

Poster #1
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Poster

Determinants of Renal Progenitor Cell responsiveness to the inductive Wnt9b signal from ureteric bud.

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Background:

Podocytes are derived from WT1-expressing cells of the metanephric mesenchyme (MM). Survival and proliferation of these early WT1(+) renal progenitor cells (RPCs) is dependent on a functional canonical Wnt-signalling pathway which initiates the differentiation cascade towards formation of a mature podocyte. Groups have generated mice harbouring null mutations in WT1 and B-catenin (specifically in the MM) which resulted in renal agenesis and severe renal hypoplasia, respectively, highlighting the importance of both molecules during nephrogenesis. As RPCs in the MM become 'fully primed' by E11.5, they must initiate response to the Wnt9b signal from the ureteric bud. However, the specific molecular components conferring responsiveness to the RPCs have yet to be identified. To address this issue, we obtained WT1-expressing M15 cells, derived from E10.5 MM and systematically analyzed WNT-receptor/signalling components required for a canonical WNT-response.

Methods:

To measure activation of the canonical WNT-pathway, we transfected our M15 cells with reporter plasmid 8XTOPFlash and measured luciferase activity using a GloMax luminometer. Exposing M15 cells to external Wnt9b resulted in minimal luciferase activity suggesting a signalling component is missing.

Results:

We analyzed M15 cells for components of the pathway and found mRNA expression of Fzd1-6, Lrp6 but neither Rspo1 or Rspo3. To ascertain whether absence of RSPO accounts for the lack of response, we transfected M15 cells with *Wnt9b* and added recombinant RSPO1 and observed a 4.77-fold increase in luciferase activity. In the presence of RSPO1, we transfected the cells with *Fzd1* to *Fzd10* and observed an additional 5-fold increase in the presence of *Fzd5* but not the other Fzds. Knockdown of *Lrp6* with siRNA resulted in a 60% reduction in mRNA levels which was mirrored by a 60% reduction in luciferase activation. To determine whether our candidate WNT-signaling genes are expressed in bona fide RPCs, we isolated CITED1-expressing RPCs from E15.5 kidneys. RNA expression of FZD5, LRP6 and RSPO1 in CITED1+ RPCs was confirmed by droplet digital PCR.

Conclusion:

These data suggest that early RPCs must acquire a specific receptor complex consisting of FZD5, LRP6 and RSPO1 to undergo an optimal B-catenin/TCF response to the inductive Wnt9b signal during nephrogenesis. We speculate that putative RPCs lacking these components are incompetent for primary nephrogenesis and will be unable to form mature podocytes.

Poster #2
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Blitz & Poster

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Transcriptional Reprogramming by Wilms' Tumor 1 and FoxC2 Mediates a Repair Response during Podocyte Injury

Background: Foot process effacement and proteinuria, representing a breakdown of the glomerular filtration barrier (GFB), may be caused by decreased expression of key podocyte proteins. We previously reported a ChIP-Seq study demonstrating that the Wilms' tumor-1 (WT1) transcription factor is a master regulator of gene expression in podocytes, binding nearly all genes known to be crucial for maintenance of the GFB. We have now identified and performed ChIP-Seq for a second transcription factor, FoxC2, that also binds many of the same target gene enhancers and transcriptional start sites as WT1, suggesting that WT1 and FoxC2 act as transcriptional activating complex in podocytes. In the present study, we demonstrate that WT1 and FoxC2 transcriptionally program a repair response after podocyte injury induced by Adriamycin.

Methods: We used Adriamycin-induced podocyte injury as a model for human Focal Segmental Glomerulosclerosis. WT1 and FoxC2 binding was determined by direct ChIP-qPCR at time points after injury using isolated glomeruli from 3, 5 and 7 days post-injection or control Balb/C mice.

Results: WT1 and FoxC2 have multiple binding sites at target genes including *Nphs2* and *Synpo*. We previously observed that after the onset of heavy proteinuria, *Nphs2* and *Synpo* expression decreases, as does WT1 and FoxC2 binding to their respective target sites. However, during the early stages of injury, we observed that WT1 and FoxC2 binding to target genes actually increases, accompanied by a transient increase in expression of target genes such as *Nphs2* and *Synpo*. Since transcription factors DNA binding is modulated by chromatin accessibility, we used immortalized podocytes as an *in vitro* model to study epigenetic reprogramming. We have observed that either Adriamycin treatment or siRNA knockdown of *Wt1* expression results in conversion of histone modifications at WT1/FoxC2 bound-enhancers from activating to repressive marks. Together, these results demonstrate that WT1 and FoxC2 mediate transcriptional reprogramming during the course of podocyte injury, and specifically that decreased levels of WT1 result in epigenetic silencing of gene expression during the course of podocyte injury.

Conclusion: Our observation that expression of WT1/FoxC2 target genes transiently increases after injury identifies a window of opportunity for treating glomerular disease in humans. These studies offer an example of how transcriptional reprogramming plays a vital role in the initiation and progression of disease.

Kidney progenitor cells with podocyte and proximal tubule cell differentiation capacity derived from urine of cystinosis patients

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Background. Cystinosis is a lysosomal storage disorder characterized by the pathological accumulation and crystallization of cystine in different cell types. If not treated, renal failure invariably develops within the first decade of life. We have shown that cystinosis patients void excessive number of podocytes and proximal tubular cells in urine. We hypothesized that in compensation for cell loss, ongoing regeneration might happen, and it could be reflected by the presence of kidney progenitor cells in urine of the patients.

Methods. We quantified the total number of cells and the number of kidney progenitor cells in urine using qPCR analysis of mRNA extracted of fresh urine samples of healthy donors (n = 10, age range 4-12 years old) and cystinosis patients (n = 8, age range 4-15 years old). None of cystinosis patients had kidney graft. The expression of vimentin was correlated to calibration curves derived from known numbers of adult kidney progenitor cells and normalized to volume of urine. We have cultured urinary cystinosis progenitor cells and characterized them by qPCR, FACS and immunofluorescent analysis.

Results. We demonstrate a significant increased excretion of kidney progenitor cells in cystinosis patients (progenituria), while in controls no progenitors were found in urine. FACS analysis showed that the progenitor cells isolated from cystinotic urine expressed mesenchymal stem cell proteins as CD73, CD44, CD105, CD29, did not express hematopoietic stem cell markers and were positive for the kidney proteins CD24 and CD133. The clones were positive for nephron progenitor markers, such as Vimentin, NCAM, *PAX2* and *CITED1* and were able to differentiate either into functional podocytes or proximal tubular cells.

Conclusion. Our data demonstrate the presence of kidney progenitor cells in urine of cystinotic patients, which might indicate a fast turnover of cells and the attempt of tissue regeneration to compensate for cell loss. Urinary cystinotic progenitor cells might have a therapeutic application in regenerative medicine once the correction of the genetic defect and consequent phenotype are successful.

Poster #4
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Poster

Title: Podocyte-specific induction of Krüppel-like factor 15 rescues podocytes from injury

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Podocyte injury resulting from a loss of podocyte differentiation markers has been implicated in many glomerular diseases. Treatment with retinoic acid and dexamethasone stabilizes the actin cytoskeleton and restores podocyte differentiation markers in cell culture and in murine models of podocyte injury. We previously demonstrated that Krüppel-Like Factor 15 (KLF15), kidney enriched zinc-finger transcription factor, is a critical regulator of retinoic acid and dexamethasone induced restoration of podocyte differentiation markers in proteinuric murine models. Furthermore, podocyte-specific loss of *Klf15* aggravates podocyte injury in proteinuric murine models. Based on these findings, we hypothesize that the restoration of KLF15 can prevent podocyte injury and eventual glomerulosclerosis.

We initially generated double-transgenic mice with *TRE-KLF15* and *Nphs2-rtTA* transgenes (*PODTA;TRE-KLF15*), which exhibit doxycycline (DOX)-inducible podocyte-specific expression of *KLF15*. To assess the salutary effects of podocyte-specific induction of *KLF15*, *PODTA;TRE-hKLF15* mice were bred with HIV-1 transgenic (*Tg26*) mice to generate *Tg26;PODTA;TRE-KLF15* and corresponding control mice. Podocyte-specific induction of *KLF15* (starting at 4 weeks of age) in *Tg26* mice attenuated podocyte injury, glomerulosclerosis, tubulointerstitial fibrosis and inflammation while improving renal function and overall survival in *Tg26* mice. This improvement in kidney injury was markedly significant with induction of *KLF15* specifically in podocytes, as compared to tubules. Furthermore, enrichment analysis of mRNA sequencing of isolated glomerular extracts from this model showed that podocyte-specific induction of *KLF15* activates pathways involved in stabilization of actin cytoskeleton, focal adhesion, and podocyte differentiation. Subsequent ChIP enrichment analysis with further experimental validation (functional promoter assay) shows that the salutary effects of KLF15 and its transcriptome is, in part, mediated by Wilms Tumor 1, transcription factor critical for podocyte differentiation. Finally, we further confirmed the salutary benefits of podocyte-specific induction of *KLF15* in the adriamycin-induced proteinuric murine model. Collectively, these data suggest that induction of *KLF15* might be a potential therapeutic target in the treatment of proteinuric kidney disease.

Poster #5
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The atypical Cyclin-dependent Kinase Cdk5 and its role on development and function of podocytes

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The atypical Cyclin-dependent kinase 5 (Cdk5) controls migration, cell adhesion and synaptic plasticity in neurons. In the kidney, Cdk5 expression is restricted to the podocytes. Three known activators, p35, p25 and Cyclin I activate Cdk5 in these visceral epithelial cells and mediate anti-apoptotic function via MEK/ERK and BCL-2. This led to the hypothesis, that Cdk5 is a master regulator of podocyte apoptosis or detachment from the glomerular basement membrane. The conventional knockout of Cyclin I and p35 as well as the conventional double knockout of these alleles were shown to cause increase susceptibility to glomerular damage in the model of nephrotoxic nephritis. Prolonged and mislocalized activity of Cdk5 by increased levels of p25 in neurons leads to neurodegeneration phenotypes and dementia.

We generated podocyte specific Cdk5 knockout mice to evaluate the role of the Cdk5 effector kinase in podocytes, by crossing Cdk5 floxed mice to a podocyte-specific Podocin:Cre line. To confirm Cdk5 knockout efficiency Cdk5 floxed mice were mated with mPodocin2Acre mice. Hence mTomato immunofluorescent labeled primary podocytes were isolated by fluorescent activated cell sorting and validated by analyzing Cdk5 mRNA expression via qPCR. Podocyte-specific Cdk5-knockout mice were born according to Mendelian rules and did not develop a proteinuria or glomerular scarring until the age of 72 weeks as documented in urine and serum analyses as well as immunohistochemical stainings. In the nephrotoxic nephritis model Cdk5-deficiency resulted in increased susceptibility to glomerular injury, aggravated proteinuria and glomerular sclerosis and a decreased number of podocytes per glomerulum.

In addition, a transgenic podocyte cell line expressing MYC-tagged p25 was generated by lentiviral gene transfer to investigate Cdk5 hyperactivity in podocytes. As compared to control podocytes, p25-transgenic cells showed an enhanced migratory phenotype, survival was not affected. Proteomic using MS/MS analysis revealed enhanced expression of proteins modulating cytoskeletal remodeling.

Whereas Cdk5 deficient mice develop no phenotype at baseline, this study highlights the anti-apoptotic effect of Cdk5 in the model of nephrotoxic nephritis. Whether activity of Cdk5 is protective in disease most likely depends on the balance of specific Cdk5-activators in the cell.

Poster #6
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The glomerulus on a chip: a new tool to study the filtration barrier

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The glomerular filtration barrier is formed by the podocyte, the glomerular basement membrane and the fenestrated endothelial cell. Damage to any of these components leads to a severe, irreversible disruption of the filtration barrier, which can lead to renal failure, requiring dialysis and transplantation. The development of new effective therapeutic approaches is limited by poor understanding of the complex cell-matrix interactions and glomerular cell crosstalk in vivo and by the absence of an in vitro model of the glomerular filtration barrier. We developed a system that mimics the complex architecture of the filtration barrier and that can be used to better study glomerular (patho)-physiology. We generated a population of renal progenitors within human amniotic fluid (hAKPC-P) that can differentiate into podocyte-like cells. Taking advantage of the peculiar characteristics of available Organ-on-a-Chip systems, we have developed an innovative Glomerulus-on-a-Chip system by co-culturing hAKPC-P and human glomerular endothelial cells in Organoplate™ microfluidic plates. We have successfully established the ideal conditions for in vitro culture of the hAKPC-P/ glomerular endothelial cell up to 21 days. Vessel formation by glomerular endothelial cells was confirmed along with marked expression of endothelial marker VE-Cadherin while hAKPC-P were positive for nephrin and podocin. De-novo deposition of collagen IV α 3 α 4 α 5 (essential for proper function of the filtration barrier) was also confirmed. Importantly, we also established the functionality of the filtration barrier since no leakage, evaluated by measuring permselectivity to physiological concentration of albumin, was detected.

Our results suggest the feasibility of Organoplates™ for the co-culture of podocytes and endothelial cells. This system might prove useful for understanding podocytes/endothelial crosstalk (or its perturbations) and how this might affect glomerular homeostasis and, ultimately, will increase our ability to individualize treatment, predict drug susceptibility among patients and avoid unwanted side effects, thus ultimately benefiting patients affected by renal failure.

Poster #7
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Coro2b is downregulated in human DKD and affects podocyte development in zebrafish but not in mice

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Podocytes play an important role in the pathophysiology of kidney disease. One of the main causes of renal disease is the loss of podocytes and the ablation of podocyte foot process structures, which play a major role in renal filtration. Podocyte dysfunction results in impairment of the glomerular filtration barrier leading to proteinuria and eventually to fibrosis. The foot processes are structurally maintained by the actin cytoskeleton and its impairment has been shown to lead to podocyte damage and loss.

Coro2b belongs to the actin cytoskeleton-associated protein family of coronins. Previously, Coro2b has been shown to be enriched in the glomerulus in microarray and RNA sequencing studies. In this study, we performed studies in man, mouse and zebrafish to get insights into the role of Coro2b in the glomerulus function and diseases.

In the kidney tissue of human and mouse, Coro2b is almost exclusively expressed in the glomerulus and podocytes. Additionally, we find that Coro2b is strongly down regulated in human diabetic kidney disease (DKD). Hence, we generated a conditional and “full” knockout (KO) mouse lines for Coro2b. Podocyte-specific KO animals did not manifest in any obvious renal phenotype. In contrast, in zebrafish the use of targeted morpholinos affected podocyte development and resulted in the edema of the cardiac chamber, distension of the urinary tract and impaired circulation.

These results indicate that Coro2b is not essential for the maintenance of glomerular filtration barrier in the mouse, but in DKD and the zebrafish they warrant for further studies.

Poster #8
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Blitz & Poster

Developmental trajectories inform the derivation of podocytes in-a-dish

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In the filtering unit of the kidney, podocytes extend foot processes wrapping around the glomerular vasculature. The derivation of podocyte-like cells has been reported by a number of groups differentiating pluripotent stem cells in a variety of approaches. Before moving to disease modeling and drug discovery, an evaluation of the properties of in vitro derived cells to their in vivo counterparts is important. In this study, we used in vivo studies, single-cell RNA sequencing and bioinformatics to explore the developmental trajectory of the human podocyte. Employing a fluorescently labeled hESC line to visualize and isolate in vitro derived podocyte-like cells, we performed a comparative analysis of in vitro generated podocytes with their human embryonic counterparts. Through high-resolution microscopy, chromatin structure profiling, single-cell and bulk transcriptional profiling, we found that hESC-derived podocytes shared molecular signatures with normal human podocytes, including the ability to form intricate interactions with their neighbors and attract blood vessels. Our analyses also identified distinct transcriptional signatures between in vitro and in vivo-derived cells that point to opportunities to improve podocyte production in culture.

Poster #9

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Blitz & Poster

Selective podocyte induction from human iPS cells

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Nephron epithelia, including podocytes and renal tubules, are derived from nephron progenitor cells (NPCs). Recently, we and others have established the induction methods of kidney organoids via NPCs from induced human pluripotent stem cells (hiPSCs), however, it remains a challenge to control the differentiation into specific nephron segment. We here established a high efficiency induction method of human podocytes from hiPSCs by examining the three steps of differentiation from mouse NPCs to nephron epithelia.

In the first step, the optimized Wnt treatment of NPCs was essential for mesenchymal-to-epithelial transition toward podocyte differentiation. The second step supported proliferation of podocyte precursors in the renal vesicle, a primitive nephron epithelium, and the last step suppressed differentiation of other nephron lineages, which resulted in an increase of podocyte proportion among the nephron epithelia *in vitro*.

These observations were applicable to human NPCs, and we successfully induced hiPSCs-derived podocytes with more than 90% purity. Induced podocytes showed high expression levels of podocyte-associated genes, such as NPHS1, NPHS2, WT1, and SYNPO, at similar levels of human adult podocytes *in vivo*. Podocyte-associated proteins were also much more abundant than the immortalized cell line, which may be applicable for proteomic analyses. Thus, by recapitulating the developmental processes in mice, we achieved the selective induction of human podocytes, which will serve as useful tools for regenerative medicine.

Poster #10
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Blitz & Poster

Mice with podocyte-specific sialylation defect represent a novel genetic model for FSGS

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All animal cells are covered by a dense glycocalyx, which is composed of glycoproteins, glycolipids, and proteoglycans. The unique structural diversity of the glycome correlates well with its position at the cellular surface, where communication processes and cellular interactions take place. Glycans are often capped by the acidic sugar sialic acid (Sia) and loss of this outermost sugar due to enzymatic removal or genetic interference with Sia biosynthesis has been shown to cause proteinuria, glomerular injury and eventually kidney failure [1-3]. Since mouse models with constitutive defect in the sialylation pathway are embryonic lethal, we generated a podocyte-specific sialylation-deficient mouse model (*PCmas*^{-/-}) to address the significance of Sia for podocyte function. Therefore we targeted CMP-Sia synthetase (CMAS), the enzyme responsible for the metabolic activation of Sia. Until postnatal day 21 (P21) *PCmas*^{-/-} mice were indistinguishable from wildtype littermates with proper kidney development, function and sialylation. Subsequently a progressive loss of sialylation was paralleled by a reduced glomerular filtration rate and the development of proteinuria. Around P60 mice died from kidney failure. Histological analyses demonstrated that the onset of proteinuria (P28) was accompanied by a mislocalisation of asialo-Nephrin. The picture observed was highly reminiscent to the nephrin mislocalisation reported in patients suffering from diabetic nephropathy and Hanta virus infection. At ultrastructural level *PCmas*^{-/-} kidneys showed drastically disorganized podocyte morphology, effacement of foot processes and loss of slit diaphragms. Moreover, the systematic analysis of kidney sections revealed severe glomerular injury: expanded capillaries, mesangial hypercellularity, synechiae between the glomerular tuft and parietal cells of the Bowman's capsule, and podocyte loss. In summary, the histological features seen in *PCmas*^{-/-} mice mimicked the renal pathology of patients suffering from focal segmental glomerulosclerosis (FSGS). Since FSGS refers to a histologic pattern which is a characteristic of several distinct and sometimes unknown underlying etiologies, we hypothesize that Sia is a new player involved in the development of FSGS. Consequently, we focus current analyses at the monitoring of sialylation patterns in biopsies taken from patients diagnosed with FSGS.

[1] Weinhold *et al.* (2012) *J Am Soc Nephrol* 23(8):1319-28.

[2] Galeano *et al.* (2007) *J Clin Invest* 117(6):1585-94.

[3] Gelberg *et al.* (1996) *Lab Invest* 74(5):907-20.

Poster #11
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Blitz & Poster

Elucidating the pathogenesis of Focal Segmental Glomerulosclerosis using CRISPR/Cas9 mediated genome engineering

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Background

NPHS2 encodes for the PHB-domain protein podocin and is the most frequently mutated gene in patients with a Steroid-Resistant Nephrotic Syndrome (SRNS) and Focal Segmental Glomerulosclerosis (FSGS). Currently, loss of podocytes is regarded as the hallmark of the pathogenesis of FSGS. Investigating the initial molecular events causing podocyte injury and depletion is of utmost importance for future preventive and therapeutic strategies.

Methods

Here, we present a mouse model for a late-onset FSGS phenotype. Using CRISPR/Cas9 mediated genome engineering we precisely integrated two point mutations in the *Nphs2* gene which, in compound-heterozygosity, represent a commonly found genotype in patients with a late-onset.

Results

Strikingly, *in vivo* data of compound-heterozygous animals reveal an early onset of mild proteinuria long before the manifestation of FSGS lesions. The proteinuria increases gradually and reaches nephrotic range in young adult mice (>10 weeks). Histologically, FSGS lesions are found at 14 weeks. Additionally, there are indications for a mesangial proliferation and an overall glomerular enlargement prior to detectable FSGS lesions. By using STED microscopy at different time points (2, 4, 8 weeks) we show a reduction of podocin signal intensities, a shortening and widening of the foot processes and a decreasing containment of the capillary surface by the secondary processes in compound-heterozygous in comparison to control mice. Quantification of podocyte numbers in kidney sections revealed a progressive decline in mutant mice.

Conclusions

In this study, we have generated a late-onset FSGS disease model resembling human genetic disease by using CRISPR/Cas9 mediated genome engineering. Mutant mice develop progressive proteinuria accompanied by a full blown morphological manifestation of FSGS. Delineation of the foot process microarchitecture by STED microscopy shows a loss of the distinct microarchitecture of the podocyte and a decreased containment of the capillary surface as an early pathological sign that precedes the onset of massive proteinuria. The reduction in signal intensities of podocin along the slit diaphragm of mutant mice suggests the inefficient membrane recruitment of podocin to be the molecular driver of disease development.

Poster #12
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Poster

Title: Nephrin signaling results in C3G-dependent Integrin β 1 activation.

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Introduction: Patients with mutations in the gene encoding the slit diaphragm protein Nephrin fail to develop functional slit diaphragms and suffer from severe proteinuria. Furthermore, Nephrin expression and function is altered in many adult-onset glomerulopathies. Nephrin signals from the podocyte slit-diaphragm to the actin cytoskeleton. We hypothesized that Nephrin also transmits signals to the Integrin receptor complex at focal adhesions.

Methods: We employed cultured human podocytes and *Drosophila* nephrocytes, which form a slit-diaphragm-like filtration barrier – the nephrocyte diaphragm - and express the Nephrin orthologue Sticks-and-stones (*Sns*) as model systems.

Results: In a discovery experiment, the rap guanine nucleotide exchange factor 1 C3G was identified to be necessary for *Drosophila* nephrocyte function. Activated Nephrin recruits Crk family proteins, which can recruit C3G in other cellular systems. In podocyte culture, activated Nephrin recruited phosphorylated C3G. Furthermore, Nephrin activation resulted in C3G-mediated activation of Integrin β 1 in cultured podocytes. *In vivo*, nephrocyte-specific gene silencing of *sns* as well as *c3g* compromised nephrocyte filtration, shown by impaired protein uptake into *Drosophila* nephrocytes as well as ultrastructural nephrocyte diaphragm defects. Gene silencing of either *sns* or *c3g* lead to altered localization of Integrin and the Integrin-associated protein Talin in nephrocytes. In *Drosophila* nephrocytes *sns* and *c3g* genetically interacted as gene silencing of *c3g* partly rescued the nephrocyte diaphragm defects of the *sns* gain of function phenotype. Immunofluorescence analysis of renal biopsy samples showed that C3G was increasingly expressed in podocytes of patients with Membranous Nephropathy.

Conclusion: Nephrin activation mediates C3G-dependent Integrin β 1 activation. This pathway may be important in subsets of human glomerulopathy.

Poster #13
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Poster

Dynamin oligomerization regulates membrane tension by orchestrating actin cortex architecture

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Background: Dynamin plays a role in the actin cytoskeleton and clathrin-mediated endocytosis. We have shown that dynamin regulates actin polymerization via direct dynamin-actin interactions. In addition, we have shown that dynamin induces actin polymerization by displacing a capping protein, gelsolin, from the fast-growing barbed ends, but only if oligomerized (Dyn^{OLIGO}). Importantly, our studies showed that pharmacological targeting of dynamin with a small molecule, BisT23 that promotes formation of Dyn^{OLIGO}, ameliorated kidney injury in animal models of human kidney disease by recovering functional actin structures in injured podocytes. Therefore, Dyn^{OLIGO} cycle plays an essential role in podocyte biology. Here, we expand the role of Dyn^{OLIGO} to include regulation of cell membrane tension.

Methods: Polarized kidney epithelial cells (IMCD) were used for this study. Atomic force microscopy was utilized to determine membrane tension. Actin polymerization assays were performed with cell lysates from IMCD or podocytes. The structure of actin filaments was evaluated by electron microscopy. Clathrin-mediated endocytosis was examined in IMCD by Total Internal Reflection Fluorescence Microscopy.

Results: Using a combination of distinct dynamin mutants and BisT23 we show that Dyn^{OLIGO} defines the length of actin filaments in conjunction with gelsolin. In addition, we show that dynamin crosslinks actin filaments into distinct structures in conjunction with its oligomerization state and the length of the actin filaments. Importantly, our data demonstrate that Dyn^{OLIGO} regulates membrane tension most likely by defining and crosslinking cortical actin. Furthermore, dynamin's effect on cell surface tension indirectly influences the speed of clathrin coated pit maturation in polarized cells.

Conclusions: Our study defines a novel role for Dynamin oligomerization cycle as a direct regulator of membrane tension and indirect regulator of endocytosis in polarized cells.

Poster #14

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Poster

Ephrin-B1 at slit diaphragm controls podocyte functions through JNK pathway independently of nephrin signaling

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Background: Ephrin and Eph are membrane-bound proteins that function as receptor-ligand pairs. B-type ephrin is reported to maintain tissue function in several organs. Previously we showed ephrin-B1 is localized at the slit diaphragm of podocytes (Kidney Int 72, 954, 2007). However, the function of ephrin-B1 at the slit diaphragm is unclear. **Methods:** We analyzed the phenotype of podocyte-specific ephrin-B1 knockout mice and assessed the molecular association of ephrin-B1 and nephrin in HEK293 cells and rats with anti-nephrin antibody-induced nephropathy. The expression of ephrin-B1 was also analyzed in a human patient with nephrotic syndrome. **Results:** Conditional ephrin-B1 knockout mice displayed the alteration of podocyte morphology, disarrangement of the slit diaphragm molecules, and proteinuria. Ephrin-B1 was interacted with nephrin via their basal regions of the extracellular domains in *cis* form. Not only nephrin but also the nephrin-binding ephrin-B1 was phosphorylated by stimulation to the extracellular site of nephrin. The phosphorylation of ephrin-B1 was inhibited by an Src kinase inhibitor. The phosphorylated ephrin-B1 enhanced the nephrin phosphorylation, and promoted the JNK phosphorylation independently of nephrin signaling. The phosphorylated JNK promoted the cell motility. Although phosphorylated JNK was detected in the glomeruli of control mice, it was not detected in that of the ephrin-B1 conditional knockout mice. The phosphorylations of ephrin-B1, nephrin and JNK were detected already at the early phase of the anti-nephrin antibody-induced nephropathy. The expression of ephrin-B1 as well as nephrin was clearly reduced in glomeruli of a patient with relapsing nephrotic syndrome. **Conclusions:** Ephrin-B1 plays an essential role in maintaining the structure and barrier function of the slit diaphragm. The nephrin-binding ephrin-B1 is phosphorylated by the stimulation to the extracellular site of nephrin and transferred the signals nephrin detected to downstream via another route of nephrin signaling. Ephrin-B1 controls podocyte function through the JNK pathway. This study provides new insights to better

understand the podocyte biology and the pathogenesis of podocyte injury.

Poster #15
Presented May 31
Poster

Functional proteomics unravels cytoskeletal signaling determinants of podocyte injury in individual animals.

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Podocyte injury is suggested to be a major cause of kidney disease. Unfortunately, the signaling pathways contributing and progressing to podocyte injury are largely unknown. Here, we used ultrasensitive sample preparation together with targeted and untargeted proteomics to investigate the response of FACS-sorted renal podocytes to injury induced by injection of LPS or Doxorubicin in individual mice. Interestingly, we identified known glomerular disease-associated pathway proteins such as Stat-1 in both models to be up-regulated. In addition, we also identified novel potential markers for glomerular diseases. In doxorubicin-induced injury, we found Filamin B, a mechanical stress sensor, to be increased. In the LPS treated animals we identified the small GTPases Cdc42 and Rab10 as significantly up-regulated proteins. Comparison of the podocyte proteomes of Doxorubicin- and LPS-induced glomerular injury revealed only 4 common proteins upregulated in both models, as well as 7 proteins decreased in both models. Transcriptomic data from FSGS patients identified common decreased proteins in human glomeruli and mice podocyte samples. More than half of gene-protein pairs shared between human glomeruli transcriptomes and mouse podocyte proteomes were related to the actin cytoskeleton. Finally, we turned to the nephrocyte, a model for podocyte function, and performed functional studies of development and filtration barrier integrity upon knock-down of altered proteins. The model also allowed for individual animal (nephrocyte-garland cells) resolved ultrasensitive proteome acquisitions together with functional validations. In conclusion, this study sets up functional proteomic studies of podocyte injury in individual animals.

Poster #16
Presented May 31
Blitz & Poster

Par3A and Par3B orchestrate podocyte polarity

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Polarity signaling through the aPKC-Par polarity complex is essential for the development and maintenance of the podocyte architecture and the function of the glomerular filtration barrier of the kidney. Despite its well-established role in aPKC-mediated signaling, Par3A appears to be dispensable for the function of the glomerular filtration barrier (Koehler et al. AJP renal 2016). Despite 14-fold increased mRNA levels of Par3B as compared to Par3A, loss of Par3B also did not result in albuminuria and glomerulosclerosis.

To study potential compensatory mechanisms between Par3A and Par3B, we employed conditional *in vivo* targeting strategies specifically in podocytes and generated podocyte-specific Par3A/B double knockout mice. Par3A/B DKO mice were born following Mendelian rules. Within 8 weeks of age Par3A/B DKO mice developed severe proteinuria and renal failure. We utilized *Drosophila* nephrocytes to further study the interplay between the different Par3 proteins. Here, we show co-localization of the Par3A/B homolog bazooka and the nephrocyte diaphragm proteins Sns (nephrin) and Duf (NEPH1) at different developmental stages. Silencing bazooka expression resulted in disturbed nephrocyte diaphragm morphology, severe filtration defects and delayed larval development. To study a potential compensatory effect between Par3A and Par3B, we re-expressed different murine Par3 variants in a *bazooka* knockdown background using the UAS-GAL4 system. Neither expression of Par3A or Par3B resulted in a complete rescue of the bazooka knockdown. As expression of both, the 100kDa Par3A isoform as well as Par3B, which are both lacking the aPKC-binding domain, also resulted in a partial rescue, we speculated on partial aPKC-independent functions for Par3 at podocyte/nephrocyte filtration barrier.

Taken together, these findings support the hypothesis of a potential compensatory mechanism between Par3A and Par3B to maintain polarity signaling at the slit diaphragm which are - at least partially – independent of aPKC.

Poster #17
Presented May 31
Poster

Regulation of contractile signaling at focal adhesions in podocytes

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The linkage of the cell to the extracellular matrix (ECM) is provided by integrin-mediated focal adhesion (FA), which transmits mechanical forces between ECM and cytoskeleton. Previously, we have demonstrated that Shp2 promotes ROCKII activation that facilitates FA maturation and stress fibers orientation to optimize cellular tension in response to the increase of matrix rigidity. In this study, we identified that α -actinin-4 interacts with Shp2 at FAs in mouse embryonic fibroblasts by an in vitro pull-down assay. This interaction was confirmed by co-immunoprecipitation and proximity ligation assay. Since α -actinin-4 plays an important role in podocytes adhesion, we then examined this molecular mechanism in the mouse temperature-inducible podocyte cells. Our data show that differentiated podocytes exhibited matured FAs and stress fibers accompany with hyperactivation of Shp2 and ROCKII those were both sensitive to the puromycin aminonucleoside-induced podocyte injury. Knockout of α -actinin-4 by CRISPR/Cas9 reduced Shp2 activation. Furthermore, treatment of cells with Shp2 inhibitor reduced ROCKII activation, FAs, stress fibers, and BSA-filtration ability of podocytes. Taken together, our results suggest the essential role of α -actinin-4 in Shp2 activation that is crucial for cell adhesion and filtration function in podocytes.

Key words: ROCK, α -actinin-4, Shp2, cell adhesion

TrkC is necessary for maintaining healthy podocytes in mice during aging.

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Background: Podocyte malfunction is central to glomerular diseases and is marked by defective podocyte intercellular junctions and actin cytoskeletal dynamics. Podocytes share many morphological features with neurons, so that similar sets of proteins appear to regulate cell process formation and maintenance. One protein that is expressed in both neurons and podocytes is tropomyosin-related kinase C (TrkC). It was shown that conventional TrkC knockout in mice leads to proteinuria in newborn mice (Lefevre et al., PLoS Genet., 2010). *In vitro*, activation of endogenous TrkC by its ligand NT-3 results in Erk-mediated WAVE2 phosphorylation leading to migration of podocytes (Gromnitza et al., FASEB J., 2018). We hypothesized that TrkC is necessary for the development or maintenance of foot processes.

Methods: C57BL/6 TrkC flox mice were crossed with C57BL/6 Six2-Cre transgenic mice which express the Cre-recombinase driven by the nephron-specific Six2 promoter. Mice were phenotyped by analyzing urine for proteinuria. Histological analysis of kidney sections and ultrastructural analysis by transmission electron microscopy were performed.

Results: Immunofluorescence analysis with antibodies specific for TrkC and the slit-diaphragm markers ZO-1 or Nephlin showed that TrkC was located at the podocyte slit diaphragm in human as well as in mice kidneys. Surprisingly, mice lacking TrkC in the nephron did not develop proteinuria in the first months of their lives, which contrasts with the phenotype of conventional TrkC knockout mice, which suffer from proteinuria early after birth. However, between 9 to 12 months of age, mice with conditional knockout of TrkC in the nephron developed mild proteinuria concomitant with foot process effacement. Histologically, glomeruli of nephron-specific TrkC knockout mice showed mesangial hypercellularity and thickening of the basement membrane.

Conclusion: These results suggest that TrkC is not necessary for foot process formation, but plays an important role in maintaining podocyte foot processes and glomerular function in aging mice.

Poster #19
Presented May 31
Blitz & Poster

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Proximity-Ligation Assay Identified the Rho Guanine Nucleotide Exchange Factor, β -PIX, as a Rac1-Interactor in Podocytes

Background: Hyperactivity of Rac1 (a small GTPase) in podocytes has been implicated in the development of proteinuria and focal segmental glomerulosclerosis (FSGS). We sought to identify guanine nucleotide exchange factors (GEFs) that activate Rac1 in podocytes.

Methods: BioID, a proximity-based ligation assay, was used to identify Rac1-GEFs in human podocytes (HP). This assay consists of HP expressing a bait, Rac1G15A (a mutant of Rac1 that has high affinity to active GEFs), conjugated to a biotin ligase, BirA (BirA-Rac1G15A). Biotinylated proteins (i.e. proteins that have come in close proximity of Rac1G15A) were isolated from HP with streptavidin-beads and identified by mass spectroscopy. Mouse podocytes (MP) with β -PIX knockdown (KD) were created using shRNA. Attachment was measured by plating cells on collagen for 1 hour, and then non-adherent cells were washed away while adherent cells were stained with crystal violet. Active Rac1 was visualized with immunofluorescence staining (IF) and quantified using CRIB pulldown (PD). β -PIX binding to Rac1 was determined by PD with GST-Rac1 or GST-Rac1G15A, followed by immunoblotting (IB). Rats were injected with puromycin aminonucleoside (PAN), and urine and glomeruli were collected 7-14 days after injection.

Results: BioID identified 5 GEFs in BirA-Rac1G15A expressing HP; β -PIX was by far the most abundant and the only one whose quantity was consistently enriched (20-fold or more) in three independent experiments, as compared with control cells expressing BirA alone. IB revealed β -PIX expression in HP and MP, while IF of kidney sections found β -PIX expression in human, mouse, and rat glomeruli, largely overlapping with nephrin and/or β 1-integrin. Compared to MP with scrambled shRNA, β -PIX KD MP had decreased active Rac1 staining, fewer projections, decreased cell area, and showed decreased attachment. Biochemically, stimulation with epidermal growth factor (EGF) increased Rac1 activity, and β -PIX binding to Rac1 by 5 and 10 minutes (5min: 1.56-fold \pm 0.24; 10min: 1.34-fold \pm 0.13, n=5, p<0.05). PAN caused proteinuria concomitant with increased β -PIX binding to Rac1G15A, suggesting increased β -PIX activity.

Conclusions: BioID using Rac1G15A as bait identified β -PIX as a predominant Rac1-interactor in HP. β -PIX is expressed in podocytes, and its binding to Rac1 is modulated by EGF and PAN. Together, this data warrants further studies on the functional role of β -PIX in podocyte health and disease.

Poster #20
Presented May 31
Blitz & Poster

Lateral and basal compartment crosstalk via Nck adaptors facilitates podocyte adhesive plasticity

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Kidney podocytes contribute to blood filtration selectivity through a network of actin-based projections termed foot processes (FP). FP morphology is critically dependent on signaling from two microdomains –basally-localized adhesive complexes, which secure the podocyte to the underlying basement membrane, and lateral cell-cell ‘slit diaphragm’ (SD) junctions, which serve as the kidney’s size-selective filter. Each are dynamic structures that must withstand significant insult while maintaining their barrier roles, although the mechanisms by which they communicate to promote plasticity are poorly understood. Mechanical force sensation is commonly transmitted through adhesive structures. Our group recently reported that signaling centered at nephrin, the SD’s core component, regulates Hippo-related mechanosignaling in the podocyte. Using several knockout podocyte cell lines and engineered adenoviruses, we now expand the role of nephrin mechanosensing to influencing focal adhesion dynamics. Live-cell imaging and biochemical analyses demonstrate that clustering of nephrin promotes focal adhesion turnover, a process that is dependent on the recruitment of the Nck1/2 cytoskeletal adaptors. In the absence of Nck, integrin β 1 activation is reduced, as is phosphorylation of the focal adhesion proteins FAK and paxillin, and adhesion complexes with decreased function accumulate. These defects are recapitulated in nephrinY3F mice, in which nephrin-Nck interactions are abolished, as well as in podocyte-specific Nck1/2 knockout mice, that furthermore display evidence of podocyte detachment. Interestingly, intermediate defects are observed in cells and animals devoid of Nck1 *or* Nck2 alone, resulting in increased susceptibility to injury and podocyte detachment *in vivo*. Collectively, our findings reveal that Nck serves as a hub to connect nephrin and integrin β 1, and further demonstrate that crosstalk between the lateral and basal compartments of the podocyte is required to coordinate the dynamics of FP remodeling in the face of strain.

Poster #21
Presented May 31
Blitz & Poster

The biologic function and intracellular localization of Crumbs2 mutants in human podocytes

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Background:

Crumbs proteins (CRB1-3) control apical membrane identity and cell polarity. In the kidney CRB2 is enriched in glomerular podocytes that form the slit diaphragm (SD). Like the SD protein Nephritin, CRB2 is a type I membrane protein with a huge extracellular region and a short intracellular tail. Defects at the SD which result in severe glomerular diseases and have been recently linked to mutants within the human *CRB2* gene. Interestingly, many disease-causing mutants are clustered in the extracellular part of CRB2. A frequent *CRB2* mutant is an Asparagine-Lysin exchange at position 800 (N800K), which carries a potential N-glycosylation site. In this study we analysed this mutant in more detail and investigated its impact on CRB2 intracellular localization, trafficking and its surface presence to obtain more insights about how CRB2 mutants cause SD defects.

Methods:

We generated stable immortalized podocytes enabling conditional expression of EGFP-tagged CRB2 WT and N800K mutant. Live cell imaging and immunofluorescence were applied to investigate intracellular trafficking and processing of CRB2 WT and the N800K mutant.

Results:

Our data show an enrichment of CRB2 in podocytes and a similar distribution as SD protein ZO-1 in glomeruli, indicating that CRB2 is predominantly exported to the SD. In cell lines overexpressed CRB2 is transported *via* the ER-Golgi route towards the plasma membrane, demonstrating that large EGFP-CRB2 fusion proteins are correctly formed and processed. The accumulation of CRB2 at overlapping regions at cell-cell contacts indicates the formation CRB2-CRB2 complexes. Moreover, CRB2 is able to recruit the scaffold protein Pals1 into these regions. By contrast, the CRB2 N800K mutant retains more inside the ER and Golgi apparatus and only a minor fraction is transported to the plasma membrane, suggesting that incorrect folding of CRB2 N800K probably prevents protein interactions and transport of binding partners to the plasma membrane.

Conclusion:

The disease-associated CRB2 N800K mutant showed striking differences in intracellular localization probably due to incorrect processing, indicating that CRB2 cell surface or SD localization is essential for podocytes function.

Poster #22
Presented May 31
Poster

Loss and Recovery of Cellular Forces upon Podocyte Injury

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In the healthy kidney, specialized cells, called podocytes, form a sophisticated blood filtration apparatus that allows excretion of wastes and excess fluid from the blood whilst preventing loss of molecules or cells. To operate effectively, this filter is under substantial hydrostatic mechanical pressure. Given their function, it is expected that the ability to apply mechanical force is crucial to the survival of podocytes. However, to date podocyte mechanobiology remains poorly understood, largely due to a lack of experimental data on the forces involved. Here, we describe data on the quantitative, continuous, non-disruptive and high-resolution measurement of the forces exerted by differentiated podocytes from both human and mouse *in vitro* models, in real time using a recently introduced functional imaging modality for continuous force mapping. Using a PAN model for podocyte injury, we find that injured podocytes experience near complete loss of cellular force transmission, but that this is reversible under certain conditions. The observed changes in force correlate with F-actin rearrangement and reduced expression of podocyte-specific proteins. In addition, we integrate cellular force measurements with immunofluorescence and perform continuous long-term force measurements of a cell population, which has not been feasible with established force mapping techniques. By introducing robust and high-throughput mechanical phenotyping and by demonstrating the significance of mechanical forces in podocyte injury, this research paves the way to a new level of understanding of the kidney.

Poster #23
Presented May 31
Blitz & Poster

The role of SH3D21 in Diabetic Nephropathy

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Introduction Diabetes is the most common cause of end-stage renal disease. The onset of albuminuria and podocyte damage is associated with disruption of the actin cytoskeleton. We identified SH3D21 as a potential key regulator of the actin cytoskeleton.

Methods Cultured murine and human podocytes under glucose- and VEGF-stimulation to mimic diabetic conditions compared to osmolality controls with mannitol were used to perform Western Blot (WB) analysis. To model type I diabetes, C57BL/6J mice were treated for 5 days either with intraperitoneal injections of streptozotocin (STZ) or sodium citrate buffer as control while blood glucose levels were determined for 16 weeks. WB analysis of STZ-injected mice and buffer-injected mice was carried out. Immunofluorescence (IF) of SH3D21 on kidney cryosections of STZ- or buffer-injected mice as well as human and murine cultured podocytes under diabetic conditions or osmolality control was carried out. Structured illumination Microscopy (SIM) was used to detect renal expression of murine Sh3d21. To identify protein interaction partner of SH3D21, we performed co-affinity purification mass spectrometry (MS) of murine whole kidneys as well as human cultured podocytes. We induced an sh3d21 knockdown in zebrafish larvae through morpholino-injection while scrambled morpholino injection served as control and detected protein loss through measurement of green fluorescent protein in retinal vessels of *Tg(l-fabp:eGFP-DBP)* zebrafish, while co-injection of human SH3D21 RNA constructs was performed to determine cross-species rescue.

Results Reduction of SH3D21 protein expression was detected in murine kidney and cultured podocytes under diabetic conditions. SIM imaging revealed Sh3d21 expression in the major foot processes. IF of diabetic murine kidney cryosections showed reduced Sh3d21 expression associated with the absence of Sh3d21-Nephrin co-expression. Murine and human podocytes revealed a shift from a membranous to a perinuclear disrupted SH3D21-expression parallel to actin disruption under glucose- or VEGF-treatment. Interactions of murine and human SH3D21 were detected primarily with cytoskeletal proteins. Knockdown of sh3d21 in zebrafish caused pericardial effusion and loss of green fluorescence protein, while a partial cross-species rescue with human SH3D21 was detected.

Conclusion SH3D21 appears to be an important regulator of the actin cytoskeleton impacting slit diaphragm functions.

Poster #24
Presented May 31
Poster

Lipid peroxidation impacts podocyte migration and cytoskeletal structure through redox sensitive RhoA signaling

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Podocytes are highly specialized cells in the glomeruli, and an integral part of a healthy glomerular filtration barrier. Any impairment in their adhesion or foot processes can lead to cell deformity, foot process effacement and ultimately to the loss of the cell, thus compromising barrier function. Early podocyte loss is characteristic of chronic kidney diseases (CKD) in obesity and diabetes. Since treatments for hyperglycemia and hypertension do not prevent podocyte loss, there must be additional factors causing podocyte depletion. The role of oxidative stress has been implicated in CKD but it is not known how exactly free radicals affect podocyte physiology. To assess this relationship, we investigated the effects of lipid radicals on podocytes, as lipid peroxidation is a major form of oxidative stress in diabetes.

In our methods, we used state-of-the-art modeling of lipid radical generation, a novel time lapse imaging approach to follow podocyte migration and site directed mutagenesis to change the cysteine residues of the migratory regulator protein RhoA to alanines.

We found that lipid radicals govern changes in podocyte homeostasis through redox sensitive RhoA signaling: lipid radicals inhibit migration and cause loss of F-actin fibers in a dose-dependent fashion while also increasing active, GTP-bound RhoA. These effects were largely mitigated when both of the redox sensitive cysteines (C16 and C20) of RhoA were mutated to alanine. We therefore suggest that in diseases associated with increased lipid peroxidation, lipid radicals can determine podocyte function independently of other conditions, with potentially pathogenic consequences for kidney physiology. Since lipid peroxidation is significantly elevated in diabetes and end-products of lipid peroxidation are known to accumulate in human diabetes, including the glomeruli, the implications of our findings may be of clinical relevance in diabetic CKDs.

Poster #25
Presented May 31
Poster

Clathrin-Dependent Nephrin Endocytosis Mediated by the Y¹¹³⁹RSL Motif is Essential for the Glomerular Slit Diaphragm Formation

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Nephrin is a critical component of the glomerular slit diaphragm. The cytoplasmic tail of human nephrin contains a canonical YxxØ-type motif Y¹¹³⁹RSL with an unknown function. Examination of genetic variation at this motif in human cases and the population support the potential importance of Y¹¹³⁹RSL. A c.C3418T nucleotide change, resulting in p.R¹¹⁴⁰C has been implicated as causal for congenital nephrotic syndrome (CNS; in compound heterozygosity) in one of the first reported patients, and was found as a single allele in siblings with CNS. From a population genetics perspective, the Y¹¹³⁹RSL sequence is very important because variation at this motif is rare, if not absent. Nephrin multi-sequence alignment demonstrated that the motif is expressed only in primates. Thus, we examined the role of Y¹¹³⁹RSL using a podocyte line and a zebrafish model expressing human nephrin.

Human nephrin co-immunoprecipitated with clathrin and the adaptor complex AP-2 and the residue Y¹¹³⁹ was phosphorylated in human podocytes. The Y¹¹³⁹F substitution, mimicking non-phosphorylated tyrosine, increased clathrin-dependent nephrin endocytosis and reduced the steady-state abundance and stability of nephrin at the podocyte plasma membrane. By contrast, the Y¹¹³⁹A substitution, disrupting the YxxØ endocytic motifs, had the opposite effects. Zebrafish embryos depleted of nephrin exhibited pericardial and yolk edema, curvature of the body axis, and amorphous glomerular and podocyte foot process organization. Co-injecting the human (hs)-Nephrin-Y¹¹³⁹F transcript with the zebrafish nephrin morpholino (dr-Nephrin-MO), partially rescued the phenotype and improved defects in glomerular and foot process organization caused by nephrin depletion, similar or superior to hs-Nephrin-WT. By contrast, morphants injected with hs-Nephrin-Y¹¹³⁹A failed to rescue, having phenotypes similar to dr-Nephrin-MO alone.

In summary, the Y¹¹³⁹RSL motif is a structural element for clathrin-dependent nephrin endocytosis and functions as a phosphorylation-sensitive signal essential for the slit diaphragm formation. We propose that the Y¹¹³⁹RSL-mediated endocytosis helps to maintain asymmetric distribution of nephrin in specialized membrane domains leading to podocyte differentiation and formation of the slit diaphragm.

Poster #26
Presented May 31
Poster

TITLE: Angiotensin Mutation Increases the Formation of Stress Fibres and Disturbs Tight Junction

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Background

Based on whole exome sequencing in a local family with X-linked recessive nephropathy, we identified a putative missense mutation in the AMOT gene which codes for angiotensin. We have used CRISPR/Cas9 system to introduce the genetic variant into rats. The transgenic animals were shown to have glomerular and cystic disease. This study aimed to reveal the effects of AMOT mutation on cell skeleton and cell junctions with ex vivo cultured podocytes.

Methods

Glomeruli were isolated with abdominal aorta injection of magnetic dynabeads ($\Phi 4.5 \mu\text{m}$) and cultured at 37 °C. Islets of cobblestone-shaped podocyte outgrowths from glomeruli were trypsin detached and subcultured. The identification of the isolated putative podocytes were affirmed by cellular morphology, as well as expression of podocyte-specific markers such as podocalyxin and synaptopodin. Immunofluorescence staining on F-actin and tight junction related proteins were next performed on the cells.

Results

In the ex vivo cultured podocytes, AMOT mutation increased the formation of stress fibres, disturbed the expression/distribution of tight junction related proteins (ZO-1, Occludin), and reduced cell focal adhesion. The perturbation on tight junction were confirmed by the FITC-albumin flux experiments using a cell monolayer formed by proximal convoluted tubular cells (PCT). The FITC-albumin concentration ratio between PCT monolayers was significantly reduced in the mutation group than in the wild type control group. On the contrary, AMOT mutation seemed to not change the formation of adherens junction.

Conclusions

AMOT appears to play important roles in regulating renal functions. AMOT mutation increases the formation of stress fibres and disturbs tight junction, leading to glomerular and cystic disease.

Poster #27

Presented May 31

Poster

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Single Cell Transcriptome Profiling of Glomeruli from Mouse Models of Glomerular Injury

Development of single-cell RNA-seq technology has led to a better understanding of cell (sub)types and heterogeneity of cellular responses to injury in various organs. Using the 10x Genomics Chromium™ technology, we profiled the major cell types of the glomeruli from mice with or without glomerular injury. Unsupervised clustering analysis based on transcriptomes clearly distinguished the three major cell types in the glomerulus (podocytes, mesangial cells, endothelial cells). We identified novel mesangial cell marker gene candidates by analyzing differentially expressed genes. Upon induction of anti-GBM glomerulonephritis, we detected accumulation of immune cells and significant cell type-specific changes in gene expression. Analysis of these changes have revealed several genes and pathways that may be targets for treating glomerulonephritis. After resolution of the heterologous phase, the global gene expression pattern of podocytes largely reverted back to its original state, whereas the transcriptome profile of mesangial cells and endothelial cells remained altered. We are currently generating single-cell RNA-seq data using glomeruli from CD2AP-deficient mice and mice with Adriamycin nephropathy. A comparative analysis of these different models may provide molecular insight into common pathways that mediate injury in various glomerular diseases.

Poster #28
Presented May 31
Poster

**Topic: Crosstalk within
glomerulus**

Podocyte activation and crosstalk with endothelial cells through Ednra leads to degradation of glomerular endothelial surface layer.

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Background. Crosstalk between activated podocytes and glomerular endothelial cells (GECs) has been demonstrated in mouse models of focal segmental glomerulosclerosis (FSGS), where podocytes' activation can result in the release of endothelin-1 (Edn1), causing GEC stress and dysfunction and concomitant albuminuria via increased Endothelin receptor A (Ednra) signaling. Consequently, this reciprocal crosstalk leads to podocyte loss. However, the link between podocyte activation, GEC injury, and albuminuria remains unclear.

Methods and Results. Ultrastructural assessment of glomeruli with scanning electron microscopy revealed that podocyte specific activation by TGF- β RI signaling affected GECs by decreasing the number of fenestrae, increasing ridges and protrusions into the lumen of capillary vessels after 4 days, and these observations preceded podocyte foot process effacement detected 7 days after of TGF- β RI activation. Transcriptomic analysis of isolated GECs revealed that target genes involved in glycosaminoglycan (GAG) metabolism and degradation of the endothelial surface layer (ESL) were significantly affected after podocyte activation, and this response coincided with microalbuminuria. We measured a significant reduction of the ESL in mice after 4 days of TGF- β RI activation using intralipid infusion and transmission electron microscopy, and by directly labeling glycoconjugates with Isolectin-B4 (IB4). This effect was prevented by Ednra antagonism. A significant reduction of the ESL was also observed in Adryamycin nephropathy, and was also prevented by blocking Ednra. Atomic force microscopy measurements showed a significant reduction of the ESL in murine GECs (mGECs) after co-incubation with Edn1 or conditioned media supernatant (SN) collected from TGF- β RI activated podocytes. There was a reduction in cell surface heparan sulfate and IB4 together with an increase in heparanase and hyaluronoglucosaminidase in mGECs co-incubated with TGF- β RI activated podocyte SN. The loss of ESL in mGECs was prevented by Ednra antagonism.

Conclusions. Our studies provide evidence of an early crosstalk between activated podocytes and GECs that results in GEC injury, loss of ESL and albuminuria via Edn1-Ednra in experimental FSGS.

Poster #29
Presented May 31
Poster

Podocyte-specific deletion of Early B Cell Factor 1 minimizes sclerotic damage following glomerular injury

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It is now understood that podocyte loss, through sloughing and/or apoptosis, is the precipitating factor driving glomerulosclerosis. We also had previously reported that podocytes express the transcription factor Early B Cell Factor 1 (EBF1), although the function of this protein in these cells was unclear. Using a floxed version of the EBF1 gene and podocin-cre we eliminated EBF1 specifically from podocytes, and found that there was no developmental necessity for EBF1 in the formation of the podocyte or the glomerulus. However, the absence of EBF1 was reno-protective. During a low nephron endowment model of congenital CKD, EBF1-deletion reduced proteinuria and delayed glomerular filtration rate (GFR) decline when measured by inulin infusion, prolonged survival, and decreased sclerotic damage. Similarly, glomerulonephritis induced through IV administration of anti-glomerular basement membrane serum was dramatically reduced at the histological and ultrastructural level seven and days post injection. Third, using L-NAME to induce reversible glomerulosclerosis GFR decline and glomerular injury were found to be equivalent during the 20-week hypertensive duration, however, recovery of the kidney for an additional ten-week period was dramatically accelerated when EBF1 was absent. This was reflected in the histological recovery of the tissue, and also functionally through GFR measurement with the conditional knockout mice recovering almost half of their lost GFR within 3 weeks and fully restoring kidney function by ten weeks. Wildtype controls, by contrast, were not improved at the three-week mark, and only partially recovered by ten weeks. These results indicate that EBF1 normally promotes injury signals detrimental to the health of the podocyte through cell-intrinsic gene regulation. To identify the signaling pathways altered by EBF1 following its deletion we induced glomerular injury in both the acute glomerulonephritis injury model as well as during and after development of the reversible hypertensive damage incurred by L-NAME. Alterations in RNA levels were compared to the chromosomal occupancy of EBF1 through CHIP-Seq where a GFP-tagged version of EBF1 was specifically expressed within on the podocytes. Pathway analysis performed with Metascape revealed EBF1 loss protects the integrity of the slit diaphragm components and that this may be occurring through loss of direct repression of STAT3 expression by EBF1.

Poster #30
Presented May 31
Poster

Title: Transcriptome of Extracellular Vesicles Derived from Podocytes, Proximal Tubule and Mesangial Cell Culture Media

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Background: Extracellular vesicles (EVs) are lipid-bilayer structures of variable sizes (30-1000um) secreted by cells. They show a distinct cell surface consisting of a variety of proteins and lipids while the intracellular content, “cargo”, consists of a rich array of proteins and distinct RNA species reflecting accurately the physiological state of the cells of origin. In diabetes, development of renal insulin resistance has a major role in Diabetic Kidney Disease (DKD) while little is still known of EV effects on DKD target cells, especially podocytes.

Objective: To characterize EVs secreted by DKD target cells, podocytes, proximal tubular cells and mesangial cells using Hydrostatic Filtration Dialysis for EV harvesting.

Methods: We used cell culture media from podocytes, proximal tubule and mesangial cells in four conditions; 1) Basal 2) Insulin resistant 3) Insulin receptor transfected 4) Insulin receptor transfected and insulin resistant. EVs were isolated from 50ml of cell culture media using the Hydrostatic Filtration Dialysis. Quality of the EV yield was analyzed with negative staining EM and Western blotting. Vesicle size distribution and concentration was determined by Nanoparticle Tracking Analysis (NTA). Isolated RNAs were profiled with Bioanalyzer Pico kit and subjected to RNAseq after cDNA library preparation using low amount protocols. RNAseq was performed using HiSeq 3000 (Illumina) pair-end (2X150) protocol. Output reads were aligned to human reference genome and counted using GENCODE gene annotations. We used gene length normalized values FKPM

(Fragments Per Kilobase of Exon Per Million) as expression measurement for genes.

Results: The isolated EVs appeared typical at EM and were positive for the EV-marker TSG101 in Western blotting. RNA quantity and quality appeared sufficient for RNAseq. Detailed results will be reported.

Conclusions: EV analysis provides a novel approach to reveal valuable pathophysiology, pathway and signaling information of cultured target cells.

Poster #31
Presented May 31
Poster

Model system with biomimetic topography promotes podocyte differentiation

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Here we present an *in vitro* culture system which allows for higher-fidelity studies of podocyte biology. Mature podocytes are distinguished by their features of arborized morphology, foot process formation and interdigitation, and expression of slit diaphragm proteins such as nephrin. However, current *in vitro* cultures of podocytes face a bottleneck, whereby many of these differentiation hallmarks lack appreciable development. This work introduces a new approach for culturing podocytes with a higher degree of maturity.

Podocytes *in vivo* experience a native environment of convex curvature in three-dimensions, as they grow and wrap their extensive process network around the glomerulus' globular and convoluted capillary loops. We hypothesized that these 3D architectural cues of the native microenvironment are integral in supporting proper podocyte development. Thus, we developed a culture platform featuring microtopography that mimics curving glomerular capillaries, in order to provide a geometric stimulus to promote podocyte differentiation *in vitro*.

We engineered a culture platform that resembles the round capillaries of the glomerulus by using spherical glass beads to generate a microhemispherical topography. Soft, PDMS-molded replicas of these platforms were inserted into 24-well plates, and used with standard cell culture and analysis techniques. We cultured podocytes on the topographic platform and compared them with cells grown on the flat counterpart. The topographic platform yielded cells with more arborized morphologies, more evolved process formation, and greater upregulation and localization of nephrin. We were also able to observe differences in barrier function by translating the topographic platform into a membrane-based system. In addition, this versatile system was amenable to testing the podocytes with molecular insults and therapeutic compounds, thereby presenting a wide range of use.

Overall, this work demonstrated the implementation of biomimetic curvature for improving the fidelity of cultured podocytes, in the effort towards creating more effective cell-based models as tools for studying biology and screening new therapeutic approaches.

Poster #32
Presented May 31
Poster

Effects of podocyte-derived microparticles on proximal tubule cell proliferation, fibrosis, and arginine metabolism

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Tubular injury is a major pathogenic component of advanced chronic kidney disease characterized by fibrosis, impaired proliferative capacity, and metabolic dysregulation. Microparticles (MPs) are 100-1000 nm vesicles shed from injured cells that are implicated in intercellular signalling. Our lab, and others, have shown that podocyte MP formation is increased in glomerular disease. The purpose of the present study was to assess the effect of podocyte MPs on proximal tubular epithelial cells *in vitro*. MPs were isolated from the media of fully differentiated, untreated human podocytes (hPODs). PTECs were treated with 1-10 $\mu\text{g}/\text{mL}$ of isolated MPs. Treatment with podocyte MPs was associated with a $\sim 70\%$ reduction in PTEC proliferation as determined by BrdU incorporation ($P < 0.0001$). In addition, podocyte MPs increased p38 and Smad3 phosphorylation and expression of the extracellular matrix (ECM) proteins fibronectin and collagen type IV. As for the arginine metabolism, treatment with podocyte MPs was shown to have no significant effect on Argininosuccinate synthetase (ASS, converts citrulline to arginosuccinate). The expression of Argininosuccinate lyase (ASL, converts arginosuccinate to arginine) and Arginase-2 (ARG-2, degrades arginine) did not change significantly following treatment. ARG-2 activity did not show any significant effect for any of the treatment time points. Taken together, our data suggest that podocyte MPs may contribute to kidney injury by promoting fibrosis and inhibiting the tubular repair process, with no significant effect on arginine metabolism.

Poster #33
Presented May 31
Poster

Role of Nephronectin in glomerular structure and function

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Our current understanding of interactions between the glomerular basement membrane and mesangial cells are quite limited. Using mouse models, we find that nephronectin, a GBM component and known ligand of $\alpha 8 \beta 1$ integrin, is produced by podocytes and deposited into the GBM, where it is required for formation of a novel GBM-mesangial cell adhesion structures. These specialized adhesions occur at sites of mesangial cell protrusion that are highly enriched in $\alpha 8 \beta 1$ integrin and appear to anchor the capillary loops. Absence of nephronectin disrupts these adhesion structures, leading to mislocalization of $\alpha 8 \beta 1$, a pronounced increase in mesangial cell number, and mesangial sclerosis. The molecular composition of the GBM is also altered. These results demonstrate a novel role for nephronectin- $\alpha 8 \beta 1$ integrin in a newly described adhesion complex and begin to uncover the molecular interactions between the GBM and mesangial cells. We are currently pursuing *in vitro* studies to examine the role of nephronectin on individual cellular behaviors as well as glomerular signaling and crosstalk.

Poster #34
Presented May 31
Poster

Identifying novel AAV capsids displaying tropism for a conditionally immortalised human podocyte cell line

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Background

Recombinant adeno-associated virus (AAV) may be used as a vector for the delivery of DNA to a target organ in gene therapy. The advent of capsid engineering techniques has yielded a range of novel AAV capsids displaying tissue-dependent specificity. The aim of this study was to define the tropism of a conditionally immortalised human podocyte (ciPod) cell line for a range of AAV capsids.

Methods

An 'AAV vector screen kit' containing 18 variant capsids, developed by the Translational Vectorology Group (Children's Medical Research Institute). Each capsid was packaged with AAV-CAG-nlsCre-eGFP to facilitate transduction and detection. 5.5×10^4 differentiated ciPod were treated with viral particles at multiplicity of infections (MOIs) ranging from 3×10^2 to 3×10^6 for 48 hours. Transduction efficiencies were assessed via flow cytometry and microscopy for green-fluorescent protein (GFP).

Results

Human podocytes *in vitro* display varied tropism for AAV based on capsid composition. For the traditional AAV capsids (AAV1-9), AAV2 displayed the strongest transduction efficiency (37.4%) with a mean fluorescence intensity of 5,111 at an MOI of 3×10^5 . However, the greatest transduction was induced by novel AAV variants, NP94 and LK03. At an MOI of 3×10^5 , transduction efficiency and MFI were 35.8% and 6,214 for NP94. At an MOI of 3×10^5 , LK03 displayed a transduction efficiency and MFI of 41.9% and 6,092, respectively. AAV NP6 and DJ achieved an MFI of > 4000 at MOI 3×10^5 . Negative (untransduced) cells displayed a transduction efficiency of 2.6% and MFI of 497.

Conclusions

This study predicts that human podocytes may be targeted by AAV vectors *in vitro*. Through the results of our screen, four novel AAV capsids (AAV DJ, LK03, NP6 and NP94) were identified that may provide similar or more favourable results in the therapeutic target of human podocytes than the traditional AAV (AAV2). Future functional gene delivery studies will help to confirm these findings.

Poster #35
Presented May 31
Poster

EVs derived from amniotic fluid stem cells as potential therapy for chronic kidney disease.

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Despite the emergence, chronic kidney disease (CKD) still is a global health threat. There is a multitude of diseases that can culminate in renal failure, and loss of glomerular cell function still remains the final outcome for nearly all of them. For this reason, the discovery of safe therapeutics designed to specifically target the glomerulus is potentially ground-breaking.

Using Alport Syndrome as an animal model for CKD, we showed that administration of amniotic fluid stem cells (AFSC) can significantly delay disease progression and can restore vascular endothelial growth factor signaling (VEGF, which plays a key role in the maintenance of the glomerular function) within affected glomeruli, thus providing therapeutic benefit. The beneficial effect of AFSC is attributed to secretion of extracellular vesicles (EVs) that contribute to the paracrine action of AFSC in the kidney. AFSC-EVs, which express exosomal and mesenchymal surface markers and contain specific miRs known to modulate VEGF/VEGFRs signaling, are capable of re-establishing VEGF/VEGFRs signaling in damaged glomeruli. They also recapitulate the protective role of their cell of origin by ameliorating renal function and morphology once injected in vivo. Using knock-out experiments, we confirmed that one of their possible mechanisms of action is that these EVs can function as a "trap" for excessive local glomerular VEGF thanks to its binding to VEGFR1 and VEGFR2 consistently expressed on their surface, thus rendering it unavailable for further downstream cellular activity.

In light of clinical translation of EVs for CKD, we also have carefully characterized EVs of human origin. Based on these data we developed specific identity and potency assays that will enable the generation of lots of EVs with very similar characteristics for therapeutic application in the field of renal disease.

In conclusion, we believe that our data would significantly advance the development of EVs as new potential glomerulus-specific targeted intervention, thus possibly minimizing disease progression by restoring homeostatic crosstalk between podocytes and glomerular endothelial cells.

A novel zebrafish model of kidney disease

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Background: Kidney disease is a major healthcare burden in New Zealand and globally. Podocytes are an essential cellular component of the kidney filtration barrier and have a complex morphology that is characterized by interdigitating extensions called foot processes. Linking the foot processes together is a protein bridge made up of transmembrane proteins, known as the slit diaphragm. Podocyte injury contributes to the onset and progression of kidney disease and is associated with a retraction and spreading of podocyte foot processes (known as effacement), a downregulation of slit diaphragm components, and loss of blood filter integrity.

Objectives: To develop an inducible model of podocyte effacement and recovery model in zebrafish using protamine **sulphate**, a positively charged compound that neutralizes the charge of the filtration barrier and induces foot process effacement. By using this model, we hope to better understand the molecular mechanisms governing the maintenance and establishment of foot processes.

Methods: Transgenic zebrafish larvae with fluorescently tagged podocytes were injected with protamine **sulphate** or water (vehicle control) and analysed at different time points post injection using confocal microscopy 3D imaging, immunostaining, electron-microscopy, qPCR and RNA-Seq. Injection of the animals with a large fluorescent dextran, which is not normally filtered under healthy conditions, was used to assess the integrity of the blood filtration barrier.

Results: protamine sulphate induced the podocytes to undergo dramatic 'rounding up' within 24 hours post injection and was associated with a leaky blood filter. Electron-microscopy revealed podocyte foot process effacement in the protamine **sulphate** injected animals. These defects were reversible with the morphology and integrity of the blood filter recovering 5 days later. Preliminary qPCR experiments indicate that protamine **sulphate** treated animals downregulate slit diaphragm genes and were supported by RNA-Seq data.

Conclusion: These data show that podocytes exhibits morphological changes (effacement), decrease of slit-diaphragm components, and loss of blood filter integrity upon Pt.SO_4 administration specifically 24 hours post injection. RNA-Seq data of induced injured podocytes suggests a future approach for potential therapeutic targets.

Poster #37
Presented May 31
Poster

A podocyte-specific knockout of the DNA repair gene *Ercc1* leads to proteinuria and focal segmental glomerulosclerosis

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Background: Chronic kidney disease with mild to severe impairment of kidney function is common among elderly patients. Yet, models investigating this functional decline are lacking. We identified the progeria mouse model of *Ercc1*-deficiency to exhibit expression profiles similar to those of glomerular aging in wild type mice. *Ercc1* facilitates the 5' incision around interstrand cross links and other bulky DNA lesions in the nucleotide excision repair pathway. Thus, a podocyte-specific knockout of *Ercc1* may help us gain new insights into aging processes.

Methods: *Ercc1*^{flox/flox} mice were bred in a mixed CD1/FVB background with CD1 mice expressing Cre recombinase under the podocin promoter. At 7, 9, 11 and 13 weeks of age weight, urine and serum were analyzed. Kidneys were fixed in paraformaldehyde and embedded in paraffin, fresh-frozen in OCT, or prepared for electron microscopy.

Results: *Ercc1*^{pk0} mice die prematurely at week 13-18. 7 week old kidneys show no morphological changes in light or electron microscopy. Male and female mice develop foot process effacement and focal segmental glomerular sclerosis at 9 weeks of age. PAS staining of 13 week old kidneys reveals sclerosed glomeruli, interstitial fibrosis with tubular atrophy and tubular protein casts. Cultured podocytes subjected to DNA damage exhibited an increase of mTOR phosphorylation and mTOR-Complex1 activation. This phenotype was rescued by rapamycin and was observed in both *Ercc1*^{pk0} and 96 week old wild type murine and human glomeruli.

Conclusion: Our study underlines the critical role of podocyte DNA maintenance. Furthermore the podocyte specific knockout of *Ercc1* depicts accelerated features of the podocyte aging process and will prove a crucial tool to investigate kidney aging. Ongoing studies focus on the rescue of this phenotype by mTOR interference.

Poster #38
Presented May 31
Poster

Leflunomide, a novel second-line agent for children with difficult-to-treat nephrotic syndrome

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Background: Idiopathic nephrotic syndrome (INS) is the most common chronic glomerular disease in children. While the precise etiology of INS remains enigmatic, indirect evidence suggests that podocyte injury is due to an altered immune response, possibly mediated by soluble factor(s). Glucocorticoid (GC) therapy induces complete remission in up to 90% of patients, but half of them experience a frequently relapsing/GC-dependent course. The disease burden to family and health care system is substantial. Several GC sparing, immunosuppressants have been tried to prevent proteinuria relapses, with variable success.

We hypothesized that leflunomide (LEF), an effective agent for the treatment of lupus nephritis and other autoimmune disorders, may reduce the frequency of proteinuria relapses by suppressing pathogenic T and B cell responses and the production of lymphocyte-derived, podocyte-targeting cytokines. Here we evaluate efficacy and adverse event profile of LEF in children with primary INS.

Methods: Retrospective study of a prospectively followed cohort of 12 children with INS who have been treated with LEF between 2011 and 2017.

Results: Patients received LEF for 31.8 months (median; range 2.8–77 months) aiming at trough levels between 40 and 80 mg/L. Ten children had GC-sensitive INS and two had GC- and calcineurin inhibitor-resistant NS. LEF monotherapy sustained long-term remission in 9/10 children with GC-sensitive INS (median 36, range 8.2–71 months) and partial remission in a patient with recurrent focal segmental sclerosis (rFSGS) after kidney transplantation. Preliminary data suggest a trough level of 40 mg/L is protective.

Four patients had mild to moderate anemia, which improved in all instances spontaneously or following LEF dose reduction or discontinuation (n=1). Anemia and skin lesions were associated

with higher LEF dosing, but no such correlation was noted for hepatic (biochemical) abnormalities or the development of oral ulcers.

Conclusions: The present series demonstrates efficacy of LEF to control GC-dependent/frequently relapsing NS and potentially, recurrent FSGS. Future studies should confirm its safety and efficacy in comparison with other second-line agents, and its therapeutic threshold.

Poster #39
Presented May 31
Poster

Neutrophil exocytosis induces podocyte cytoskeletal reorganization and proteinuria in
experimental glomerulonephritis

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Background:

Acute glomerulonephritis is characterized by rapid glomerular neutrophil recruitment, proteinuria, and glomerular hypercellularity. The current study tested the hypothesis that release of neutrophil granule contents plays a role in both loss of filtration barrier leading to proteinuria and the increase in glomerular cells.

Methods:

Acute glomerulonephritis was induced in C57BL/6J mice using nephrotoxic serum (NTS) followed by administration of an inhibitor of neutrophil degranulation, TAT-SNAP-23. Proteinuria, kidney pathology, glomerular leukocyte accumulation, and glomerular inflammatory mediator expression were assessed. Cytoskeletal injury was assessed by confocal imaging of actin in immortalized cultured human podocytes incubated with neutrophil granule contents. The ability of supernatants from resting and activated cultured podocytes to alter chemotaxis, exocytosis, and respiratory burst activity of primary human neutrophils was tested.

Results:

Inhibition of neutrophil exocytosis with TAT-SNAP-23 prevented proteinuria, attenuated podocyte foot process effacement and loss of WT-1 staining, and reduced endothelial cell injury.

Inhibition of exocytosis had no effect on glomerular hypercellularity. Cultivation of podocytes with neutrophil granule contents disrupted cytoskeletal organization, an *in vitro* model for podocyte effacement.

TNF-activated cultured podocytes released cytokines that stimulated neutrophil chemotaxis, primed respiratory burst activity, and stimulated or primed exocytosis.

We conclude that crosstalk between podocytes and neutrophils contributes to disruption of the glomerular filtration barrier in acute glomerulonephritis. Neutrophil granule products induce podocyte injury, but do not participate in the proliferative response of intrinsic glomerular cells.

Poster #40
Presented May 31
Poster

Interleukin (IL)-27 induced podocytes migration in minimal change nephrotic syndrome (MCNS) in relapse

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Background

We have previously reported decreased monocyte CD14 expression with downregulation of pro-inflammatory cytokines, IL-8 and tumor necrosis factor (TNF)- α in lipopolysaccharide (LPS)-stimulated monocytes during nephrotic relapses in MCNS patients, suggestive of a monocytes M2 polarisation effect by Th2 cytokines. This study aimed to identify the monocyte 'gene signature' in MCNS patients and subsequently to validate the findings in human podocytes.

Methods

Monocytes were isolated from 5 MCNS patients in relapse and remission using monocytes isolation kit II (miltenyibiotec). Monocyte transcription profile was performed using Illumina Human Ref8 chips. Plasma IL-27 levels were measured in 14 MCNS patients in relapse and remission and 20 healthy controls. The role of IL-27 in human podocytes was studied using cell migration assay (cultrex). Podocyte RhoA/Rac1 activity were measured using ELISA and STAT1/3 levels were studied using Western blot. Statistical analysis was done using Mann-Whitney test and Wilcoxon signed rank test for paired data.

Results

Monocyte transcriptome in MCNS patients in relapse demonstrated involvement of genes in IL-1 signaling, RhoGTPases regulation of actin cytoskeleton, toll-interleukin receptor (TIR)-domain-containing adapter-inducing interferon- β (TRIF) and IFN-induction (IRF4, IRF7, IFI6, IFI27, IFI35, IFI44, SERPING1, OAS1, OAS2, OAS3, OASL, CXCL9, CXCL10, DDX58) pathways. Analysis of the monokines showed 2.7-fold increased in IL27 gene expression in MCNS patients in relapse. Consistent with the microarray results, plasma IL-27 levels were significantly higher in MCNS patients in relapse (1.56 ± 0.19 pg/ml) compared to remission (0.95 ± 0.13 pg/ml) ($P < 0.05$) and controls (0.89 ± 0.14 pg/ml) ($P \leq 0.01$). IL-27 stimulation in human podocytes resulted in phosphorylation of both STAT1 and STAT3. RhoA activity in IL-27 stimulated podocytes remained largely unchanged whereas activated Rac1 levels in podocytes were 1.56-fold higher compared with unstimulated podocytes at 20 minutes. Moreover, IL-27 induced 6.35% podocytes migration, comparable to the 6.96% podocytes migration observed in LPS-stimulated podocytes.

Conclusions

During relapses in MCNS, there was an increased monocyte production of IL-27 which could result in activation of STAT1, STAT3 and Rac1 as well as induction of cell migration in human podocytes.

Poster #41

Presented May 31

Poster

Cilengitide Inhibits $\alpha\beta3$ Integrin Activation and Functional Changes in Human Podocytes Exposed to Focal Segmental Glomerulosclerosis Disease Plasma

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Background: Focal segmental glomerulosclerosis (FSGS) is a rare form of nephrotic syndrome, and carries a poor renal prognosis. A ‘circulating factor’ has been implicated to activate $\alpha\beta3$ integrin on glomerular podocytes that results in enhanced podocyte motility and dysfunction of the filtration barrier. Inhibition of an over-activated $\alpha\beta3$ integrin in FSGS could modulate the pathologic process characterized by increased podocyte motility observed at the cellular level. Cilengitide is a clinically validated compound that is based on the cyclic RGD peptide which binds to RGD recognition site of $\alpha\beta3$ integrin and inhibits its activity. We examined whether cilengitide inhibits $\alpha\beta3$ integrin activation and whether it reduces the increased motility of human conditionally immortalized podocytes (CiPODs) incubated with diseased plasma in vitro.

Methods: After full differentiation, CiPODs were incubated for 30 minutes in serum free medium with no stimulant, 2mM manganese (positive control), 10% of post-transplant relapse and remission plasma samples obtained from the same patients and from a healthy control, pre-treated with or without 0.025 μ M of cilengitide. Cells were fixed and stained using antibody to active- $\alpha\beta3$. After imaging with total internal reflection fluorescence microscope (TIRFM), the signal intensity per cell was quantified and the differences among stimuli were compared. Podocyte motility was evaluated using a real-time electric cell-substrate impedance sensing (ECIS) wound healing assay. Cells were pretreated with or without 0.025 μ M of cilengitide. Stimulants were added before electrical wound making. The data was analyzed with curve fitting using Prism 6.0 and Hill slope was taken as the cell migration velocity.

A novel protein permeability assay on isolated rat glomeruli was performed for *ex vivo* functional assessment, using the same stimuli (Desideri et al, Kidney International 2018).

Results: Significant increase of $\alpha\beta3$ integrin activation and motility of CiPODs incubated with relapse samples was observed compared to vehicle and remission samples. Cilengitide treatment inhibited $\alpha\beta3$ integrin activity and reduced motility of CiPODs incubated with relapse samples to the same levels of that observed in vehicle and remission samples.

Finally relapse plasma significantly and consistently elevated glomerular protein permeability, which was reduced to remission plasma levels by Cilengitide.

Conclusion: These results strengthen Cilengitide as a potential new drug candidate in FSGS.

Poster #42
Presented May 31
Poster

The novel $\alpha\beta3$ integrin binding protein, inducible co-stimulatory ligand (ICOSL), protects podocytes from kidney injury.

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The ligand for the inducible co-stimulatory (ICOS) receptor, ICOSL, is constitutively expressed on antigen presenting cells (APCs), and plays an essential role in T-cell mediated immune responses. Previous studies have reported that the expression of ICOSL is restricted in the immune system, and as such, its potential role in non-hematopoietic cells under disease conditions remains completely unexplored. Using both *in vitro* and *in vivo* approaches, we show that LPS-induced injury rapidly up regulates ICOSL expression levels on podocytes. ICOSL-deficient mice (*ICOSL*^{-/-}) subjected to either LPS-induced kidney injury or STZ-induced diabetic nephropathy (DN) show severe proteinuria and have a greater susceptibility to the progression of kidney damage. 3D modeled of the ICOSL protein reveals exposed loop regions containing an arginine-glycine-aspartic acid (RGD) motif, known to be involved in integrin binding. Surface plasmon resonance (SPR) shows that ICOSL directly binds active $\alpha\beta3$ integrin, and *in vitro* adhesion assays reveal that podocyte-to-substrate attachment is modulated by ICOSL. Knockdown of ICOSL in human podocytes results in decreased adhesion and subsequent detachment from vitronectin-coated surfaces. Finally, exogenous ICOSL injection into *ICOSL*^{-/-} mice is sufficient to counter LPS-induced kidney injury. In conclusion, the rapid induction of ICOSL during kidney injury acts as a protective mechanism for podocytes to mitigate excessive $\alpha\beta3$ integrin activation and prevent the development of kidney disease.

Poster #43
Presented May 31
Blitz & Poster

APOL1 risk variant induced kidney damage in podocytes is mediated by inflammasome-dependent injury

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Background: Coding variants of APOL1 (termed as G1 and G2) are associated with increased risk of kidney disease in African Americans. Recently, we developed a new mouse model that recapitulates human APOL1 associated renal disease by conditional inducible expression of G1 and G2 APOL1 variant in podocytes. While these animals develop albuminuria, glomerulosclerosis, and azotemia, the exact pathomechanism of APOL1 risk variant induced kidney damage remains poorly understood. Here, we tested whether Nlrp3/caspase-1/IL-1 beta (IL-1B) mediated inflammatory cell death pathway plays a role in disease development.

Materials and Methods: Podocyte specific G2 APOL1 transgenic mice were generated by crossing TRE-G2 APOL1 transgenic animals with mice carrying nephrin rtTA. Transgene expression was regulated by doxycycline. To test the role of Nlrp3 and caspase1, we crossed nephrin rtTA/TRE-G2APOL1 with Nlrp3 knock-out (KO) and caspase-1 KO mice respectively. To test the role of IL-1B, we treated mice with IL-1B neutralizing antibody Anakinra. Histological changes were evaluated by PAS staining and the level of proteinuria was determined by albumin specific ELISA. The mRNA levels of kidney injury markers (vimentin, fibronectin, Coll1, Coll3) were analyzed by qPCR.

Results: Anakinra injection for 21 days failed to significantly reduce albuminuria or glomerulosclerosis of G2 APOL1 mice. In line with these findings, no changes in the transcript level of kidney injury markers were observed after the neutralization of IL-1B as compared to their control saline injected littermates. In contrast, renal disease was ameliorated in G2 APOL1 Nlrp3 and caspase-1 KO mice as evidenced by decreasing level of proteinuria over the 21 day doxycycline treatment as opposed to their wild type littermates. Additionally, caspase-1 KO mice displayed marked reduction of kidney fibrosis confirmed by PAS staining.

Conclusion: Our initial data suggest that inflammasome-mediated cell injury plays a crucial role in the development of G2 APOL1 induced kidney damage, albeit the mechanism still needs to be elucidated.

Poster #44
Presented May 31
Poster

High content screening assays identify paullone derivatives as novel podocyte protective compounds.

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Background:

Podocyte dysfunction and loss is an early event and a hallmark of proteinuric kidney diseases. Targeting podocytes is an excellent approach for the kidney-directed therapeutics. We have set up high content screening (HCS) assay for detecting damaged podocytes based on their cellular morphology and cytoskeleton structure. To identify small molecules that protect podocytes from injury, we performed HCS using a phenotypic assay.

Methods:

To induce podocyte injury, puromycin aminoglycoside (PAN) was treated on mouse podocytes. Cell images taken by automated HCS were used for cellular phenotype quantification and multiparametric analysis to identify podocyte protective compounds. In vitro protection of small molecules on podocytes was tested by various assays (immunofluorescence, qPCR, western blotting, scratch assay for cell motility, caspase 3/7 activity measurement, and annexin V staining assay). Adriamycin-induced kidney injury model of zebrafish was used for in vivo protection of small molecules. Candidate compounds were added in growth medium and pericardial edema of zebrafish was measured.

Results:

HCS assay revealed that kenpaullone reversed damaged phenotypes of PAN-induced podocyte injury. Kenpaullone and 2 more paullone derivatives, 1-azakenpaullone and alsterpaullone, showed dose-dependent protection of F-actin structure. The paullones also enhanced acetylated tubulin that was reduced by PAN. Alsterpaullone showed the most protective effects among the paullones. Alsterpaullone reduced the expression of a podocyte damage marker, inhibited podocyte migration, and reduced apoptosis of podocytes. In vivo, alsterpaullone protected zebrafish from Adriamycin-induced kidney injury.

PAN increased activation of GSK3 β and p38, but alsterpaullone reduced their activation in podocytes. Because paullones are GSK3 β inhibitors, p38 inhibition was not expected. Another GSK3 β inhibitor, SB216763 also inhibited p38 activation. Alsterpaullone and

SB216763 reduced anisomycin(p38 activator)-induced apoptosis of podocytes. SB203580, p38 inhibitor showed in vitro and in vivo podocyte protection like paullones.

Conclusion:

Multiparametric analysis of podocyte-based HCS assay identified paullone family compounds as novel podocyte-protective compounds. Paullones showed podocyte protection from injury by targeting GSK3 β in both in vitro and in vivo assays. p38 signaling is also involved in the GSK3 β -mediated podocyte protection.

Poster #45
Presented May 31
Poster

Protease-activated receptors as a potential therapeutic target in circulating factor type nephrotic syndrome

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Background

This study utilised a conditionally immortalised human podocyte (ciPod) cell line (gift of Prof MA Saleem, Bristol, UK) and plasma from paediatric patients with active steroid resistant nephrotic syndrome (gene negative, pre-transplant) to investigate whether the podocytotoxic effects of active disease plasma was mediated through protease-activated receptors (PARs).

Method

Cell viability assays using the xCELLigence™ Real-Time Cellular Analysis (RTCA) system (ACEA Biosciences) were used to quantify podocyte damage. Differentiated ciPod were pre-treated with PAR1 selective antagonist RWJ56110 (Tocris) for 30 minutes. Cells were then treated with i) 10% patient plasma in ciPod media without fetal calf serum or ii) with complete media/no patient plasma (control group). The cellular dose-response to 10 µM, 20 µM or 30 µM RWJ56110 was measured for either 24 or 48 hours. Expression of the four PARs (PAR1-4) in ciPod was evaluated through RT-PCR. FITC-phalloidin staining was used to qualitatively assess patient plasma-mediated actin cytoskeleton changes.

Results

10% patient plasma treatment for 24 hours ($p=0.0001$, $n = 2$) and 48 hours led to a significant loss in viability compared to the control group ($p\leq 0.05$, $n = 2$). Podocyte damage caused by patient plasma was reduced in RWJ56110 pre-treated groups compared to those without drug pre-treatment. This response was dose-dependent with 30 µM RWJ56110 yielding a significant improvement in cell viability after 24 hours ($p\leq 0.01$, $n=2$). No significant change in cell viability was observed in (no-plasma) control group upon RWJ56110 administration. The human podocyte cell line displayed PAR1 and PAR2 expression. Cells treated with 10% patient plasma displayed weaker actin filaments under immunofluorescence at 24 and 48 hours.

Conclusions

Our study confirms that SRNS plasma effects damage to human podocytes in vitro. Additionally, the effect may be ameliorated by selective blockade of the receptor PAR1. Further studies are required to support these findings and to extend the mechanistic role of PAR1 and PAR2 in podocyte damage.

Poster #46
Presented May 31
Blitz & Poster

SMPDL3b Modulates Insulin Signaling in Podocyte Lipid Rafts

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Background. SMPDL3b is a recently identified phosphodiesterase localized in lipid raft domains that regulates lipid composition and plasma membrane fluidity. Although the crystal structure of murine SMAPLD3b was recently reported, the specificity of its phosphodiesterase activity and the possibility of other lipid-modifying functions have not been investigated yet. As SMPDL3b is upregulated in diabetes while insulin receptor signaling is impaired, our study was aimed at testing the hypothesis that SMPDL3b affects the generation of sphingolipids involved in the regulation of insulin receptor signaling.

Methods. For *in vitro* studies, control and SMPDL3b overexpressing human podocytes were used. Cells were treated with insulin (0.1 and 1 nM, 30 min) or ceramide-1-phosphate (C1P; 100 uM, 1h) and analyzed by Western blotting or PCR. Lipidomic analysis was performed using LC-MS analysis and TLC plates. Co-immunoprecipitation experiments were performed using HEK293 cells. For *in vivo* studies, podocyte-specific *Smpdl3b* deficient diabetic db/db mice were produced using Cre-LoxP technology. Starting at 4 weeks of age, vital parameters (weight, glycemia, urine) were measured bi-weekly. For C1P replacement therapy, diabetic db/db mice were IP injected with 30 mg/kg C1P daily for 28 days. Mice from all experiments were sacrificed for in-depth phenotypical analysis. All animal studies were performed in accordance with the NIH IACUC Guide. For statistical analysis One-Way ANOVA followed by Bonferroni's posttest or Student t-test were used (GraphPrism, Version 7).

Results. SMPDL3b binds to both IR isoforms (IRA and IRB) and, when in excess, competes with the binding of IRB to caveolin-1 and alters pro-survival insulin signaling in podocytes. We identified a novel phosphatase enzymatic function of SMPDL3b converting C1P to ceramide. *In*

in vivo, we demonstrated that kidneys of diabetic db/db mice are characterized by SMPDL3b excess and C1P deficiency, whereas podocyte-specific *Smpdl3b* deficient diabetic db/db mice show a normal C1P content and are protected from the development of diabetic kidney disease (DKD). Exogenous administration of C1P is sufficient to restore proper IR signaling *in vitro* and to protect from DKD *in vivo*.

Conclusion. Taken together, we identified new sphingolipid modulators of insulin signaling and demonstrated that replacement of deficient active sphingolipid species such as C1P may represent a novel approach to treat diabetic complications such as DKD.

Poster #47
Presented May 31
Poster

A small molecule inhibitor of TRPC5 protects against podocyte injury and proteinuria in hypertensive FSGS

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The Ca²⁺ permeable TRPC5 channel is a critical mediator of proteinuria in FSGS. Inhibition of TRPC5 channel activity protects against proteinuria and podocyte loss in AT1R transgenic and DAHL salt sensitive rats. Here we report the identification of a potent and selective small molecule inhibitor of TRPC5. We demonstrate that TRPC5 inhibition protects against protamine sulfate induced loss of stress fibers. TRPC5 inhibition also restores stress fibers in podocytes after knockdown of synaptopodin. We further show that inhibition of TRPC5 suppresses pathogenic podocyte motility in a scratch assay. Finally, we show that inhibition of TRPC5 suppressed proteinuria in hypertension-induced FSGS in uninephrectomized DOCA salt rats without altering blood pressure. In summary, we have identified a new selective small molecule inhibitor of TRPC5 for the treatment of proteinuria in FSGS.

Nephrotic Syndrome-Associated Hypercoagulopathy is Alleviated by Both Pioglitazone and Steroid Treatment

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Venous thromboembolism (VTE) is a common complication of nephrotic syndrome (NS). We previously demonstrated that hypercoagulopathy is proportional to NS severity in rat models. Thus, NS severity markers may enable VTE-risk stratification. We previously showed that pioglitazone (Pio) reduced proteinuria in rats independently and in combination with glucocorticoids (GC). However, the effect of these treatments on hypercoagulopathy remains unknown. The aim of this study was to determine the effects of Pio and/or steroids on global markers of thrombotic risk in (1) a rat NS model and (2) children with incident NS.

Puromycin aminonucleoside-induced rat NS was treated with sham, low- or high-dose GC, Pio, or combination (low-GC +Pio; n=8-13/group). Plasma anticoagulated with 0.32% sodium citrate was collected after 11 days of treatment. Plasma samples were also collected from a cohort of children with steroid-sensitive NS (SSNS; n=24) and steroid-resistant NS (SRNS; n=14) at disease presentation and again after ~7 weeks of standard-of-care GC therapy (Pio not yet investigated in children). Steroid responsiveness was defined as complete resolution of proteinuria. Endogenous thrombin potential (ETP) was determined by thrombin generation assay. Plasma antithrombin (AT) activity and albumin (Alb) were measured by amidolytic and colorimetric methods, respectively.

In rat NS, high-GC, Pio, and Pio+GC improved proteinuria and also corrected hypoalbuminemia, ETP and AT activity ($P<0.05$); whereas low-GC did not. Proteinuria ($P<0.001$) and hypoalbuminemia ($P=0.001$) were correlated with ETP. In the pediatric cohort, ETP was not different at presentation. Steroid therapy improved proteinuria, hypoalbuminemia, and ETP in children with SSNS (4,200 nM*min) but not SRNS (5,050 nM*min; $P=0.002$). AT activity improved with treatment response ($P<0.05$) and was correlated with ETP ($P<0.001$) in the children.

Both Pio and GC diminish NS disease severity and significantly improve NS-associated hypercoagulopathy. Importantly, Pio enabled a steroid-sparing treatment strategy for proteinuria reduction in rats. These experiments confirm our previous observation that hypercoagulopathy is proportional to disease severity and extends these findings to (1) Pio-induced proteinuria reduction in rats, and (2) GC-induced proteinuria reduction in rat and children. Thus, even a partial reduction in proteinuria may reduce the risk of thrombosis.

Poster #51
Presented June 1
Blitz & Poster

Diagnostic Biomarkers of Endoplasmic Reticulum Stress in Glomerular Disease

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Introduction

Podocytes are vital in maintaining glomerular permselectivity. Podocyte injury and endoplasmic reticulum (ER) stress have been implicated in the pathogenesis of various glomerular diseases. Diagnostic and therapeutic strategies aiming at ameliorating the health of podocytes represent a promising avenue to address chronic kidney disease. ERdj3 and mesencephalic astrocyte-derived neurotrophic factor (MANF) are ER chaperones lacking the KDEL motif, and can be secreted extracellularly. ER stress in injured podocytes can lead to accumulation of KDEL-deficient chaperones in the urine.

Methods

ER stress was induced in cultured glomerular epithelial cells (GECs) with tunicamycin (TM). Complement-induced ER stress in podocytes was also studied in passive Heymann nephritis (PHN), a rat model of human membranous nephropathy. Intracellular expression and secretion of ER chaperones was monitored by immunoblotting. The protective effect of the chemical chaperone, 4-phenyl butyric acid (4-PBA), on complement-mediated podocyte injury was examined by adding 4-PBA to the drinking water of rats with PHN.

Results

In cultured GECs, TM upregulated ER chaperones, ERdj3 and MANF, intracellularly and in culture medium, whereas GRP94 (KDEL chaperone) increased only intracellularly. ERdj3 and MANF secretion was blocked by the secretory trafficking inhibitor, brefeldin A. ERdj3 and MANF appeared in the urines of PHN rats on days 7-14 after injection of nephritogenic antibody, and coincided with the onset of proteinuria on day 7. Moreover, in PHN, there were concomitant increases in glomerular ER chaperones, GRP94, ERP57 and MANF, compared to control. Rats with PHN were treated with 4-PBA starting at the time of disease induction, or on day 7, until day 14. In both protocols, 4-PBA reduced proteinuria (on days 7-14) and urinary ER chaperone secretion, compared to PHN rats treated with saline.

Conclusions

ERdj3 and MANF secreted into the urine reflect glomerular ER stress. 4-PBA protected against complement-mediated podocyte injury and the therapeutic response could be monitored by ERdj3 and MANF secretion. Secreted ER chaperones may potentially serve as diagnostic biomarkers for identifying and treating patients with glomerular ER dysfunction.

Poster #52

Presented June 1

Poster

Heparanase Increases Autophagy in Podocytes Following Adriamycin-Induced Injury

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*Equal contribution

Introduction: Heparanase (Hpa) is an endoglycosidase that cleaves heparan sulfate (HS). HS proteoglycans are present in the extracellular matrix and cell surface of all components of the kidney glomerular filtration barrier (GFB). Recently, an association between Hpa and glomerular damage was demonstrated. Previously, we showed that Hpa-transgenic mice were resistant to Adriamycin (ADR)-induced kidney injury and preserved their normal GFB structure and function, despite disruption of anionic charges. Thus, we hypothesize that constitutive Hpa overexpression in podocytes may possess a protective effect in response to ADR exposure.

Methods: To test this hypothesis, we utilized conditionally immortalized human podocyte cell line (AB8/13, a kind gift of M. Saleem). Undifferentiated AB8/13 cells were infected with pLenti6/V5-DEST control vector (V) or vector containing Hpa cDNA (H). Differentiation and growth arrest were induced by transferring the cells to 37°C for two weeks. Subsequently, cells were exposed to various concentrations of ADR to induce injury. Cell survival, expression of specific markers for cellular pathways, and morphology were studied using methylene blue assay, Western blot (WB) and immunofluorescence (IF) analyses, and transmission electron microscopy (TEM), respectively.

Results: The survival of differentiated podocytes overexpressing Hpa (H) was significantly higher compared with control mock-infected cells (V) following ADR injury. Programmed cell death, studied by the expression of cleaved caspase-3 and cleaved PARP, was significantly enhanced in response to ADR treatment in both V and H cells. WB and IF analyses of Microtubule-associated protein 1A/1B-light chain (LC)3II protein revealed a similar basal expression of autophagy, an adaptive response to stress, in cultured V and H podocytes. However, LC3II was significantly upregulated in H compared to V podocytes after ADR exposure. Addition of chloroquine (Q), an autophagosome degradation inhibitor, confirmed augmented Hpa-associated autophagy flux in H cells, which was further increased in cells treated with a combination of ADR and Q. This trend was further supported by TEM micrographs, which showed an increased number of autophagosomes in H cells.

Conclusion: Collectively, our results support a protective role for Hpa in the survival of podocytes following ADR-injury. Enhanced autophagy flux is suggested as a stress rescue mechanism, which warrants further investigation.

Poster #53
Presented June 1
Poster

Podocyte susceptibility to Angiotensin II induced calcium signals is increased in Adriamycin nephropathy

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Background

Angiotensin II (AngII) signaling has been shown to regulate glomerular perfusion and influence progression of chronic kidney disease. The treatment of patients with ACE inhibitors and AT1R blockers is well established. Further, AngII has shown to be able to directly trigger calcium signals in podocytes *ex vivo*. Here we studied AngII induced calcium signaling in healthy and diseased podocytes *in vivo*. **Methods**

Kidney disease was induced in 4 week old mice expressing the genetic calcium indicator GCaMP3 in podocytes (Pod:cre) by injecting 25 mg/kg Adriamycin (ADR) into the jugular vein. 4 days after injection we performed *in vivo* imaging on these mice. Mice were anaesthetized, an arterial catheter was placed into the right carotid artery and the left kidney was exteriorized. The vasculature was labelled with a 70 kDa dextrane. Untreated GCaMP3 Pod:cre animals were used as controls. AngII was infused with 0.1 µg/g BW/min. Losartan (10 mg/kg) was injected as AT1R blocker and PD123319 (10 mg/kg) as AT2R blocker respectively. Calcium transients were recorded as time lapse videos with one frame per second. The percentage of podocyte area showing an increase in calcium levels was determined using the Imaris Software.

Results

Our data shows that AngII triggers a calcium signal in 23 % of healthy (untreated) glomeruli. In these cases single podocytes respond with a calcium transient. The probability of inducing a calcium transient by AngII stimulation increases by two-fold in diseased (ADR) glomeruli. Furthermore, the number of podocytes per glomerulus showing a calcium transient is significantly increased. These findings correlate with a rise in the percentage of podocyte area showing a calcium signal from 17 to 40 %.

The AngII induced calcium transient can be completely blocked by injection of Losartan, while PD123319 has no significant effect.

Conclusion

Our study shows that AngII induced calcium signals in podocytes increase upon injury. We observed that not all podocytes react to AngII with a calcium signal which points to a heterogeneity in the podocyte population during health and disease. Further we could show that the calcium effects of AngII are only mediated through the AT1 receptor since blocking the AT1 receptor with Losartan completely abolished the calcium signals. Therefore our results strongly emphasize the need of a RAAS blockade in glomerular diseases to protect podocytes from high calcium levels.

Poster #54
Presented June 1
Blitz & Poster

ABCA1 deficiency-mediated mitochondrial dysfunction contributes to podocyte injury and the progression of diabetic kidney disease

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Diabetic kidney disease (DKD) is the most common cause of end-stage kidney disease in the US. In DKD patients, decreased podocyte number and glomerular lipid accumulation are associated with albuminuria. We demonstrated that ATP Binding Cassette A1 (ABCA1)-mediated cholesterol efflux is impaired in patients with T1D DKD and that patients with T2D DKD have reduced glomerular ABCA1 expression. Alterations in the mitochondrial oxidative phosphorylation complexes (OXPHOS) and reactive oxygen species (ROS) have been implicated in DKD and may contribute to lipid oxidation. However, the role of ABCA1-mediated cholesterol/phospholipid efflux in mitochondrial dysfunction and podocyte injury in DKD remains elusive.

Pima Indian patients were separated in two groups based on their change in glomerular filtration rate between over 10 years: progressors (P, n=15) and non-progressors (NP, n=16). Normal, scrambled control (siCO) and siRNA *ABCA1* podocytes (siABCA1p) were treated with P and NP patient sera. ABCA1 expression, cholesterol efflux, cytotoxicity and ROS were measured. ABCA1 overexpressing podocytes (ABCA1OE), and Elamipretide (cardiolipin peroxidase inhibitor) treated siABCA1p, were treated with patient sera and cytotoxicity was measured. Diabetic mice with podocyte specific ABCA1 deficiency (DKO) were analyzed for albuminuria, mesangial expansion, podocyte foot process effacement (FPE), podocytopenia and podocyte mitochondrial morphology.

P sera treated podocytes show reduced ABCA1 mRNA expression, reduced cholesterol efflux, increased cytotoxicity and ROS compared to NP sera treated podocytes.

siABCA1p are more susceptible to NP and P sera induced cytotoxicity when compared to siCO. siABCA1p show decreased endogenous and CII driven OCR, and increased ROS, associated with alterations in OXPHOS complexes. ABCA1OE and Elamipretide treated siABCA1p are protected from P and NP sera mediated cytotoxicity. DKO mice are more susceptible to develop DKD compared to diabetic mice as indicated by increased albuminuria,

mesangial expansion, podocyte loss, FPE and mitochondrial damage. Diabetic mice are currently being treated with elamipretide.

Our data indicate that a reduction of *ABCA1* expression is a susceptibility factor for DKD progression. These data also indicate that ABCA1 deficiency contributes to lipid peroxidation and mitochondrial dysfunction and that treatment strategies to restore ABCA1 function or mitochondrial function may be beneficial to prevent podocyte injury in DKD.

Poster #55
Presented June 1
Blitz & Poster

A novel podocyte phenotype of TRPC6 KO mice and the importance of the protein interaction between TRPC6 and calpain.

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Background

Podocyte pathology of TRPC6 mutations has previously been thought to be due to increased calcium conductance. However, various disease-causing mutations have shown unaltered/decreased calcium conductance, suggesting a more complex role of podocyte TRPC6. We have investigated whether mutations are affecting TRPC6 protein interactions.

Methods

Glomerular albumin permeability assay and electron microscopy (EM) were performed on young (12-16 weeks) and old (10-14 month) WT and TRPC6 KO C57Bl/6 mice. TRPC6 KO conditionally immortalised podocytes were generated. Lentiviral transfection stably reintroduced GFP tagged WT/mutant TRPC6 into the KO cells. Cell lines were characterised using motility and adhesion assays. Novel binding partners were identified using GFP TRAP and proteomics and confirmed through co-immunoprecipitation (Co-IP). Calpain assays were performed using a commercially available kit. TIRF and confocal microscopy were used to assess protein localisation.

Results

Old TRPC6 KO mice had significantly increased glomerular albumin permeability compared to age matched controls, suggesting TRPC6 KO mice have a pathological phenotype of increased permeability, but that the albumin is being reabsorbed before excretion. EM demonstrated an increased foot process width and glomerular basement membrane thickness in TRPC6 KO mice compared to controls.

TRPC6 KO (T6K) podocytes are less motile and more adhesive than those expressing WT TRPC6. Proteomics identified calpain 1 and 2, ERK 1/2 and caldesmon as novel TRPC6 binding partners and this was confirmed through co-IP. Calpain is a protease and T6K cells have decreased protein cleavage and calpain activity compared to cells containing WT TRPC6, suggesting that TRPC6 is responsible for calpain activation. The TRPC6 mutant K874* is disease causing and has normal calcium conductance, however cells expressing this mutant also have decreased calpain activity and cleavage of calpain targeted proteins. Co-IP experiments have shown that TRPC6 K874* does not bind to calpain. Confocal and TIRF microscopy showed a loss of calpain from the membrane in T6K and K874* podocytes.

Conclusions

TRPC6 KO mice glomeruli have altered structure and increased albumin permeability compared to WT mice. Physical interaction allows TRPC6 mediated activation of calpain which plays an important role in podocyte motility and detachment. Loss of this interaction leads to podocytes which have increased adhesion and decreased motility.

Poster #56
Presented June 1
Blitz & Poster

Title: Podocyte expressed miR-146a protects against Diabetic Glomerulopathy via suppression of ErbB4 and Notch-1

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Background: Diabetic glomerulopathy is a major complication of Diabetes Mellitus (DM) and is the leading cause of end stage renal disease (ESRD). MicroRNA-146a (miR-146a) is a negative regulator of inflammation and is highly expressed in myeloid cells and in podocytes. We have previously shown that miR-146a levels are significantly reduced in the glomeruli of diabetic nephropathy (DN) patients. To study its role in podocyte function, we have generated mice with selective deletion of miR-146a in podocytes. Here we will present our results from studies in these animals.

Methods: We generated and characterized podocyte-specific miR-146a deficient mice. To investigate the role of miR-146a in glomerular function in vivo, we induced hyperglycemia in C57BL/6 wildtype mice (WT), global miR-146a knockout mice (miR-146a^{-/-}) and podocyte-specific miR-146a knockout (KO) animals using streptozotocin (STZ).

Results: We further confirmed that podocytic miR-146a expression decreased in the glomeruli of type 2 diabetes (T2D) patients and correlated with increased albuminuria and glomerular damage. Mice lacking miR-146a globally or selectively in podocytes showed accelerated development of glomerulopathy upon STZ-induced hyperglycemia. miR-146a targets, Notch-1 and ErbB4, were significantly upregulated in the diseased glomeruli and TGF β signaling was induced. Treatment of podocytes in vitro with TGF β resulted in increased levels of Notch-1 and ErbB4, increased ErbB4 phosphorylation, and increased expression of inflammatory chemokine MCP-1, which suppresses miR-146a via an autocrine loop. Similarly, administration of low-dose LPS to podocyte-specific miR-146a KO mice resulted in increased albuminuria as compared to the WT mice, further suggesting that podocyte-expressed miR-146a protects from glomerular damage.

Conclusions: We suggest a novel role for miR-146a in protecting against glomerular injury via protecting podocytes from injury and cell death. This indicates that miR-146a might have a therapeutic potential in DN.

Poster #57
Presented June 1
Poster

Podocyte-specific HIF-1 α deletion prevents Adriamycin-induced glomerulopathy in mouse

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Background: Hypoxia contributes to the progression of glomerulopathy. Under hypoxia, activities of the ubiquitin-ligase von Hippel Lindau (VHL) protein that degrades hypoxia-inducible factors (HIF) is suppressed and HIFs are stabilized. Mice lacking VHL specifically in podocytes showed high HIF-1 α expression in podocytes and various degree of glomerulosclerosis, while no apparent proteinuria was detected (Brukamp and Haase, 2007, AJP renal). These data suggest that HIFs play critical roles in development of glomerulopathy. Here, we tested whether podocyte-specific deletion of HIF-1 α protects mice from glomerulopathy.

Methods: Mice in which exon 2 of HIF-1 α gene is floxed were backcrossed to 129 strain using the SpeedBax strategy (Charles River). HIF-1 α ^{fl/fl} mice (129) were then mated with mice expressing Cre-recombinase under control of the podocin promoter. At 8 weeks old, Adriamycin (ADR, 10 mg/kg) was intravenously administered to HIF-1 α ^{fl/fl}; podocin-Cre (129) or their wild type (WT) littermates. Mice were sacrificed 14 days later and the kidneys were harvested. Urine albumin and creatinine levels were determined by ELISA kits. Glomeruli or WT-1 positive podocytes were isolated from cryosectioned samples by laser-capture microdissection (LCM) and glomerulus-specific mRNA expression was analyzed by qPCR after pre-amplifying target genes using the Bio Rad prime-PCR system.

Results: HIF-1 α ^{fl/fl}; podocin-Cre mice are viable and live normal life spans, and no proteinuria was observed basally. ADR-induced proteinuria was significantly less in HIF-1 α ^{fl/fl}; podocin-Cre mice compared to WT (Albumin/Creatinine; 18.9 \pm 2.3 vs. 7.5 \pm 2.3, p=0.04). PAS staining showed obliteration of capillary loops and accumulation of extracellular matrix in glomeruli in WT mice administered with ADR, while capillary loops are open and little fibrosis is observed in HIF-1 α ^{fl/fl}; podocin-Cre mice. COL1A1 mRNA levels were elevated in glomeruli of WT mice with ADR but not in HIF-1 α ^{fl/fl}; podocin-Cre mice. Conversely, synaptopodin mRNA expression was significantly reduced in WT mouse glomeruli, but not in HIF-1 α ^{fl/fl}; podocin-Cre mice. RNAs prepared from WT1-positive podocytes are being analyzed by RNA seq to determine differential expression of HIF-1 α target genes.

Conclusions: Deletion of HIF-1 α protects podocytes from proteinuria and consequent glomerulosclerosis. HIF-1 α or genes regulated by HIF-1 α could be a novel therapeutic target for glomerulopathy.

Poster #58
Presented June 1
Poster

Rg3 inhibits puromycin induced apoptosis in human podocyte through suppression of ER stress.

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Background

Podocyte is a key player in glomerular disease. Recent studies have shown that podocyte endoplasmic reticulum (ER) stress leads to severe proteinuria. However there is little information about therapeutic strategies to overcome ER stress in podocyte. In this study, we investigated the effect of Ginsenoside Rg3 on puromycin induced apoptosis and ER stress in podocytes.

Methods

We used human podocyte cell lines. Rg3 toxicity and therapeutic concentrations was examined by XTT test. Puromycin induced podocyte injury is known as a cellular stress model of focal segmental glomerular sclerosis. We stimulated podocyte with puromycin and induced apoptosis in this cell. The protein expression of ER stress markers such as glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) were evaluated by western blot.

Results

In human podocyte, puromycin increased apoptotic protein, cleaved PARP in a dose-dependent manner at 48 hours. ER stress marker, C/EBP homologous protein (CHOP) was also increased by puromycin stimulation. Rg3 pretreatment prevented puromycin induced apoptosis and decreased expression of CHOP on western blotting.

Conclusions

Our results suggest Ginsenoside Rg3 prevents puromycin induced apoptosis in human podocyte through suppression of CHOP pathway. Rg3 might be a potential therapeutic option for glomerular disease.

Poster #59
Presented June 1
Blitz & Poster

The Role of DDR1 in podocyte lipotoxicity and progression of Alport Syndrome

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The GBM is primarily composed by laminin and Collagen type IV. *de novo* production of the $\alpha 1$ chain of collagen type I (Col I) has been observed mouse models of Alport Syndrome (AS, Col4a3KO). Discoidin domain receptor1 (DDR1) is a unique receptor tyrosine kinase that is activated by collagens. Deletion of the DDR1 in Col4a3KO mice was shown to improve survival and renal function. However, how DDR1 activation by aberrant collagen production contributes to podocyte injury and proteinuria is poorly understood.

Differentiated human podocytes were serum starved, followed by 18hrs treatment with 50ug/mL Col I (Corning). Following collagen treatments, podocyte lipid content was determined by BODIPY 493/503 and Cell Mask Blue staining. Free Fatty acid (FFA) uptake assessed using the fluorometric free fatty acid uptake kit (abcam). Mice in which exon 5 of α chain of collagen type IV is deleted (Col4a3KO), a model for AS, were obtained from the Jackson Laboratory for the determination of DDR1 phosphorylation.

DDR1 phosphorylation was increased in kidney cortex from Col4a3KO mice whereas the expression of podocin and synaptopodin was decreased. pDDR1 correlated with blood urine nitrogen (BUN, $R^2 = 0.7$, $p < 0.01$). *In vitro*, DDR1 was phosphorylated by collagen type I (50 μ g/mL, 18hr) in cultured human podocytes. Increased intracellular lipid accumulation ($p < 0.05$) and FFA uptake ($p < 0.001$) were also observed in Col I treated podocytes. DDR1 DA transfected HEK293 cells showed increased expression of CD36, a protein involved in FA uptake, and FFA uptake compared to cells transfected with DDR1 WT and DN ($p < 0.05$). Knock down of the CD36 reduced in FFA uptake when compared to cells transfected with scramble siRNA control ($p < 0.05$). Glomeruli isolated from Col4a3KO mice showed increased lipid deposition and expression of CD36. Col I induced DDR1 activation is associated with podocyte lipotoxicity, FFA uptake and intracellular lipid droplet deposition.

Our data suggest that col I-induced/DDR1-mediated lipotoxicity may represent a novel mechanism leading to podocyte injury in AS.

Poster #60
Presented June 1
Blitz & Poster

Heterogeneous Nuclear Ribonucleoprotein F Deficiency Aggravates Podocyte Loss via Down-Regulation of Sirtuin-1 Expression in Adriamycin-Induced Nephropathy in Mice.

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Background: We reported previously that overexpression of heterogeneous nuclear ribonucleoprotein F (hnRNP F) enhances sirtuin-1 expression and attenuates renal proximal tubular cell (RPTC) apoptosis in db/db hnRNP F-transgenic mice (Diabetes 2017). In the present study, we investigated whether selective deletion of hnRNP F in podocytes could aggravate podocyte injury in adriamycin (ADR)-induced nephropathy in mice.

Methods: Podocyte-specific hnRNP F knockout (KO) mice were generated by crossbreeding podocin (Pod)-Cre mice with floxed hnRNP F mice on a C57BL/6 background. To induce nephropathy, male 10-week old non-KO littermates (controls) and Pod-hnRNP F KO mice were given ADR (doxorubicin) (18 mg/kg BW) via the tail vein. Urinary albumin/creatinine ratio (ACR) was assessed one and two weeks later. Mice were euthanized 2 weeks post-ADR. Kidneys were processed for histology. Podocyte numbers were identified and counted by immunofluorescence staining for p57 and WT-1. Primary podocytes isolated from Pod-hnRNP F KO mice and controls \pm ADR were also studied in vitro. In addition, male adult controls and Pod-hnRNP F KO mice were studied at age 10 to 20 weeks. Urinary ACR was monitored bi-weekly. Freshly isolated glomeruli were assessed for mRNA and protein expression by real time-qPCR and Western blotting, respectively.

Results: Administration of ADR for 1-2 weeks significantly increased urinary ACR and reduced podocyte number in both Pod-hnRNP F KO and control mice with more pronounced changes in Pod-hnRNP F KO mice. In vitro, primary podocytes isolated from hnRNP F KO mice exhibited lower sirtuin-1 level and higher acetylated p53 expression after ADR treatment. Furthermore, non-treated Pod-hnRNP F KO mice were phenotypically normal with a slight increase in ACR at the age of 20 weeks as compared to controls. Glomeruli isolated from Pod-hnRNP F KO mice exhibited significantly lower mRNA and protein levels of sirtuin-1 and reduced staining for the podocyte markers WT1 and synaptopodin as compared to controls.

Conclusions: HnRNP F deficiency aggravates podocyte loss and urinary ACR in ADR-induced nephropathy in mice, indicating a protective role for hnRNP F against podocyte injury.

Poster #62
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Blitz & Poster

The paraoxonase PON2 regulates TRPC6 channel activity via modulation of lipid composition of the plasma cell membrane

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The understandings regarding the implications of disturbed TRPC6 signaling on podocyte function and its role in hereditary and sporadic glomerular disease is increasing. However, regulatory and gating mechanisms of TRPC6 are still obscure.

The mammalian protein complex at the slit diaphragm is orthologue to neuronal mechanosensory complexes of *C. elegans*. The Podocin homologue, MEC-2 coordinates protein-lipid super complexes in the plasma membrane of *C. elegans* touch neurons that allow proper signalling via the degenerin

cation channel MEC-4/-10. Another protein in this mechanosensory complex is the lipid modifying enzyme MEC-6, which interacts with MEC-4/-10 and enhances its channel activity.

Previously, we have identified the MEC-6 orthologue paraoxonase PON2 as a type II transmembrane protein that protects the plasma membrane from lipid peroxidation. Our current study focuses on investigating PON2 localization at the slit diaphragm and its biochemical and functional interaction with TRPC6. Using immunofluorescent staining of human kidney sections, we were able to identify PON2 as a podocyte protein that may localize to the plasma membrane, including the slit diaphragm. Co-precipitation studies showed interaction of PON2 with slit diaphragm proteins, Podocin, Nephlin, and TRPC6. In addition, PON2 was found to localize into detergent-resistant membrane domains. In order to investigate the effect of PON2 *in vivo*, we performed studies on PON2-deficient mice in the adriamycin-induced nephropathy model. Adriamycin persistently increases oxidative stress. We were able to observe that under adriamycin stimulation, PON2 knockout animals are more susceptible to proteinuria and glomerulosclerosis in comparison to control animals. The phenotype is partially rescued in the PON2/TRPC6 double knockout.

The knockdown of PON2 in cultured mouse podocytes affected the activity and refractoriness of the TRPC6 channel both after chemical stimulus and after activation by membrane stretch following hypoosmotic stimulation. Moreover, data from mass spectrometry and atomic force microscopy showed altered lipid composition and altered physical properties of the plasma membrane in PON2-deficient cells.

Poster #63
Presented June 1
Poster

Composite diagnostic assay identifies suPAR- β 3 integrin pathogenic pathway in FSGS

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Background: The membrane bound urokinase-type plasminogen activator receptor (uPAR) undergoes cleavage to release its soluble form suPAR, which is present in body fluids. There are three different forms of suPAR: suPAR_{I-III}, suPAR_{II-III} and suPAR_I. These are designated as inflammatory and lifestyle risk biomarkers. Elevated suPAR levels in the plasma predict rapid decline in kidney function and onset of chronic kidney disease (CKD). However, elevated suPAR levels are also correlated with other diseases such as cancer and sepsis. Therefore, only the suPAR level may not be sufficiently specific as a strong diagnostic tool for CKD. suPAR drives kidney injury by activating β 3 integrin on podocytes, which provides a potential downstream pathogenic pathway that could be used to develop kidney-specific diagnostic tools. We propose that a composite scoring system, which considers multiple CKD associated biomarkers and β 3 integrin activation pathway, may generate an efficient and robust diagnostic approach. **Methods:** Urine and serum samples obtained from healthy individuals and patients with recurrent and non-recurrent focal segmental glomerulosclerosis (FSGS) were analyzed. The levels of suPAR, IL-6 and β 3 integrin activation were determined. Presence of suPAR_{II-III} fragment in the serum samples was also evaluated. The data were statistically analyzed to develop a pathway-specific aggregate biomarker score that can aid in diagnosis of suPAR signaling axis in CKD.

Results: Combination of biomarkers and bioassays generated a diagnostic score that predicted β 3-integrin driven kidney injury. The majority of recurrent FSGS patients exhibited high levels of suPAR and IL-6. The plasma from these patients exhibited the presence of suPAR_{II-III} fragment. Additionally, the serum from these patients induced an increase in β 3-integrin activation in human podocytes in culture.

Conclusion: The weighted analysis of distinct biomarkers and bioassays was able to predict FSGS recurrence after transplant with greater than 80% accuracy.

Poster #64
Presented June 1
Poster

GH induces TGF β 1 in podocytes: Implications in podocytoathy

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Background: Podocytes are the specialized cells that offer epithelial coverage to glomerular capillaries. Podocytes contribute to glomerular permselectivity and any injury to podocytes results in proteinuria and poor renal outcome. Podocyte injury is a characteristic of many clinical conditions including diabetes mellitus (DM). Elevated growth hormone (GH) secretion during type1 DM is implicated in the podocyte injury and glomerular sclerosis. TGF β , a pro-sclerotic molecule is also implicated in the development of glomerulosclerosis, and podocyte injury. The mechanism by which TGF β 1 secretion is regulated remains incompletely understood. Our study identified that GH induces TGF β 1 expression in podocytes and alters its biology and function.

Methods & Results: We observed an increased expression of TGF β 1 propeptides in both cell lysates and conditioned media from GH treated human podocytes. LC-MS/MS analysis identified the presence of specific isoform, TGF β 1 in the secretome of podocytes treated with GH. Both the GH and conditioned medium from GH treated podocytes revealed the activation of SMAD binding element as evidenced by reporter assay. Furthermore, we observed activation of SMAD2&3 in podocytes exposed to GH-conditioned media, suggesting activation of TGF β /SMAD pathway. Increased expression of extracellular matrix molecules including CTGF, MMP2 and integrins was observed in podocytes treated with GH or with GH-conditioned media. We also observed the expression of TGF β 1 propeptides in glomerular lysates of mice administered with GH (2mg/kg) for two weeks. ELISA analysis revealed increased TGF β 1 levels in plasma samples of GH injected mice. Mice injected with GH showed massive albuminuria. Interestingly, plasma TGF β 1 levels were decreased in mice treated with inhibitors of either JAK2 or TGF β R1. GH receptor (GHR) knockout (KO) mice have reduced plasma

TGF β 1 levels compared to wild-type mice. Mice which is a conditional KO for GHR in podocytes protected from glomerulosclerosis and albuminuria.

Conclusion: The detrimental effect of GH is mediated, at least in part by aberrant TGF β /SMAD pathway in podocytes that manifested in podocyte injury and proteinuria. Our long-term goal is to determine the role of JAK2 or TGFBR1 inhibitors to improve renal function in diabetic settings.

Poster #65
Presented June 1
Blitz & Poster

Hypoxia induced proteinuria: A role of HIF1 α -ZEB2 axis in Podocytes

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Background: The glomerular filtration barrier (GFB) plays an essential role in the filtration of blood, ensuring protein free urine. The integrity of the GFB is compromised in hypoxia which prevails during extreme physiological and certain pathological conditions which progresses to “proteinuria”. However, the mechanism by which permselectivity of GFB is abrogated is poorly established. In the present study, we investigated the mechanisms leading to altered glomerular permeability under chronic hypoxic condition.

Methods and Results: We observed an increased expression of HIF1 α and ZEB2 in hypoxic setting, both *in vitro* and *in vivo* from cultured human podocytes and glomerular extracts from rats. There was an increase in HIF1 α occupancy on the ZEB2 promoter under hypoxia, suggesting that ZEB2 is a direct transcriptional target of HIF1 α . Further, we observed an altered expression of ZEB2 transcriptional targets E-cadherin and P-cadherin, which are responsible for epithelial to mesenchymal transition (EMT) of podocytes. We also observed an increase in ZEB2-natural antisense transcript (NAT), with HIF1 α stabilization in human podocytes leading to increased translation efficiency of ZEB2 mRNA. Ectopic expression of ZEB2 in podocytes altered the podocyte permselectivity, whereas knockdown of ZEB2 abrogated this phenotype. Similar to hypoxia-induced HIF1 α , ZEB2 over expression also led to the loss of E- cadherin and P-cadherin expression thereby augmenting podocyte migration. In addition to these observations, we also found that rats subjected to hypoxia also showed decreased glomerular filtration rate (GFR), effacement of podocyte foot-process leading to proteinuria. Our results suggest that hypoxia induces over-activity of HIF1 α - ZEB2 axis in podocytes and consequent loss of the epithelial phenotype of podocytes leading to “proteinuria”.

Conclusion: Our study reports how chronic hypoxia lead to glomerular dysfunction/ proteinuria. The hypoxia-dependent increase in ZEB2, mediates EMT, foot-processes effacement and alters permeability of podocytes leading to “proteinuria”.

Poster #66
Presented June 1
Poster

Yeast deleted strains that lack the translocon gene products are protected from APOL1 toxicity

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Background: Two variant at the APOL1 gene (G1 and G2) account for more than 70% of the increased risk for chronic kidney disease (CKD) in individuals of African ancestry. In our previous work, we described differential toxicity of the APOL1 risk compared to non-risk variants in *S. cerevisiae*, and showed that yeast strains defective in endosomal trafficking or organelle acidification displayed augmented APOL1 toxicity. This pattern of differential injury by the APOL1 variants, which occurs across evolutionarily divergent species makes the yeast a model system highly amenable to genetic interrogation for further investigating basic mechanisms of APOL1 cell biology.

Methods: We used a barcoded yeast deletion mutant library, in order to identify strains lacking genes which are involved in a pathway that mediates APOL1 toxicity.

Results: APOL1-G2 lethality was partially abrogated in strains deleted of genes encoding 2 key Endoplasmic Reticulum translocon proteins e.g. SBH2 and SEC72 and in a strain deleted of HUT1, which is involved in protein folding in the ER. Immunolocalization of those mutants with APOL1 fused to mCherry, showed altered staining, compared to the WT strain, with scant ER staining.

Conclusions: Those results suggest that APOL1 proper trafficking to the ER is essential for its localization and toxicity.

Poster #67
Presented June 1
Poster

Role of Serine Proteases and Protease-Activated Receptors in Control of Intracellular Calcium Levels in Podocytes in Type 2 Diabetic Nephropathy

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Multiple clinical reports revealed that serine proteases such as thrombin and corresponding protease-activated receptors (PARs) signaling cascade, could be involved in the development of diabetes and nephrotic syndrome in humans. Moreover, the elevation of concentrations of serine proteases in plasma and urine is associated with the development of albuminuria and proteinuria in both patients and experimental rodent models. However, the direct cell-specific mechanisms, as well as the type of serine proteases and PARs involved in the development of diabetic nephropathy, are not well defined. Here using our established technique of kidney glomeruli isolation and confocal ratiometric imaging of intracellular calcium dynamics in podocytes, we explored the involvement of PARs and serine proteases in the development of type 2 diabetic nephropathy (T2DN). The T2DN rat model strain was developed in MCW by crossing diabetic Goto-Kakizaki (GK) and FHH (Fawn Hooded Hypertensive) rats. The histological changes in the kidney of T2DN rats closely mimic the changes seen in human diabetic nephropathy. Various serine proteases including thrombin, urokinase, and kallikrein with or without selective inhibition peptides for PARs were applied to test the changes in intracellular calcium concentrations in glomeruli podocytes. In addition to the T2DN rats, PAR signaling was assessed in the GK, Wistar, and Dahl salt-sensitive (SS) rats fed both low and high salt diets. Therefore, PAR signaling was tested under control, diabetic and hypertensive conditions when rats exhibit symptoms of FSGS. Our results indicate that T2DN rats have a significant elevation in calcium signaling in response to tested serine proteases compared to control or hypertensive strains. As an example, urokinase produced a high sustained elevation of intracellular calcium, which resulted in significant calcium overload and possibly leading to podocyte apoptosis. Furthermore, we determined that kallikrein/urokinase effect on calcium signaling in T2DN podocytes was presumably facilitated through the PAR-4, but not PAR1 dependent pathway. Collectively, these data implicate the direct involvement of serine proteases in the modulation of intracellular calcium signaling, which might lead to podocytes injury, apoptosis and the development of nephrotic syndrome in diabetes. Therefore, PARs and corresponding serine proteases become a new potential therapeutic target for the prevention of diabetic nephropathy.

Poster #69
Presented June 1
Poster

Single glomerular proteomes connect morphology and function in proteinuric kidney disease

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In diseases of many parenchymatous organs, heterogeneous deterioration of individual functional units determines the clinical prognosis. However, the molecular characterization at the level of such individual subunits remains a technological challenge that needs to be addressed in order to better understand pathological mechanisms. Proteinuric glomerular kidney diseases are frequent and assorted diseases affecting a fraction of glomeruli and their draining tubules to variable extents, and for which no specific treatment exists. Here, we developed and applied an ultrasensitive mass spectrometry-based methodology to investigate heterogeneity of proteomes from individually isolated nephron segments from mice with proteinuric kidney disease (“One glomerulus, one proteome”). This method can quantify up to 2000 proteins per individual microdissected glomerulus and allows unprecedented insights into kidney disease heterogeneity. In single glomeruli from two different mouse models of sclerotic glomerular disease, we identified a coherent protein expression module across individual glomeruli that consisted of extracellular matrix protein deposition (reflecting glomerular sclerosis), glomerular albumin (reflecting proteinuria) and LAMP1, a lysosomal protein. This module was associated with a loss of podocyte marker proteins while genetic ablation of LAMP1-correlated lysosomal proteases could ameliorate glomerular damage *in vivo*. Furthermore, proteomic analyses of individual glomeruli from patients with genetic sclerotic and non-sclerotic proteinuric diseases revealed increased abundance of lysosomal proteins, in combination with a decreased abundance of mutated gene products. Thus, altered protein homeostasis (proteostasis) is a conserved key mechanism in proteinuric kidney diseases. Moreover, our technology can capture intra-individual variability in diseases of the kidney and other tissues well beyond a sub-biopsy scale.

Poster #70
Presented June 1
Poster

A multi-layered quantitative *in vivo* expression atlas of the podocyte unravels kidney disease candidate genes

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Damage to and loss of glomerular podocytes has been identified as the culprit lesion in most progressive kidney diseases that result in end stage renal failure. Here, we combine deep quantitative mass-spectrometry based proteomics with next generation RNA sequencing technologies, bioinformatics and hypothesis-driven studies to provide a comprehensive and quantitative map of mammalian podocytes that identified several unanticipated signaling pathways. Comparison of the comprehensive *in vivo* data sets with quantitative proteomics data from podocyte cell culture models showed a limited value of available cell culture models. Moreover, *in vivo* stable isotope labeling by amino acids uncovered surprisingly rapid synthesis of mitochondrial proteins under steady state conditions in podocytes, a finding perturbed in autophagy-deficient, disease-susceptible conditions. Integration of acquired omics dimensions suggested FARP1 as a novel candidate essential for podocyte function, which could be substantiated by genetic analysis in humans and knock-down experiments in zebrafish. This work exemplifies how the integration of multi-omics datasets can set a framework for the systematic understanding of cell type specific features relevant for organ health and disease.

Podocyte proteostatic networks and protein dynamics are regulated by protein half-lives during podocyte differentiation.

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Background: Podocytes need to accurately maintain their proteome when adapting to metabolic and mechanical stress. Immortalized podocyte cell lines undergoing temperature dependent differentiation induced by cell cycle delay represent a widely-utilized model to study podocyte biology *in vitro*. Methods: We performed functional in-depth omics analyses of the cultured human podocyte proteome in order to characterize changes regarding protein expression, proteostatic mechanisms and metabolism during differentiation of the cells. The comprehensive dataset included mass spectrometry based deep proteomic analyses, proteomic analyses of protein half life and degradation via pulsed stable isotope labeling by amino acids and cycloheximide assays and bioinformatics. In addition, we performed integration with public available transcriptome and newly generated metabolomic data.

Results: Similar to mouse podocytes, human podocyte differentiation involved a proteostatic shift: While expression of proteasomal proteins was high in the undifferentiated state, lysosomal proteins were predominant after differentiation. Additional analyses using pulsed stable isotope labeling by amino acids in cell culture (pSILAC) and protein degradation assays showed protein dynamics and half-lives. These studies unraveled a globally increased stability of proteins in differentiated podocytes. In particular membrane,

Importantly, protein expression levels were influenced by protein half-lives in each state. In addition, systems-level integration with untargeted metabolomic data demonstrated a strong increase of metabolites of the pyrimidin and purine deoxyribonucleotide salvage pathways during differentiation, and an increase in lysosome-associated lipid species. These data suggest that coregulation of protein turnover of particular cellular functions determines podocyte differentiation, a paradigm possibly involving mitophagy and potentially of importance in conditions of increased podocyte stress.

Conclusion: Podocyte differentiation *in vitro* is largely associated with a proteostatic shift, and the deep proteomic mapping approach utilized here may demonstrate the limitations, but also the potential of podocyte cell culture. Finally, functional coupling of lysosome abundance with cytoskeletal and mitochondrial turnover suggests a role for unanticipated molecular mechanisms such as mitophagy, as regulators of the posttranscriptional protein landscape in podocytes.

Poster #72
Presented June 1
Poster

Localized mRNA translation mediated by Staufen modulates podocyte actin cytoskeleton and matrix adhesion

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Background: Irreversible damage to podocytes and their detachment from the glomerular basement membrane, result in glomerular scarring and progression to End Stage Renal Disease.

How injury affects the biological pathways that regulate podocyte architecture and matrix adhesion are not fully understood. Here, we provide evidence that podocytes utilize localized mRNA glomerular filtration barrier during injury. eton and cell-matrix adhesion, thereby maintaining the

Methods: A cell biological and gene targeting approach was used to study the role of local mRNA translation in podocytes.

Results: We show that the RNA-binding protein Staufen2, previously demonstrated to regulate mRNA transport and stability in neurons, is expressed in podocytes and localizes predominantly to primary and secondary processes. During injury, Staufen2 and translating ribosomes increase in areas of foot process effacement. Next, we show that Staufen2-bound RNAs are enriched for cytoskeletal assembly and cell-matrix adhesion regulators, one example being the GEF *Dock5*. Consequently, *Stau2* knockdown in immortalized podocytes affects *Actb* mRNA localization and *Dock5* mRNA stability and results in cell detachment as well as impaired re-establishment of actin stress fibers upon recovery from injury. Lastly, we generated *Stau2* single and *Stau1/2* double knockout (DKO) mice; these mice had normal baseline kidney function but DKO mice developed massive proteinuria and extensive foot process effacement in response to Adriamycin, far greater than observed in control, or *Stau1* and *Stau2* single knockouts.

Conclusion: Our results establish mRNA transport and localized translation as newly recognized mechanisms in the kidney to preserve the glomerular filtration barrier during injury.

Poster #73
Presented June 1
Poster

Transforming growth factor-beta receptor 3: a new regulator in the TGF- β pathway in podocytes

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Background; Podocytes are injured in many renal diseases and this plays a key role in the progression of these disorders. Transforming growth factor beta (TGF β) pathway activation occurs virtually in all chronic kidney disorders, both in animal models and in human disease. TGF β pathway is considered to be one of the most important pathways driving podocytopathies. In this study, we identified transforming growth factor beta receptor 3 (TGFB3) as a podocyte-enriched protein and we aimed to analyze its role in both normal and disease conditions.

Methods and results; Our results show that TGFB3 protein is highly expressed and enriched in both human and mouse glomeruli. In the glomerulus, TGFB3 co-localizes with the podocyte marker nephrin. To understand the role of TGFB3 in podocytes, we generated a stable human podocyte cell line that over-expresses TGFB3 and treated the cells with TGF β 1. Interestingly, the over-expression in podocytes constitutively activates the Smad1/5/8 pathway and prevents the activation of Smad2/3 pathway after TGF β 1 treatment. Next, we generated mice with conditional knock-out (KO) and knock-in (KI) alleles for TGFB3. These lines were crossed with podocin-cre line to inactivate/activate TGFB3 specifically in podocytes. The glomerular filtration barrier developed normally in the transgenic mice and so far no functional defects have been detected. We challenged the conditional KO mice with nephrotoxic serum (anti-GBM), and somewhat surprisingly, they showed significantly less glomerular lesions compared with the control animals.

Conclusion; TGFB3 is a novel podocyte-enriched protein. TGFB3 is playing a role in the development of glomerular lesions in mice, although its mechanism of action in podocytes is unknown. We speculate that the regulation of the activity of this receptor in podocytes can offer us a novel tool to promote glomerular health and treat chronic kidney diseases.

***APOL1* RNA is Differentially Spliced in Nephrotic Syndrome and with Glucocorticoid Therapy**

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Introduction: Understanding the mechanistic action of APOL1 risk alleles (G1 and G2) has become a subject of intense research since the discovery of its association with heightened risk for chronic and end stage kidney disease in African Americans. The presence and/or absence of exons 2 and 4 distinguish some of the splice forms of *APOL1*, and recent evidence indicates that exon 4 is a major contributor of APOL1 cytotoxicity. However, the potential role of alternate splicing of *APOL1* in podocyte biology and in the regulation of kidney disease is unknown. We hypothesized that *APOL1* RNA is differentially spliced in nephrotic syndrome (NS) and with glucocorticoid (GC) therapy, thus playing a potential pathophysiological role in disease and health, regardless of APOL1 haplotype.

Methods: *APOL1* RNA and splice variant expression was analyzed in podocytes injured with puromycin aminonucleoside (PAN), and treated with dexamethasone (Dex) by RT-PCR using primers specific for each splice variant. Splice variant analyses were also performed in circulating leukocytes of children with steroid sensitive (SSNS) and steroid resistant NS (SRNS), before and following initial oral GC therapy (N =8 SSNS and 8 SRNS paired samples). T-test and 2 way ANOVA statistical analyses were performed to measure variant ratios.

Results: Podocytes expressed all 5 known splice variants of *APOL1* [v.A, variant3 v.A, v.B1,v.B3 and v.C]. Higher relative expressions of exon 4 (+) [v.A, v.B1] and exon 2 (-) [v.A, v.C] forms were observed, with variant 1 [v.A] the most predominantly expressed form. Dex increased the expression of all variants, which was otherwise decreased with PAN-induced injury. Also, the ratio of variants with exon 4 (+) and exon2 (-) increased with Dex treatment during PAN-induced injury. Although RNASeq data analysis was unable to detect these variants, in-depth RT-PCR analyses revealed that both exon 4 (+) and (-) variants were expressed in the circulating leukocytes of both SSNS and SRNS patients, while exon 2 (+) forms were only minimally expressed. SRNS patients had increased levels of almost all *APOL1* variants [except exon 2 (+)] compared to SSNS patient prior to GC treatment. With GC treatment, expression of most of the variants was increased in SSNS, but decreased in SRNS.

Conclusion: Cultured podocytes and circulating human leukocytes express different APOL1 splice variant patterns and differential splicing of *APOL1* is associated with NS and with GC therapy.

Poster #75
Presented June 1
Poster

Novel Role for Albumin and its Modification in Glomerular Injury

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Introduction: Albuminuria is both a characteristic hallmark and a known risk factor for progressive glomerular disease. We previously identified that both albumin deficiency and albumin overload resulted in enhanced glomerular injury. We thus hypothesized that glomerular and podocyte injury can be regulated by modifications of albumin levels, binding to free fatty acid (FFA) and associated factors, and molecular charge.

Methods: *In vitro* podocyte injury was studied following exposure to albumin, delipidated albumin, FFA, and cationic albumin. The ability of plasmapheresis effluents (PE) from nephrotic patients to induce podocyte injury *in vivo* and *in vitro* was also analyzed. Additional analyses included testing the ability of various clinically applicable matrices to ameliorate albumin- and PE-induced podocyte injury.

Results: While exposure of podocytes to albumin or FFA (lauric acid, oleic acid and arachidonic acid) alone reduced podocyte viability, supplementation of FFA with delipidated albumin restored podocyte viability. Albumin exposure also activated the kinases p38 MAPK, JNK/SAPK and AKT. While arachidonic acid and delipidated albumin alone did not activate these kinases, activation was moderately increased when podocytes were exposed to their combination. Additionally, cationic albumin induced greater podocyte toxicity vs. albumin, even at 400x lower concentration. Translational studies in nephrotic PE revealed diverse signs of direct podocyte injury, including reduced viability, actin cytoskeletal disruption, increased lipid staining, activation of Erk1/2 and JNK/SAPK, and induction of COX-2. Importantly, some of these responses were attenuated by pretreatment of PE with various matrices (charcoal, blue sepharose, liposorber gel and dextran sulfate). Finally, while PE injections alone did not induce significant albuminuria in rats, combined treatment with albumin induced greater albuminuria than albumin overload alone.

Conclusion: Albumin modifications, including altered levels, binding to FFA and associated factors, and altered ionic charge can alter albumin-induced podocyte and glomerular injury. Moreover, treatment with clinically applicable matrices appears to be able reduce this injury, thus representing a potential novel mechanistic approach to reduce glomerular injury and disease progression.

Poster #76
Presented June 1
Poster

Essential Involvement of Purinergic Signaling in Calcium Handling in the Podocytes of Type 2 Diabetic Nephropathy Rats

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Purinergic signaling is one of the major triggers of intracellular calcium $[Ca^{2+}]_i$ influx in podocytes. Growing evidence suggests that the activation of extracellular ATP signaling may play a crucial role in the development of diabetic nephropathy. The goal of this study was to characterize purinergic signaling in the podocytes of type 2 diabetic rats. Here we used 12 weeks old and aged (40-60 weeks old) Wistar, Goto-Kakizaki (GK) and Type 2 Diabetic Nephropathy (T2DN) rats. Using real-time confocal microscopy, we measured acute effects of ATP on $[Ca^{2+}]_i$ dynamics in the podocytes of glomeruli freshly isolated from these strains. We have shown that basal $[Ca^{2+}]_i$ concentration was within normal range in Wistar and GK podocytes (150.1 ± 10.8 and 174.7 ± 29.7 nM, respectively), whereas in T2DN rats it was pathologically elevated up to 232.9 ± 20.1 nM. Effect of ATP in Wistar rat (in the range of 1 to 200 μ M) was dose dependent and similar to what we previously reported for Sprague Dawley rats. However, ATP-triggered calcium influx in GK rats was substantially enhanced with dose-response curve shifted towards stronger activation of calcium influx with lower concentrations of ATP. Importantly, the response to ATP was further increased in T2DN compared to GK rat podocytes, and was found to be dose-independent within the same ATP concentration range. The same phenomenon was observed in aged (approximately 12 months old) GK and T2DN animals. Furthermore, experiments in calcium-free solutions and studies using alpha,beta-methylene-ATP revealed a major role of extracellular calcium influx as distinct from intracellular depot depletion, as well as a likely involvement of the ionotropic P2X receptors, or calcium channels activated by P2Y-evoked metabotropic GPCR cascades. Western blotting revealed a significantly higher expression of P2X₇ and P2X₄ receptors in the cortex of T2DN and GK strains compared to Wistar rats, and a decrease in P2Y₁ expression. Thus, our data indicate that in T2DN podocytes ATP-triggered calcium influx is enhanced compared to control animals with even low concentrations of ATP causing activation of calcium flux and augmented $[Ca^{2+}]_i$ concentration, correspondingly. This mechanism might be an important determinant of podocyte injury resulting from pathological remodeling from metabotropic P2Y to ionotropic P2X receptors occurring in DN.

Poster #77
Presented June 1
Poster

Loss of ADCK4 causes mitochondrial defects in podocytes

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Mutations in ADCK4 (aarF domain containing kinase 4, also known as COQ8B) usually manifest as steroid-resistant nephrotic syndrome (SRNS) and sometimes accompany medullary nephrocalcinosis or extrarenal symptoms including seizure. ADCK4 has a helical domain, ABC1 domain, and a kinase-like domain and is similar to yeast Abc1/Coq8 which is required for CoQ10 biosynthesis. ADCK4 localizes to mitochondrial matrix and associates with the inner membranes. Interestingly, patients with ADCK4 mutations exhibit reduced cellular CoQ10 contents. However, the role of ADCK4 at the molecular level is unclear. To address this, we knocked out ADCK4 in cultured podocytes and HK-2, a proximal tubule cell line using CRISPR/Cas9. ADCK4 knockout did not affect cell viability in both cell lines. The basal levels of coenzyme Q10 (CoQ10) levels of podocytes were three-fold higher compared to those of HK-2 cells. The levels of CoQ10 were severely decreased in ADCK4 knockout podocytes compared to control podocytes, whereas CoQ10 contents in HK-2 cells were not different. As CoQ10 is required for electron transfer from complex I and II to complex III of respiratory chain, we measured complex II+III activities. Complex II+III activities were defective only in podocytes, but not in HK-2 cells. In addition, transmission electron microscopy showed that ADCK4 knockout resulted in disintegrating mitochondria and loss of cristae formation in cultured podocytes, but not in HK-2 cells, suggesting that ADCK4 is indispensable for maintaining mitochondrial function in podocytes. In conclusion, our results showed that podocytes are more vulnerable to loss of ADCK4 than HK-2 cells and this may explain why individuals with ADCK4 mutations mostly manifest only SRNS.

Poster #78
Presented June 1
Blitz & Poster

Integrated Epigenomic Mapping and Expression Profiling Identifies Novel Glomerular Disease Pathways from Kidney GWAS Loci

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Though powerful, analysis of gene expression signatures alone has not fully illuminated the mechanisms underlying complex human phenotypes such as kidney disease. Genome wide association studies (GWASs) often correlate complex and non-specific kidney phenotypes (e.g. elevated eGFR) to specific genetic loci, but cannot identify the relevant kidney cell types that are contributing to the disease process.

It is now clear that genetic susceptibility to kidney diseases and traits is concentrated in epigenetic regulatory DNA elements (e.g. enhancers) that can act at great distances to control gene expression in an exquisitely cell-type specific fashion. Systematic identification of regulatory DNA is now possible with genome-wide chromatin accessibility profiling methods such as DNase-seq and ATAC-seq. Integrated analysis of gene expression together with these epigenomic maps promises to uncover new kidney disease mechanisms and to identify specific cell types which are the pathogenic targets of the disease process. However, efforts to apply these powerful techniques to the study of kidney disease have been hindered by the lack of epigenomic maps for most important adult human kidney cell types, notably glomerular podocytes.

To address this need, we generated first-of-kind, reference quality genome-wide epigenomic maps (DNase-seq) and paired gene expression profiles (RNA-seq) from primary human glomerular outgrowth cultures composed mainly of podocytes and mesangial cells. Similar datasets were generated from human cortical tubule cultures to enable comparison with the glomerular cultures.

Our analysis of these data revealed thousands of dynamically regulated enhancers and hundreds of differentially expressed genes that differed between the glomerular cell and cortical tubular cell cultures. Importantly, we cross-correlated differential activity at both the chromatin accessibility and gene expression levels to functionally connect kidney disease-associated loci to

novel target genes. Surprisingly, this identified several genes whose contribution to genetic risk for complex kidney phenotypes may be functioning in glomerular podocytes. Our findings enrich the current understanding of the complex genome regulation of podocytes and reveal novel candidate kidney disease genes and pathways.

Poster #79
Presented June 1
Poster

Public-Private Partnerships: Bridging Gaps to Advance Glomerular Disease Research

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Background:

The landscape of renal research is changing in academia and the private sector. Traditionally, research in academia emphasized publication, not rapid drug development. By working cooperatively, both the public and private sectors can share responsibility in the drug development process. Academia can offset long-term challenges that the private sector may not be set up to sustain, while the private sector may keep critical, translational projects on track through targeted support. Thus, research performed in the public sector can more quickly benefit patients through partnership with private entities.

Methods:

Numerous models exist to achieve successful public-private partnership in nephrology. Software tools are developed that increase universal access to publically-available data; contractual arrangements expedite data-analysis, and bilateral collaborations between the academic and pharmaceutical sector enhance understanding of analytical results. New models of partnership between the public and private sectors within a pre-competitive space integrate complimentary approaches in research direction and priorities. These efforts center around enriching the renal therapeutic target pool by identifying key molecular pathways, expediting detection of high-value targets for therapeutic development.

Results:

Critical success factors of these arrangements are based on establishment of a research space that allows partners to engage in a mutually-supportive environment; equally important is to define expectations on data transparency and sharing from the outset. Exquisite attention to contractual details for research direction, work scope, intellectual property protection, data sharing, publication rights, and project governance are key operational aspects. Meticulous project management, and effective communication between all parties can lead to progress across kidney research fields for all members of consortia.

Conclusions:

By bridging gaps in the translational research pipeline through public-private partnership, academic labs can expand the potential of medical research and significantly accelerate the drug development pipeline.

Poster #80
Presented June 1
Poster

Podocyte meeting, 2018, Montreal

***PRDM15* mutations cause steroid-resistant nephrotic syndrome with microcephaly, polydactyly, and heart defects**

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Steroid-resistant nephrotic syndrome (SRNS) causes 15% of chronic kidney disease in children and young adults. First insights into the pathogenesis of SRNS came from identification of >50 single-gene causes. PRDM proteins contain SET domain and multiple zinc fingers domains, and are involved in transcriptional regulation. We performed whole exome sequencing (WES) to identify novel monogenic causes of SRNS in >1,000 individuals with SRNS.

We identified 3 different recessive mutations in *PRDM15* (PR domain containing 15) (p.M483K, E519K and C1173Y) in 6 unrelated families. Interestingly, 4 affected individuals with the C1173Y mutated allele exhibited SRNS (childhood-onset) with microcephaly, polydactyly, and heart defects, while the 2 mutations in the SET domain of PRDM15 only caused isolated SRNS. C1173 is a critical "cysteine" residue at the "knuckle" of the Cys-x-x-Cys sequence necessary to complex the zinc ion. We tested the stability of wild type (WT) protein *versus* two mutations (M483K and E519K) in the SET domain of PRDM15, using thermal stability assay by tryptophan absorption. We demonstrate that the M483K mutant was significantly less stable than WT, while the E519K mutant was insoluble. Further, we show that stable knockdown of *PRDM15* results in decreased cell migration and severe proliferation defects in cultured human podocytes. WT, but not 3 mutant, constructs rescue the migration defects in podocytes, confirming deleteriousness of the mutations that we identified in SRNS patients. By immunofluorescence studies, we find that PRDM15 localizes at nucleoli of human podocytes.

We have identified *PRDM15* mutations as a novel cause of childhood-onset SRNS with microcephaly, polydactyly and heart defects. Our findings may implicate a defect in a transcriptional program as a new cause of SRNS.

Relation between G1 and G2 haplotypes in APOL1 gene and progression of chronic renal disease in children

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Background: In children the most frequent cause of Chronic Kidney Disease (CKD) is due to malformations of the urinary tract. Blacks are three times more likely to progress to end stages of renal disease (ESRD) compared to europeans. Researchers have shown that kidney disease in Africans is associated with two haplotypes, G1 and G2, present in exon 7 of the APOL1 gene. Therefore the objective of this study was to evaluate the association of these haplotypes with the faster evolution of CKD in Brazilian children.

Methods: 327 Brazilian children diagnosed with CKD in preparation for renal transplantation or already transplanted were selected for the study. The analysis was performed by extracting genomic DNA from the blood, amplification by PCR and Sanger sequencing.

Results: After sequencing analysis, it was possible to observe the presence of the high-risk variants in 12 of the 327 individuals analyzed. Of the 12 alterations found, 10 were homozygous for G1 (G1/G1) and 2 were compound heterozygosis (G1/G2). When we divided the chronic kidney population into subgroups we found 120 nephrotic patients, in whom we observed 10 high-risk variants, 8 G1/G1 and 2 G1/G2. In this group the age of onset of the disease was about 11.1 years and the duration of ESRD was about 2.5 years. These data are consistent with the current literature, in which it is observed that individuals who have high-risk variants in the APOL1 gene have later Nephrotic Syndrome (NS) onset and faster time of evolution to final stages, when compared to individuals who do not possess these variants.

Conclusions: Because this population is very broad and diverse, the high-risk variants for ESRD were found predominantly in nephrotic individuals. These results apparently do not have direct correlation with the progression to ESRD in individuals who did not have NS diagnosis, for example, in children with malformation urinary tract. However, although few variants were found in the group without NS diagnosis, this study was important to map part of the pediatric chronic renal population in Brazil, since the participating patients are from the whole country and this type of study was never performed before in this population.

Poster #82

Presented June 1

Blitz & Poster

Disease-causing mutations within *NPHS1* induce dominant-negative effects on nephrin signaling

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Nephrotic syndrome (NS) is a non-specific kidney disorder characterized by disruption of the glomerular blood filtration barrier. A main target of injury is the specialized glomerular podocytes, which normally encircle the capillaries with their foot process extensions and contribute to filtration through a unique intercellular junction known as the slit diaphragm. Nephrin (*NPHS1*) is an essential structural component of the slit diaphragm. Mutations in this transmembrane protein lead to loss of foot process morphology (effacement) and proteinuria. Numerous different nonsense, frameshift, splice-site or missense mutations have been reported in *NPHS1* and are classified according to predicted effects on nephrin protein expression, variably impacting the age of onset and disease progression. In addition to its structural role, nephrin also serves as a signaling scaffold, wherein its tyrosine phosphorylation promotes recruitment of key regulators of podocyte foot process morphology such as Nck, as well as activation of transcription factors including AP-1. Tyrosine phosphorylation of nephrin is essential in mice for podocyte maintenance and restoration of injured foot processes, and this phosphorylation is reduced in human and experimental renal diseases. To date however, the tyrosine phosphorylation status of inherited nephrin variants and its impact on nephrin function is not well understood. Here, we have characterized a novel nephrin sequence variant, A419T, expressed in a patient presenting with recurring NS. The patient is a compound heterozygote for the novel mutation and a previously characterized mutation, C623F, which is not properly expressed on the podocyte cell surface. We show altered trafficking and tyrosine phosphorylation of A419T and C623F, revealing dominant negative effects of both mutations on wildtype nephrin signaling. Our findings uncover a potential molecular mechanism by which mild mutations in nephrin can perturb filtration barrier integrity.

Poster #83
Presented June 1
Blitz & Poster

Title: Novel *MYO1E* Homozygous Stopgain Mutation and Rare Heterozygous Variants in Steroid Resistant Nephrotic Syndrome Associated Genes Segregate in a Family Modulating Different Phenotypes

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Background: Nephrotic syndrome (NS) is defined by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Approximately 20% of the patients do not respond to treatment with steroids and are classified as steroid-resistant (SRNS). At least 53 genes are currently known as the monogenic cause of SRNS. The aim of this study was to search for pathogenic variants in a SRNS familial case using whole exome sequencing (WES), as this method has become a tool of choice for genetic screening in diseases with high genetic heterogeneity and phenotypic variability such as SRNS.

Patients and Methods: We performed whole exome sequencing in one familial patient who was diagnosed with SRNS at 4 years of age. She presented FSGS on renal biopsy and 7 years later progressed to end-stage renal disease. She

received kidney transplant when she was 21 years old. *NPHS1*, *NPHS2* and *WT1* pathogenic variants had been excluded by Sanger sequencing before WES. We used strict genetic criteria for reduction of variants. Sanger sequencing was performed in order to confirm the variants identified.

Results: We found a novel homozygous stopgain variant c.505C>T (p.Arg169*) in the *MYOIE* gene. Only a few pathogenic *MYOIE* variants were reported so far associated with SRNS, mostly in familial cases. We also identified two heterozygous missense variants in *cis* in the *COL4A4* gene: c.2276C>T (p.Pro759Leu) and c.2008G>A (p.Val670Ile). We screened those variants in both parents and 12 siblings and identified the same genotype in two sisters with nephrotic proteinuria. Along with those variants, four other rare heterozygous variants are segregating in this family: two heterozygous missenses in *KANK1* gene c.511A>G (p.Tre171Ala) and c.3772G>T (p.Ala1258Ser), a missense variant c.3071C>T (p.Pro1024Leu) in *LAMB2* gene and a splicing variant c.2476+9G>A in *ANLN* gene.

Conclusions: The genetic and phenotypic heterogeneity identified in the family presented here illustrate how monogenic diseases such as NS may be associated with putative epistatic variants acting in the background of the disease. The patient and her family will benefit of the unequivocal etiology of the disease and of genetic counselling.

Poster #84
Presented June 1
Poster

Steroid treatment exacerbates nephrotic syndrome in *magi2a* knockout zebrafish larvae

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Background

Mutations in more than 55 genes may cause monogenic forms of nephrotic syndrome (NS) in humans. Their relevant expression in renal glomerular podocytes established the central role of podocytes in the pathogenesis of NS.

Recessive mutations of *MAGI2* cause steroid resistant NS in humans and mice.

Methods

To further delineate the pathogenesis of *MAGI2* loss of function, we generated knockout zebrafish lines for the duplicated genes *magi2a* and *magi2b* by CRISPR/Cas9. Pronephric glomeruli were assessed by light and electron microscopy. Drug treatments were performed from 2 to 9 days post fertilization.

Results

Whereas *magi2b* knockout did not yield a phenotype in zebrafish larvae, *magi2a*^{-/-} larvae developed a distinct edema phenotype between 4 to 15 days post fertilization (dpf). Electron microscopy showed podocyte foot processes effacement consistent with NS. Interestingly, as in human NS there was genotype/phenotype correlation with truncating alleles causing edema in 50% of all larvae between 5-6 dpf and hypomorphic alleles causing edema in 50% of all larvae between 19-20 dpf. Additionally, we provide genetic evidence that the PDZ0 domain of Magi2a plays an important role for Magi2a function in the zebrafish kidney.

To better understand their pharmacological effects, we tested drugs, typically used in the treatment of NS. We found that, paradoxically, steroid treatment exacerbated the phenotype with earlier onset by 4-4.5 dpf in 50% of larvae. Cyclosporine A and Tacrolimus did not yield this effect. RhoA activity has been implicated in the pathogenesis of *MAGI2*. However, drug targeting of the RhoA pathway did not modify the phenotype in *magi2a*^{-/-} larvae.

Conclusions

We here present a new zebrafish model for nephrotic syndrome, and demonstrate that steroid treatment might be harmful in certain monogenic forms of steroid resistant nephrotic syndrome. Thus, rapid genetic assessment by whole exome sequencing may be indicated before initiating steroid therapy in pediatric patients with certain monogenic forms of nephrotic syndrome.

Poster #85
Presented June 1
Poster

Single-cell Transcriptomics of Wildtype Mouse Glomeruli

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The glomerular filter is composed of three different cell types, the mesangium, endothelium and podocytes. Whether physiologic cues acting in glomeruli such as pressure gradients and blood flow result in cellular heterogeneity has thus far not been studied in detail. Here, we used nanodroplet-based, highly parallel transcriptional profiling to characterize the cellular content of purified wildtype mouse glomeruli.

Unsupervised clustering of 13,000 single-cell transcriptomes identified the three known glomerular cell types. Within each cell type, we confirmed established cell-type specific marker genes and identified novel marker genes. Most novel markers were corroborated by immunohistochemistry stainings obtained from the Human Protein Atlas (HPA). Novel markers reflect a wide variety of molecular functions, including transcription factors, ubiquitin ligases, and RNA-binding proteins.

We addressed cellular heterogeneity in glomeruli by subclustering of endothelial cells and identified a subset expressing marker genes related to endothelial activation. Pathway and gene set overdispersion analysis showed enrichment of terms related to proliferation and cell adhesion within this subset. Several of the subcluster markers indeed showed heterogeneous endothelial staining in HPA immunohistochemistry. While subclustering of podocytes showed a more uniform cellular population as compared to endothelium, three small subclusters of podocytes were robustly identified. Marker genes of podocyte subclusters included *Cald1* and *Lars2*, which in immunofluorescence staining colocalized only with a subset of podocytes marked by GFP in vivo.

In summary, our study comprehensively characterizes gene expression in individual glomerular cells. Furthermore, we are preparing an online atlas of gene expression in glomerular cells, which can be queried and visualized using an interactive and freely available database.

Poster #86
Presented June 1
Poster

CRISPR/Cas9 zebrafish model of human nephrotic syndrome due to Advillin loss of function

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Background: Steroid-resistant nephrotic syndrome (SRNS) is characterized by proteinuria due to disruption of the glomerular filtration barrier. It is a frequent cause of chronic kidney disease. We performed whole exome sequencing and homozygosity mapping in a cohort of SRNS patients and identified recessive mutations in the gene encoding actin-binding protein advillin (*AVIL*) in three unrelated families with SRNS. By a series of cell-based experiments we implicated in the pathogenesis of *AVIL* loss of function EGF signaling, *PLCE1* recruitment by *AVIL*, *IP3* and *DAG* generation by *PLCE1*, actin bundling by *AVIL*, and assembly of the *ARP2/3* complex to form podocyte lamellipodia (Rao J. et al, *J Clin Invest*, 127:4257, 2017). To further study the pathogenesis of *AVIL* loss of function we generated a zebrafish animal model.

Methods: To study larval-onset developmental phenotypes by CRISPR/Cas9 acute knockdown of the zebrafish orthologue *avil*, we performed injections of multiple guide RNAs. We generated stable knockout zebrafish lines for *avil* by breeding selected zebrafish. Survival curves were generated for acute knockdown and stable knockout zebrafish models by monitoring larvae twice a day for 14 days.

Results: Neither the acute knockdown nor the stable knockout developed a larval-onset phenotype of nephrotic syndrome. There was no difference in survival after 14 days when compared with wildtype. We now aim to evaluate fish for chemical induction of a nephrotic syndrome phenotype by use of pharmacologic inhibitors directed at different targets of the EGF/*AVIL* pathway.

Conclusion: CRISPR/Cas9 zebrafish models of *AVIL* protein defects do not recapitulate the human phenotype of nephrotic syndrome.

Poster #87
Presented June 1
Poster

Apolipoprotein L1 Dynamics in Human Parietal Epithelial Cell Molecular Phenotype Kinetics

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Background: PECs do not express APOL1. We hypothesize that APOL1 expression emerges in PECs for podocytes' (PDs) renewal (PECs' transition) in adverse milieus. We further hypothesize that the absence of apolipoprotein (APO) L1 favors the PEC phenotype and that the induction of APOL1 transitions to PD renewal.

Methods: Immortalized human PECs, which proliferate at 33°C and differentiate (transition) after 14 days after incubation in special media were used. To evaluate the time course effect, PECs' expression of APOL1 was evaluated at different time intervals (0, 4, 8, 14 days) during their transition (Tr). Hepatocytes and podocytes were used as a +ve control and HEKs as a negative control. Effects of a miR193a inhibitor and overexpression of miR193a were examined on PECs' expression of APOL1. Similarly, the effect of silencing and overexpression of APOL1 was evaluated on PECs' expression of miR193a. Effects of vitamin D receptor agonist (VDA), IFN- γ , and HIV were examined on the induction of APOL1 and associated expression of PD markers in HEKs and PECs. Luciferase assay was used to establish a putative interaction between

miR193a and APOL1 in PECs. To confirm the PEC induction of APOL1 *in vivo*, renal biopsy specimens of HIVAN patients were co-labeled for APOL1 and synaptopodin.

Results: During PECs' transition, APOL1 expression coincided with the expression of PD markers (PEC transition) along with down regulation of miR193a. The induction of APOL1 down regulated miR193a and induced PD markers in PECs/HEKs; whereas, the APOL1-silencing in Tr-PECs/HepG2s up regulated miR193a expression suggesting a reciprocally linked feedback loop relationship between APOL1 and miR193a. HIV, IFN- γ , and vitamin D receptor agonist (VDA) down regulated miR193a expression as well as induced the expression of APOL1 and PD markers both in PECs and HEKs. Since silencing of APOL1 both in PECs and HEKs partially attenuated HIV-, VDA-, and IFN- γ -induced expression of PD makers, it appears that APOL1 is an important functional constituent of miR193-APOL1 axis. This notion was further confirmed by enhanced expression of PEC markers in APOL1 silenced Tr-PECs despite down regulation of miR193a. HEKs overexpressing pCMV-miR193a displayed significantly decreased pEZX-MT06-APOL1 luciferase signal compared to empty vector, suggesting a putative interaction between miR-193a and APOL1. Renal biopsy specimen from HIVAN patient revealed PECs' expression of APOL1 and synaptopodin.

Conclusion: APOL1 absence favors PECs' phenotype but its expression facilitates PECs transition

Poster #88
Presented June 1
Blitz & Poster

APOL1 Preserves Human Podocyte Adherence Junctional Complex (PAJC) and the integrity of Podocyte Actin Cytoskeleton (PAC)

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Background: Gain or loss of function by APOL1 risk alleles (G1 and G2) in podocytes (PD) remains a controversial issue. We hypothesize that APOL1G0 (wild type) maintains the organization of the integrity of PD actin cytoskeleton (PAC) through the stabilization of PJAC (APOL1-Nephrin-CD2AP-Dendrin), whereas APOL1 risk alleles lack this function.

Methods: Stable human PD cell lines expressing vector (PDV), APOL1G0 (PDG0), APOL1G1 (PDG1) and APOL1G2 (PDG2) were developed. PDs were differentiated (DPDs) and evaluated for composition of PJAC, levels of Cathepsin (CTS) L, and the status of the PAC. Cellular lysates of DPDV, DPDG0 were immunoprecipitated (IP) with anti-APOL1 antibody and IP fractions were analyzed for PJAC constituents. To determine the role of APOL1 on the stability PJAC, DPDs were silenced for APOL1 and were evaluated for PJAC constituents. To explore mechanistic insights, the status of the APOL1-miR193 axis was evaluated in DPDG0, DPDG1s and DPDG2s. *In vivo* studies, renal cortical sections of control, APOL1G0, and APOL1 G1 transgenic mice were evaluated for the status of PJAC.

Results: DPDG0 displayed an intact PJAC, PAC, and low levels of CTSL, whereas, DPDG1 and DPDG2 exhibited a destabilized complex, a higher expression of CTSL, and disorganization of the PAC. IP studies confirmed the presence of APOL1 as a constituent of a stabilized PJAC in DPDG0s but DPDG1 and DPDG2s showed a destabilized PJAC. DPDs-silenced for APOL1 also displayed destabilized PJAC and an enhanced expression of CTSL. Renal cortical sections of APOL1G0 transgenic mice displayed intact PJAC, whereas, APOL1G1 transgenic mice displayed destabilized PJAC in the form of attenuated nephrin expression and nuclear import of dendrin. Mechanistic studies revealed that APOL1 stabilizes PJAC through down regulation of miR193a, the latter facilitates the expression of PD molecular markers; on the contrary, APOL1 risk alleles are not able to down regulate miR193a efficiently, contributing to the attenuated expression of nephrin and CD2AP. Puromycin aminonucleoside (PAN) enhanced PD expression of miR193a, nonetheless, both miR193a inhibitor and vitamin D receptor agonist (VDA) attenuated this effect of PAN; moreover, VDA provided protection against APOL1 risk alleles/PAN-induced down regulation mRNA expression of PJAC constituents and upregulation of CTSL.

Conclusion: APOL1G0 stabilizes but APOL1 risk alleles (G1 and G2) destabilize PJAC through modulation of APOL1-miR193a axis.

Poster #90
Presented June 1
Poster

Expanding the Phenotype of Nephrotic Genes *MYOIE* and *TRPC6*

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Background

From 2014 Children's Hospital at Westmead offered massively parallel sequencing of a targeted panel of 30 genes associated with nephrotic syndrome. Use of the panel for all children presenting with congenital or steroid resistant nephrotic syndrome has the power to detect genetic aetiology not predicted by clinical phenotype.

Methods

All children presenting to the Sydney Children's Hospitals Network with either congenital or steroid resistant nephrotic syndrome now undergo genetic testing routinely. A review of recent cases identified unexpected genetic results.

Results

Case 1. 9month old male with no family history or consanguinity, presented with typical nephrotic syndrome but resistant to prednisolone. Light microscopy suggestive of mesangial expansion/early FSGS, EM showed thickening/wrinkling of glomerular basement membrane. Audiology and ophthalmological assessment always normal. Identified as carrying *MYOIE* (NM_004998.3): c.[1567C>T];[3094_3097del] p.[(Arg523Trp)];[(Thr1032Profs*73)] (parental segregation confirmed) with no collagen variants of note. At 13yrs of age, controlled with ACE inhibition only, ongoing proteinuria with serum albumin of 30g/L and eGFR 106mls/min/1.73m².

Case 2. 9 year old female, presents with nephrotic syndrome but with reduced GFR (60mls/min/1.73m²), resistant to prednisolone. Histology of FSGS but EM showing subendothelial deposits. Rapid decline to end stage over 3 months despite supportive therapy. History of father developing therapy resistant nephrotic syndrome at 6years of age progressing within 6months to end stage (biopsy FSGS), no relapse post 2 transplants. Both identified as carrying *TRPC6* (NM_004621.5): c.[523C>T];[=] p.[(Arg175Trp)];[=]. At 4months follow up, almost anuric, establishing on peritoneal dialysis.

Discussion

It is not possible to determine likelihood of genotype by phenotype alone and yet the results described above have the power to alter clinical management. This confirms the need for genotyping in all paediatric patients with steroid resistant nephrotic syndrome as routine during diagnostic work-up.

Poster #91
Presented June 1
Poster

Title: Panel sequencing distinguishes monogenic forms of nephritis from nephrosis in children

Authors' names:

Makiko Nakayama, David Schapiro, Ankana Daga, Jennifer A. Lawson, Amar Majmundar, Svjetlana Lovric, Weizhen Tan, Jillian Warejko, Innes Fessi, Jia Rao, Merlin Airik, Heon Yung Gee, Ronen Schneider, Eugen Widmeier, Tobias Hermle, Shazia Ashraf, Tilman Jobst-Schwan, Amelie Van Der Ven, Shirlee Shril, Daniela A. Braun and Friedhelm Hildebrandt

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Text of the abstract:

Background: Alport syndrome (AS) and atypical hemolytic-uremic syndrome (aHUS) are rare forms of chronic kidney disease (CKD) that can lead to a severe decline in renal function. In children and young adults, steroid resistant nephrotic syndrome (SRNS) is more common than AS and aHUS, causing 10% of childhood-onset CKD. In recent years, multiple monogenic causes of AS, aHUS and SRNS have been identified, but their relative prevalence has yet to be studied in a typical pediatric cohort of children with proteinuria and hematuria. We hypothesized that identification of causative mutations by whole exome sequencing in known monogenic nephritis and nephrosis genes would allow etiologically distinguishing nephritis from SRNS in a typical pediatric group of patients with both proteinuria and hematuria at any level.

Methods: We therefore conducted panel exon sequencing for 11 AS, aHUS and thrombocytopenic purpura-causing genes in an international cohort of 371 patients from 362 different families who presented with both proteinuria and hematuria before age 25. In parallel, we conducted either whole exome sequencing or panel exon sequencing for 23 SRNS-causing genes analysis in all patients.

Results: We detected pathogenic mutations in 18 of the 34 genes analyzed, leading to a molecular diagnosis in 14.1% of families (51 of 362). Disease-causing mutations were detected in 3 AS-causing genes in 17 families (4.7%), 3 aHUS-causing genes in 5 families (1.4%), and in 12 SRNS-causing genes in 29 families (8.0%). We observed a much higher mutation detection rate for monogenic forms of CKD in consanguineous families (35.7% vs. 10.1%).

Conclusion: We present the first estimate of relative frequency of inherited AS, aHUS (6.1%) versus SRNS (8.0%) in a typical pediatric cohort with proteinuria and hematuria. Important therapeutic and

preventative measures may result from mutational detection in individuals with proteinuria and hematuria.

Poster #92
Presented May 1
Poster

Mutations in type 4 α collagen chains are a significant contributor to late onset focal segmental glomerulosclerosis

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Introduction: Focal and segmental glomerulosclerosis (FSGS) is a descriptive clinical and pathologic entity characterized by proteinuria and partial glomerular scarring in the kidney. More than 50 genes, including mutations in the type 4 α collagen genes, have been reported to be causal for FSGS. The type 4 collagen $\alpha3\alpha4\alpha5$ heterotrimer, secreted by podocytes, comprises the major component of the glomerular basement membrane. Mutations in *COL4A3/4/5* (referred to as *COL4A* herein) which encode for these proteins were classically associated with Alport Syndrome or Thin Basement Membrane Disease (TBMD). This study aimed to identify mutations in *COL4A* genes associated with late onset FSGS.

Methods: Familial and sporadic cases of FSGS were recruited from Toronto General Hospital. Our cohort comprised of 14 families and 90 sporadic cases with a total of 122 patients. Whole exome sequencing (WES) was performed on DNA samples collected from participants. We evaluated ~50 FSGS genes for rare variants including *COL4A*. Previously reported disease causing rare variants were designated as pathogenic while unreported rare variants predicted to be damaging by *in silico* prediction programs were categorized as variants of unknown significance (VUS). Rare variants were defined as having a minor allele frequency (MAF) ≤ 0.00005 for autosomal dominant genes and MAF ≤ 0.005 for autosomal recessive genes.

Results: Sex distribution within the cohort was 55% males, 43% female and 2% unknown. Population substructure revealed that 73% of patients were of non-Finnish European descent. The mean age of onset of disease was 36 ± 16 years and mean age at end stage renal disease (ESRD) was 47 ± 17 years. Of all FSGS genes, we found the highest proportion of pathogenic variants in one of the 3 *COL4A* genes. Pathogenic *COL4A* variants were identified in 2/14 (14%) families and in 3/90 (3%) sporadic FSGS cases. Additionally, 9/90 (10%) sporadic cases had a VUS in *COL4A*.

Conclusion: We found that pathogenic *COL4A* mutations was the largest contributor to familial and sporadic disease of all FSGS genes in our predominantly European late onset cohort. In sporadic cases, up to 13% of disease is potentially explained by mutations in the *COL4A* genes if variants of unknown significance are considered. Our findings highlight an unmet need to characterize *COL4A* variants of unknown significance found in FSGS cases, which has clinical implications in terms of diagnosis, prognosis and likely therapeutics in the future.

Poster #93
Presented June 1
Poster

An eQTL landscape of kidney tissue in human nephrotic syndrome

Christopher Gillies¹, Rosemary Putler¹, Rajasree Menon², Edgar Otto³, Kalyn Yasutake¹, Viji Nair³, Paul Hoover^{4,5}, David Lieb⁵, Shuqiang Li⁵, Sean Eddy³, Damian Fermin¹, Nephrotic Syndrome Study Network, Nir Hacohen^{4,5}, Krzysztof Kiryluk⁶, William Wen⁷, Matthias Kretzler^{2,3}, Matthew G. Sampson¹

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Background

Expression quantitative trait loci (eQTL) studies illuminate the genetics of gene expression and, in disease research, are particularly illuminating when using the tissues directly impacted by the condition. Currently, there are few kidney eQTLs studies, with most using bulk renal cortex. Here, we integrated whole genome sequencing (WGS) and microdissected glomerular (GLOM) & tubulointerstitial (TI) transcriptomes from 187 patients with nephrotic syndrome (NS) to describe the eQTL landscape in these functionally distinct kidney structures.

Methods

Using MatrixEQTL, we performed *cis*-eQTL analysis on 136 GLOM and 166 TI transcriptomes from NEPTUNE cohort participants. We used the Bayesian “Deterministic Approximation of Posteriors” (DAP) to fine-map these signals, eQTLBma to discover GLOM- or TI-specific eQTLs, and single cell (sc)RNA-Seq data of adult human kidney tissue to identify cell-type specificity of significant eQTLs. We integrated eQTL data with an IgA Nephropathy (IGAN) GWAS to perform a transcriptome-wide association study (TWAS).

Results

We discovered 894 GLOM eQTLs and 1767 TI eQTLs at FDR <0.05. 14% and 19% of GLOM & TI eQTLs, respectively, had > 1 independent signal associated with its expression. 12% and 26% of eQTLs were GLOM-specific and TI-specific, respectively. scRNA-Seq of 4,734 cells identified 14 clusters of cell types. We identified genes that were significantly differentially expressed in, or specific to, particular clusters. GLOM and TI eQTL genes were most significantly enriched in podocytes (OR: 2.5, $p=8 \times 10^{-11}$) and proximal tubule cells (OR: 3.4, $p=1 \times 10^{-43}$), respectively. Functional annotation of the 79 podocyte-specific GLOM eQTLs identified the top three biologic processes as “vesicle-mediated transport”, “endocytosis”, and “regulation of locomotion”, the top molecular function was “extracellular matrix binding”, and the top signal transduction pathway was “integrin signaling” ($p < 5 \times 10^{-05}$). The TWAS identified decreased predicted expression of *HLA-DRB5* in GLOM & TI as most associated with IGAN.

Conclusions

Here, we discovered GLOM & TI eQTLs, deconvoluted them into tissue & cell-specific signals, and used them to characterize known GWAS alleles. These data are released openly and publicly for the benefit of the wider community at <http://nephqtl.org>. NephQTL has a searchable browser of the summary-level MatrixEQTL and DAP output. The full MatrixEQTL output is available for download and secondary use.

Poster #94
Presented June 1
Poster

GAPVD1 and ANKFY1 mutations implicate RAB5 regulation in nephrotic syndrome R. Schneider¹, T. F. Hermle¹, D. Schapiro¹, S. M. El desoky², J. A. Kari², D. A. Braun¹, F. Hildebrandt¹

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Background: Steroid Resistant Nephrotic syndrome (SRNS) is a frequent cause of end-stage renal disease within the first 3 decades of life. The discovery of more than 50 different monogenic causes has helped to elucidate the pathogenesis of SRNS.

Methods: To identify novel monogenic causes of NS we performed homozygosity mapping and whole Exome Sequencing (WES) in a worldwide cohort of ~600 individuals with nephrotic syndrome. Co-immunoprecipitation using HEK cells were employed to analyze protein interaction.

Results: By WES we identified two homozygous missense mutations of GAPVD1 (c.1240C>G, p.L414V and c.2810G>A, p.R937Q) in two patients from unrelated families with early-onset nephrotic syndrome. Both mutated amino acids are conserved to *C. intestinalis*. One patient did not respond to steroids while the other showed the unusual combination of congenital nephrotic syndrome and spontaneous remission. This has previously been observed in few alleles of NPHS1. The histology in both cases was characterized by mesangial hypercellularity while electron microscopy revealed podocyte foot process effacement. GAPVD1 is a known regulator of endosomal trafficking and interacts with RAB5. GAPVD1 harbors both, a GTPase activating and an inactivating domain.

We further identified a mutation of ANKFY1 (c.284G>T, p.R95L, conserved to *D. melanogaster*) in a patient with SRNS and FSGS with an affected sibling sharing the mutation. ANKFY1 is also an interaction partner of RAB5 and serves as a RAB5-effector. Western blotting revealed expression of GAPVD1 and ANKFY1 in a human podocyte cell line. Using co-immunoprecipitation we observed physical interaction between both proteins. We further found interaction of GAPVD1 and the slit diaphragm protein NPHS1. Mapping experiments suggest that both functional domains of GAPVD1 bind to NPHS1. Podocyte migration experiments showed slower migration rate for shRNA knock-down of either gene in cultured podocytes and rescue of the phenotype by the over-transfection of the wild-type gene, but not the mutated gene, both in GAPVD1 and ANKFY1 KD models.

Conclusion: We discovered mutations of GAPVD1 and ANKFY1 as novel monogenic causes of nephrotic syndrome. Interestingly, both proteins interact with each other and RAB5. GAPVD1 further interacts with NPHS1, mutations in which cause nephrotic syndrome.

Poster #95
Presented June 1
Poster

The renoprotective role of Deiodinase 3 in podocytes

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Deiodinase 3 (D3) is a membrane-bound, catabolic enzyme that regulates cellular metabolism by deactivating tri-iodothyronine (T3), the metabolically active thyroid hormone. As evident by the embryonic lethality of D3 knockout animals, proper regulation of thyroid hormone activity is vital in nearly all cell types, including podocytes. Despite the prevalence of overlapping complications of thyroid hormone disorders and kidney disease, a unifying mechanism regulating T3 homeostasis in the kidney is absent. To address this void, we aimed to determine the significance of D3 dysfunction in glomerular kidney disease using podocytes, which have mechanisms that respond to both glomeruli derived and circulating changes in hormone levels. We found D3 was highly expressed in podocytes, downregulated in injury models, and podocyte specific D3 KO mice responded poorly to LPS-induced acute kidney injury, resulting in heavy proteinuria compared to control. Our data demonstrates D3 plays a renoprotective role against thyroid hormone associated kidney disease in podocytes, and we propose D3 reduces energy expenditure to prevent podocyte exhaustion and death.

Kidney organoids: a model system for hereditary glomerular diseases

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Background: Kidney organoids differentiated from induced pluripotent stem cells (iPSCs) have recently emerged as powerful tools for *in vitro* disease modelling. Differentiation of iPSCs towards the kidney lineage is directed by applying specific stimuli that are spatiotemporally integrated by cells, thus recapitulating the sequential stages of embryonic kidney development. Since iPSCs can be directly derived from easily accessible patient cells, this approach allows studying the pathogenic effect of mutations involved in hereditary renal diseases in the patient's own genetic background.

Methods: We differentiated iPSCs derived from healthy controls and patients affected by diverse forms of hereditary nephrotic syndrome into kidney organoids using an adapted version of *Morizane's* protocol. In particular, we obtained patient's cells harbouring the homozygous p.R138Q substitution in the podocin encoding *NPHS2* gene, that is, the most frequent mutation leading to early-onset steroid resistant nephrotic syndrome. We investigated the structure and organization at both organoid and podocyte levels using immunofluorescence and electron microscopy in order to unveil a pathogenic phenotype.

Results: iPSCs-derived kidney organoids display the same characteristics of *in vivo* nephrons, with the ordered succession of glomeruli, proximal tubules, Henle's loop and distal tubules. Glomerular podocytes are organized in clusters of polarized goblet-shaped cells extending cellular protrusions at their basal side. We confirmed the expression and localization of key podocyte markers, with localization of WT1 in the nucleus, podocalyxin at the apico-lateral surface and nephrin, podocin and synaptopodin at the basal side. Importantly, mutant organoids show altered subcellular localization of podocin with a diffuse perinuclear staining indicative of endoplasmic reticulum retention as previously described *in vivo* in human renal biopsies and knock-in mouse model.

Conclusions: Together, our results underscore the relevance of this system in the modelling of glomerular diseases. In order to better reproduce the glomerular filtration barrier features and investigate the podocyte structure and its potential alterations under pathological conditions, we are currently vascularizing kidney organoids by transplanting them on the chick embryo chorioallantoic membrane.

Session 1: Development
Presented May 31
Short Oral

Derivation of functional podocytes from human iPS cells and the establishment of a kidney glomerulus chip

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Abstract

Glomerulopathy is a leading cause of end-stage renal disease. An *in vitro* model of human glomerulus could facilitate therapeutic discovery and illuminate kidney disease mechanisms. Efforts to develop such models are limited by the lack of functional human podocytes, the specialized epithelial cells that encase glomerular capillaries and regulate selective permeability in the glomerulus. Human induced pluripotent stem (iPS) cells have a remarkable capacity to self-renew indefinitely and differentiate into almost any cell type under appropriate conditions, and could potentially serve as an unlimited source of podocytes. Despite recent advances in the derivation of nephron progenitor cells, a method for specific differentiation of human iPS cells into podocytes remains elusive. We developed a highly efficient method for directed differentiation of human iPS cells into mature podocytes that exhibit primary and secondary foot processes, and integrate into forming glomeruli when injected into developing mouse embryonic kidneys. The podocytes also recapitulate the natural tissue-tissue interface and exhibit the selective molecular clearance of the glomerular capillary wall when co-cultured with human glomerular endothelial cells in an organ-on-a-chip microfluidic device. The engineered human glomerulus-on-a-chip also replicates kidney disease phenotypes *in vitro*. This work could illuminate the mechanisms of human kidney development and disease, and provide a functional *in vitro* system for therapeutic development.

Session 3: Cytoskeleton
Presented May 31
Short Oral

Effects of SRNS-associated mutations on Myo1e stability, localization, and functions in podocytes

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Non-muscle Myosin 1e (Myo1e) is an actin-dependent motor protein enriched in the glomerulus. Mutations in *MYO1E* are associated with early onset steroid-resistant nephrotic syndrome (SRNS), which is characterized by massive proteinuria due to the disruption of glomerular filtration. Exon sequencing in SRNS patients identified 5 individuals aged between 2 months to 7-year-old with novel *MYO1E* mutations (Sadowski et al., *JASN*, 2015, **26**(6): 1279-89). This study aims to determine the effects of SRNS-associated mutations on Myo1e protein expression and activity. Myo1e localizes to podocyte cell-cell junctions and to clathrin-coated vesicles (CCVs) and has been proposed to be necessary for slit diaphragm dynamics and for endocytosis. We hypothesized that mutations in Myo1e lead to its misfolding and instability and/or to the loss of its activity at cell-cell junctions and CCVs. Using immortalized podocyte cell lines and adenovirus-mediated transduction of EGFP-tagged Myo1e constructs, we quantified Myo1e expression level by Western blotting and measured Myo1e protein turnover rate using ribosome and proteasome inhibitors. Myo1e mutants T119I, D185G, D388H and del3094-7 were unstable compared to the wild type (wt) Myo1e. Myo1e mutants T119I and del3094-7 did not localize to podocyte junctions while Myo1e-D185G exhibited junctional localization similar to the wt. Myo1e D388H localized at the cell-cell junctions but its junctional enrichment was lower than for the wt. By analyzing EGFP-Myo1e co-localization with CCVs in mouse fibroblasts, we found that Myo1e T119I and del3094-7 lost the ability to localize to the CCVs. Similarly to the wt, D185G, R523W, and L822M were co-localized with CCVs. Interestingly, Myo1e D388H showed enhanced CCVs localization and remained at the CCVs longer than the wt. In conclusion, mutations T119 and del3094-7 disrupt Myo1e stability and localization while mutants D185G and D388H are not rapidly degraded but nevertheless exhibit decreased protein expression in podocytes. In addition, the D388H mutation may affect Myo1e functions in endocytosis. This assessment of the impact of specific *MYO1E* mutations provides new information for evaluating their pathogenic effects and may lead to better understanding of the activity of Myo1e and the mechanisms leading to podocyte dysfunction in disease. Supported by NIH-NIDDK R01DK083345.

Session 4: Friends
Presented May 31
Short Oral

Podocyte recruitment in the adult kidney induced by stimulation of macula densa Wnt signaling

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The recruitment of new podocytes from precursor cells including parietal epithelial cells (PECs) and cells of the renin lineage (CoRL) in response to glomerular injury has been established, however the mechanism remains elusive. Here we tested the hypothesis that macula densa (MD) cells localized at the glomerular entrance are master regulators of endogenous glomerular repair, including podocyte recruitment. The combination of time lapse intravital multiphoton imaging and genetic cell fate tracking provided “smoking gun” direct visual evidence for the migration of single CoRL from the PEC layer to the glomerular tuft (sometimes within 15 s) in the intact mouse kidney, especially in disease models that are associated with MD stimulation (UO, 5/6 Nx). Results from MD gene profiling, immunolocalization of active β -catenin, and TCF/Lef:H2B-GFP reporter mice all strongly suggested that the renal cell type with the strongest Wnt activity is the MD. The newly established, exclusively MDn specific secretome includes several glycoproteins with well known angiogenic, patterning, growth factor, and extracellular matrix remodeling properties, including Pappalysin 2, Ccn1, Sfrp1, Cxcl14, Wnt10a, Sema3c, Bmp3, Egfl6, Fgf9, Vegfd, Pdgfr, Frem1, Thsd4, Adamts18, etc. In a new MDn Wnt transgenic mouse model (nNOS-CreERT2; Ctnnb1^{(ex3)fl/fl}), tamoxifen inducible MDn specific stimulation of Wnt signaling increased glomerular size, and podocyte number (from control 17 ± 1.1 to 25 ± 1.3 per unit glomerular volume) within 4 weeks. Treatment of Ren1dn Confetti mice with GSK3 β and ROCK inhibitors for only 5 days caused a 3n fold increase in the density of CoRL at the glomerular vascular pole and in PEC layer. MDn Wnt mice treated with podocyte cytotoxic IgG, and Ccn1n treated NEP25 mice after LMB2 (two podocyte specific injury models of FSGS) were protected from proteinuria. These results suggest that MD cells are central players in the physiological remodeling of the glomerulus via Wnt signaling and secreted paracrine factors that act on glomerular precursor cells (including CoRL). This new mechanism protects from glomerular injury when augmented, and may be developed further to specifically treat glomerular diseases.

Session 4:
Friends Presented
May 31 Short
Oral

Imaging the podocyte and the filtration barrier using super-resolution fluorescence light microscopy

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Background: Historically, the glomerular filtration barrier and its nanometer-scale constituents could only be visualized using electron microscopy (EM). Although EM is a powerful imaging tool for many purposes, it has some limitations regarding labelling of multiple epitopes and three-dimensional imaging. Confocal fluorescence microscopy offers both spectral separation of multiple epitopes as well as 3D optical sectioning, but the diffraction-limited resolution of around 300 nm is not sufficient for imaging the fine morphology of the podocyte. However, novel super-resolution techniques such as stimulated emission depletion (STED) microscopy and expansion microscopy both push the resolution to around 50 nm, opening up the possibility to perform nanometer scale imaging of podocytes using light microscopy.

Methods: By applying an optical clearing protocol, we show that multi-color imaging of podocyte foot processes and the slit diaphragm can be performed at sub-70 nm resolution. Further, we apply an expansion protocol to spatially expand kidney tissue a factor of 5, thereby resolving foot processes and the slit diaphragm using confocal microscopy at sub-70 nm effective resolution. Using both of these methods, we can study and quantify podocyte morphology in health and disease. We also show the possibility to combine expansion microscopy and STED microscopy to further push the resolution into the sub-20 nm regime.

Results: We show that both STED microscopy and expansion microscopy can both be used

to visualize parts of the filtration barrier such as foot processes and the slit diaphragm. We apply both these methods to disease models, such as passive Heymann nephritis and a mouse model of anti-GBM nephropathy. We also apply the analysis to two different genetic mouse models. Two compound-heterozygous point mutations in the podocin gene (which leads to FSGS), and one where the Med22 is inactivated (leading to a severe phenotype in podocytes with heavy proteinuria). In all of the models, we describe and quantify the changes in podocyte morphology and protein topology as compared to healthy controls.

Conclusions: We conclude that using super resolution methodologies, imaging of podocyte foot processes is no longer restricted to EM. Super-resolution light microscopy techniques can now serve as a compliment to EM when imaging foot processes and the filtration barrier, especially when protein topology and 3D morphology is studied.

Session 5: Immune
Presented June 1
Short Oral

Generation of a humanized mouse model for SLE/LN for studying human lupus and testing potential therapies

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Lupus Nephritis (LN) is a common complication of systemic lupus erythematosus (SLE) with unclear etiology and limited treatment options. Approximately 50-60% of SLE patients develop LN over the course of the disease, making LN the common cause of morbidity and mortality. Leukocyte infiltration into the kidneys, a hallmark of LN, triggers tissue damage and proteinuria. We recently discovered novel small molecule CD11b agonists termed leukadherins (model agonist “LA1”) that binds and activates CD11b/CD18 integrin reducing leukocyte migration and inflammation and protects kidney function in several inflammatory diseases including SLE in MLR/lpr lupus prone mice. While studies of spontaneous or induced mouse models of SLE have advanced our understanding of lupus, each strain has unique advantages and disadvantages and none of them are a phenocopy of human SLE. Here, I present a humanized mouse model of lupus as a new experimental tool to understand the cellular and genetic requirements for human SLE/LN and a method to screen potential therapies.

For a pilot study, peripheral blood mononuclear cells (PBMCs) were transferred from SLE patients into NSG mice. The exclusion criteria were pregnancy and acute infection. There was 100% successful engraftment in NSG mice transferred with healthy PBMCs (Healthy-NSG) or SLE PBMCs (SLE-NSG) with an average of 78% human CD45+ cells in the spleens at 6 weeks post engraftment. Interestingly, mouse CD11b+Gr1+ cells and human CD14+ cells were significantly increased *in vivo* in response to the engraftment of SLE PBMCs, but not healthy PBMCs. Within 1 week, SLE-NSG mice, but not the healthy-NSG mice, lost whiskers and developed skin lesions and alopecia. Histopathological analysis of nasal dermis showed increased epidermal thickness and leukocyte. Interestingly, SLE-NSG mice that were treated with LA1 did not display skin lesions or alopecia. Further studies also indicated that like SLE human PBMC donors, SLE-NSG mice have significantly elevated levels of serum anti-dsDNA and suPAR. The SLE-NSG mice also had significantly reduced kidney function, as indicated by elevated levels of proteinuria, renal leukocyte infiltration, renal fibrosis and glomerular damage, which was not evident in healthy-NSG or LA1 treated SLE-NSG mice. This novel humanized SLE/LN model exhibits several clinical manifestation of human lupus, most importantly kidney injury and is an important tool to test therapies.

Session 6: Therapeutics
Presented June 1
Short Oral

Podocyte Meeting, Montreal, May 2018

Title: Nephrotic Syndrome Outcome Prediction Using Tissue Transcriptomic Profiles

Authors: Laura H. Mariani¹, Habib Hamidi¹, Huateng Huang¹, Sean Eddy¹, Yuanfang Guan¹, Matthias Kretzler¹ for the Nephrotic Syndrome Study Network

Affiliations: University of Michigan

Background: Clinical parameters do not accurately predict outcomes and treatment response in nephrotic syndrome (NS) due to the biologic heterogeneity underlying the disease classifications. Machine learning techniques can identify predictors from the many potential parameters across the genotype-phenotype continuum, including tissue gene expression levels which may better capture biologically relevant predictors.

Methods: NEPTUNE is a prospective cohort study of NS patients enrolled at the time of clinically indicated biopsy. Clinical data, pathology features and kidney tissue genome wide mRNA expression levels are collected. Weighted gene co-expression network analysis was used to cluster genes into modules based on expression level across samples. Elastic net regularization was used to build Cox proportional hazards models for time to (1) composite of ESRD/40% eGFR decline and (2) complete remission (UPCR <0.3 mg/mg) using gene expression modules, clinical and pathology data. Expression modules selected by the algorithm as important predictors were analyzed for their functional significance and biological processes using PANTHER and KEGG pathway enrichment analysis.

Results: 197 patients were used to derive models including clinical and pathology variables and gene expression modules [median age 35(15-56), 65% male, 28% black, eGFR 84 (62-107), UPCR 1.92 (0.5, 4.0)], 24% MN, 26% MCD, 14% IgA and 36% FSGS]. In the full elastic net model, 23 tubular and 27 glomerular modules were selected predictors for remission (tAUC 0.72) and 12 tubular and 11 glomerular modules were selected for eGFR loss (tAUC 0.77). An enrichment of genes and signaling pathways associated with specific modules. Pathway enrichment analysis demonstrate that the top glomerular modules that are predictor of remission are characterized by specific molecular signaling pathways such as integrin, interleukin, FGF, EGF receptor, JAK/STAT and VEGF which can be perturbed by known pharmacological agents and thus serve as potential therapeutic targets.

Conclusions: Machine learning elastic net models have high accuracy for outcome prediction and identify novel predictors. Tissue mRNA expression modules identified novel biological processes which are predictive of clinical outcome and thus potential therapeutic targets.

High throughput screening for drug discovery against ApoL1 toxicity in HEK cells

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Background: APOL1 risk variants (G1 and G2) are associated with progressive Chronic Kidney Disease (CKD) in the African ancestry population. In order to identify drugs that will rescue APOL1 toxicity, we generated T-REx 293 stable cell lines that express FLAG- tagged APOL1 variants under control of tetracycline. We were able to recapitulate APOL1 renal risk variants differential toxicity, following induction at equal expression levels of the APOL1 risk variants compared to the non-risk variant.

Methods: High throughput screening (HTS) was conducted at the Nancy & Stephen Grand Israel National Center for Personalized Medicine (G-INCPM), at the Weizmann institute, using rescue of T-Rex cells expressing the APOL1-G1 variant. Approximately 100,000 compounds were screened, these compounds can be divided into two categories: bioactive compounds, which are approved medications or modulators of known biological pathways, or chemical diversity compounds, which are designed to cover a chemical space with properties that are considered favorable for medicinal chemistry. Cells were dispersed to 384 well plates, that were already containing compounds dissolved in DMSO, and induced for APOL1 expression for 16 hours. Induction of G1 variant in this system is sufficient to cause cell death, and cell viability was measured using quantification of the ATP content by luminescence assay.

Results: The HTS was performed in three steps: pre-screen, screen, validation. The pre-screen was designed to investigate the suitability of the cell platforms for HTS and estimate compound concentration to use in the screen. The pre-screen included 2,505 different compounds, each at two different concentrations. Based on the pre-screen results the screen included approximately 100,000 compounds tested at one concentration for replicated rescue of 20% or greater. For both pre-screen and screen Z' values greater than 0.5, were obtained, pointing to a robust capacity to detect rescue. Following the validation, 125 compounds are considered as hits for further study.

Conclusions: We have established an induced-cell system which allows us to screen for compounds that will rescue APOL1-cell toxicity. Among other follow up steps, these hits will be examined in other systems including podocytes, yeast, Drosophila and cell-free biochemical assays.

Session 7: Complement
Presented June 1
Short oral

Targetable mechanisms identified from shared molecular profiles in ANCA-associated vasculitis and nephrotic syndrome

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ABSTRACT

Background: Clinical trials in glomerular diseases typically test therapeutic efficacy in a disease defined by common clinical or structural features. Understanding shared molecular pathways across different rare diseases may lead to novel treatment approaches and classification strategies independent of descriptive diagnoses. Renal biopsies from patients with nephrotic syndrome (NS) or ANCA-associated vasculitis (AAV) were evaluated for molecular programs cutting across conventional disease categories as candidates for therapeutic targets. **Methods:** Patients with NS (minimal change disease, focal segmental glomerulosclerosis, or membranous nephropathy) (n=187) or AAV (granulomatosis with polyangiitis or microscopic polyangiitis) (n=80) were recruited from Nephrotic Syndrome Study Network (NEPTUNE) and the European Renal cDNA Bank (ERCB). Transcriptional profiles were assessed in renal biopsies for cross-cutting disease mechanisms and potential therapeutic targets shared across diseases. **Results:** In the ERCB discovery cohort, 10-25% transcripts were differentially regulated versus controls in both NS and AAV (fold change > 1.3, q-value<0.05, tubulointerstitium and glomeruli). The majority (90%) of transcripts differentially expressed in NS were also regulated in AAV and 60-77% cross-validated in independent samples from a replication dataset (samples from patients with NS from NEPTUNE and samples from patients with AAV from ERCB) profiled on a different platform. Functional analysis of validated transcripts in the glomeruli identified therapeutically relevant networks in the glomeruli including: complement activation, glucocorticoid signaling, IFNG activation, IL2 activation, TLR7 activation, NfκB activation, and JAK-STAT activation. Transcripts causally downstream of JAK-STAT1 were used to develop an activation signature that was correlated with predictors of CKD progression, interstitial fibrosis (r=0.41, p<0.001), and a prognostic CKD biomarker, urinary EGF (r=-0.51, p<0.001).

Conclusion: AAV and NS share common intra-renal molecular pathways cutting across conventional disease classification. Pathway activity can be modeled to develop patient-centric targeted therapies. This approach provides a starting point for *de novo* drug development, and repurposing efforts in these rare kidney diseases.

Session 8: Metabolism
Presented June 1
Short Oral

ER targeting is a determinant of APOL1-linked cytotoxicity

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[§]) contributed equally

Background: In individuals of recent African ancestry, two renal risk variants in the human *APOL1* gene called *G1* and *G2* (RRVs) have been associated with certain forms of chronic kidney disease (CKD). APOL1 mediated CKDs involve disruption of the structure and function of podocytes, which are essential cells of the renal filtration barrier. So far, the molecular and cellular mechanisms of APOL1-linked cell injury are poorly understood. In this study, we compared the intracellular localization and cellular functions of the cytotoxic APOL1 variants with highly homologous APOL2 to obtain more insight about the sequences that mediate the toxic effect.

Methods: We generated stable podocyte and HEK293T cell lines allowing an inducible overexpression of EGFP-tagged APOL1, APOL2 and chimeric proteins. Moreover, we used mito-EGFP-APOL1 fusion proteins that were directly targeted to the outer mitochondrial membrane. Live cell imaging and cell viability assays were applied to analyze intracellular localization and cytotoxic effects of the different cell lines.

Results: APOL1 and APOL2 were predominantly targeted to the ER. APOL2 had no cytotoxic effects, even if combined with the C-terminal SRA-interacting domain (SID) of APOL1 RRVs, showing that the SID alone is unable to transfer the toxic effect. Interestingly, these chimeric proteins were targeted to mitochondria although the SID alone had no membrane binding properties. Furthermore, APOL1-G2 with the BH3-only sequence motif (BSM) of APOL2 showed no toxicity, suggesting that the BSM is an essential determinant required for the cytotoxic effect. However, direct targeting of APOL1 to the outer mitochondrial membrane did not result in an enhanced cytotoxicity in contrast to ER-localized full length APOL1 RRVs.

Conclusion: Our data indicate that ER rather than mitochondrial localization is a precondition for APOL1 associated cell injury.

Session 8: Metabolism
Presented June 1
Short Oral

Critical Role of NOX4/TRPC6 Pathway in Podocyte Calcium Regulation and Kidney Damage in Diabetic Nephropathy

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One of the main indicators of diabetic nephropathy (DN) is the loss of glomerular podocytes. The damage to these cells has been attributed in part to elevated intrarenal oxidative stress. The primary source of the renal reactive oxygen species (ROS), and particularly H_2O_2 , is the NADPH oxidase 4 (Nox4). We hypothesized that Nox4-derived H_2O_2 contributes to podocyte damage in DN via elevation of podocyte calcium. Using patch-clamp analysis on freshly isolated mouse glomeruli, we found that H_2O_2 acutely activates TRPC channels in the podocytes. H_2O_2 also produced a robust increase in calcium level in wild type mouse podocytes, same as was observed earlier. Using freshly isolated glomeruli the present study shows that in podocytes H_2O_2 stimulates calcium influx via TRPC channels and this increase in calcium level within the podocytes is dose-dependent, with an EC_{50} of $56.4 \pm 6.2 \mu M$. Importantly, application of lower (below $50 \mu M$) concentrations of H_2O_2 results in a short calcium transient, whereas H_2O_2 above $50 \mu M$ produces a transient followed by a long-term plateau-like increase in calcium, that should likely be attributed to apoptotic rather than signaling events. This response was attenuated in a similar manner in both $Trpc6^{-/-}$ and $Trpc5/6^{-/-}$ mice. Electron microscopy revealed podocytes of glomeruli isolated from $Trpc6$ knockout mice are protected from damage induced by H_2O_2 . Type 1 streptozotocin-induced diabetes was then induced in Dahl Salt-sensitive (SS) rats with a null mutation for the *Nox4* gene ($SS^{Nox4^{-/-}}$) and which exhibit reduced production of H_2O_2 compared to SS rats. $SS^{Nox4^{-/-}}$ rats exhibited a significant reduction of basal podocyte $Trpc6$ dependent $[Ca^{2+}]_i$ levels and an attenuation of DN-associated damage compared to SS rats. Ang II-elicited calcium flux in podocytes was also blunted in $SS^{Nox4^{-/-}}$ rats. We further created the TRPC6 knock-out on a SS rat background ($SS^{TRPC6^{-/-}}$), and demonstrated that the deletion of TRPC6 protected podocytes and glomeruli during the development of type 1 DN. Taken together, these data reveal a novel mechanism involving Nox4 and $Trpc6$ that could be pharmacologically targeted to abate the development of DN.

Session 8: Metabolism
Presented June 1
Short oral

Diabetic conditions enhance the phosphorylation of PACSIN2/syndapin II in podocytes

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Background: Nephtrin localization at the slit diaphragm is critical for podocyte function. We previously found that PACSIN2 (also called syndapin II), which regulates endocytosis and intracellular trafficking, is elevated in glomeruli of Zucker Diabetic Fatty (ZDF) rats showing aberrant localization of nephtrin and severe albuminuria. We also showed in vitro that PACSIN2 overexpression reduces nephtrin insertion at the plasma membrane. Interestingly, PACSIN2 can be phosphorylated at serine 313 (pS313-PACSIN2) by Protein Kinase C alpha (PKC α) which in turn regulates the endocytic activity of PACSIN2. Importantly, PKC α is elevated in diabetes and is associated with loss of nephtrin expression.

Goal: In the present study, we aim to evaluate the phosphorylation status of PACSIN2 in different models of diabetes and diabetic kidney disease (DKD). We also assess the regulation of pS313-PACSIN2 and the presence of nephtrin at the plasma membrane after treating cultured podocytes with palmitate, elevated in patients with diabetes.

Methods: PKC α , total PACSIN2 and pS313-PACSIN2 levels are assessed by Western blot. On-Cell Western, a 96-well plate -based surface labeling assay, is used to determine the insertion of nephrin at the plasma membrane. Glomeruli are isolated from patients with or without T2D and lean or obese ZDF rats by graded sieving. Cultured podocytes are incubated with palmitic acid or with sera from patients with T2D having normoalbuminuria or microalbuminuria.

Results: In human, pS313-PACSIN2, normalized to total PACSIN2, is upregulated in glomeruli isolated from patients with T2D compared to people without diabetes. PKC α , pS313-PACSIN2 and total PACSIN2 are elevated in obese and diabetic ZDF rats compared to lean controls. Increased pS313-PACSIN2 and a trend for an increase of total PACSIN2 are observed when human podocytes are incubated with sera from patients with T2D with microalbuminuria compared to normoalbuminuric patients. Treating podocytes with palmitic acid results in a time-dependent phosphorylation of PACSIN2 on S313. Palmitic acid treatment also resulted in a reduction of nephrin inserted at the plasma membrane.

Conclusions: Our results indicate that PACSIN2 and its phosphorylation at serine 313 are elevated in diabetes and DKD. This could be a consequence of elevated circulating palmitate and result in a decreased presence of nephrin at the plasma membrane, therefore causing the destabilization of the slit diaphragm and albuminuria.

Poster #72
Presented June 1
Poster

Localized mRNA translation mediated by Staufen modulates podocyte actin cytoskeleton and matrix adhesion

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Background: Irreversible damage to podocytes and their detachment from the glomerular basement membrane, result in glomerular scarring and progression to End Stage Renal Disease.

How injury affects the biological pathways that regulate podocyte architecture and matrix adhesion are not fully understood. Here, we provide evidence that podocytes utilize localized mRNA glomerular filtration barrier during injury. eton and cell-matrix adhesion, thereby maintaining the

Methods: A cell biological and gene targeting approach was used to study the role of local mRNA translation in podocytes.

Results: We show that the RNA-binding protein Staufen2, previously demonstrated to regulate mRNA transport and stability in neurons, is expressed in podocytes and localizes predominantly to primary and secondary processes. During injury, Staufen2 and translating ribosomes increase in areas of foot process effacement. Next, we show that Staufen2-bound cytoskeletal assembly and cell-matrix adhesion regulators, one example being the GEF *Dock5*. Consequently, *Stau2* knockdown in immortalized podocytes affects *Actb* mRNA localization and *Dock5* mRNA stability and results in cell detachment as well as impaired re-establishment of actin stress fibers upon recovery from injury. Lastly, we generated *Stau2* single and *Stau1/2* double knockout (DKO) mice; these mice had normal baseline kidney function but DKO mice developed massive proteinuria and extensive foot process effacement in response to Adriamycin, far greater than observed in control, or *Stau1* and *Stau2* single knockouts.

Conclusion: Our results establish mRNA transport and localized translation as newly recognized mechanisms in the kidney to preserve the glomerular filtration barrier during injury.

Session 9: Genetics
Presented June 2
Short Oral

Mutations in new genes as a cause of familial FSGS through a vesicular recycling defect.

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Background: Steroid-resistant nephrotic syndrome (SRNS) is characterized by a high range proteinuria and more often focal and segmental glomerulosclerosis (FSGS) on kidney biopsy. Identification of genes causing FSGS has improved our understanding of disease mechanisms and points to defects in the glomerular epithelial cell, the podocyte, as a major factor in disease pathogenesis.

Methods: Mutations in new causative genes were searched by exome sequencing. Functional analyses were performed both *in vivo* in zebrafish and *in vitro* in cultured cells (immortalized podocytes).

Results: We first identified a dominant missense mutation (c.82C>T) in *Tripartite Motif Containing 3* gene (*TRIM3*) in a four affected family members with late onset FSGS. In neurons, TRIM3 is known to act in vesicular trafficking along actin fibers in a complex that include α -actinin 4. Mutations in *ACTN4* encoding α -actinin 4 have been previously described in autosomal dominant FSGS. We first showed that mutated mRNA injection in zebrafish embryos led to pericardial edema, a surrogate of NS in fish. Electron microscopy revealed foot-process effacement and focally irregular morphology of preserved foot processes in fish expressing the mutant protein. Glomerular protein leakage was confirmed by fluorescence in tubular cells six hours after a high molecular weight FITC-dextran injection at 96hpf, revealing increased glomerular permeability. Despite a normal interaction between TRIM3 and α -

actinin 4 in cultured podocytes, the vesicular trafficking along actin fibers was altered as shown by a delay in the transferrin recycling. Another mutation p.R433C gene has recently been found in a sporadic late-onset FSGS. As preliminary results, zebrafish exhibited the same pericardial edema when injected with the mutated mRNA.

More recently, we identified mutations in a new gene called “X” in two families with early onset FSGS. Knockout of the unique “X” ortholog in zebrafish revealed a loss of glomerular filtration barrier integrity, podocyte foot process effacement, and an edematous phenotype linked to glomerular protein leakage as revealed by the dextran injection. This phenotype was almost completely rescued by human “X” mRNA injection but not by both mutated mRNA.

Results: Altogether, these data confirm the involvement of two new genes in FSGS and suggest that new mechanisms including defects in podocyte trafficking may lead to proteinuria and FSGS in patients.

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Title: Novel *MYO1E* Homozygous Stopgain Mutation and Rare Heterozygous Variants in Steroid Resistant Nephrotic Syndrome Associated Genes Segregate in a Family Modulating Different Phenotypes

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Background: Nephrotic syndrome (NS) is defined by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Approximately 20% of the patients do not respond to treatment with steroids and are classified as steroid-resistant (SRNS). At least 53 genes are currently known as the monogenic cause of SRNS. The aim of this study was to search for pathogenic variants in a SRNS familial case using whole exome sequencing (WES), as this method has become a tool of choice for genetic screening in diseases with high genetic heterogeneity and phenotypic variability such as SRNS.

Patients and Methods: We performed whole exome sequencing in one familial patient who was diagnosed with SRNS at 4 years of age. She presented FSGS on renal biopsy and 7 years later progressed to end-stage renal disease. She

received kidney transplant when she was 21 years old. *NPHS1*, *NPHS2* and *WT1* pathogenic variants had been excluded by Sanger sequencing before WES. We used strict genetic criteria for reduction of variants. Sanger sequencing was performed in order to confirm the variants identified.

Results: We found a novel homozygous stopgain variant c.505C>T (p.Arg169*) in the *MYOIE* gene. Only a few pathogenic *MYOIE* variants were reported so far associated with SRNS, mostly in familial cases. We also identified two heterozygous missense variants in *cis* in the *COL4A4* gene: c.2276C>T (p.Pro759Leu) and c.2008G>A (p.Val670Ile). We screened those variants in both parents and 12 siblings and identified the same genotype in two sisters with nephrotic proteinuria. Along with those variants, four other rare heterozygous variants are segregating in this family: two heterozygous missenses in *KANK1* gene c.511A>G (p.Tre171Ala) and c.3772G>T (p.Ala1258Ser), a missense variant c.3071C>T (p.Pro1024Leu) in *LAMB2* gene and a splicing variant c.2476+9G>A in *ANLN* gene.

Conclusions: The genetic and phenotypic heterogeneity identified in the family presented here illustrate how monogenic diseases such as NS may be associated with putative epistatic variants acting in the background of the disease. The patient and her family will benefit of the unequivocal etiology of the disease and of genetic counselling.