS indicates that serological testing for nephrin autoantibodies may aid in the prognostication of INS patients.

12 PODOCYTE SOFTENING AND PROTEINURIA: CAUSE OR CONSEQUENCE?

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Background/Aims: Podocytes are a critical component of the glomerular filtration barrier, and as such podocyte injury is a major cause of proteinuria. Historically, investigators have focused on biochemical changes in podocytes that occur following injury. Comparatively little is known about changes in biophysical properties such as cell stiffness, despite growing evidence suggesting that stiffness can change dramatically when cells are damaged and that such changes can alter intracellular signaling with important consequences.
Methods: Using atomic force microscopy (AFM), we profiled podocyte stiffness in 3 different murine disease models (Akita+/- Ren+/- mice, a murine model of diabetes; Myo1e-/- mice, a mouse model of genetic FSGS characterized by a deficiency of a non-muscle myosin; Col4a5-/- mice, a mouse model of Alport's syndrome) as well as in healthy and diseased human biopsy tissue. Moreover, we were able to demonstrate that alterations in podocyte stiffness were associated with important changes in the mechanosensitive signaling components of the cells.
Results: AFM measurements revealed that glomerular stiffness increased in Akita+/- Ren+/- and Col4a5-/- mice, a finding that correlated with the degree of glomerulosclerosis. GBM stiffness was increased in Akita+/- Ren+/- and Myo1e-/- mice. In all 3 mouse models, as well as in human FSGS biopsies, podocytes were softer than in healthy control kidneys.

To understand how podocytes of Akita+/- Ren+/- mice could be softer despite sitting on a stiffer underlying GBM, we stained for alpha3 and beta1 integrins, the main proteins used by podocytes to attach to the GBM. In these mice, alpha3 integrin levels did not change, but beta1 integrin levels were reduced. Interestingly, the activity of the mechanosensory transcription co-factor YAP, which has been shown to be critical for podocyte survival, was also reduced in Akita+/- Ren+/- mouse podocytes.

Conclusion: Taken together, these are the first data demonstrating that podocytes soften in proteinuric glomerular disease. Our data suggest that podocyte softening may be a common manifestation of podocyte injury. Further work is ongoing to understand whether this softening is solely a consequence of podocyte injury, or if it also contributes to ongoing podocyte damage.

13 QUANTIFIABLE PHENOTYPING OF A NPHS1-DEFICIENT MOUSE MODEL

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Background/Aims: Steroid-resistant nephrotic syndrome is a frequent cause of chronic kidney disease. The most severe form is caused by biallelic mutations in NPHS1. Since therapeutic options for a molecular target remain challenging, gene replacement therapy (GRT) has recently been considered. To set the ground for GRT studies in vivo, we established phenotypic assessment of a novel Nphs1 knockout (KO) mouse model
Methods: We established a floxed nephrin mouse model that was previously studied for pancreatic β-cell survival. We bred Nphs1fl/fl mice with a Cre recombinase under a podocin promoter, to generate podocyte specific nephrin deficiency. We then performed survival, transmission electron (TEM) and light microscopy (LM) analyses, and proteinuria assessment to quantitatively phenotype these mice.
Results: We observed median survival of 5 days in Nphs1fl/fl Nphs2-Cre KO mice as compared to 100% survival in controls. Quantification of proteinaceous casts by LM in proximal tubules revealed a ratio of 0.9 casts/glomeruli in KO mice, and none in controls. Counting the number of foot process (FP) per tuft length (µm) by TEM, control mice revealed an average of 2.3 FP/µm of GBM, KO mice showed 0.8 FP/µm, indicating FP effacement. Finally, KO mice had significant albuminuria compared with controls.
Conclusion: Here, we quantitatively phenotyped Nphs1fl/fl Nphs2-Cre KO mice in a model that can provide reliable phenotypic read-outs for GRT in this respective mouse model.

14 QUANTIFIABLE PHENOTYPING OF AN EXISTING NPHS1 KNOCKOUT MOUSE MODEL

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Background/Aims: Congenital nephrotic syndrome Finnish Type 1 (CNS-1) is the most severe cause of monogenic steroid resistant nephrotic syndrome (SRNS), caused by biallelic loss-of-function mutations in NPHS1, encoding nephrin. To develop gene replacement therapy strategies for CNS-1, suitable mouse models are required that offer quantifiable phenotypic assessment as a reproducible reference for treatment effects.
Methods: We used a previously published unconditional Nphs1 knockout (KO) mouse model (Nphs1tm1Rk/J, Hamano JBC 277:34, 2002) performing phenotypic evaluation by comparing KO mice to littermate controls in 3 quantitative independent conditions: a) Kaplan-Meier survival analysis, b) number of dilated proximal tubules upon light microscopy, c) foot process effacement upon electron microscopy.
Results: Homozygous segregation of the Nphs1-KO allele led to an a) perinatally lethal phenotype with a median survival of 1.0 day (n=19, $p<0.0001$, Mantel-Cox test), b) elevated number of tubular cysts (68.5) in KO mice (n=10) vs. 6.6 cysts in controls (n=10, $p<0.0001$, t-test) and c) reduced number of podocyte foot processes (FP), quantified by counting FP over a defined length of the glomerular basement membrane, with 0.98 FP/µm in KO (n=10) and 2.15 FP/µm in controls (n=11, $p<0.0001$, t-test).
Conclusion: We established 3 different quantifiable and reproducible phenotyping methods for the Nphs1tm1Rk/J mouse model, as a prerequisite to study effects of gene replacement strategies in CNS-1.

15 ULTRASTRUCTURAL GLOMERULAR CHANGES IN AN EXPERIMENTAL MODEL OF WARFARIN RELATED NEPHROPATHY

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Background/Aims: Anticoagulant related nephropathy (ARN) presents as acute kidney injury (AKI) in patients on anticoagulation therapy or underlying coagulopathy. Kidney biopsy in such patients shows acute tubular necrosis (ATN) with red blood cell (RBC) tubular casts, indicating glomerular hemorrhage. 5/6 nephrectomy (5/6NE) rats treated with anticoagulants develop ARN. The mechanisms of ARN are not clear.
Methods: Sprague Dawley 5/6NE rats were treated with vehicle or warfarin for 3 weeks at 3, 8 and 19 weeks after the ablative surgery (5 rats per group, animals were sacrificed at 6, 11 and 22 weeks after the surgery). Warfarin dose was 0.20 mg/kg/24h for the first week of treatment, 0.34 mg/kg/24 for the second week of treatment and 0.75 mg/kg/24h for the third week of treatment.
Results: The glomerular basement membrane (GBM) thickness was increased in 5/6NE rats after the ablative surgery. Warfarin treatment did not affect the GBM thickness in neither study group. Ultrastructurally, 5/6NE rats had a segmental loss in the fenestration of the glomerular capillary loop endothelial cells. Podocyte foot process effacement was mild to segmentally moderate. Similar findings were observed in all vehicle-treated and warfarin-treated 5/6NE rats regardless of the time after 5/6NE.
Conclusion: Our studies did not reveal ultrastructural changes in the podocytes or the glomerular capillary endothelial cells in 5/6NE rats that could explain the development of glomerular hematuria in warfarin related nephropathy. Podocytes do not play a significant role in the pathogenesis of WRN.

16 WHOLE EXOME SEQUENCING REVEALS A MONOGENIC CAUSE OF STEROID RESISTANT NEPHROTIC SYNDROME IN 27.5% OF 320 FAMILIES

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Background/Aims: Steroid-resistant nephrotic syndrome (SRNS) is a leading cause of end-stage renal disease before the age of 25. More than 65 monogenic genes have been identified as causes of SRNS. In this study, we employed whole exome sequencing (WES) to identify monogenic causes of SRNS and potential novel causes for SRNS in a large international cohort of 320 families.
Methods: From 1998 to 2018, we recruited 343 individuals with SRNS from 320 different families who had an age of onset of SRNS under 25 years of age. WES data were evaluated for 59 monogenic SRNS genes known at the time. In 111 families out of 320 families for whom we had one or both parental DNA available, we also employed 'Duo' or 'Trio' analysis for the detection of compound heterozygous or de-novo variants in potential novel candidate genes.
Results: In 88/320 families (27.5%) we identified a pathogenic variant using WES in one of 59 genes known to cause SRNS. We detected a causative variant in a known SRNS gene in 50.4% of consanguineous families and 66.6% in children with infantile nephrotic syndrome. In 60 out of 320 families (18.8%), we detected variants in a single potential novel candidate gene for SRNS. Duo/trio analysis had a higher detection rate of deleterious variants in potential novel candidate genes for SRNS at 28.9% (22 out of 76).
Conclusion: Our study confirms that WES is effective in detecting causative genetic variants in the 65 SRNS genes and has a high yield for establishing a molecular diagnosis in SRNS patients with onset before age 25 years. In a research setting, the inclusion of parental DNA in WES analysis will be important for the detection of novel candidate genes for SRNS.

17 X-LINKED RECESSIVE VARIANTS IN X-PROLYL AMINOPEPTIDASE 2 (XPNPEP2) AS A POTENTIAL NEW CAUSE OF NEPHROTIC SYNDROME

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Background/Aims: Steroid-resistant nephrotic syndrome (SRNS) is the second most frequent cause of chronic kidney disease in children. Major insights into its pathogenesis came from discovery of 65 monogenic causes that contribute to ~12-30% of SRNS with onset at <25 years of age. We hypothesized that additional monogenic causes of nephrotic syndrome (NS) exist, which may be identified by exome sequencing.
Methods: To identify novel potential monogenic causes of SRNS, we performed exome sequencing in a worldwide cohort of 1,283 different families with NS. We evaluated potential pathogenicity of bi-allelic hemizygous genetic variants with minor allele frequency (MAF) <1% by predefined criteria: in-silico deleteriousness prediction scores, evolutionary conservation, and allele frequency in gnomAD database.
Results: We discovered 3 X-linked recessive hemizygous, likely deleterious variants in gene XPNPEP2 in 3 unrelated males with childhood onset of SRNS: nonsense variant c.670C>T, p.(Arg224*), splice variant c.1107+1G>A, and missense c.346C>T, p.(Arg116Cys) with the arginine part of a highly evolutionary conserved DXRY motif that plays central role in the enzymatic center of the protein, and deemed as likely disease-causing by prediction programs. All variants are absent hemizygously from gnomAD database.
Conclusion: By exome sequencing, we here identified 3 different potentially deleterious X-linked recessive variants in the gene XPNPEP2 in 3 affected individuals, as a potential novel monogenic cause of steroid-resistant nephrotic syndrome.

Discovery “omics” approaches for glomerular disease or therapeutic development

1 COMPREHENSIVE SINGLE-NUCLEUS TRANSCRIPTIONAL AND METABOLOMIC PROFILING DEFINES UNIQUE PODOCYTE INJURY RESPONSES DURING OBESITY RELATED GLOMERULOPATHY

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Background/Aims: The underlying cellular events driving kidney podocyte injury and metabolic dysfunction during obesity are incompletely understood. This study was conducted to identify pathophysiologic factors in podocyte of obesity related glomerulopathy (ORG).
Methods: We employed single- nucleus RNA sequencing(snRNA-seq) to analyze 3 human ORG kidney specimens, 3 simple obesity without albuminuria kidney specimens and 3 controls. Respectively, we also performed snRNA-seq analyses of isolated glomerular cells and metabolomics analyses of kidney cortex from high fat feeding-induced ORG mice and control mice.
Results: In human kidney specimen experiments, snRNA-seq analysis identified diverse injury states of podocyte between ORG group and obesity without albuminuria group, ORG group and control group, including two distinct populations with dysregulated gluconeogenesis and lipid metabolism. Gluconeogenesis was defective in simple obesity without albuminuria kidney but was extremely activated in ORG state. Consistently, we discovered significantly up-regulated gluconeogenesis metabolism in podocytes of ORG mice, where we identified increased lipid and multiple intermediate products of citrate cycle accumulation according to metabolomics. Next, a gene-metabolite interaction network was made, we show that activated phosphoenolpyruvate carboxykinase 1 (PCK1), the rate-limiting enzyme in gluconeogenesis, in ORG podocyte, and its overexpression in vitro disrupted cell energy state maintenance and reactive oxygen species production during lipid and byproducts of citrate cycle deposition.
Conclusion: We integrated snRNA-seq and metabolomics data to generate a high-throughput landscape of dysregulated energy metabolism in podocyte during ORG. PCK1 as key pathophysiologic factors that might contribute to ORG progression.

2 DEVELOPING A SMALL MOLECULE DRUG TO TREAT DIABETIC KIDNEY DISEASE (DKD)

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Background/Aims: DKD is a leading cause of kidney failure. We demonstrated that the intracellular accumulation of lipid droplets (LDs) injures podocytes and mediates DKD pathology. Drugs that lower circulating lipids do not rescue podocytes from LD accumulation and cell death. We hypothesize that a drug that specifically treats LD accumulation in podocytes can protect them from injury and prevent DKD progression.
Methods: We developed a phenotypic assay using immortalized human podocytes and deployed it to screen a combinatorial library representing over 45 million unique compounds. We performed mechanistic analyses on hit compounds using transcriptomic, proteomic, and phenotypic techniques. We conducted medicinal chemistry to improve drug-like properties and tested one compound in a mouse model of DKD.
Results: We discovered and developed a series of novel compounds that prevent LD accumulation in podocytes and protect them from injury and cell death. RNAseq analysis revealed that the compounds modulate autophagy and downregulate inflammatory signaling. Cell-based studies demonstrated that the compounds strongly induce lipophagy and cytotoxic lipid clearance in podocytes. We tested one compound with improved drug-like properties in db/db mice and found that it dramatically prevented DKD progression.
Conclusion: Our drug discovery campaign successfully identified compounds that protect podocytes from LD accumulation by inducing lipophagy. We developed a lead candidate that demonstrated very high efficacy in preventing DKD progression in vivo. Our work provides a roadmap for developing a clinical candidate to treat DKD.

3 DIGITAL SPATIAL PROFILING OF MINIMAL CHANGE DISEASE

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Background/Aims: Patients with minimal change disease (MCD) can present clinically with heavy proteinuria and the nephrotic syndrome. Despite this severe presentation, there are few changes seen by light microscopy and immunofluorescence studies are also negative. The only change that may be seen is diffuse podocyte foot process effacement detected by electron microscopy. The clinical presentation of MCD overlaps with focal and segmental glomerulosclerosis (FSGS). However, in contrast to FSGS, MCD generally (but not always) is associated with a good prognosis and little kidney scarring. The mechanisms underlying these differences remain poorly understood.
Methods: We have previously characterized collapsing glomerulopathy, an aggressive variant of FSGS, using digital spatial profiling (DSP). This technology permits profiling of expression of thousands of genes simultaneously within individual glomeruli from clinically sourced biopsy sections. We therefore applied whole transcriptome DSP to understand the gene expression profiles of glomeruli from n=10 patients with MCD and n=4 controls, to determine if inter-patient variability was detectable and whether pathways of disease were distinct from those of collapsing glomerulopathy.
Results: Whole transcriptome DSP of glomeruli successfully quantified >4,600 genes in individual glomeruli across our patient cohort. Unbiased clustering, principal component and pseudotime analysis identified at least two different cohorts of MCD patients with distinct gene expression profiles. In silico cellular deconvolution revealed upregulation of canonical podocyte genes in one MCD patient cohort with relative downregulation in the other patient cohort. Pathway analysis revealed dysregulation of actin cytoskeleton, focal adhesion and metabolic pathways which differed from those seen in our previous study of FSGS.
Conclusion: Even though no changes were apparent by light microscopy, DSP was able to identify gene expression changes in MCD glomeruli and reveal heterogeneity among patients. DSP also uncovered differences in gene expression between MCD and FSGS patients identifying subgroups with distinct mechanisms of injury. In the future, DSP of glomerular disease cohorts and correlation with clinical parameters and outcome data could inform mechanistic understanding of disease pathogenesis and inform treatment decisions.

4 EPIGENOMIC DYNAMICS OF PARIETAL EPITHELIAL CELLS IN PROLIFERATIVE GLOMERULOPATHY

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Background/Aims: Proliferative glomerulopathies are devastating chronic kidney disorders characterized by increased proliferation of certain glomerular epithelial cells. They arise from predisposing kidney ailments such as diabetes, sickle cell disease, HIV infection and drug toxicities - just to name a few. Currently there are no curative treatments for this category of chronic kidney diseases, therefore a more mechanistic understanding of pathogenesis is sorely needed. Podocytes are specialized epithelial cells that are integral to glomerular filtration, and it is their dysfunction and death in proliferative glomerulonephritis (GN) and cellular/collapsing focal segmental glomerulosclerosis (FSGS) that perpetuates glomerular crescent formation. A primary patho-proliferative cell type in this context is the parietal epithelial cell (PEC), which lines the basement membrane of Bowman's capsule, and there is considerable evidence that it is a causal agent of glomerular injury following podocyte foot process effacement and eventual cell death.
Methods: I have utilized two distinct mouse models of glomerular crescentic disease: (1) deletion of the transcription factor Klf4 specifically in podocytes in-vivo, (2) the nephrotoxic serum nephritis (NTS) model which utilizes an injection of anti-murine glomerular basement membrane (GBM) anti-sera to cause injury. With these two disease models I wanted to understand the conserved and unique epigenomic landscapes underlying glomerular crescentic disorders – and how these shared/varying features are borne out in PEC behavior. To this end, I have generated single-nucleus RNA-seq (snRNA) and snATAC-seq datasets to monitor PEC dynamics during disease progression.
Results: My preliminary studies reveal conserved/unique PEC sub-types across these disease models, whose pathogenic transitions are synchronized across time and pseudo-time, wherein quiescent PECs once activated via a pathologic insult will transition into unique sub-populations in-vivo. I have identified Fos-Related Antigen 2 (FOSL2)-containing heterodimeric activator protein (AP-1) complexes which may be critical in orchestrating the pathogenic expansion of activated PECs. Additionally, I have uncovered a putative PEC-Podocyte-Macrophage autocrine/paracrine signaling loop involving Integrin Subunit Alpha V (ITGAV)-containing heterodimers and ligands such as Secreted Phosphoprotein 1 (SPP1).
Conclusion: Understanding the delicate epigenomic and intercellular PEC-centric mechanisms involved in these models of proliferative glomerulopathy may prove to be critical for uncovering a therapeutic Achilles heel for this devastating class of chronic kidney disorders.

5 HIGH-THROUGHPUT DISCOVERY OF NOVEL PODOCYTE-PROTECTIVE SMALL MOLECULES

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Background/Aims: The best treatment options for patients experiencing advanced stages of Chronic Kidney Disease (CKD) include dialysis or kidney transplantation. In addition to comorbidities and predisposition factors, CKD occurrence can be aggravated by the administration of prescription drugs. These compounds, such as the chemotherapeutic Adriamycin (ADR), exhibit off-target nephrotoxic effects in podocytes.
Methods: Since podocytes are vital to kidney fluid filtration and are primarily targeted during nephrotoxic-induced CKD, delivering reno-protective drugs could
benefit patients. Employing our established protocol for iPSC-derived podocytes, we have developed a high-throughput screening (HTS) platform to discover new protective molecules and therapeutics for podocytes.
Results: We have identified three new compounds that protected human stem cell-derived podocytes from severe injury by ADR treatment.
Conclusion: There is an urgent need for novel therapies that address the underlying causes of kidney dysfunction and disease progression. Ultimately, a podocyte-directed therapeutic, as a small molecule, could protect cells against known nephrotoxic drugs, potentially reducing the need for dialysis or kidney replacement in the future and alleviating the economic healthcare burden.

6 MULTI-POPULATION GENOME-WIDE ASSOCIATION STUDY IMPLICATES BOTH IMMUNE AND NON-IMMUNE FACTORS IN THE ETIOLOGY OF PEDIATRIC STEROID SENSITIVE NEPHROTIC SYNDROME

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Background/Aims: Pediatric steroid-sensitive nephrotic syndrome (pSSNS) is the most common childhood glomerular disease. Previous genome-wide association studies (GWAS) identified a risk locus in the HLA Class II region and three additional independent risk loci. But the genetic architecture of pSSNS, and its genetically driven pathobiology, is largely unknown.
Methods: We conducted a multi-population GWAS meta-analysis in 38,463 participants (2,440 cases). We then conducted conditional analyses and population specific GWAS followed by HLA fine mapping, colocalization with eQTLs and generation of a polygenic risk score (PRS).
Results: We discovered twelve significant associations – eight from the multi-population meta-analysis (four novel), two from the multi-population conditional analysis (one novel), and two additional novel loci from the European meta-analysis. Fine-mapping implicated specific amino acid haplotypes in HLA-DQA1 and HLA-DQB1 driving the HLA Class II risk locus. Non-HLA loci colocalized with eQTLs of monocytes and numerous T-cell subsets in independent datasets. Colocalization with kidney eQTLs was lacking b
Conclusion: Altogether, these discoveries expand our knowledge of pSSNS genetic architecture across populations and provide cell-specific insights into its molecular drivers.

7 PLASMA PDGFRB AND URINARY A2M PREDICT AN INTRA-RENAL SPARSENTAN RESPONSE PROFILE IN KIDNEY BIOPSIES FROM PATIENTS WITH GLOMERULAR DISEASE

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Background/Aims: Sparsentan is a dual endothelin receptor A inhibitor and angiotensin blocker that demonstrated reduced proteinuria in patients with FSGS in Phase II studies (DUET). Gene expression data from Adriamycin (ADR) treated rats treated with sparsentan were used to develop a response profile for translation into human kidney disease biopsies.
Methods: An interventional dose-response study was performed in rats challenged with Adriamycin (ADR). UPCR randomization was performed at day 7 post-challenge, with daily dosing of sparsentan across a dose range (6-60 mg/kg) carried out for 35 days. Kidneys were harvested, stored in FFPE, and kidney cortex RNAseq profiles were generated. Candidate biomarkers were measured using validated ELISA assays.
Results: Attenuation of proteinuria ($p=0.053$) and glomerulosclerosis ($p<0.05$) was observed in the high dose group at Day 33. Differential expression profiles were consistent with this. A large number of genes (388) reversed directionality with sparsentan intervention. These genes were carried forward as a sparsentan response signature and mapped onto human kidney biopsy transcriptomes. The sparsentan response signature from animals was elevated in patients with FSGS. Plasma PDGFB and urinary A2M predicted activation of the sparsentan response signature in human kidney biopsy transcriptomes.
Conclusion: Sparsentan treatment attenuated gene expression and activity of disease-related networks in a rat model of FSGS. The sparsentan response signature from rats was elevated in human FSGS kidney tissue and associated with disease severity. PDGFB and A2M were identified as candidate non-invasive biomarkers able to predict intra-renal pathway activation. These markers warrant further validation.

Glomerular disease clinical studies methods/trial development

1 NEPHROTIC SYNDROME STUDY NETWORK

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Background/Aims: The Nephrotic Syndrome Study Network (NEPTUNE) is a collaborative, investigational infrastructure of 34 North American sites for conducting clinical and translational research on Focal and Segmental Glomerular Sclerosis (FSGS), Minimal Change Disease (MCD), and Membranous Nephropathy (MN). NEPTUNE recruits adults and children with biopsy-proven FSGS, MCD, or MN and children/youth under 19 years of age with incident NS but without a diagnostic kidney biopsy. NEPTUNE recently expanded its scope to now include adults and children with Alport syndrome. All participants provide blood, urine, and clinical data at enrollment, at the 4-month visit, and annually thereafter.

NEPTUNE's newest study, Match, is designed to develop and test a mechanism for evidence-based clinical trial selection and effective communication aimed to match the highly heterogeneous nephrotic syndrome patient population to clinical trials targeting specific mechanisms activated in their disease.

Through NEPTUNE Ancillary Studies Program, investigators can apply to obtain biosamples, kidney biopsy images, clinical data, patient-reported outcomes, and datasets derived from biosamples. The web-based tranSMART interface provides opportunity for exploratory analyses with NEPTUNE data. NEPTUNE integrates a Career Enhancement Program and a competitive fellowship to attract promising scientists to research in rare diseases.

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Methods: (see above)
Results: (see above)
Conclusion: (see above)