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Abstracts
11th International Podocyte Conference:

Podocyte Biology in Glomerular Health and Disease

A Global Scientific Perspective

April 3–6, 2016, Haifa and Jerusalem, Israel

Editors
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The prescient understanding by a kernel of research, academic and clinical leaders correctly predicted the centrality of understanding the intricacies of the podocyte in unlocking many secrets of glomerular function in health and disease. The extent to which investigating the podocyte continues to inform broader aspects of biology and medicine may even have been underestimated at the time of the 1999 first International Podocyte Conference in Freiburg.

Late comers to the field of Podocyte Biology, such as the local Israeli organizers of the 11th International Podocyte Conference, are privileged to be welcomed into this community of exciting scientific and medical discovery.

With the advice and help of seasoned and veterans in the field, we have tried our best to put together an attractive program that will highlight the frontiers of new knowledge, much of it as yet unpublished, and combine this with clinical relevance and guidance for future research directions. Accordingly, the program encompasses a large spectrum of topics relevant to podocyte functional and structural integrity in health and disease. Notably, unlike tubule function, where physiology often predicted the identification of genes and proteins – in the case of the podocyte, much of our detailed understanding was informed and enabled by recent conceptual and technological breakthroughs ranging from exome-based podocyte disease gene discovery to advanced imaging capabilities. Since so much remains to be discovered, we also look forward to lively discussions that will attempt to delineate research challenges, formulate testable hypotheses, and suggest models that can guide future research and therapeutic discovery.

We are cognizant of the time and travel constraints under which fully engaged scientists and clinicians operate, and which limit availability to participate in highly specialized conferences. Yet, despite the availability of webinars and other electronic means of interaction, we still feel that like the healthy glomerulus, so too healthy podocyte research requires the direct interaction of individual specialized components, enabled by such a meeting. Therefore, we are especially grateful to all of the participants from numerous countries worldwide, and are doing our best to ensure a gratifying and secure experience at the scientific, medical, personal and social levels. To this end, we express our appreciation for the support of many organizations and sponsors listed in the program book, together with the invaluable contribution of the International Scientific Organizing Committee and the significant input provided by Tobias Huber and Thomas Benzing based on their most recent success in Freiburg 2014. Nephcure Kidney International, whose patient health promotion agenda converges well with podocyte research, has been a strategic partner for many of the recent conferences. We are grateful for this continued alliance, as well as for the pleasure of a seamless interaction with our local conference organizers and host institutions.

We will be conveying some logistical and technical points at the opening and throughout the course of the conference, in order to optimize the experience. Finally, a major goal of the conference is to engage the best and the brightest young minds to the exciting world of the podocyte, and its cardinal role as a key to a deeper understanding of human biology and human health.

With many thanks,

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Disclosure Statement

The Editors have no conflicts of interest to declare.

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Scientific Program
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SUNDAY, APRIL 3, 2016

17:00	Registration Opens	*Dan Carmel Hotel, Haifa*
17:30	Welcome Reception	*Dan Carmel Hotel, Haifa*
19:00	**OPENING REMARKS**	*Dan Carmel Hotel, Haifa*
	Karl Skorecki & Suheir Assady, Conference Co-Chairs, Israel **Yona Yahav**, Mayor of Haifa, Israel **Eliezer Shalev**, Dean, Faculty of Medicine, Technion, Haifa, Israel **Rafael Beyar**, Director of Rambam Health Care Campus, Haifa, Israel	
19:15–20:00	**PLENARY LECTURE 1**	*Dan Carmel Hotel, Haifa*
	SCIENTIFIC DISCOVERY AGAINST ALL ODDS **Dan Shechtman**, Israel	

MONDAY, APRIL 4, 2016

07:30–08:15	**'MEET THE PROFESSOR'** *Students and young scientists to meet established investigators*	*Rambam Health Care Campus, Haifa*
	There will be 4 individual groups: **Hans-Joachim Anders**, Germany **Paola Romagnani**, Italy **Mario Schiffer**, Germany **Katalin Susztak**, USA	
08:30–09:00	**PLENARY LECTURE 2** **Chair: Suheir Assady**, Israel	*Rambam Health Care Campus, Haifa*
08:30	MANY WAYS TO DIE; MECHANISMS OF CELL DEATH **Adi Kimchi**, Israel	
09:00–10:10	**SESSION 1** **PODOCYTE ORIGINS AND GLOMERULAR DEVELOPMENT AND DIFFERENTIATION** **Chairs: William Smoyer**, USA; **Paola Romagnani**, Italy	*Rambam Health Care Campus, Haifa*
09:00	INTRINSIC AGE-DEPENDENT CHANGES AND CELL-CELL CONTACTS REGULATE NEPHRON PROGENITOR LIFESPAN **Raphael Kopan**, USA	
09:20	THE ROLE OF WNT SIGNALING IN THE SPECIFICATION OF GLOMERULAR PODOCYTES **Tom Schultheiss**, Israel	
09:40	SORTING OUT HUMAN KIDNEY DEVELOPMENT **Benjamin Dekel**, Israel	
10:00	***Abstract for Oral Communication*** DNA METHYLATION REGULATES NEPHRON PROGENITOR CELL FATE IN EARLY KIDNEY DEVELOPMENT **Nicola Wanner**, Germany	
10:10	*Discussion, Coffee and Networking*	

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11:00–12:30	**SESSION 2** **GLOMERULAR STRUCTURAL AND FUNCTIONAL INTEGRITY** **Chairs: Jeffrey Miner**, USA; **Wilhelm Kriz**, Germany	*Rambam Health Care Campus, Haifa*
11:00	MOLECULAR PRINCIPLES OF GLOMERULAR PROTEINURIA **Tobias Huber**, Germany	
11:20	ROLE OF ECM FOR GLOMERULAR FUNCTION AND MAINTENANCE **Rachel Lennon**, UK	
11:40	MITOCHONDRIAL FUNCTION IN PODOCYTES **John He**, USA	
12:00	PROTECTIVE PODOCYTE GROWTH FACTORS THAT MAINTAIN GLOMERULAR ENDOTHELIAL INTEGRITY **Rebecca Foster**, UK	
12:20	***Abstract for Oral Communication*** AT2R DEFICIENCY IMPAIRS PODOCYTE FUNCTION VIA THE ALTERATION OF HEDGEHOG INTERACTING PROTEIN (HHIP) GENE EXPRESSION **Shao-Ling Zhang**, Canada	
12:30	*Discussion, Lunch and Poster Session I*	
14:00–15:30	**SESSION 3** **PODOCYTE CELL SIGNALING AND METABOLISM IN HEALTH AND DISEASE** **Chairs: Agnes Fogo**, USA; **Ken Inoki**, USA	*Rambam Health Care Campus, Haifa*
14:00	mTOR AND GLOMERULAR FUNCTION **Ken Inoki**, USA	
14:20	NEW INSIGHTS INTO THE PODOCYTE ADHESOME **Christoph Schell**, Germany	
14:40	EMERGING ROLES FOR TRPC6 IN PROTEINURIC KIDNEY DISEASES **Robert Spurney**, USA ***In Memoriam of Michelle Winn***	
15:00	MITOCHONDRIAL METABOLISM AND THE ROLE OF PROHIBITINS IN PODOCYTES **Paul Brinkkoetter**, Germany	
15:20	***Abstract for Oral Communication*** ZEB2-NATURAL ANTISENSE TRANSCRIPTS MEDIATE EPITHELIAL MESENCHYMAL TRANSITION OF PODOCYTES: IMPLICATIONS IN THE PATHOGENESIS OF NEPHROPATHY **Anil Kumar Pasupulati**, India	
15:30	*Discussion, Networking and Poster Session II*	
16:30–17:20	**SESSION 4** **IMAGING AND INTRACELLULAR TRAFFICKING** **Chairs: Thomas Benzing**, Germany; **Peter Mathieson**, Hong Kong	*Rambam Health Care Campus, Haifa*
16:30	THE CELL BIOLOGY OF FOOT PROCESS EFFACEMENT **Andrey Shaw**, USA	
16:50	MULTIPLE ROLES OF ACTIN IN ENDOCYTOSIS **Sanja Sever**, USA	
17:10	***Abstract for Oral Communication*** 4-D IN VIVO IMAGING OF PODOCYTES IN A ZEBRAFISH INJURY MODEL **Florian Siegerist**, Germany	
17:20	*Tour of Rambam Health Care Campus*	
Evening	*Festive Dinner and Awards (Knights Hall-Acre)*	

TUESDAY, APRIL 5, 2016

07:30–08:15	**'MEET THE PROFESSOR'** *Students and young scientists to meet established investigators*	*Rambam Health Care Campus, Haifa*
	There will be 5 individual groups: **Friedhelm Hildebrandt**, USA **Tobias Huber**, Germany **Ken Inoki**, USA **Marcus Moeller**, Germany **Martin Pollak**, USA	
08:30–09:00	**PLENARY LECTURE 3** **Chair: Talia Weinstein**, Israel	*Rambam Health Care Campus, Haifa*
08:30	MEMBRANOUS NEPHROPATHY-PLA2R AND BEYOND? **David Salant**, USA	
09:00–10:30	**SESSION 5** **ORGANISMAL AND STEM CELL EXPERIMENTAL PLATFORMS FOR GLOMERULAR AND PODOCYTE FUNCTION IN HEALTH AND DISEASE** **Chairs: Stuart Shankland**, USA; **Susan Quaggin**, USA	*Rambam Health Care Campus, Haifa*
09:00	MODELING PODOCYTE DISEASE WITH HUMAN KIDNEY ORGANOIDS **Benjamin Freedman**, USA	
09:20	A HUMANIZED MOUSE MODEL OF FSGS **Eunsil Hahm**, USA	
09:40	STUDYING PODOCYTE MORPHOLOGY AND FUNCTION IN LIVING ZEBRAFISH **Karlhans Endlich**, Germany	
10:00	DROSOPHILA NEPHROCYTE AS A MODEL FOR PODOCYTE CYTOSKELETON AND MEMBRANE TRAFFICKING **Zhe Han**, USA	
10:20	***Abstract for Oral Communication*** ASSESSING THE EFFECT OF SGPL1 MUTATIONS USING THE DROSOPHILA MODEL **Sara Gonçalves**, France	
10:30	*Discussion, Coffee Break and Networking*	
11:10–12:30	**SESSION 6** **MECHANISTIC INSIGHTS FROM THE GENETICS OF GLOMERULAR DISEASE** **Chairs: Martin Pollak**, USA; **Friedhelm Hildebrandt**, USA	*Rambam Health Care Campus, Haifa*
11:10	MUTATIONS IN 6 NOVEL GENES DEFINE A PATHOGENIC PATHWAY IN PARTIALLY STEROID-SENSITIVE NEPHROTIC SYNDROME **Friedhelm Hildebrandt**, USA	
11:30	APOL1 RENAL RISK VARIANTS: FIRE IN THE PODOCYTE **Jeffrey B. Kopp**, USA	
11:50	ROLE OF COL4A1 IN EMBRYONIC PODOCYTE DIFFERENTIATION AND IN ADULT PECS MAINTENANCE **Emmanuelle Plaisier**, France	
12:10	INTRAVITAL IMAGING OF THE ORIGIN, MIGRATION AND FATE OF GLOMERULAR STEM CELLS **János Peti-Peterdi**, USA	
12:30	*Discussion, Lunch and Poster Session III*	

14:00–15:20	**SESSION 7** **ACQUIRED PODOCYTE AND GLOMERULAR INJURY STATES AND REPAIR** **Chairs: Hans-Joachim Anders**, Germany; **Richard Coward**, UK	*Rambam Health Care Campus, Haifa*
14:00	OBESITY-RELATED GLOMERULOPATHY **Michal Herman-Edelstein**, Israel	
14:20	PODOCYTES AND APOL1 ASSOCIATED KIDNEY DISEASE **Katalin Susztak**, USA	
14:40	AUTOCRINE AND PARACRINE FUNCTIONS OF SDF-1/CXCL12 IN PODOCYTE HOMEOSTASIS AND INJURY **Hans-Joachim Anders**, Germany	
15:00	***Abstract for Oral Communication*** TRANSGLUTAMINASE 2 INTERACTS WITH IGG AND AFFECTS IMMUNE-COMPLEX DEPOSITION AND PODOCYTE LOSS IN MEMBRANOUS NEPHROPATHY **Christina Papista**, France	
15:10	***Abstract for Oral Communication*** PODOPLANIN AND SYNAPTOPODIN BEING UPREGULATED IN ALLERGIC INFLAMED ESOPHAGEAL EPITHELIUM LINKS EPITHELIAL RESPONSES IN ATOPY TO THOSE IN KIDNEY DISEASES **Mark Rochman**, USA	
15:20	*Discussion, Networking and Poster Session IV*	
16:30–17:45	**SESSION 8** **PANEL: THE CHANGING LANDSCAPE OF SCIENTIFIC PUBLICATION**	*Rambam Health Care Campus, Haifa*
16:30	PLENARY STATEMENT BY JOURNAL EDITOR **Richard Horton**, UK	
17:00	PLENARY DISCUSSION **Moderator: Randy Levinson**, USA	
	Participants: **Thomas Benzing**, Germany, **Agnes Fogo**, USA, **Lawrence Holzman**, USA, **Richard Horton**, UK, **Tobias Huber**, Germany, **Paul Kimmel**, USA, **Susan Quaggin**, USA	

WEDNESDAY, APRIL 6, 2016

Podocyte Conference, Katzir Satellite, Jerusalem

09:30	*Coffee Break and Networking*	
10:00–10:30	**PLENARY LECTURE 4** **Chair: Katalin Susztak**, USA	*Mishkenot Sha'ananim, Jerusalem*
10:00	GENOME EDITING, PROSPECTS FOR THERAPEUTIC INTERVENTION **Sara Selig**, Israel	
10:30–12:00	**SESSION 9** **microRNAs AND PODOCYTES** **Chairs: Mario Schiffer**, Germany; **Matthew Sampson**, **USA**	*Mishkenot Sha'ananim, Jerusalem*
10:30	PODOCYTE AND GBM-CROSSTALK VIA miRNAs **Mario Schiffer**, Germany	
10:50	ROLE OF PODOCYTE microRNAs IN DIABETIC NEPHROPATHY **Vineet Gupta**, USA	
11:10	miRNA-92a-INDUCED LOSS OF PODOCYTE QUIESCENCE CAUSES CRESCENTIC GLOMERULONEPHRITIS **Pierre-Louis Tharaux**, France	
11:30	miRNA-30 FAMILY MEMBERS REGULATE CALCIUM/CALCINEURIN SIGNALING IN PODOCYTES **Zhi-hong Liu**, China	

11:50	***Abstract for Oral Communication*** EVALUATION OF miRNA EXPRESSION AND TARGETING IN CULTURED HUMAN AND MOUSE PODOCYTES **Iddo Z. Ben-Dov**, Israel
12:00	*Networking and Lunch Break*
13:30–14:15	**SESSION 10** *Mishkenot Sha'ananim, Jerusalem* **OXFORD STYLE DEBATE – RESOLVE THAT DEPLETED ADULT PODOCYTES CAN BE REPLENISHED/REPLACED?**
13:30	**Moderator: Karl Skorecki**, Israel **Pro: Stuart Shankland**, USA; **Con: Marcus Moeller**, Germany
14:15–15:30	**SESSION 11** *Mishkenot Sha'ananim, Jerusalem* **ADVANCES IN GLOMERULAR AND PODOCYTE DIAGNOSTICS & THERAPEUTICS** **Chairs: Jochen Reiser**, USA; **Yaacov Frishberg**, Israel
14:15	TARGETING PODOCYTE LIPIDS IN GLOMERULAR DISEASES **Alessia Fornoni**, USA
14:35	MOLECULAR PHENOTYPING FOR PRECISION MEDICINE IN GLOMERULAR DISEASE **Matthias Kretzler**, USA
14:55	***Abstract for Oral Communication*** GLUCOCORTICOID RECEPTOR ANTAGONISM IS EFFECTIVE IN EXPERIMENTAL MODELS OF MINIMAL CHANGE DISEASE **Christoph Kuppe**, Germany
15:05	***Abstract for Oral Communication*** ARCTIGENIN (ATG) ATTENUATES PODOCYTE INJURY IN DIABETIC KIDNEY THROUGH INHIBITION OF PP2A/NFKB/NOX4 PATHWAY **Yifei Zhong**, China
15:15	CLOSING REMARKS

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1

Potential of Urinary Nephrin as a Biomarker Reflecting Podocyte Dysfunction in Various Kidney Disease Models

Masaki Abe[1], Yusuke Wada[1], Hiroshi Moritani[1], Hikaru Mitori[2], Mitsuhiro Kondo[1], Keiko Tanaka-Amino[1], Megumi Eguchi[1], Akira Imasato[1], Yutaka Inoki[1], Hiroshi Kajiyama[3], Toshihide Mimura[3], Yuichi Tomura[1]

[1]Drug Discovery Research, Astellas Pharma Inc., Tsukuba, [2]Drug Discovery Research, Astellas Pharma Inc., Osaka, [3]Department of Rheumatology and Applied Immunology, Faculty of Medicine, Saitama Medical University Faculty of Medicine, Moroyama, Japan

Urinary nephrin is a potential non-invasive biomarker of disease. To date, however, most studies of urinary nephrin have been conducted in animal models of diabetic nephropathy, and correlations between urinary nephrin-to-creatinine ratio (uNCR) and other parameters have yet to be evaluated in animal models or patients of kidney disease with podocyte dysfunction. We hypothesized that uNCR can be up-regulated and is negatively correlated with renal nephrin mRNA (rNRNA) levels in animal models of kidney disease, and that increased uNCR levels are attenuated following administration of glucocorticoids. In the present study, rNRNA, uNCR, urinary protein-to-creatinine ratio (uPCR) and creatinine clearance ratio (Ccr) were measured in animal models of adriamycin nephropathy, puromycin aminonucleoside (PAN) nephropathy, anti-glomerular basement membrane glomerulonephritis, and 5/6 nephrectomy. The effects of prednisolone on uNCR and other parameters in PAN (single injection) nephropathy rats were also investigated. In all models tested, uNCR and uPCR increased, while rNRNA and Ccr decreased. uNCR exhibited a significant negative correlation with rNRNA in almost all models, as well as a significant positive correlation with uPCR and a significant negative correlation with Ccr. uPCR exhibited a significant negative correlation with rNRNA. Following the administration of prednisolone to PAN (single injection) nephropathy rats, uNCR significantly was suppressed and exhibited a significant positive correlation with uPCR. In addition, the decrease in number of glomerular Wilms tumor antigen-1-positive cells was attenuated, and uNCR exhibited a significant negative correlation in these cells. In conclusion, these results suggest that uNCR level is a useful and reliable biomarker for predicting the amelioration of podocyte dysfunction by candidate drugs in various kidney disease models with podocyte dysfunction. It will be also validated in a clinical setting in future studies.

2

SULF2 Is Associated with Podocyte Pathophysiology and Steroid Resistance in Nephrotic Syndrome

Shipra Agrawal[1], Richard Ransom[1], Saras Saraswathi[2], Amy Webb[3], Esperanza Garcia-Gonzalo[4], Juan Fernandez-Martinez[4], Melinda Chanley[1], Milan Popovic[1], Audrey Papp[5], Adam Guess[1], Andrzej Kloczkowski[2], Wolfgang Sadee[5], The Midwest Pediatric Nephrology Consortium (MWPNC)[6], William Smoyer[1]

[1]Center for Clinical and Translational Research, The Research Institute at Nationwide Children's Hospital, Columbus, [2]Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, [3]Biomedical Informatics, The Ohio State University, Columbus, United States; [4]Mathematics, University of Oviedo, Oviedo, Spain; [5]Pharmacology, The Ohio State University, Columbus, [6]The Midwest Pediatric Nephrology Consortium, (Mwpnc), Columbus, United States

Introduction and Aim: SULF2 has been reported to have a genetic association with nephrotic syndrome (NS), although its biologic role in NS remains unknown. SULF2 is an enzyme that removes 6-O-sulfates and modulates the sulfation pattern on heparan sulfate proteoglycans (HSPGs), which are present on cell surfaces and in extracellular matrix of the glomerular basement membrane and regulate the availability and activity of molecules such as VEGF, PDGF and FGF. In particular, VEGF-A has a well-established role in glomerular physiology and pathology, with its altered expression leading to glomerular injury. We thus hypothesized that SULF2 is associated with podocyte health and disease and steroid resistance in NS, likely via regulation of VEGF-A.

Methods/Results: Deep RNA-seq and in-silico analyses of leukocyte samples from children with NS obtained before and after ~8 weeks of steroid therapy identified SULF2 as part of a panel of 12 candidate genes able to distinguish between steroid sensitive NS and steroid resistant NS (SSNS vs. SRNS). Validation and biochemical analyses revealed that both SULF2 leukocyte gene expression and plasma sulfatase activity ratios (after/ before therapy) were greater in SSNS vs. SRNS. Plasma VEGF-A levels were also increased after steroid therapy but did not differ between children with SSNS and SRNS. Moreover, puromycin aminonucleoside (PAN)-induced proteinuria in rats resulted in decreased glomerular SULF2 gene expression. Furthermore, in differentiated human podocytes, PAN injury also reduced SULF2 gene expression, while glucocorticoid treatment induced expression. However, these changes did not result in measurable changes in VEGF-A secretion (normalized to cellular protein).

Conclusions: We conclude that relative reductions in plasma SULF2 expression correlated with clinical steroid resistance dur-

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ing childhood NS. Moreover, reductions in glomerular and podocyte SULF2 expression also corresponded with injury, while glucocorticoid treatment restored SULF2 in podocytes. Our data suggest that SULF2 plays an important role in glucocorticoid response in NS.

3

Reversal of Podocyte Loss by Imatinib Treatment in Mice with Membranoproliferative Glomerulonephritis (MPGN)

Noppanit Pattanachaiwit[1], *Masayuki Iyoda*[1], *Tomasz Wietecha*[1], *Kelly Hudkins*[1], *Charles E. Alpers*[2]

[1]Department of Pathology, University of Washington, Seattle, [2]Pathology, University of Washington, Seattle, United States

Background: Transgenic thymic stromal lymphopoietin mice (TSLP-Tg) develop cryoglobulinemia and MPGN. Platelet-derived growth factor (PDGF)-D, expressed by podocytes in humans, with its receptor (PDGFR-β) are likely mediators of mesangial expansion in MPGN. Inhibition of PDGFR-β by imatinib, a tyrosine kinase inhibitor, reduces mesangial proliferation in TSLP-Tg mice. The importance of podocyte loss in MPGN is currently unknown. This study sought to identify possible podocyte loss and investigate the effect of imatinib on podocyte density in mice with MPGN.

Methods: Three-week old TSLP-Tg mice treated with imatinib (50 mg/kg) intraperitoneally daily for eight weeks; controls included TSLP-Tg mice treated with sterile water, and wild type (WT) mice. Podocytes were identified by p57 immunostaining, and their density morphometrically quantitated. Expression of PDGF-D and PDGFR-β was detected by immunohistochemistry.

Results: Podocyte density in TSLP-Tg mice was significantly less than WT controls (182.67 ± 21.15 vs 278 ± 6.19 podocytes/106 μm3, p = 0.005), and was markedly increased by imatinib (249.17 ± 14.49, p = 0.029). TSLP-Tg mice had significantly increased mesangial (but not podocyte) expression of PDGF-D (248.23 ± 40.75 μm^2) and slightly increased PDGFR-β expression (192.91 ± 17.31) compared to WT controls (124.17 ± 10.46, p = 0.028 and 175.55 ± 6.61, p = 0.388 respectively). The overexpression of PDGF-D was reduced (129.02 ± 10.95, p = 0.025) whereas the expression of PDGFR-β was increased after imatinib treatment (260.51 ± 18.62, p = 0.024). Albuminuria in TSLP-Tg mice was higher than WT controls (16.6 ± 4.66 vs 4.23 ± 0.76 μg/24 h, p = 0.036), and was decreased by imatinib (4.97 ± 1.64, p = 0.049).

Conclusions: Podocyte loss may be an unrecognized feature of MPGN. Restoring podocyte number may be a key to reversal of MPGN. Imatinib largely reversed podocyte loss and reduced mesangial injury, PDGF-D overexpression and albuminuria in TSLP-Tg mice.

4

Pharmacologic Interventions for Diabetic Nephropathy (DN) That Are Efficacious for the Restoration of Podocyte Density

Charles E. Alpers[1], *Minseob Eom*[2], *Noppanit Pattanachaiwit*[2], *Anna Batorsky*[2], *Tomasz Wietecha*[2], *Floor Steegh*[2], *Julia Shankland*[2], *Kelly Hudkins*[2]

[1]Pathology, University of Washington, Seattle, [2]Department of Pathology, University of Washington, Seattle, United States

Background: Leptin deficient BTBRob/ob mice develop type 2 diabetes and DN including podocyte (podo) loss. We tested whether atrasentan (A), an endothelin-1 receptor antagonist, with or without concurrent RAAS inhibition by losartan (L), or infusion of the novel mitochondrial targeting peptide SS-31 (S) could restore podocyte number in DN.

Methods: 18 week old BTBRob/ob mice and BTBR WT littermates were treated with A (5 mg/kg/day), A plus L (25 mg/kg/day), or normal drinking water for 6 weeks. Separately, 18 week male BTBRob/ob and WT mice were infused with SS-31 via osmotic pump for 6 weeks. Controls included ob/ob infused with saline, untreated ob/ob, WT, and WT with SS-31 infusion.

Results: There were decreased podocytes (p57 expressing cells) in BTBRob/ob (149.7 + 6.7) compared to WT mice (188.7 + 9, $p < 0.05$). Podocytes increased in BTBRob/ob mice receiving A (167.0 + 7, ns, p = 0.093) and were restored to WT numbers in mice receiving A plus L (190.4 + 5.7, $p < 0.001$). Expression of CD44, a marker of activated parietal epithelial cells (PECs), increased in BTBRob/ob mice compared to WT (27.5% of glomeruli with PEC staining vs. 1.5%, $p < 0.05$). This was further increased in both A (37.2%), and A plus L treated BTBR ob/ob mice (45.7%, $p < 0.01$). Podocyte density, diminished in BTBR ob/ob mice, was significantly restored with SS-31 infusion (147.2 ± 6.33 podocytes/106 μm^3) compared with saline treated mice (107.6 ± 4.78) or untreated mice (100.5 ± 6.14) (p = 0.0026 & 0.0002). SS-31 increased CD44 expression in PECs similar to atrasentan.

Conclusions: Treatment with A, A plus L, and S significantly increased podocyte density in diabetic BTBRob/ob mice. Podocyte restoration correlated with PEC activation, with increased CD44 expression in A and A plus L mice ($r^2 = 0.98$). Benefits of combined A and L treatment in patients with DN may occur through a previously unrecognized restoration of podocytes, perhaps derived from niches of activated PECs.

5

Precision Medicine: Using *Nephroseq* and *TranSMART* Data-Exploration Tools to Define Glomerular Disease Mechanisms

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Precision Medicine, identifying the right treatment for the right patient at the right time, must be applied in glomerular diseases to advance the field beyond the current 'one size fits all' approach. Comprehensive genetic data from patient cohorts and animal model systems are presently generated for many disorders, including those of the glomerulus. The challenge of precision medicine, however, is designing an 'informational commons'; a virtual space for researchers to share and explore large-scale datasets.

One of the goals pursued by the Applied Systems Biology Core is to establish an effective way for scientists to interrogate molecular data without requiring specific expertise in bioinformatics or statistics. To this end, *Nephromine* was developed as a web-based, systems-biology search engine, focused on renal gene-expression datasets. The next generation, *Nephroseq*, was released in early 2016. *Nephroseq* has an intuitive interface, accessing all publicly-available, human, renal gene-expression datasets and a growing number of model-system expression datasets. It allows exploration of differentially-regulated transcripts using predefined cohorts and datasets with an extensive suite of systems-biology tools.

The data exploration platform *TranSMART* goes beyond preset analyses and allows user-specified exploration of cohort-study datasets along the entire genotype-to-phenotype continuum. Researchers define cohort strata and, using a simple drag-and-drop function, explore interactions in data from cohort study participants; these can range from prospective clinical phenotypes, histological descriptors, genotypic information, gene and protein expression profiles to environmental exposures. *TranSMART* instances, using shared data ontologies, are currently deployed for multiple cohorts, including NEPTUNE, CureGN, ERCB and EURenOmics. Within these networks, *TranSMART* serves as an outreach tool for ancillary study investigators, enabling dynamic access to complex datasets from cohort studies.

Future goals for *Nephroseq* and *TranSMART* are to further facilitate integration of glomerular disease datasets and to empower geographically-distributed research networks to jointly implement precision medicine in glomerular diseases.

6

Recessive Mutations in 5 Novel Genes of Interaction Partners Elucidate Steroid Sensitivity in Nephrotic Syndrome

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Idiopathic nephrotic syndrome is a common pediatric kidney disease. 80% of all cases are steroid sensitive (SSNS). First insights into the pathogenesis of steroid-resistant nephrotic syndrome (SRNS) came from identification of ~30 single-gene causes. However, mechanisms of treatment sensitivity vs. resistance remain unknown. We performed homozygosity mapping (HM) and whole exome sequencing (WES) to identify novel disease-causing genes in a worldwide cohort of ~2,000 individuals with severe NS.

By WES and high-throughput sequence analysis, in families who mostly had steroid-dependent NS (SDNS), we identified multiple recessive mutations in the following genes: *MAGI2*, *TENC1*, *DLC1*, *CDK20*, and *ITSN1* in 2, 5, 4, 1, and 3 unrelated families, respectively. Knockout mice of *Magi2* or *Tenc1* have been previously shown to develop NS. By Co-IP, we now show that MAGI2 interacts with TENC1 and DLC1 and these interactions are abrogated by the two MAGI2 mutants. Knockdown of *MAGI2*, *DLC1* or *ITSN1* in cultured podocytes exhibited a decreased podocyte migration rate. Immunofluorescence studies showed that TENC1 and DLC1 colocalize with phosphotyrosine at focal adhesions in human podocytes. We discover CDK20 as a novel renal regulator of DLC1. In addition, we discover ITSN as a novel GEF for Rho/Rac/Cdc42, relevant for podocyte function.

Thus, by identification of 5 novel monogenic causes of NS we define a functional network of proteins at the intersection between steroid sensitivity vs. steroid resistance of NS. These findings for the first time may elucidate cell autonomous podocytic mechanisms of treatment response in NS and will make specific genetic variants of NS amenable to treatment.

7

Evaluation of MicroRNA Expression and Targeting in Cultured Human and Mouse Podocytes

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Background: In 2008, researchers described the effects of selective deletion of Dicer in podocytes upon mouse kidney development and function. They found podocyte disruption, leading to collapsing glomerulopathy clinically characterized by proteinuria, kidney failure and premature death. Subsequently another group described similar phenotype of inducible podocyte deletion of Drosha at a postnatal stage. While these studies collectively testify for a crucial role for miRNA in podocyte development and homeostasis, they do not identify the causative miRNA and miRNA targets responsible for the phenotype. Hence, the objective of our study is to identify specific miRNA and miRNA targets affecting podocyte differentiation and homeostasis.

Methods and Results: Using small RNA deep-sequencing and photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) of AGO2 we have studied miRNA expression and targeting in conditionally immortalized human and mouse podocytes. These experiments have allowed us to generate ranked lists of miRNA abundance in podocytes, miRNA regulated during podocyte differentiation, miRNA crosslinked to AGO2 in PAR-CLIP experiments, and mRNA targets of miRNA in PAR-CLIP experiments. Applying Sylamer (seed-enrichment analysis) on AGO2 PAR-CLIP-derived mRNA clusters we found the most enriched 6–8-mer sequences complimentary to miRNA sequence families that are expressed in podocytes, with extensive overlap between findings in human and mouse. Surprisingly, the most strikingly enriched sequences are complementary to the seed common to a miRNA sequence family which is not among the most abundant miRNA in podocytes, and thus cannot have been predicted based on miRNA profiles alone.

Conclusions: We have generated a prioritized list of miRNA and miRNA:mRNA interaction candidates likely crucial to podocyte integrity and hypothesize that manipulating them would produce striking effects upon gene expression and cell phenotype. If the functional relevance of our ranked candidates is confirmed by ongoing experiments, they will steer further studies into the role of miRNA in podocyte biology and disease.

8

Successful Treatment of Idiopathic Nephrotic Syndrome and Recurrent FSGS (rFSGS) with Leflunomide

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Introduction: Idiopathic nephrotic syndrome (INS), mostly due to minimal change disease (MCD) and focal segmental glomerulosclerosis (rFSGS), are important pediatric renal disorders causally linked to podocyte injury. They are clinically characterized by a chronic-relapsing (MCD) or progressive course (majority of FSGS), with risk of recurrence after kidney transplantation (KT). B-cell aided T-cell activation and TNFα have been postulated to target podocytes and have been implicated, among other factors, in the pathogenesis of INS and rFSGS.

We *hypothesized* that leflunomide (LEF), which promotes anti-inflammatory Th2-cells and suppresses T- and B-cell expansion and production of pro-inflammatory cytokines, including TNFα and IFNγ, may afford podocyte protection and disease suppression.

Objectives: To report our initial experience with LEF in pediatric INS and rFSGS.

Method: Observational study/retrospective review. Results are described as medians (and ranges).

Results: Twelve children age 11.5 (3.2–18.4) years with glucocorticoid-dependent (responsive) NS (n = 8 GCD-NS, 5 MCD per biopsy; n = 2 FSGS; n = 1 treatment-recalcitrant collapsing glomerulopathy, and n = 1 rFSGS post-KT) received LEF over 24 (3–58) months. LEF starting dose 20 mg per day was titrated to steady-state trough levels of 40–80 mg/L. All patients with INS/MCD and two with GC-responsive FSGS remained in remission with LEF monotherapy over a total of 24 (6–52) months. Current (or last) LEF dose in this group was 30 (10–50) mg/day (22.0 [10.9–50.3] mg/m^2), trough levels 51.0 (10.9–50.3) mg/L. Four patients with persistent proteinuria stopped LEF due to lack of efficacy (one with GCD-MCD, one with collapsing glomerulopathy) or treatment-emergent adverse effects (palmar eczema, chronic anemia). The rFSGS patient achieved stable, partial remission (urine protein/creatinine 1–2 g/g, GFR >110 mL/min/1.73 m^2) with LEF added to KT immunosuppression.

Conclusions: LEF represents an effective, mechanistically interesting therapeutic agent in GCD-NS and potentially, rFSGS. Future studies are directed at the mechanism of LEF-mediated podocyte protection and, indirectly, understanding podocyte injury in INS and rFSGS.

9

LATS-Dependent Activation of Hippo Signaling Leads to Cytoplasmic YAP Localization and Induces Apoptosis in Podocytes

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The Hippo signaling pathway is known to regulate the balance between proliferation, differentiation and apoptosis of cells. YAP (Yes-associated protein) is the best characterized effector protein of this pathway and functions as a co-transcription factor. A phosphorylation of YAP at Serin 127 by LATS (large tumor suppressor kinase), the key regulator of the Hippo pathway, inactivates YAP and mediates its retention in the cytoplasm. In contrast to other cells, podocytes, which are crucial components of the glomerular filtration barrier, display a predominant nuclear YAP localization resulting from a largely inactive Hippo signaling pathway.

To investigate the role of Hippo signaling in podocytes, lentiviral transductions were used to establish inducible human podocyte cell lines to increase LATS or to silence YAP expression. Induction of LATS2 kinase overexpression activates the Hippo pathway depending on Threonin 1041 phosphorylation. In contrast to the constitutively inactive form (LATS T1041A), the overexpression of wild-type LATS2 as well as a dominantly active mutant (LATS T1041E) leads to YAP phosphorylation (inactivation) and its translocation to the cytoplasm, shown by immunofluorescence experiments. Surprisingly, this translocation of YAP leads to an increased apoptosis. Interestingly, the knockdown of YAP in stable podocyte cell lines neither leads to YAP translocation into cytoplasm nor induces apoptosis. This suggests that the amount of YAP is less important for podocyte survival than its nuclear localization.

By contrast to other differentiated cells, postmitotic podocytes show a predominant nuclear distribution of YAP, indicating that Hippo signaling is inactivated in podocytes. An activation of Hippo signaling by an increased LATS kinase expression leads to a robust nuclear export of YAP accompanied by an increased apoptosis, whereas lowering of endogenous YAP expression alone has no lethal effect. For the podocyte an inactive Hippo signaling and thereby nuclear localized YAP seems to be of particular importance for homoeostasis and survival.

10

mTOR: Metabolic Control of Podocytes

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Introduction: Podocytes play a critical role in the formation and maintenance of the kidney filtration barrier and show active mTOR signaling regulating podocyte adaption, differentiation and size-control. However, the underlying molecular mechanisms of active mTOR promoting these essential cellular effects in podocytes remain elusive.

Methods: Mice bearing podocyte specific TSC1 knock-out (model for mTOR hyperactivation) and WT mice were phenotypically analyzed and further crossed to a Tomato/eGFP reporter line to efficiently isolate podocytes for primary cell culture studies. Out of these primary cells deep proteome analysis was performed. Furthermore, mitochondrial respiration and ATP synthesis was assessed in vitro and in vivo using Seahorse bioanalyzer with specific inhibitors of glycolysis and ß-oxidation.

Results: Our proteomics data reveal that active mTOR signaling pathway massively promotes the expression of glycolytic enzymes in podocytes. Functional studies show strikingly elevated basal metabolic activity in TSC1 deficient podocytes including both upregulation of the capacity for oxidative phosphorylation as well as increased anaerobic glycolysis. However, despite increased mitochondrial capacity glucose is exclusively used for anaerobic glycolysis bypassing any mitochondrial oxidative phosphorylation. Glucose thereby provides podocyte major ATP supply.

Conclusion: mTOR signaling is a key regulator of podocyte metabolism. By massively promoting anaerobic glycolysis mTOR signaling regulates podocyte metabolic activity thereby providing high amounts of ATP for potentially energy demanding downstream effects of mTOR such as hypertrophy and podocyte adaptation. Targeting anaerobic glycolysis as the predominant metabolic pathway of podocytes might help to ameliorate features of diabetic nephropathy or podocyte ageing.

11

Podocyte Specific GSK3 Is a Master Regulator of Kidney Function during Development and in Maturity

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Introduction: GSK3 is a multi-functional kinase existing as two isoforms; α and β that show 97% protein homology. However, GSK3 isoforms have different functions in different cell types.

Methods: We have studied the role of Glycogen Synthase Kinase 3 (GSK3) in the glomerular podocyte both in development and maturity using transgenic mouse models and conditionally immortalized glomerular cells.

Results: Within the glomerulus GSK3 α and β are preferentially phosphorylated and deactivated in the podocyte in response to insulin and lithium (a common treatment for bipolar disease, and associated with glomerulosclerosis and an increased risk of end-stage-renal-failure in some patients). Developmental deletion of both GSK3 isoforms in the podocyte results in death at postnatal day 10–16 with massive albuminuria, renal failure and acidosis. However, deleting 3 out of 4 alleles of GSK3α/β causes no phenotype demonstrating a high level of compensation within this system. In the fully developed kidney, knockout of both GSK3 isoforms also results in severe renal disease with a spectrum of pathology ranging from focal and segmental glomerulosclerosis to crescentic glomerulopathy.

A loss of podocyte GSK3α/β results in massive β-catenin activation developmentally and in maturity. However, this is not the major mechanistic factor responsible for disease progression as contemporaneous specific genetic knock down of beta catenin in the podocyte together with GSK3α/β does not alter pathology.

Other signaling pathways affected by podocyte loss of GSK3 are currently being investigated by a proteomic approach, using conditionally immortalized podocytes derived from GSK3α/β floxed mice treated with soluble cre recombinase.

Conclusion: GSK3 is a critical complex in the podocyte which controls kidney function in development and maturity. It may explain why some patients on lithium therapy develop glomerulosclerosis and renal failure.

12

TGF-β1/mTOR Axis Controls Expression of Plasminogen Activator Inhibitor Type 1 in Mouse Podocytes

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Transforming growth factor-β is a well-known profibrotic cytokine which is elevated in most of the fibrotic kidney diseases. Increased expression of PAI-1 has been previously reported in patients suffering from focal segmental glomerulosclerosis (FSGS) and membranous nephropathy (MN). PAI-1 is a physiologic inhibitor of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activators (u-PA) and thereby attenuates fibrinolysis favoring matrix accumulation. Moreover, increased secretion of PAI-1 can lead to podocyte injury mediated by u-PA receptor (u-PAR) activation. Recently we reported that activation of mammalian target of rapamycin (mTOR) by TGF-β1 controls translation of NADPH oxidase 4 (Nox4) which accounts for oxidative stress and podocyte apoptosis. Here we report that expression of PAI-1 is regulated in a similar manner by TGF-β1-mTOR pathway in podocytes. In immortalized mouse podocytes, TGF-β1 treatment dose dependently stimulated PAI-1 mRNA and protein synthesis as well as its secretion, which could easily be detected from culture media. Silencing of either Smad2 or Smad3 abrogated PAI-1 secretion. Inhibition of mTORC1 by rapamycin or knock-down of p70S6K also significantly inhibited TGF-β1-induced PAI-1 secretion. However, transcription of PAI-1 was not influenced by the treatment of rapamycin. These observations clearly indicate that only translation of PAI-1 mRNA is governed by mTORC1 in podocytes. In mouse adriamycin nephropathy model which resembles human FSGS, early activation of mTOR was clearly noticed in podocytes and it was blocked by TGF-β receptor-I inhibitor, SB431542. In addition, SB431542 and rapamycin treatment significantly reduced PAI-1 expression and glomerulosclerosis. Therefore, we conclude that inhibition of TGF-β1-mTOR axis might be one of the therapeutic targets to attenuate PAI-1 expression in glomerular fibrotic diseases.

13

Podocytes' in vitro Answer to Complement Challenge

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Background: In membranous nephropathy unrestricted complement (C) activation affects podocytes directly, but local C-activation may also damage podocytes secondary and worsen the progression in other C-mediated glomerulopathies. Little is known about podocytes defense against C-activation. Complement-regulators (CR) seem to be critical. The aim of this study was the evaluation of podocytes' expression of CR and their answer to complement-challenge (CC) in immortalized human podocytes.

Methods: Podocytes were examined in immunofluorescence (IF), flow cytometry (FACS) and qPCR for expression of CR (CD46, CD55, CD59, factor H (CFH)), and compared to blood grown endothelial cells (BOEC). Activity of secreted CFH was tested in cofactor-assay. CC was induced with an antibody against CD59 and human serum (NHS) as a source of C-factors. As proof of cell-damage, phosphorylation of mitogen-activated protein-kinases (p38α, JNK1/2 and ERK1/2) and LDH-release were measured. Deposition of C3c and C5b-9, as C-activation markers, was determined.

Results: Podocytes and BOECs showed similar mRNA-levels for membrane-bound CR (CD46, CD55, CD59), but podocytes expressed higher levels of CFH (1.03 ± 0.25 vs. 0.25 ± 0.09, relative expression). Podocytes' CFH was secreted into the supernatant and lead to cleavage of C3b, a central step in C-inactivation, in a cofactor-assay. CC induced C3c-fixation (FACS (MFI) CC vs. NHS: 2800 ± 1189 vs. 660 ± 189, $p = 0.05$) and deposition of C5b-9 (IF (ID): CC vs. NHS: 16110 ± 3964 vs. 3303 ± 1304, $p = 0.0037$). LDH-release was induced (CC vs. NHS 2.3 ± 0.89 vs. 1.93 ± 0.98) and p38α was phosphorylated.

Conclusion: We showed that podocytes are damaged by C-activation in vitro. They express membrane bound CR in similar levels like BOECs. Nevertheless, podocytes express higher levels of CFH. In vivo they should not have contact to serum-derived CFH, so they are most likely dependent on their own production to protect themselves against local C-activation.

14

The Calcium-Sensing Receptor Leads to Reorganization of Focal Adhesions in Podocytes

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Background: The G-protein coupled calcium-sensing receptor (CaSR) is responsible for measurement of extracellular calcium in various cells. We have shown, that the receptor is expressed in podocytes and that the calcimimetic compound R-568, stabilizes the actin-cytoskeleton in podocytes. In addition, it had an anti-proteinuric effect in an animal model. However, it is unclear how the activation of the CaSR influences the cytoskeletal organization and the binding to extracellular matrix.

Method: Knockdown (KD) of CaSR was induced in immortalized murine podocytes with siRNA. The expression of the receptor and proteins of focal adhesions (FA) (Paxillin, FAK, Testin, Vinculin) were measured in western blot, the cytoskeleton and FA were examined in immunofluorescence. The dynamic migration capacity is critical for podocytes' function. Migration is dependent from detachment and new formation of FA. We used a wound healing assay to assess migration and an adhesion assay for quantifying the formation of FA.

Results: The KD of CaSR lead to reduction of cell-size compared to the wildtype (WT) (18.80 ± 4.53 vs. 56.06 ± 8.37 μm^2 $p < 0.0001$) and to reorganization of the cytoskeleton, with reduction of actin stress fibers (4.60 ± 0.76 vs. 17.00 ± 2.52, $p < 0.0001$) and the formation of filamentous processes. The KD reduced the number (12.50 ± 1.99 vs. 20.33 ± 2.96, $p < 0.05$) and changed the morphology of FA. Additionally the KD enhanced the ratio of phosphorylated paxillin to total paxillin (WB (arbitrary units 7.61 ± 1.87 vs. 2.31 ± 0, 74, $p = 0.015$). After treatment with trypsin KD-podocytes showed slower adhesion compared to WT (cells fixed on surface after 1 h: 3.43 ± 1.18 vs.13.68 ± 1.4, $p = 0.007$, after 6 h: 10.98 ± 3.02 vs. 30.80 ± 2.72, $p = 0.014$). Migration was reduced in KD-podocytes ($p < 0.01$).

Conclusion: The CaSR-KD impairs the organization of the actin-cytoskeleton, the binding capacity of FA, and induces an activation of paxillin. This might lead to reduced adherence and migration. This finding may explain the anti-proteinuric effect of CaSR-stimulating R-568.

15

The Full-Length 60 kDa and 45 kDa Fragment of Calcineurin Involved in Podocyte Injury

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Calcineurin is a serine/threonine protein phosphatase and firstly identified and widely known in nerve system diseases. During these years, its inhibitors (cyclosporine A and tacrolimus) have already been widely used in renal diseases as anti-proteinuric agents. However, the accurate status of calcineurin and its upstream have not been completely identified in proteinuric diseases as well as podocyte injury. So, in the present study, we aimed to examine the activity and protein expression of calcineurin, and also to identify regulators of calcineurin in podocyte injury. In puromycin aminonucleoside induced podocyte injury model, the activity of calcineurin was abnormally increased, while the protein expression of full-length 60 kDa calcineurin was decreased. To investigate whether calpain was involved in regulating calcineurin, we applied both pharmacological inhibitors of calpain and specific siRNAs against calpain in PAN treatment podocytes. As a result, blockade of calpain could reduce the enhanced activity of calcineurin and restored the down-regulated protein expression of 60 kDa calcineurin. Incubation purified calpain with podocyte extracts identified a 45 kDa fragment of calcineurin and also confirmed in PAN-induced podocyte injury and inhibition of calpain experiments. We concluded that calcineurin activity was abnormal increased in PAN-induced podocytes injury while the full-length of 60 kDa calcineurin was down-regulated because of over-activated calpain involved in cleaving calcineurin to 45 kDa fragment.

16

The Glomerular Cytoskeleton Protein-Protein Interaction Network

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Glomerular diseases are mainly caused by structural abnormalities and dysfunction of the glomerular filtration barrier. The cytoskeletal system in the glomerulus plays a key role in this process of kidney filtration. Although previous genetics and animal studies have revealed many cytoskeletal proteins (e.g., actinin 4, synaptopodin, actin-related protein (Arp) 2/3 complex, cofilin, and myosin IE) with involvement in glomerular diseases, few studies have addressed the cytoskeleton network or performed systematic analyses of the interactions between each cytoskeletal protein based on bioinformatics technology. In the present study, several available bioinformatics tools, datasets and methods were applied for analyzing interactions between glomerular cytoskeletal proteins and for predicting potential key glomerular cytoskeletal components to advance the biological understanding. In this study, genes/proteins that were annotated to the cytoskeleton were identified from Gene Ontology data, and glomerulus-enriched genes were selected from nine available glomerular expression datasets. Then, a Glomerular Cytoskeleton Network (GCNet) was generated by combining these two sets of information. In addition, the genes in the GCNet were examined in relation to five existing glomerular diseases based on high-throughput microarray data. Twenty-one new potential candidate key cytoskeletal components were highlighted. These candidates were consistently down-or up-regulated in all five glomerular diseases, which provide important insights into the core of the glomerular cytoskeleton. Moreover, these candidates were examined in relation to existing known glomerular diseases genes to determine their possible functions and interactions, and some detailed information is presented, providing a foundation for further studies.

17

Cyclosporine A Protects Podocytes by Regulating WAVE1 Phosphorylation

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Accumulating evidence suggests that podocytes are direct targets of many classic antiproteinuric drugs. The immunosuppressive drug cyclosporine A (CsA), which is a calcineurin inhibitor, is used to treat proteinuric kidney diseases. One novel mechanism by which CsA reduces proteinuria is by directly stabilizing the podocyte cytoskeleton. Previous studies showed that calcineurin can directly regulate WAVE1 within mouse striatal slices. In this study, WAVE1 was expressed in podocytes and was localized in the podocyte cell bodies and foot processes (FPs). WAVE1 expression increased in both in vivo and in vitro models of puromycin aminonucleoside (PAN)-induced podocyte injury. CsA restored WAVE1 expression and also partially rescued the disordered F-actin arrangement after PAN injury. Co-immunoprecipitation assays showed that calcineurin directly interacted with WAVE1 and regulated WAVE1 phosphorylation in podocytes. Synaptopodin is a well-characterized target of CsA. WAVE1 overexpression and synaptopodin knockdown experiments directly demonstrated that WAVE1 expression is not dependent on synaptopodin expression, and vice versa. Overexpression of WAVE1 using a WAVE1 plasmid disrupted F-actin structure and promoted podocyte migration compared with the empty vector group. Therefore, WAVE1 may be a novel molecular target for the maintenance of podocyte FPs and for antiproteinuric treatment in the future.

18

The Expression Profile of Complement Components in Podocytes

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Podocytes are critical for maintaining the glomerular filtration barrier and are injured in many renal diseases, especially proteinuric kidney diseases. Recently, reports suggested that podocytes are among the renal cells that synthesize complement components that mediate glomerular diseases. Nevertheless, the profile and extent of complement component expression in podocytes remain unclear. This study examined the expression profile of complement component in podocytes under physiological conditions and in abnormal podocytes induced by multiple stimuli. In total, 21/32 complement component-associated genes were detected in podocyte primary culture by conventional RT-PCR. Both primary cultured podocytes and immortalized podocytes expressed the complement factors C1q, C1r, C2, C3, C7, MASP, CFI, DAF, CD59, C4bp, CD46, Protein S, CR2, C1qR, C3aR, C5aR, and Crry (17/32), whereas no specific C4, CFB, CFD, C5, C6, C8, C9, MBL1, or MBL2 (9/32) bands were observed. C3, Crry and C1q-binding protein were detected by tandem mass spectrometry. Podocyte complement gene expression was affected by several factors (puromycin aminonucleoside (PAN), angiotensin II (Ang II), interleukin-6 (IL-6) and transforming growth factor-β (TGF-β)). Representative complement components were detected using fluorescence confocal microscopy. iTRAQ analysis, western blot and fluorescence showed increased C3 expression post-PAN treatment. In conclusions, it is the first time that the expression profile of complement was confirmed in primary cultured as well as immortalized murine podocytes at the mRNA and protein levels. The complement gene expressions were affected by several podocyte injury factors. The abnormal expression and distribution of complement were revealed in PAN rat nephropathy and human kidneys.

19

Genetic Analysis of Chinese Childhood Steroid-Resistant Nephrotic Syndrome

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Genetic steroid-resistant nephrotic syndrome, caused by at least 30 genes, is an important cause of end-stage renal disease. However, there is a scarcity of data for the frequency of single-gene causes of childhood steroid-resistant nephrotic syndrome in large cohorts of Chinese patients using targeting next generation sequencing. Using a multigene next generation sequencing panel of nephrotic syndrome-related 28 genes, we performed genetic analysis of 80 patients, from our single center, with SRNS or hereditary proteinuria manifested before 18 years of age. Disease-causing mutations for 9 genes were detected in 27/80 patients, with the mutation detection rate of 33.75%. Of the 80 patients, 5 patients had ADCK4 mutations (6.25%), 5 patients had NPHS1 mutations (6.25%), 6 patients had WT1 mutations (7.5%), 4 patients had NPHS2 mutations (5%) and 7 patients had other 5 genes mutations together (8.75%). A de novo mutation in TRPC6 was detected in a patient with infantile-onset nephrotic syndrome. In conclusion, ADCK4 was one of most frequent causative genes in Chinese childhood steroid-resistant nephrotic syndrome.

20

Scanning Electron Microscopy of Thin Sections: New Perspectives for Imaging Podocyte Ultrastructure

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Introduction and Aims: Changes in podocyte function are strongly correlated with morphological changes on the ultrastructural level. Transmission electron microscopy (TEM) of podocytes is therefore still a major technique in research and diagnosis. Ultrastructural examination, however, is hampered by time-consuming, demanding preparation, laborious documentation, and, often enough, limited access to equipment. Recent innovation in scanning electron microscopy (SEM) has led to improved technology in preparation and examination of biological tissues. The potential of large-scale datasets, acquired by SEM, has been studied with respect to podocyte ultrastructure. Data were compared to conventional TEM.

Methods: Standard perfusion-fixation, embedding, and sectioning techniques were performed with mice. Thin sections were placed either on grids for TEM or on silicon substrates for SEM and stained with heavy metals. Imaging for TEM was performed using a 'Zeiss 906'. Imaging for SEM was performed using a 'Zeiss Ultra', by acquiring multiple high resolution tiles that were stitched together to obtain large-scale datasets of glomeruli.

Results: Large-scale datasets of glomeruli offered a coherent 'google earth' view from very low to very high magnification in a quality comparable or superior to classical TEM. The cellular detail of podocytes was well-resolved by SEM, with clear structure identifiable even at the nanometer range. Use of silicon substrate drastically reduced preparation artefacts, permitting digitization of very large tissue areas. After acquisition, datasets could be examined on the computer, independent of an EM.

Discussion and Conclusion: We have produced high quality, large-scale datasets of glomeruli by SEM technique. Quality was comparable or superior to classical TEM imaging. Technical limitations of TEM preparation and examination have been largely overcome with SEM. Decentralized examination of datasets will further allow expert consultation via tele-pathology. Our approach sheds new light on EM in biomedical applications.

21

Tyrosine-Phosphorylation-Dependent Nephrin Signaling

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Introduction: Mutations in the human NPHS1 gene encoding the slit diaphragm protein Nephrin lead to malformation of podocyte foot processes followed by proteinuria. Nephrin binds to another cell adhesion molecule Neph1 forming a signaling hub. Phosphorylation of Nephrin by src-kinase Fyn activates the protein and mediates signaling in a phosphotyrosine dependent manner to the actin cytoskeleton that leads to lamellipodia formation and actin polymerization in cultured podocytes. The developing eye of *D. melanogaster* is a well-established model to study Nephrin biology. Ommatidial cells of the fly eye express the Nephrin orthologues Hibris and Sticks-and-stones, interommatidial cells express Neph1 orthologues Kirre and Roughest. Together, they mediate correct cell sorting. The aim of this study is to elucidate molecular mechanisms of Nephrin signaling with focus on novel phosphorylation-dependent pathways involved in rearrangement of the actin cytoskeleton.

Methods: An *in vivo* model was established to analyze signal transduction of murine Nephrin in the Drosophila developing eye. Wild-type and mutants of cytoplasmic tyrosine residues of murine UAS Nephrin transgenes were integrated into the Drosophila genome by ΦC31-based transformation. In addition, a human podocyte line was established that inducibly expresses chimeric Nephrin that can be activated by clustering with an extracellular antibody. Mass spectrometry will be performed with podocytes where Nephrin was activated compared to controls to discover proteins that are phosphorylated following Nephrin activation.

Results: Flies that overexpress wild-type Nephrin under control of a GMR-Gal4 driver show a rough-eye phenotype due to incorrect cell sorting during eye development. However, upon expression of Nephrin tyrosine mutants, the phenotype was much weaker.

Conclusion: The Drosophila model is a valid system for studying Nephrin biology. Cytoplasmic tyrosine residues are necessary for Nephrin signaling in the *D. melanogaster* developing eye.

22

The Combined Deficiency of ABCA1 and SOAT1 Is Required to Cause Podocyte Injury in Diabetic Kidney Disease

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In patients with Diabetic Kidney Disease (DKD), decreased podocyte number and glomerular cholesterol accumulation are associated with albuminuria. Among several genes involved in cholesterol metabolism, glomerular expression of ATP Binding Cassette A1 (ABCA1) is reduced in DKD when compared to healthy controls. However, the relative contribution of free and esterified cholesterol to podocyte injury in DKD remains unknown. We hypothesize that a combination of decreased ABCA1 expression and sterol-o acyltransferase 1 (SOAT1) activity is required to cause free cholesterol mediated podocyte apoptosis in DKD.

Sera obtained from Pima Indian patients with T2D and separated in two groups based on the rate of decline in GFR (progressors, DGFR -97.39 ± 8.2, $n = 15$; non-progressors DGFR $+40.62 \pm 8.6$, $n = 16$) were utilized to culture human podocytes. ABCA1 expression, cholesterol efflux and SOAT1 activity were measured. Cholesterol content, cholesterol efflux and caspase 3 activity were also measured in siRNA ABCA1 (siABCA1) and siRNA control (siCO) podocytes in the presence or absence of a SOAT inhibitor (SI) and/or cyclodextrin (C), a cholesterol sequestering agent. A mouse for the conditional deletion of ABCA1 in podocytes was also developed.

Podocytes exposed to sera from patients with progressive DKD revealed significantly reduced ABCA1 mRNA expression ($p < 0.05$), ABCA1 mediated cholesterol efflux ($p < 0.01$) and SOAT1 activity ($p < 0.05$) when compared to non-progressors. siABCA1 podocytes demonstrated an increase in esterified cholesterol content and in lipid droplet number compared to siCO. SiABCA1 podocytes did not undergo apoptosis unless treated with SI (4 μM dose for 24 hours, $p < 0.05$), a phenomenon that was prevented by pretreatment with CD ($p < 0.05$). Mice with the podocyte conditional deletion of ABCA1 remained normoalbuminuric.

Our data indicate that the concomitant reduction of ABCA1 expression and SOAT1 activity are required to cause free-cholesterol mediated podocyte injury. This suggests that treatment strategies to restore ABCA1 and SOAT1 function may be beneficial to inhibit podocyte loss in DKD.

23

Effects of APOL1 on Autophagy Are a Manifestation of a General Disruption of Vesicle Trafficking That Is Exacerbated by the Renal Risk Variants

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Background: C terminal variants of APOL1 (G1 and G2, compared to non-risk G0) are strongly associated with glomerular diseases. In a proposed model, VAMP8 inhibits APOL1 pore formation and subsequent autophagic cell death, and the risk variants have decreased affinity for VAMP8, resulting in increased toxicity.

Methods: Experiments employed transiently transfected HeLa and HEK293 cells, and HEK293 cells stably infected with inducible APOL1-mCherry variants. Toxicity was measured by SYTOX exclusion dyes and ATP assay. Autophagy and autophagic flux were measured by quantification of GFP-LC3 puncta or LC3B-II Western blot, each with and without chloroquine to inhibit autophagosome-lysosome fusion. Vesicle pH was measured as the ratio of endocytosed pH-sensitive FITC-dextran to pH-insensitive AF647-dextran by flow cytometry and fluorescence microscopy.

Results: Following overexpression of APOL1, toxicity was observed in a pattern G0 <G2 <G1. C-terminal truncation abrogated all toxicity. Greater toxicity in G1 and G2 cells was associated with decreased autophagic flux, but not with increased induction of autophagy. These data suggest the G1 and G2 variants impair autophagosome-lysosome fusion. Vesicle fusion events are pH-mediated. Dextran uptake assays revealed that APOL1 expression increased mean vesicle pH (G0 <G2 <G1). Further, these experiments demonstrated that APOL1 decreased total internalized dextran (G1 <G2 <G0), which is dependent on the net effects of endocytosis and exocytosis. APOL1-mCherry co-localized with GFP-VAMP8 (+) late endosomes/lysosomes, but contrary to the proposed model, VAMP8 co-expression increased APOL1 toxicity.

C-terminal truncated APOL1 trafficked to VAMP8 (+) vesicles, but lacked toxicity and was associated with normal autophagic function. These data suggest the risk variants do not alter trafficking of APOL1 to vesicles, but instead alter trafficking of APOL1(+) vesicles.

Conclusions: Alterations in autophagy, vesicle pH, and dextran uptake suggest that APOL1 over-expression confers a global defect in vesicular trafficking, with an effect G0 <G2 <G1. Agents that block APOL1 trafficking to vesicles might ameliorate APOL1 nephropathy.

24

PKCε Is Identified as a Novel Binding Partner of B-Catenin in Podocytes

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Background: PKCα, one conventional isoform of PKC, is well known as a binding partner of β-catenin. However, a link between PKCε, a novel isoform, and β-catenin remains unclear. PKCε regulates the cytoskeleton by phosphorylating IQGAP1, a protein involved in the regulation of β-Catenin in the cell-cell adhesion complex. Therefore, we investigated the association between PKCε and β-catenin.

Methods: Immunofluorescent staining was performed on murine kidney sections and podocytes to examine the β-catenin expression. Time courses were performed in murine wild type and PKCε–/– podocytes. 7 promising phospho-motifs in β-catenin were selected and site-specific mutations were produced. The interaction between the β-catenin mutants and PKCε were verified by immunoprecipitation. The mutants were overexpressed in murine podocytes using adenovirus. Zebrafish larvae were injected with mutant β-catenin RNA to investigate their function in vivo.

Results: During the development of mice β-catenin showed increasing expression level in the glomeruli. However, the upregulation of β-catenin in wild type mice was much higher than those of PKCε–/– mice. While in wild type podocytes, β-catenin showed a translocation from the perinuclear areas to the nuclei during differentiation, the distribution of β-catenin switched the reverse way in PKCε–/– podocytes from the nuclei to the perinuclear areas. During the time course, PKCε–/– podocytes displayed both decreased expression level of active β-catenin and total β-catenin. 3 of 7 mutant β-catenin exhibited decreased interaction with PKCε in immunoprecipitation, suggesting these phospho-motifs as important binding sites. These 3 mutant β-catenin were not able to recover the phenotype of the β-catenin knockdown in zebrafish. Further, overexpression of wildtype ß-Catenin in PKCε–/– podocytes rescued the phenotype of the impaired actin cytoskeleton, while the mutant unable to reestablish the zebrafish showed no rescue.

Conclusion: It is the first time to indicate PKCε as an important binding partner of β-catenin and they are involved in the process of glomerular disease and podocytes differentiation. 3 significant phospho-motifs in β-catenin are proved as interaction sites for PKCε.

25

Assessing the Effect of SGPL1 Mutations Using the Drosophila Model

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Introduction: Sphingosine-1-phosphate lyase (SGPL1) is a highly conserved enzyme, responsible for the irreversible degradation of sphingosine-1-phosphate, a bioactive lipid that controls cell survival, proliferation and migration. We have recently found nonsense and missense recessive mutations in *SGPL1* in several families in which the affected individuals presented with steroid-resistant nephrotic syndrome (SRNS), ichtyosis and adrenal insufficiency (see S.Lovric abstract). We sought to determine the pathogenicity of *SGPL1*missense mutations using the *Drosophila* model and patient fibroblasts.

Methods: Using the knock-out (KO) of the *SGPL1* ortholog, *sply*, in flies, we re-expressed the *sply* gene WT or carrying the corresponding human mutations E132G, R222Q and S346I. The effect of the KO and the human mutations on the function of nephrocytes, the fly counterparts of podocytes, was examined by assessing the macromolecule filtration, expression of slit diaphragm proteins and ultrastructural morphology. Patient fibroblasts were used to assess SGPL1 activity, protein levels and mRNA expression.

Results: The absence of *sply* greatly reduced fly viability. In nephrocytes, *sply* KO reduced the expression of the slit-diaphragm protein Kirre, a Neph1 orthologue, and led to decreased filtration, effacement of the nephrocyte foot processes and slit diaphragm number reduction. Unexpectedly, *sply*KO nephrocytes had a striking reduction of lipid droplets. Re-expression of the *sply* WT and E132G transgenes rescued these phenotypes but not the mutant forms R222Q and S346I. Additional studies in patient fibroblasts carrying the c.395 A>G p.E132G mutation revealed the absence of SGPL1 protein and enzymatic activity, and RT-PCR revealed that the pathogenic mechanism is the skipping of exon 5, leading to a frameshift and a stop codon 24 amino-acids ahead.

Conclusions: Loss of SGPL1 in nephrocytes leads to a phenotype reminiscent of the nephrotic syndrome in humans. Altogether, our data demonstrates that the mutations found in SGPL1 are pathogenic.

26

Renal Risk Variants of APOL1 Mediate Mitochondrial Dysfunction and Cell Death in Podocytes

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The G1 and G2 variants of the human *APOL1* gene are associated with an increased risk for renal diseases, including HIV- and hypertension-associated nephropathy, FSGS, and progressive CKD, which all lead to ESRD. However, so far the molecular and cellular mechanisms are poorly understood. Here, we examined the role of the renal risk variants (G1/G2) on cellular signaling pathways and their impact on cellular integrity. For this purpose, we established stable HEK293T and AB8 podocyte cell lines by lentiviral transduction, which allowed the inducible overexpression of N-terminally GFP-tagged *APOL1* G0, G1 and G2 variants. Live cell imaging of the generated cell lines revealed co-localization of all *APOL1* variants with membranes of the ER, the Golgi-apparatus and mitochondria. However, by contrast to *APOL1* G0, overexpression of *APOL1* risk variants G1/G2 caused an altered expression of ER stress (e.g. pEIF2α, pJNK) and autophagy marker proteins (LC3-II). Interestingly, in all experiments G2 overexpression resulted in a higher and faster, Caspase-independent cytotoxicity compared to G1. Moreover, in stable podocytes overexpressing the G2 variant, cell death was accompanied by progressive vacuolization, the disruption of the Golgi-apparatus, and fragmented mitochondria. Metabolic measurements showed a strong decrease of the mitochondrial respiration rate in podocytes expressing the G2-variant, whereas the rates in G0 and G1 expressing podocytes showed only minor effects or remained unchanged. Taken together our data reveal a novel strong association of *APOL1* to ER-, Golgi- and mitochondria-membranes and confirmed the cytotoxic effect due to an increased *APOL1* expression, especially for *APOL1* G2. Thus, our data support a model in which an increased *APOL1* expression causes cytotoxicity by enhanced disturbances of ER-, Golgi- and mitochondria-membrane associated functions.

27

The Neurotrophic Receptor Kinase TrKC Signals to the Podocyte Actin Cytoskeleton

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Introduction: Podocyte malfunction is central to glomerular disease and is characterized by defective podocyte intercellular junctions and actin cytoskeletal dynamics. Recently, TrkC was shown to be located at the podocyte slit diaphragm. TrkC knockout mice developed proteinuria shortly after birth (Lefevre et al., PLoS Genet., 2010). The aim of this study is to further investigate the role of TrkC in podocytes and its relevance in glomerular disease.

Methods: Mouse podocyte lines were generated that inducibly express wild-type TrkC or TrkC where specific tyrosine-residues known to mediate TrkC signal transduction were mutated to phenylalanine. Glomeruli were isolated from murine kidneys and treated with Lipopolysaccharide (LPS) or Adriamycin (ADR) to induce podocyte injury.

Results: We confirmed that TrkC was expressed in mouse glomerular podocytes and co-localized with the slit diaphragm marker Nephrin. Activation of endogenous TrkC by adding the ligand Neurotrophin 3 (NT3) to the culture media of mouse podocytes resulted in tyrosine-phosphorylation of TrkC as well as activation of the downstream target proteins Erk and Akt. Activation of inducibly expressed TrkC or endogenous TrkC by NT3 lead to increased filopodia formation in cultured mouse podocytes while increased filopodia formation could not be observed in podocytes that express kinase dead TrkC. Injury induced by treating isolated murine glomeruli with ADR or LPS resulted in a significant increase in tyrosine-phosphorylation of endogenous TrkC.

Outlook and Conclusion: Currently, genetic mouse models with the potential to conditionally express or knockout TrkC in podocytes or nephrons are generated to test whether TrkC is essential for the podocyte and to establish a model to dissect TrkC signaling in vivo. These mouse models will be employed to test whether TrkC can be therapeutically-targeted to treat glomerular disease. Our results imply that TrkC is activated during glomerular injury and signals to the podocyte foot process actin cytoskeleton.

28

Drosophila Nephrocyte as a Model for Podocyte Cytoskeleton and Membrane Trafficking

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Drosophila nephrocytes and mammalian podocytes share striking structural, molecular and functional similarities. Both cell types have highly specialized cytoskeletal and vesicular architectures essential for renal function. We designed and performed the first genetic screen for renal genes in Drosophila nephrocytes. Using this powerful genetic system, we are able to study the genetic control of this unique cytoskeleton and membrane trafficking system in a systematic way for the first time. We screened thousands of genes in nephrocytes and identified over 30 genes essential for nephrocyte cytoskeleton and membrane trafficking. Among them are 13 Rab GTPases, 8 Exocyst genes, as well as small GTPases Rho, Rac and Cdc42. Detailed studies using organelle and cytoskeleton markers, as well as electron microscopy, revealed the essential roles of these cell trafficking and cytoskeleton genes in Drosophila nephrocytes, and illustrated the major cell trafficking paths in nephrocytes for the first time. Since all these genes in this study are highly conserved from Drosophila to humans, it is expected that these genes play similar roles in mammalian podocytes, and mutations of these genes are likely to be associated with podocyte-related renal diseases.

29

Generation of Multiple Nephronal Cell Types Using Human Pluripotent Stem Cells

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Introduction: Chronically damaged nephrons deteriorate progressively towards end stage renal disease, owing to the limited regenerative capacity of adult mammalian kidneys. The generation of renal cells and tissues from human pluripotent stem cells (hPSC) is a promising strategy to develop regenerative therapies for ESRD. In this study, we established a protocol to differentiate hPSCs to the renal progenitors, capable of producing nephronal cell types and structures *in vitro* and *ex vivo*.

Methods: Pluripotent stem cell culture, quantitative Polymerase Chain Reaction (qPCR), RNA sequencing, High content screening (HCS) of immunofluorescence, *in vivo*angiogenesis on chick embryo – Chorioallantoic Amniotic Membrane (CAM), embryonic mouse kidney re-aggregation assay.

Results: An effective combination of factors obtained after intensive screening, was used to create an 8-day-protocol that

steered hPSCs to the renal lineage by a step-wise process outlining renal organogenesis. Six days after growth factor treatment, a mixture of SIX2+/CITED1+ cells representing metanephric mesenchyme and an HOXB7+/GRHL2+ population indicative of ureteric bud progenitors was obtained that developed into renal vesicle cells by the eighth day. Prolonged cultivation of these progenitor cells in three inductive media resulted in generation of WT1+/PODXL+/SYNPO+ podocyte-precursors, PDGFRß+/DESMIN+/αSMA+-mesangial cells and fractions of proximal, distal and collecting duct tubular epithelial cells were observed *in vitro*. Moreover, when day 8 cells were allowed to differentiate spontaneously, renal organoids have been observed in culture. PSC-derived renal progenitors exhibited vascularization, when grafted on the chorioallantoic membrane of a 7-day old chick embryo and also integrated into embryonic kidney re-aggregations forming tubular networks.

Conclusion: An efficient and fast protocol for nephronal cell generation was developed. Further studies need to be performed to investigate the contribution of human PSC derived renal progenitors *in vivo* to renal injury, assessing their suitability as candidates for cell based therapies.

30

Y Isoform of PI3K Mediates Podocyte Injury and Is a Potential Marker for Glomerular Sclerosis

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We recently reported that, in the Adriamycin (ADR)-induced mouse glomerular sclerosis model, the gamma isoform of the p110 catalytic subunit (p110gamma) of phosphoinositide 3 kinase is selectively upregulated in podocytes of the sclerosing glomeruli, and that a specific inhibitor to p110gamma (AS605240) prevented proteinuria and glomerular sclerosis. Here, we evaluated the molecular mechanisms by which p110gamma mediates podocyte damage in vitro and a possible role for p110gamma in mouse genetic models of FSGS in vivo.

When podocin was deleted at age of 6 weeks in Nphs2f/f, b-Cre-ERT2 mice by Cre recombinase activation with tamoxifen administration, mice demonstrated proteinuria starting as early as 1 week after Cre activation and developed FSGS-like lesions by 4 weeks as previously reported (Mollet, JASN, 2009). p110gamma protein was detected specifically in podocytes of diseased mice. In pooled urine samples, p110gamma mRNA was detected only in mice with disease induction, but not in healthy controls. Mice treated with AS606240 (5 mg/kg, i.p., 3x.week) starting from 3 days after the completion of tamoxifen injection, showed significantly less proteinuria and improved glomerular sclerosis. Similarly, in CD2AP+/–, synaptopodin+/– mice that develops FSGS-like lesion to a variable degree at age 8–9 months, p110gamma-positive cells per total nuclei in glomeruli correlated significantly with glomerular COL1A2 mRNA content.

In culture, p110gamma kinase-dead podocytes, but not wild type cells, demonstrated increased membrane ruffling along with distinct Rac1 staining at the membrane, and kinase-dead podocytes were resistant to in vitro ADR treatment. Wild type podocytes expressing constitutively active p110gamma showed decreased cytoskeletal assembly and cytoDEATH early apoptosis marker staining was detected, suggesting that p110gamma activity directly mediates podocyte dysfunction.

Together, these results suggest that p110gamma expression in podocyte correlates with the degree of glomerular sclerosis, and therefore, is a potential maker for progression. p110gamma activity mediates podocyte injury by affecting signals critical for cytoskeletal structure.

31

Functional Aspects of Podocyte Biology Are Conserved in Drosophila Nephrocytes

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Introduction: The Drosophila garland cell nephrocyte is a podocyte-like cell that bridges membrane invaginations called labyrinthine channels by auto-cellular slit diaphragms. These are formed by the orthologues of mammalian slit diaphragm proteins, providing a potential in vivo model to study mechanisms of nephrotic syndrome. However, current knowledge is fragmentary and the extent of functional evolutionary conservation remains unclear. Well-known etiologies of human nephrotic syndrome such as actin-dysregulation, laminin-loss or CoQ10-depletion have not been studied in nephrocytes.

Methods: Gene silencing by RNAi in larval nephrocytes, ultrastructure-analysis by transmission electron microscopy (TEM), assessment of slit membrane proteins by immunofluorescence and confocal imaging of endocytosis of 66 kDa fluorescent tracers (FITC-Albumine, Texas-Red-Avidin) as a functional read-out.

Results: To study the slit diaphragm proteins *sticks and stones* (*sns*, orthologue of Nephrin) and *kirre* (NEPH1) we performed immunostainings revealing a fingerprint-like pattern correlating with labyrinthine channel morphology. Removal of kirre or sns results in a punctate staining of the respective unsilenced protein. Uptake of the 66 kDa fluorescent tracers is saturable and competitive, suggesting receptor-mediated endocytosis. Rapid endocytosis is diminished by sns/kirre-knockdown but entirely abrogated upon silencing of *Cubilin/Amnionless*, implicating the latter proteins as the respective receptors.

Then we used the tracer-endocytosis-assay and sns/kirre-staining in combination with TEM-imaging to analyze orthologues of further genes involved in the pathogenesis of human nephrotic syndrome. Knockdown of the LAMB2-orthologue *LanB1* and the Drosophila CoQ10-synthesis gene *Coq2* result in loss of labyrinthine channels and slit diaphragms, partial internalization of sns/kirre and reduced tracer-endocytosis. Furthermore, TEM-data indicates that Rho1 silencing results in narrow/elongated labyrinthine channels while expression of constitutively active Rho1,

Cdc42-RNAi and a Rac1-dominant negative construct lead to dilated/fused labyrinthine channels. Expression of constitutively-active Rac1 results in internalization of sns/kirre and loss of labyrinthine channels.

Conclusion: Roles of CoQ10-depletion, laminin-interaction and actin-dysregulation seem analogous between nephrocytes and podocytes.

32

Mutations of Genes Encoding for the Nuclear Pore Proteins NUP93, NUP205, or EXPORTIN-5 Link Nephrotic Syndrome to Alterations in BMP7/SMAD Signaling

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Steroid resistant nephrotic syndrome (SRNS) is a frequent cause of end-stage renal failure in children and young adults. To date, mutations in more than 30 genes have been identified as monogenic causes of SRNS. Discovery of these monogenic genes has helped identifying signaling pathways that are relevant for podocyte development and maintenance.

We here utilized whole-exome sequencing, genetic mapping, and next-generation sequencing to identify mutations of the genes encoding for the nuclear pore proteins *NUP93*, NUP205, and the nuclear transport protein exportin-5 in 8 unrelated families with SRNS. To identify the pathogenic link between nucleoporin mutations and SRNS, we demonstrate that knockdown of *NUP93* affects cell viability of human podocytes in-vitro as shown by reduced cell proliferation and impaired oxidative stress resistance. Furthermore, we show that podocyte migration rate, a well-established surrogate phenotype of SRNS, is significantly impaired by knockdown of *NUP93*. We observed that *NUP93* strongly localizes to WT1 positive podocyte precursor cells in developing rat kidney, and therefore tested whether knockdown of *NUP93* impairs the BMP7-SMAD signaling pathway that is particularly relevant for podocyte development and maintenance. We show that NUP93 interacts with SMAD4 and its nuclear import protein importin-7, and that some of the mutations of *NUP93* identified in individuals with SRNS abrogated this interaction. We furthermore show by immunofluorescence and by utilizing a SMAD responsive luciferase construct that BMP7-SMAD signaling is impaired upon knockdown of NUP93. While transfection of wild-type *Nup93* successfully rescued the cellular defects in both assays, constructs reflecting any of the 5 alleles identified in individuals with SRNS failed to rescue the defects.

We identify mutations of genes encoding for the nuclear pore proteins NUP93, NUP205, or exportin-5 as novel monogenic causes of steroid-resistant nephrotic syndrome in humans and show that NUP93 is required for podocyte survival and BMP7-SMAD signaling in-vitro.

33

Podocyte-Selective Overexpression of the TGF-ß1 Receptor Type II (TßRII) Promotes Diabetic Nephropathy in STZ Induced Transgenic Rats

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Glomerular TGF-ß1 is increased early in the course of diabetic nephropathy. The podocytes are sources and targets of TGF-ß1. This study addresses the hypothesis that increased TGF-ß1 in diabetic rats stimulates the development of diabetic nephropathy via selective signalling in podocytes.

Transgenic rats (TGR) carrying the TßRII driven by the podocin promoter were generated. Transgene expression was verified by Northern blotting, in-situ hybridization and Western blotting. Glomerular expression profiling was performed by real time RT-PCR, Western blotting and immunohistochemistry. Podocyte density was determined by counting WT-1 stained podocytes per glomerular area, which was determined morphometrically in 200 glomeruli per rat. At 2 months of age rats received STZ (40 mg/kg i.v.) or diluent, respectively. Body weight and kidney function were evaluated by urinary albumin excretion in the 24 h-urine and by creatinine clearance at monthly intervals.

TGR expressed the transgenic receptor specifically in podocytes. Glomerular TßRII protein levels were almost twice that of WT. At 5 months of age 40% of TGR exhibited moderately increased albumin excretion up to 2.2 mg/24 hr vs. 0.11 mg/24 hr in WT. 3 months after STZ, albumin excretion was significantly increased in 80% of TGR with an average of 4 mg/24 hr (max: 7.3) but only slightly elevated in 40% of the WT with in average 0.7 mg/24 hr (max: 2.2). Expression profiling in isolated glomeruli revealed that in TGR PAI-1 was significantly upregulated and the survival marker blc-2 and podocyte differentiation marker synaptopodin, podocin and nephrin were significantly down-regulated relative to WT. Podocyte number per glomerulus was significantly decreased in diabetic TGR vs. diabetic WT.

Podocyte-selective TßRII-overexpression contributes to the leakage of the glomerular filter in the course of STZ induced diabetic nephropathy which might be mediated by podocyte loss due to dedifferentiation, downregulation of bcl-2 and due to PAI-1 upregulation.

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34

Autophagy Inhibition by Chloroquine Disrupts the Actin Cytoskeleton of Human Podocytes

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Background: Actin cytoskeleton rearrangement is a key pathological feature of minimal change nephrotic syndrome. It has been considered as the consequence of cytokines disruption or gene mutations of cytoskeleton proteins. Recently autophagy has been described as a vital cytoprotective mechanism for keeping podocyte homeostasis. Our previous published data suggested that autophagy could be manipulated for attenuating podocyte injury in vitro. However, it remains unknown whether the actin cytoskeleton could be disrupted by autophagy inhibition in podocytes. Thus, we investigated the changes of actin cytoskeleton after autophagy inhibition by chloroquine which is a well-known autophagy inhibitor.

Methods: Human conditional immortalized podocytes were treated with chloroquine. Autophagy was investigated by western blotting for LC3 and P62. We performed the podocyte migration and adhesion assays to analyze podocyte injury. In addition, podocyte cytoskeleton was evaluated by filament actin immunofluorescence staining as well as the mRNA expression of nephrin, CD2AP and alpha actinin-4. Furthermore, B7-1 which is a key regulator of podocyte cytoskeleton has also been measured.

Results: The number of podocytes with disrupted cytoskeleton increased significantly after chloroquine treatment as well as its mobility. However, no obvious changes were observed in adhesion assay and the mRNA expression of nephrin, CD2AP and alpha actinin-4. More importantly, B7-1 increased significantly when autophagy was inhibited in podocytes after chloroquine treatment.

Conclusions: These results suggested that autophagy inhibition by chloroquine could disrupt the actin cytoskeleton of human podocytes.

35

A Novel Assay to Study Podocyte (De)Differentiation in situ

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Podocyte dedifferentiation is the major event during the development of glomerulopathies and the underlying process is poorly understood. However, the detailed knowledge of the (de)differentiation process is necessary to develop suitable therapies and medications. We have established an assay to study podocyte (de)differentiation by isolated glomeruli of mice expressing cyan fluorescent protein (CFP) under control of the nephrin promoter (Cui et al. 2005). Since dedifferentiation of podocytes is accompanied by the downregulation of nephrin we used the decrease of fluorescence of CFP-podocytes (CPod) as an indicator for dedifferentiation.

To be sure that the loss of fluorescence is not caused by the preparation, we confirmed the cellular integrity by propidium iodide assay and immunohistochemistry of podocyte-specific proteins. Electron microscopy showed that CPod have well-formed foot processes.

Since it is known that podocytes dedifferentiate in culture, we followed the decrease of the fluorescence of CPod cultured in RPMI/FBS up to 9 d. To quantify podocyte fluorescence, z-stacks of glomeruli (50–80) were taken by confocal laser scanning microscopy and after background correction the mean total fluorescence intensity per glomerulus (MFG) was calculated. We found that MFG remained stable for 5 d and decreased to 10% of the initial value on day 9. Similar results were obtained by RNA-seq.

To investigate whether CPod dedifferentiation can be pharmacologically accelerated, we used doxorubicin in our assay, which is known to reduce nephrin expression. In line with our expectations, incubation of CPod with 50 μM doxorubicin for 3 d decreased podocyte fluorescence by about 50%.

Taken together, we have established an assay to determine the influence of drugs/small molecules on the differentiation of podocytes *in situ*. This assay enables us to screen for small molecules that inhibit the dedifferentiation of podocytes and could be helpful for therapy.

36

Nphs2.T2A.iCre.T2A.mtTomato: A Tricistronic Podocyte Reporter Mouse That Combines Enhanced Cre Recombinase Activity and Fluorescent Labeling

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The Nphs2.Cre mouse model introduced by Moeller et al. in 2003 has been a valuable tool to study podocyte biology. It has been widely used not only to genetically manipulate but also to stably label podocytes e.g. with fluorescent proteins. However, there are certain limitations as the podocin:cre transgene is randomly inserted and Cre efficiency might vary or even be lost over time. In addition, the combination of a podocyte reporter and the simultaneous genetic manipulation of a certain gene of interest often requires multiple time and cost-consuming crossings. To overcome these weaknesses, we generated a novel podocyte-specific Cre mouse model combining enhanced Cre efficiency and fluorescent cell labelling. To this end, we targeted the Nphs2 locus to generate a tricistronic mRNA linking Nphs2 to a codon improved Cre recombinase (iCre) via a viral 2A sequence followed by a second 2A sequence and mtTomato allowing direct podocyte labelling. Podocin, iCre and mtTomato are expressed in equimolar amounts under the control of the endogenous Nphs2 promoter. Immunofluorescence and FACS-analysis revealed exclusive mtTomato expression in podocytes. Nphs2.T2A.iCre.T2A.mtTomato mice did not develop glomerular disease confirming that the knock-in per se was not harmful. We assessed Cre recombinase efficiency by mating the mice to Phb2fl/fl mice. Phb2fl/fl;Pod2ACretg/wt mice presented with aggravated glomerular injury already evident after 2–3 weeks of age and premature lethality after 4 weeks while the onset of disease in conventional Phb2fl/fl;Pod.cretg/wt mice was delayed by approximately 7 days.

Taken together, we generated a tricistronic podocyte reporter mouse that combines improved Cre recombinase activity and expression of a membrane-tagged tomato for easy visualization and identification of podocytes.

37

Functional Analysis of Myo1E Mutations Associated with FSGS

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Introduction: Sequencing of the genes implicated in FSGS has resulted in identification of many novel mutations in podocyte-expressed proteins. Application of these findings to diagnosis and treatment of FSGS requires robust functional tests that can be used to distinguish pathogenic mutations from the normal sequence variants. We have developed two complementary model systems for characterizing functional effects of mutations in Myo1E, a motor protein that is expressed in podocytes and required for glomerular filtration. One system utilizes podocytes derived from the Myo1e-KO mice, which can be reconstituted with the wild type or mutant Myo1E. The other uses a simple model organism, fission yeast *Schizosaccharomyces pombe*, to test how mutations affect the activity and stability of the yeast class I myosin, Myo1, which is related to Myo1E both structurally and functionally.

Methods: Myo1e-null podocytes were infected with adenoviral vectors encoding EGFP-Myo1E along with mCherry-tagged cytoskeletal and slit diaphragm markers and examined using confocal imaging and calcium switch assays. For yeast experiments, mutations equivalent to those found in the FSGS patients were introduced into the yeast *myo1* gene, and the resulting mutant strains were used for functional analysis, including growth and endocytosis assays, fluorescence imaging and Western blotting.

Results: Two FSGS-associated mutations, A159P and T119I (and their analogs in yeast, A181P and T140I), were found to disrupt myosin localization to actin-containing structures (cell junctions in podocytes and endocytic actin patches in yeast) and result in functional defects. The A181P mutant also exhibited increased degradation, while the stability of the T140I mutant was similar to that of the wild type myosin-1.

Conclusions: Motor domain mutations tested in this study impair myosin functional activity and actin binding, confirming that these mutations are likely pathogenic. These findings highlight the relationship between the myosin motor activity and its ability to support normal glomerular filtration.

38

Mesangial Matrix Expansion in Diabetic Nephropathy (DN) Is Due to Accumulation of Worn-Out GBM Material

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Mesangial matrix expansion is a hallmark of DN and is generally believed to emerge from an overproduction by mesangial cells. Re-evaluation of 918 biopsies of DN from the years 2007–2015 (archive: Dep. of Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg) revealed strong evidence that a major part of the accumulated mesangial matrix in DN is derived from the deposition of undegraded GBM-material. The developmental process seems to be as follows.

The overproduction of GBM-material by podocytes leading to a greatly thickened GBM in DN has been known for a long time. The thickened GBM causes a narrowing of the clefts within the GBM-infoldings seemingly compromising the space for the podocyte portions extending into these clefts. They retract out of these clefts frequently sequestering part of their cytoplasm as matrix vesicles. In consequence, the innermost portions of the GBM, i.e. the bends of the GBM-infoldings are deprived from any podocyte support and become incorporated into the mesangium. Strong evidence for this process comes from the sequestered podocyte matrix vesicles that survive within the engulfed GBM material for some time.

By IF it is evident that the α3 and α5 chains of collagen IV are widely found within the accumulated mesangial matrix. In addition, the α1 chain of collagen IV is, compared to controls prominently expressed in the GBM in DN and is later deposited in large amounts in the expanded mesangium.

Due to these findings the turnover of the GBM takes centre stage in the pathogenesis of DN. Actually, we do not know where under normal conditions the worn-out GBM is degraded. We also do not know whether in DN overproduction or insufficient degradation of GBM-material are primarily responsible for the thickening of the GBM and the subsequent accumulation in the mesangium. In our view, the dramatic deposition of worn-out GBM in the mesangium represents the crucial damage in DN that accounts for most of further pathologies, such as the outgrowth of vessels at the vascular pole and the loss of podocytes by detachment.

39

Not Pressure and Distension, But Filtrate Flow and Shear Stress, Challenge the Attachment of Podocytes to the GBM

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Podocytes are lost by detachment from the GBM as viable cells. Impaired attachment to the GBM, or rheological overstress are crucial causes. Here, we will discuss the rheological challenges to the filtration barrier by pressure and filtrate flow. Pressure causes distension depending on the elastic resistance of the GBM, both other layers, endothelium and podocyte cover, adapt to area changes of the GBM. The most critical structure in this process is the slit membrane (SD). It seems to adjust to changes in GBM area by a shuttling system of SD components between the SD and the adjacent foot processes (FPs). Thus, length and total area of SD change in parallel with pressure.

Filtrate flow permanently tends to drag podocytes towards the urinary orifice by shear forces, which increase with the velocity of flow. Podocytes that have come to lie within the urinary orifice are exposed to high shear forces and lost into the urine; this can directly be seen. The estimation of shear forces on FPs within the filtration slits is fraught by the slit membrane. Calculation neglecting the SD results in improbably high values of 8 Pascal (Endlich and Endlich, Sem Nephrol 2012) more than 10fold higher than those acting on cell bodies. This leads to the conclusion that a major function of the SD (likely in addition to others) must be to counteract the shear forces on FPs. As a modified adherens junction the SD mechanically interconnects the cytoskeleton of opposing FPs likely mutually balancing the shear stress on the opposite FP.

If under pathological conditions, increased filtrate flows locally overstress the stabilizing function of the SD, the SD is replaced by occluding junctions sealing the slits and, second, the attachment of podocytes to the GBM is reinforced by foot process effacement. If these temporary adaptive mechanisms fail, a slow process of podocyte detachment follows based on uncontrolled filtrate flows through bare areas of GBM and, subsequent, labyrinthine subpodocyte spaces presenting as pseudocysts.

40

A Novel NPHP3 Mutation May Leads to Early Presentation of Cystic Kidneys in Infantile NPHP

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Nephronophthisis (NPHP) is an autosomal recessive cystic and tubulointerstitial kidney disease that represents the most frequent genetic cause of end-stage renal disease (ESRD) in children and

young adults. Infantile NPHP, often in combination with other features like *situs inversus*, are commonly caused by mutations in the NPHP2 gene. In this article, we present a one-year-old boy with ESRD, anemia, complete transposition of viscera, hepatic dysfunction, and central nervous system defects. Therefore, we sequencing 13 genes (NPHP1-11, AHI1, and CC2D2A) related to NPHP using exon combined targeted region capture sequencing chip. Interestingly, we detect two heterozygous frameshift mutation of NPHP3 (c.2719_2720ins GCTGAGTT, p.K1234fsX1246 in exon 20 and c.3697-7delT in IVS 25) instead of INVS gene. Among there, c.2719_2720ins GCTGAGTT as a novel mutation, which may lead to truncated protein, or protein degradation; surprisingly, the mutation identified in the patient was found to be inherited from the healthy mother. Therefore, further research still need to clarify and validate the mechanism of these mutations.

41

Glucocorticoid Receptor Antagonism Is Effective in Experimental Models of Minimal Change Disease

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Background: High-dose glucocorticoids (GCs) are the primary treatment for minimal change disease. However, GCs are associated with serious side effects, especially in children and after prolonged treatment. Moreover, the mechanism of action of GCs in minimal change disease is still incompletely understood.

Results: Two alternative models for minimal change disease were used: protein-overload in mice and puromycin aminonucleoside-induced nephropathy in rats. Both models are responsive to GC treatment, mimicking minimal change disease. Notably, high-dose GC as well as inactivation of the glucocorticoid receptor in podocytes in Pax8-Cre/GRfl/fl mice ameliorated proteinuria. Cell-specific inactivation of the glucocorticoid receptor in endothelial cells in Tie2-ERT2/GRfl/fl mice did not have any effect. Similar anti-proteinuric effects were achieved with the pharmacologic glucocorticoid receptor antagonist, mifepristone. Furthermore, treatment with both GCs or the antagonist mifepristone ameliorated proteinuria in puromycin aminonucleoside injected rats

Summary: These results show for the first time *in vivo* that the therapeutic effects of GCs are mediated by glucocorticoid receptor signaling in podocytes. Of note, deletion of glucocorticoid receptor signaling specifically in podocytes, as well as pharmacological inhibition of glucocorticoid receptor activity, exerts similar anti-proteinuric effects. Our findings in proteinuric disease models are consistent with our previous findings in activated parietal cells, where mifepristone ameliorated glomerulopathies associated with parietal cell activation (i.e. crescentic glomerulonephritis and FSGS) similar to high-dose prednisolone. The results of the present study suggest the potential that minimal change disease might be able to be treated with GR receptor inhibition with mifepristone.

42

Mechanisms of Podocyte Dysfunction in Nephropathic Cystinosis

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Introduction and Aim: Cystinosis is an autosomal recessive disorder caused by mutations in the CTNS gene that encodes a lysosomal cystine transporter and results in lysosomal cystine accumulation. Apart from proximal tubular dysfunction, the disease is characterized by glomerular damage, with high molecular weight proteinuria and morphological changes of podocytes. We have previously shown increased urinary loss of podocytes by cystinosis patients. Here we provide the insight on the possible mechanisms of podocyte dysfunction in cystinosis.

Methods: Podocyte loss by cystinotic patients was demonstrated using qPCR analysis of urine sediments and immunofluorescent analysis of urine sediment. Mechanisms of podocyte dysfunction were studied on cultured human podocytes deriving from healthy donors and cystinotic patients with different mutations: homozygous 57 kb deletion resulting in a complete absence of protein expression or heterozygous 57 kb deletion + c.198-218 deletion, affecting part of the luminal tale of the protein, which causes juvenile form of the disease. siRNA downregulation of the CTNS gene in control podocytes was used to confirm the results.

Results: Cystinotic podocytes demonstrated increased motility in wound-healing and individual cell tracking assays. Moreover, cystinotic podocytes had impaired ability to adhere to the substrate, with paxillin-positive focal adhesion sites severely perturbed. Akt kinase is one of the key regulators of cytoskeleton and migration in podocytes. Enhanced phosphorylation of Akt1 and Akt2 kinases (p-Ser473) was detected in cystinotic cells on the Western blot. Microscopic analysis also revealed phosphorylated Akt (p-Ser473) at the leading edge of the migrating cells. The increased motility of cystinotic podocytes could not be rescued by cysteamine, the drug used for treatment of cystinotic patients, which depletes the lysosomal cystine storage. By contrast, inhibition of Akt with specific inhibitor resulted in dose-dependent decrease of motility, as shown by the individual cell tracking assay.

Discussion and Conclusions: We demonstrate podocyte dysfunction in cystinosis that can be associated with progression of this disease and can be a target of therapeutic interventions.

43

SGPL1 Mutations Lead to Sphingosine-1-Phosphate Lyase 1 Deficiency with Nephrotic Syndrome, Adrenal Insufficiency, and Ichthyosis in Humans

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Introduction: SGPL1 (sphingosine-1-phosphate lyase) is an intracellular enzyme responsible for the final step in sphingolipid breakdown, converting S1P into ethanolamine phosphate and hexadecanal. S1P functions as a ligand for G protein coupled receptors that mediate autocrine and paracrine signals controlling cell migration and proliferation. *Sgpl1* knockout mice exhibited glomerular proteinuria, mild acanthosis with orthokeratotic hyperkeratosis, and platelet activation [1].

Methods: We performed homozygosity mapping (HM) and whole exome sequencing (WES) to search for disease-causing mutations in >200 individuals with steroid resistant nephrotic syndrome (SRNS). The function and localization of SGPL1 protein were examined in cultured podocytes and mesangial cells.

Results: By HM and WES, we identified 6 recessive mutations in *SGPL1* (p.S3Kfs*11, R222Q, S346I, Y416C, E132G and R278Gfs*17) in 5 families. Affected individuals exhibited SRNS, acanthosis and orthokeratosis with facultative adrenal insufficiency. Two mutant proteins (p.R222Q and p.S346I) had decreased expression compared to wild type; however they do not affect dimerization. SGPL1 is expressed in podocytes and mesangial cells. Knockdown of *SGPL1* in mesangial cells yielded decreased migration, but did not cause apoptosis or proliferation defects. Knockout of *sply*, the *SGPL1* orthologue in Drosophila, leads to nephrocyte foot process effacement (see S. Goncalves abstract).

Conclusions: We have identified *SGPL1* mutations as a novel cause of SRNS, skin involvement and adrenal insufficiency. *SGPL1*mutations seem to affect mainly mesangial cells.

Reference

1 Schumann J, Grevot A, et al: Toxicol Pathol 2015;43:694–703.

44

A Homozygous Missense Variant in *ARHGAP24* in a Child with End Stage Renal Disease Due to Steroid Resistant Nephrotic Syndrome

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Introduction: Steroid resistant nephrotic syndrome (SRNS) accounts for 10–20% of idiopathic nephrotic syndrome of childhood, and is a leading cause of pediatric end stage renal disease (ESRD). Renal histology of SRNS is often compatible with focal segmental glomerulosclerosis (FSGS). Although the pathogenesis of SRNS is mostly idiopathic, approximately 30% of cases presenting before 25 years of age are attributed to single gene defects associated with podocyte disruption. We report for the first time a homozygous variant in *ARHGAP24*, encoding the Rho GTPase Activating Protein 24, in a 10-year-old boy with ESRD due to SRNS.

Methods: Mutation analysis was employed on Illumina NextSeq500, using the TruSight One (Illumina) sequencing panel, targeting 4813 disease-related genes. Data analysis focused on SRNS/FSGS related candidate genes. Rare nonsynonymous variants were prioritized using the VarElect tool, and verified by conventional Sanger sequencing.

Results: The patient was born to healthy, first-degree cousins of Arab-Muslim origin, with no family history of kidney disease. He presented with SRNS at 4 years of age, and rapidly progressed to ESRD. Kidney histology was compatible with IgM nephropathy and mesangial hypercellularity. Sequence analysis in the patient ruled out mutations in most genes related to SRNS/FSGS, but revealed a homozygous missense variant in *ARHGAP24* (c.1442C>T; p.T481M), that was carried in a heterozygous state in his healthy parents. The p.T481M variant was observed in The Exome Aggregation Consortium (ExAC) database in 118 heterozygous alleles, with no reported homozygotes.

Discussion: *ARHGAP24* encodes a RhoA-activated Rac1 GTPase-activator implicated in podocyte actin remodeling and membrane dynamics. A heterozygous mutation of functional significance in *ARHGAP24* (Q158R) has been reported in autosomal dominant familial FSGS. Additionally, heterozygous nonsynonymous *ARHGAP24* variants, including the p.T481M variant identified in our patient, were reported in individuals with biopsy-prov-

en FSGS. Nevertheless, functional analysis of these variants was not performed, and phenotypic segregation in familial cases could not be reliably established. Hence, the role of most *ARHGAP24* variants in the pathogenesis of SRNS/FSGS remains obscure. Our finding of the homozygous p.T481M variant in *ARHGAP24*, in association with early-onset SRNS is intriguing. Further functional analysis is needed in order to establish the role of bi-allelic *ARHGAP24* mutation, in general, and the homozygous p.T481M variant, in particular, in the pathogenesis of hereditary SRNS.

45

Podocytes Micro-RNA-143 Dependent Down Regulation of the Glycocalyx Proteins Causes Impairments in Glomerular Filtration Barrier

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Introduction: Next to podocytes, fenestrated endothelial cells and the glomerular basement membrane (GBM) the glycoclayx is an important component of the glomerular filtration barrier. General loss of the glycocalyx can cause proteinuria. However, about the regulation and function of individual glycocalyx components is not much known yet. Micro-RNAs (miRs) play an important role in gene regulation by downregulating their targets.

Methods: Baseline and stress-induced miR-profiles of cultured human glomerular cells as well as miR-profiles of urine samples of patients with different glomerular diseases were screened for overlapping expression of miRs. Regulation of miR target genes was analysed concerning their importance for the glomerular filtration barrier in cultured glomerular cells as well as in a zebrafish model.

Results: MiR-143 was not only specifically upregulated in cultured human podocytes after TGF-beta stimulation but was also detectable in high amounts in urine samples of patients with membranous glomerulonephropathy. Targets of miR-143 are the glycoclayx proteins versican, syndecan 1, 3 and 4. In cell culture syndecan 1 and 4 were predominantly expressed in podocytes, while syndecan 3 was predominantly expressed in glomerular endothelial cells. Versican could be detected in both cell types.

After injection of a miR-143 mimic in zebrafish at one to four cell stage, syndecan 3, syndecan 4 and versican were significantly down regulated. Moreover, the miR-143 mimic caused proteinuria, edema as well as impairment on the epithelial and endothelial side of the glomerular filtration barrier. Injection of a specific versican morpholino also caused proteinuria and edema but the main ultrastructural glomerular changes were seen on the epithelial side with podocyte effacement. In contrast, knockdown of syndecan 3 and syndecan 4 had no effects on glomerular filter function or ultrastructure.

Conclusion: A tight regulation of versican and syndecan isoforms is needed for proper barrier function that can be regulated by podocyte descending miRs. Downregulation of glycocalyx proteins by miR-143 not only can cause proteinuria but also glomerular endothelial cell and podocyte damage.

46

Chemical Induction of Proteinuria in Larval Zebrafish Using PAN and ADR

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Background: Zebrafish have become a widely used model organism in glomerular kidney research. Developing a method that produces a standardized glomerular proteinuria phenotype in zebrafish through treatment via the fishwater would be an asset to high throughput testing of potential beneficial drugs. Thus far such a method does not exist.

Methods: We induced glomerular proteinuria phenotypes in zebrafish of different genetic backgrounds by treating the zebrafish embryos with Puromycin Aminonucleoside (PAN) or Adriamycin (ADR) in the fishwater at varying timepoints. Treatment with PAN and ADR was conducted at timepoints from 44hpf to 50hpf. Different crosses of Tg(l-fabp:DBP-EGFP) zebrafish backcrossed onto AB or *nacre* background were examined.

GFP fluorescence content was measured in 96hpf embryos' eyes as a readout of their proteinuria phenotype.

Results: Embryos homozygous for the *nacre* mutation were more susceptible to PAN and ADR treatment compared to embryos with an AB background. Moreover, we noted that a treatment at 46hpf reliably yields consistent phenotypes. Treatments at later timepoints were less effective in proteinuria induction. This specific line crossing is a good starting point for testing of drugs potentially beneficial for the treatment of proteinuria.

Conclusion: The basis of *nacre* is a mutation in the *Mitf* transcription factor regulating the formin-homology protein Dia1. Further studies are on the way to examine the relation between the *Mitf* mutation and a higher susceptibility for a disruption of the filtration barrier.

47

Congruency and Mechanism of APOL1 Toxicity in *S. cerevisiae*

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Background: APOL1 risk variants (G1 and G2) are associated with progressive Chronic Kidney Disease (CKD) in the African ancestry population. We demonstrate that APOL1 G1 and G2 human disease risk variants display a congruent pattern of differential toxicity in *S. cerevisiae* and explore the mechanism of APOL1 toxicity by genetic intervention.

Methods: Plasmids inducible for expression of the human APOL1 variants – G0, G1, G2, the artificial APOL1 constructs C-terminalΔ (C-tr), S342G and I384M, under the yeast GAL1 promoter, were transformed into WT and to various specific endocytic, autophagic, Vma and Trk deleted yeast strains, to dissect the pathways that enhance APOL1 toxicity. APOL1 variants were tagged with mCherry for localization studies. Vacuole acidification was demonstrated by quinacrine staining. The VPS pathway was characterized by the localization of GFP-tagged Vph1.

Results: Induction of human APOL1 caused reduced yeast viability, with a markedly greater effect of the G1 and G2 variants compared to G0. The C-terminal truncated construct was innocuous to yeast viability and the S342G mutation alone without the I384M mutation was sufficient to mimic full G1 increased toxicity. Impairment of endocytic organelle acidification was most evident in the G1 and G2 variants. *trk2Δ* which disrupts potassium influx, also aggravates the yeast viability loss in the kidney risk variants. Variants which disrupt APOL1 trafficking to the vacuole display markedly enhanced toxicity of G0, G1 and G2.

Conclusions: We demonstrate that differential toxicity of APOL1 kidney disease risk and non-risk variants is conserved in *S. cerevisiae* and is dependent on an intact C-terminus. APOL1 impairs endocytic trafficking and the kidney disease risk variants impair endocytic organelle acidification more than the G0, and in turn, diversion from or interruption of vacuole acidification augment toxicity. These findings place the endocytic trafficking and the acidifying compartment of the cell at a key juncture in APOL1 eukaryotic cell toxicity.

48

Biological Mechanisms Underlying African American Kidney Disease Risk: APOL1 Messenger RNA Renal Risk Variants Activate Protein Kinase R (PKR) and Reduce Cell Protein Synthesis

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Background: Coding variants in APOL1 S342G and M384I (G1) and NYK388K (G2), compared to ancestral (G0) are strongly associated with focal segmental glomerulosclerosis (FSGS). APOL1 (apolipoproteinL1) circulates as HDL component and is also expressed in podocytes and microvascular cells. Wiggins and colleagues showed that in transgenic rats, suppression of protein translation via expression of a dominant-negative 4E-BP1 in podocytes leads to FSGS. We asked whether APOL1 variants might affect global protein synthesis.

Methods: For *in vitro* assay, we generated stable HEK293 cell lines with recombinant *APOL1 variants*. Using synthetic RNA, we conducted PKR activation assay and selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) for functional RNA experiments. For *in vivo* studies, nephrin-promoter driven G1 stem-loop only RNA transgenic mice were generated.

Results: In APOL1 over-expressing HEK293 cells, phosphorylated PKR (the active form) and inactive eIF2α (a key molecule for protein translation initiation) were increased, and global protein synthesis was reduced with the *G1* and *G2 variants* (G1,–20.0%, G2, –27.6%) compared to cells that were transfected with the *G0 variant*. PKR was activated by long dsRNA, which is absent in normal eukaryotic cells but is present following certain viral infections. *G0 mRNA* did not activate PKR, but *G1* and *G2 mRNA* was able to activate PKR *in vitro* independent of APOL1 protein expression. We used selective SHAPE to probe RNA secondary structure and the results suggested that there are long double-strand RNA (dsRNA) structures (> 32 nc) in *G1* and *G2 mRNA* that are absent in *G0 mRNA*; these dsRNA may activate PKR. Finally, *APOL1-G1 stem-loop* only (non-protein coding) transgenic mice, showed increased proteinuria in the puromycin-FGF2 nephrotic model.

Conclusions: These results suggest that the risk *APOL1 variant mRNAs* activate PKR and reduce protein synthesis in podocytes and enhance podocyte susceptibility to stress in vivo. These effects appear to be mediated by RNA structure. The reduced protein synthesis plausibly contributes to impaired podocyte function.

49

Transglutaminase 2 Interacts with IGG and Affects Immune-Complex Deposition and Podocyte Loss in Membranous Nephropathy

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Idiopathic Membranous Nephropathy (iMN), the most common cause of nephrotic syndrome in adults, is characterized by the subepithelial deposition of circulating IgG_4 antibodies targeting podocyte surface proteins. This leads to thickening of the glomerular basement membrane (GBM) and podocyte injury following complement activation. However, the mechanism by which subepithelial immune-complexes are retained to the GBM is still unknown. We found that the crosslinking enzyme transglutaminase 2 (TG2) was overexpressed in the glomeruli of iMN patients, specifically in podocytes, where it colocalized with IgG. The interaction between TG2 and human IgGs was further validated *in vitro* by ELISA, surface acoustic wave technology and co-immunoprecipitation. Using a mouse model of anti-podocyte nephritis (APN), we showed that deletion of TG2 attenuated disease progression with reduced albuminuria, podocyte loss and GBM thickening. APN mice also demonstrated overexpression of TG2 in the GBM and subepithelial space, where it closely interacted with IgG as observed by fluorescence resonance energy transfer. Interestingly, IgG deposition was delayed in TG2KO mice. Moreover, stimulation of cultured primary podocytes with mouse serum containing anti-podocyte IgG showed decreased binding of IgG in the surface of podocytes in the absence of TG2. TG2 was also involved in complement deposition as demonstrated by diminished C3 glomerular deposition in TG2KO animals than in wild type counterparts during APN. C3 markedly colocalized with TG2 in APN kidneys and biopsies from iMN patients. In conclusion, these results suggest that TG2 binds to IgGs and plays a role in the retention of immune-complexes in the subepithelial space, thus influencing podocyte loss and renal dysfunction. The mechanisms involving the impact on C3 deposition are still to be deciphered. These data open new perspectives in the modulatory role of TG2 in the pathophysiology of iMN and would suggest the use of TG2 as target for therapeutic intervention.

50

ZEB2-Natural Antisense Transcript Mediate Epithelial Mesenchymal Transition of Podocytes: Implications in the Pathogenesis of Nephropathy

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The podocytes are highly specialized, terminally differentiated cells offering epithelial coverage to the glomerular capillaries. These podocytes form a major size selective barrier for the filtration of serum proteins whereas reduced podocyte number is a critical event in the pathogenesis of proteinuric diseases including nephropathy. Several cellular and endocrine factors whose levels are elevated during diabetes mellitus including growth hormone (GH), Transforming growth factor, beta-induced (TGFBI), and carboxymethyl lysine (CML) were implicated as causative factors in the development of nephropathy. However, the precise mechanism of their action on podocytes remains to be elucidated. To exemplify the molecular basis for the effects of GH, TGFBI and CML on the podocytes, we conducted PCR-array analyses of immortalized human podocytes exposed to these factors independently and identified that zinc finger E-box-binding homeobox2 (ZEB2) to be up-regulated. ZEB2 orchestrates epithelial-mesenchymal transformation (EMT), during which cell-cell and cell-extracellular matrix interactions are feeble and enable epithelial cells to become invasive. We established that increase in ZEB2 levels is associated with increased transcription of ZEB2 natural antisense transcript (ZEB2-NAT), which is required for efficient translation of the ZEB2 transcript by preventing the cleavage of internal ribosomal entry site. Elevated ZEB2 expression suppressed E- & P-cadherins levels, whereas mutation of ZEB2 binding sites on the E-cadherin promoter abolished this effect of ZEB2 on the E- & P-cadherin expression. ShRNA-mediated knockdown of ZEB2 expression abrogated altered podocyte permeability to albumin in a paracellular albumin influx, thus preserving podocyte function. We also have noticed elevated expression of ZEB2 and decreased podocyte count in diabetic rat glomeruli. We conclude that GH, TGFBI and CML induce expression of ZEB2-NAT and ZEB2; consequent switch in cadherins, which implicate in EMT and decreased podocyte count that observed during nephropathy.

51

The Regulation of AMP-Activated Protein Kinase Signaling by Protein Kinase G in Cultured Rat Podocytes

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AMP-activated protein kinase (AMPK) is postulated to be a cellular energy sensor that plays a role in restoring energy homeostasis by activating ATP generation and inhibiting ATP consuming pathways. AMPK also play an important role in modulating cell polarity, permeability and immune responses independent of intracellular energy levels. AMPK is central in controlling the metabolism of glucose and fatty acids, its role in obesity and type-2 diabetes is a major importance. The AMPK is expressed in various mammalian, including the kidney and podocytes; however, its pathophysiological role in podocytes, especially in the context of insulin resistance, is not well recognized. Insulin increases activation of protein kinase G type Ia (PKGIa) subunits, leading to podocyte dysfunction. Here we investigated whether AMPK is involved in regulation of filtration barrier permeability in PKGIα-dependent manner.

We measured glomerular permeability by measuring glomerular capillary permeability to albumin in isolated glomeruli from Wistar rats and transmembrane albumin flux in cultured rat podocytes. Expression of AMPKα, PKGIα and upstream proteins was confirmed in the podocytes using RT-PCR, Western blot and immunofluorescence. The AMPKα-PKGIα interaction was confirmed by co-immunoprecipitation.

We demonstrated that PKGIα and AMPK are mutually regulated in podocytes. Using siRNA directed against PKGIα we observed degrease in AMPKα expression about 32% (from 0.44 ± 0.02 to 0.30 ± 0.02, n = 3, $P < 0.05$). We also observed increase AMPKα protein co-immunoprecipitated with PKGIα in lysates after PKGIα activation (oxidative stress, H2O2 or activator PKGI, 8-Br-cGMP). We confirmed the role of AMPK in insulin-evoked increases in albumin permeability in podocytes with PKGIα siRNA. Overall we have identified a potentially important new mechanism that may be injurious during diabetes in podocytes and affect filtration barrier permeability.

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52

The Role of VASP in Regulation of Cytoskeleton and Filtration Barrier Permeability in Cultured Rat Podocytes

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Podocytes are dynamic polarized cells that lie on the surface of glomerular capillaries and comprise an essential component of the glomerular filtration barrier. Vasodilator-stimulated phosphoprotein (VASP) family members have subsequently emerged as important regulators of actin assembly and cell migration. A little is now about VASP signaling in podocyte function. Podocyte morphology, function and the actin cytoskeleton are closely linked. Disruption of this highly specialized podocyte cytoskeleton is closely associated with a disease phenotype, resulting in foot process effacement. We demonstrated recently that insulin increases activation of protein kinase G type Ia (PKGIa) subunits, leading to podocyte dysfunction. Here we investigated whether VASP is involved in insulin- or high glucose-dependent regulation of cytoskeleton and filtration barrier permeability in PKGIα-dependent manner.

We assessed changes in insulin-induced glomerular permeability by measuring transmembrane albumin flux in cultured rat podocytes. Expression of VASP, PKGIα and upstream proteins was confirmed in the podocytes using RT-PCR, Western blot and immunofluorescence.

In this study we showed that podocytes exposure to exogenous insulin (300 nM) caused an increase in VASP protein expression about 46% (from 0.80 ± 0.02 to 1.17 ± 0.06, n = 4, $P < 0.05$). When podocyte were exposed to high glucose concentration we also observed increase in VASP expression about 34% (from 0.68 ± 0.06 to 0.91 ± 0.03, n = 4, $P < 0.05$). This effect was abolished in the presence of PKG inhibitor (Rp-8-cGMPS; 100 μM). Moreover we confirmed the role of VASP in insulin-evoked increases in albumin permeability in podocytes with VASP siRNA.

Thus, we propose that an insulin or high glucose induced imbalance in PKGI activity may affect in the VASP expression. These effects appeared VASP as a possible target witch phosphorylation or dephosphorylation through PKGIα may affect podocyte permeability.

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53

Whole Exome Sequencing Identifies Advillin Mutations as a Novel Single-Gene Cause of Nephrotic Syndrome

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Introduction: Steroid resistant nephrotic syndrome (SRNS) is a frequent cause of end-stage renal disease in the first decades of life. Identification of single-gene causes of SRNS has furthered the understanding of its pathogenesis.

Methods: We combined homozygosity mapping with whole exome sequencing (WES) in 100 families with SRNS. To identify additional mutations, we screened our cohort of ~800 individuals with SRNS by microfluidic multiplex PCR (Fluidigm Access Array™) and next generation sequencing. We performed functional analysis of wild type and mutant proteins in human podocytes.

Results: By WES and next generation sequencing, we identified 4 mutations of the AVIL (advillin) gene in three unrelated families with SRNS. A homozygous missense mutation in AVIL was found in an individual of consanguineous parents with SRNS, deafness, cataracts, microcephaly, mental retardation and renal histologic identification of diffuse mesangial sclerosis. The other two individuals had compound heterozygous mutations in AVIL. Advillin is a member of the gesolin superfamiy of actin binding proteins with 6 domains, a C terminal cap and three PIP2 binding motifs. It is known to be involved in neurite outgrowth and morphogenesis. We show that advillin localizes to WT1 positive podocytes in adult rat kidney. When the truncation mutant allele (p.Val656fs.7*) transfected in human podocytes, advillin failed to reorganize F-actin in podocytes which are comparable to the mutant construct AVIL-Δ628-819 (deletion of the C terminal domains). The mutant allele p.Arg135Gln results in redistribution of F-actin with minimal colocalization of advillin in podocytes consistent with the phenotype when transfected with the truncation mutant of AVIL-Δ 135-143(PIP2 binding motifs) construct.

Conclusion: We identified mutations of AVIL as a novel monogenetic cause of SRNS. Further genetic and functional studies will shed light on the gesolin superfamily of actin binding proteins in the pathogenesis of NS and will provide further the understanding its disease mechanism.

54

High Affinity Interaction of Apolipoprotein L1 (APOL1) and Integrin β3 Is Linked to Soluble Urokinase Plasminogen Activator Receptor (suPAR)

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Recent clinical reports show that genetic variants, G1 and G2, of the Apolipoprotein L1 (*APOL1*) gene in African Americans are strongly associated with an increased risk of non-diabetic chronic kidney disease. However, the underlying mechanism associated with the genetic variants in developing a certain kidney disease remains unclear. Several reports suggested APOL1-associated modifiable environmental factors (i.e., HIV, interferon) as secondary, immunological factors for developing APOL1-associated kidney disease. Soluble urokinase plasminogen activator receptor (suPAR) is typically a three Lys-6 domain containing protein characterized as immunological signaling molecule but two domain versions and splice variants additionally exist. High suPAR levels are found in the blood of a subset of FSGS patients and can cause focal segmental glomerulosclerosis (FSGS) like manifestations in mice by activating podocyte integrin αvβ3. To examine a possible association of APOL1 and suPAR, we transfected *APOL1 wild type*, *suPAR*, or *integrin β3* in HEK-293T cells, and measured the interaction of APOL1:suPAR and APOL1:integrin β3 by co-immunoprecipitation. APOL1 wild type protein binds both suPAR and integrin β3. To further investigate whether purified APOL1 proteins (wild type and variants; WT and G1, G2) directly interact with suPAR (D1D2 or D1D2D3) or integrin αvβ3, we performed surface plasmon resonance (SPR). Experiments show that (1) APOL1 proteins, irrespective of APOL1 variants, directly bind suPAR (D1D2 and D1D2D3), and (2) APOL1 proteins bind strongly to integrin αvb3. Interestingly, the strength of the binding affinity between APOL1 and integrin αvb3 depends on the activation status of integrin αvb3. Once integrin αvβ3 is activated, the binding is increased by more than a 100-fold (K_D = ~6.6 nM), compared with inactivated αvβ3. The binding of APOL1 protein to integrin αvβ3 is specific to αvβ3, and was not found to bind α3β1. Taken together, these results identify integrin αvb3 as a novel high affinity binding partner of APOL1. Furthermore, our data suggest a cooperation of genetic risk (APOL1) and immunological risk (suPAR) to both converge on integrin αvβ3 activation in human podocytes.

55

Podoplanin and Synaptopodin Being Upregulated in Allergic Inflamed Esophageal Epithelium Links Epithelial Responses in Atopy to Those in Kidney Diseases

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The pro-allergic Th2 cytokine IL-13 has been implicated in the pathogenesis of both allergy and minimal-change nephrotic syndrome, suggesting that common molecular mechanisms are implicated in their pathogenesis. Focusing on eosinophilic esophagitis (EoE), an IL-13–driven allergic inflammation of the esophagus, we show that synaptopodin (SYNPO), an actin-associated kidney epithelium-specific gene, is constitutively expressed in normal esophageal epithelium, induced during allergic inflammation (13.4 fold, $p < 10^{-5}$), and is epigenetically regulated by IL-13 in esophageal epithelial cells. Similarly, podoplanin (PDPN), another actin-associated protein relatively specific to kidney epithelium, was significantly upregulated in the esophageal biopsies of patients with active EoE compared with control individuals (2.6 fold, $p < 10^{-2}$). Immunofluorescence analysis revealed that the most basal layer of esophageal epithelium in contact with the lamina propria expressed both proteins regardless of disease status. In EoE, despite both proteins having elevated expression in the basal layer, only SYNPO was detected in suprabasal layers of the epithelium. Western blot of esophageal biopsies revealed markedly elevated expression of SYNPO protein in EoE samples (~25 fold vs. controls, $p < 0.004$). *SYNPO* was transcriptionally induced by IL-13 in esophageal epithelial cells in a STAT6-dependent manner. Moreover, epigenetic analysis revealed significant increases in activating epigenetic marks (histone H3 lysine 9, 27 acetylation and lysine 4 trimethylation) in the promoter of *SYNPO* following IL-13 stimulation. Immunofluorescence studies in primary esophageal epithelial cells and cell lines showed that SYNPO was co-localized with actin filaments. Accordingly, shRNA-mediated gene silencing of *SYNPO* in esophageal epithelial cells reduced wound closure in a wound healing model *in vitro*.

In summary, we propose that the classic kidney markers, SYNPO and PDPN, are IL-13–regulated genes involved in allergic inflammation. Epithelial responses to immune stimuli involving these proteins unify pathogenic kidney responses with allergic responses and begin to unravel early linkage between certain kidney diseases and atopy.

56

Insulin Induces Changes in RhoA Phosphorylation through Activation of PKGIA-Dependent Signaling Pathways in Cultured Rat Podocytes

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Insulin could dynamically remodel the actin cytoskeleton of podocytes, and this was critically important in maintaining the integrity of the glomerular filtration barrier. Recently, we have demonstrated that insulin increases albumin permeability of both isolated rat glomeruli and podocytes. Rho family GTPases, modulators of cytoarchitecture, is expressed in podocytes. Thus changes of Rho GTPases activity may affect glomerular permeability for albumin leading to albuminuria/proteinuria, a sign of kidney disease and independent risk factor for the progression of renal failure. Here we investigated whether RhoA is involved in insulin-dependent regulation of cytoskeleton and filtration barrier permeability in PKGIα-dependent manner.

We evaluated changes in insulin-induced glomerular permeability measuring glomerular capillary permeability to albumin in isolated glomeruli from Wistar rats, and transmembrane albumin flux in cultured rat podocytes. Expression of RhoA, PKGIα and upstream proteins was confirmed in the podocytes using Western blotting and immunofluorescence.

The PKGIa induces phosphorylation of RhoA at Ser188, leading to the reduction of RhoA kinase activity. We hypothesized that insulin induces changes in RhoA phosphorylation through activation of PKGIα-dependent signaling pathways. To test this hypothesis, podocytes were incubated with insulin (300 nM) and PKGI activators (8-Br-cGMP and H2O2, 100 μM). Insulin treatment increased the basal level of phosphorylated RhoA by 107%. H2O2 and 8-Br-cGMP also increased phosphorylation of RhoA in podocytes by 89% and 56%, respectively. Moreover, the quantitative analysis confirmed that insulin and PKGIα activators directly increase the F-actin immunostaining in the vicinity of the plasma membrane while having little effect on the intracellular labeling.

These data may suggest that the inhibition of RhoA has a positive impact on podocyte morphology and function. We therefore hypothesized that PKGIα regulates the balance between contractility and relaxation (permeability) of the podocyte barrier by the regulation of actin cytoskeleton and that this mechanism could be disrupted in diabetes.

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57

SDF-1/CXCL12 Regulates Notch Signaling in Podocytes and Podocyte Progenitors and Is a Therapeutic Target to Accelerate Podocyte Regeneration after Glomerular Injury

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Stromal-derived factor (SDF)-1/CXCL12 is a homeostatic chemokine facilitating the homing and activation of stem cells. It is unclear, why podocytes constitutively secrete CXCL12 and why CXCL12 blockade can attenuate progressive glomerulosclerosis. We speculated on a role of CXCL12 in regulating podocyte survival and podocyte regeneration. In vitro CXCL12 protected human podocytes from toxic injury and in vivo blockade of intrinsic CXCL12 aggravated proteinuria and podocyte loss in two different mouse models of toxic podocyte injury. However, continued blockade of intrinsic CXCL12 during the course of adriamycin nephropathy significantly attenuated podocyte loss, proteinuria, and glomerulosclerosis. Indeed, CXCL12 blockade significantly increased podocyte numbers not only as compared to vehicle-treated controls but also versus the earlier time point of injury, implying that intrinsic CXCL12 suppresses podocyte regeneration. To study this aspect we used human renal progenitor cells with the capacity to differentiate into mature podocytes. CXCL12 suppressed progenitor growth and their induced differentiation into podocytes, which could be reversed by the same CXCL12 inhibitor that improved outcomes in mice. This effect depended on CXCL12 suppressing of Notch signaling in podocyte progenitors. We conclude that podocyte-derived CXCL12 has different biological functions during podocyte injury. First, CXCL12 serves as a podocyte survival factor. Second, CXCL12 promotes podocyte progenitor quiescence, vice versa, CXCL12 inhibition might podocyte regeneration, as it improves outcomes in toxic (as shown here) and diabetic (as shown by us before) podocyte injury. This finding may have important clinical implications.

58

APOL1 High Risk Genotypes Are Associated with Preterm Birth in Patients with Proteinuric Disease

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Individuals harboring a high risk (HR) apolipoprotein L1 (*APOL1*) genotype have greatly increased risk of focal segmental glomerulosclerosis (FSGS). The FSGS phenotype associated with a HR genotype is incompletely penetrant. Identifying factors that modify the penetrance of the risk alleles may be important clinically while also providing clues to the biological mechanisms that mediate their harm.

We studied characteristics associated with *APOL1* risk genotypes in 110 black participants with proteinuric disease enrolled in the Nephrotic Syndrome Study Network (NEPTUNE). Those with a HR genotype had 4.1 increased odds of preterm birth (CI: 1.2, 16.5). This association was replicated in an independent cohort of 56 black children with glomerular disease from the Chronic Kidney Disease in Children (CKID) cohort (OR [CI] = 9.6 [1.1, 85]). The HR genotype was not associated with lower birthweight.

These results raised questions about the relationships between prematurity, proteinuric disease, and *APOL1* genotypes. Does prematurity act as a 'second hit' to an *APOL1*HR genotype, increasing risk of proteinuric disease? Or, given the known racial disparities in preterm birth, Apol1's high placental expression, and maternal complications in model systems, is a HR genotype directly involved in prematurity? To address this, we analyzed available *APOL1* genotype and epidemiologic data from two population cohorts of African-American mothers and their term or preterm infants (GENEVA Study of Preterm Delivery & Boston Medical Center GWAS of Preterm Birth; n = 2418). We performed multivariable analyses testing for association between HR APOL1 and birth outcomes. We found no association between maternal

and/or infant HR *APOL1* genotype and prematurity, gestational age, or birth-weight in these population cohorts.

We conclude that our observed association between HR APOL1 and prematurity appears specific to those with glomerular disease. Further work is needed to identify mechanisms underlying this association and to determine if preterm children with a HR genotype are at increased risk of proteinuric disease.

59

The Role of miRNA-636 in Glomerular Diseases

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In an extended urine and glomerular cell screen we detected miRNA-636 specifically regulated in the urine of patients suffering from membranous nephropathy, FSGS and Alport's disease. We therefore suspected a pathophysiological role for this miR in these diseases. To determine the relevance in developing glomerulopathies, we used our zebrafish model for proteinuria. The injection of miRNA-636 at 0 hours post fertilization (hpf) lead to loss of high molecular weight proteins from the circulation leading to pericardial effusion and yolk sac edema suggesting proteinuria as the underlying cause. Cardinal vein injection of miRNA-636 at 48 hpf resulted in an equivalent loss of fluorescence confirmed a non-developmental cause. Electron microscopy imaging revealed foot process effacement after treatment with miRNA-636 supporting its role in affecting the glomerular filtration barrier. The underlying mechanism is not fully understood so far. Promising predicted targets of the miRNA-636 are Cdc42se2 (Cell division cycle 42 small effector 2) and SENP1 (SUMO-specific protease 1). The small GTPase Cdc42 is an important player in the organization of the actin cytoskeleton in podocytes in the network of other small GTPases, while Cdc42 mediates predominantly the motility phenotype. Small ubiquitin-related modifier (SUMO) is a transient and reversible posttranslational dynamic protein modifier which effects activity and localization of receptors and membrane proteins. SENPs can be reverse SUMOylation and thus have an important impact on podocytic protein functions. SENP1 in particular has been shown to be highly expressed in podocytes along with HIF-1α and VEGF under hypoxia conditions. Therefore we conclude that miRNA-636 may play a relevant role in the development of glomerular diseases and hence might function as a target of podocyte directed therapeutic strategies.

60

Proteinuria Detection in Zebrafish Larvae Using High Sensitivity Protein Chip Analysis and Tandem Mass Spectrometry

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Introduction: Proteinuria is a hallmark of glomerular diseases revealing damage to the glomerular filtration barrier. To assess the degree of proteinuria, we previously used our transgene based zebrafish model with an indirect detection method. Our current approach using proteomics techniques is to detect impairment of the glomerular filtration barrier by measuring excreted plasma proteins in the fish water after genetic or pharmacological challenge.

Methods: Zebrafish larvae were either injected with CD2AP morpholino or control morpholino or were treated with either puromycin, adriamycin or DMSO in fish water. To measure proteinuria indirectly, we used a transgenic fish expressing a fluorescent plasma protein that can easily be quantified in retinal vessels. In addition, fish water was analyzed using high sensitivity protein chips (Bioanalyzer, Agilent Technologies) to directly measure proteinuria and determine the molecular weight of the excreted proteins as well as tandem mass spectrometry to identify excreted proteins.

Results: Knockdown of CD2AP and treatment with adriamycin and puromycin caused a phenotype with edema at 120 hours post fertilization. In our indirect assay, the circulating fluorescence decreased in CD2AP morpholino injected fish as well as after treatment with adriamycin and puromycin compared to controls. In the direct assay, we were able to detect the loss of proteins of different molecular weights in water of zebrafish that showed decreased fluorescence in our indirect method. A loss of higher molecular weight proteins was detected in CD2AP knockdown larvae as well as in adriamycin and puromycin treated fish. Mass spectroscopy analysis revealed that the most abundant protein group was the 150 kDA fraction of vitellogenin subtypes identifying vitellogenin 1 as predominant protein. Serotransferrin was measured as the most abundant protein in the 70 kDa fraction.

Conclusions: Glomerular and tubular proteinuria in zebrafish larvae can be detected reliably using a direct proteomics techniques or an indirect assay with a transgenic fish expressing a fluorescent plasma protein.

61

PAR3A Is Dispensable for the Function of the Glomerular Filtration Barrier of the Kidney

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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. To study the contribution of Par3A in this complex we generated a novel Par3A podocyte-specific knockout mouse model by targeting exon 6 of the Par3A gene. Genetic deletion of Par3A did not impair renal function neither at birth nor later in life. Even challenging the animals did not result in glomerular disease. Despite its well-established role in aPKC-mediated signaling Par3A appears to be dispensable for the function of the glomerular filtration barrier. These results raised the question, if another Par3 variant might compensate for the Par3A loss. Our mRNA seq data from FACsorted primary podocytes revealed high levels of Par3B in podocytes, which are much higher than Par3A levels. Interestingly, Par3B seems to localize to the slit diaphragm, proven by immunohistological analysis and immunogold-labelling. Furthermore, we were able to show increasing Par3B levels in murine immortalized mouse podocytes during differentiation, which implies a crucial function of Par3B in differentiated cells. In addition, we study effects of a nephrocyte specific bazooka (Par3-homolog) knockdown in the model organism Drosophila melanogaster. Here we can show, that decreased levels of bazooka in nephrocytes cause severe filtration defects.

In conclusion, Par3A function is either dispensable for slit diaphragm integrity or compensatory mechanisms through its homolog Par3B maintain the function of the glomerular filtration barrier even in the absence of Par3A.

62

APOL1 Kidney Risk Variants Toxicity Is Conserved in *Drosophila melanogaster* via an Intracellular Rather Than Systemic Mechanism

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Background: APOL1 risk variants (G1 and G2) are associated with progressive chronic kidney disease (CKD) in the African ancestry population. The molecular pathogenesis underlying this increased risk has not yet been fully elucidated. We demonstrate that APOL1 G0, G1 and G2 human disease risk variants display a congruent pattern of differential toxicity in the *Drosophila melanogaster* experimental system which is readily amenable to genetic interrogation and screening.

Methods: We generated transgenic *Drosophila melanogaster* expressing wild type (G0) and mutants (G1 and G2), as well as the artificial C-terminal truncated variant under the control of UAS/GAL4 system. We used the ubiquitous Daughterless (*da*-GAL4) driver as a model for systemic APOL1 expression and the eye Glass multiple reporter (*GMR*-GAL4) driver, as a model for tissue specific expression. We investigated the specific phenotypes under each driver.

Results: Expression of human APOL1 under strong ubiquitous driver (*da*-GAL4) causes >90% lethality of the flies expressing risk variants (G1 and G2) versus G0 or C-terminus truncated APOL1, which displayed no such phenotype. Expression of APOL1 under *GMR*-GAL4 caused a rough eye phenotype with severely disrupted retinal structure in ~65% of G1 and G2 expressing flies compared to 8% in G0 flies. APOL1 G0, G1, and G2 gene products were expressed in the heads and not in the bodies of adult flies, under the *GMR*-GAL4, implicating an intracellular rather than circulating source of APOL1 in pathogenesis of cell injury.

Conclusions: APOL1 G1 and G2 human disease risk variants display a congruent pattern of differential toxicity in the *D. melanogaster* experimental system, with an intracellular mechanism. The findings suggest that APOL1 mediated cell injury involves perturbation of core pathways which are highly conserved across eukaryotic evolution. This sets the stage for genetic modifier and high throughput compound screens to ameliorate APOL1 risk allele disease.

63

4-D in vivo Imaging of Podocytes in a Zebrafish Injury Model

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In the past, it was postulated that podocytes are able to migrate along the glomerular basement membrane (GBM), especially in podocytopathies. Recently, we have shown that podocytes in healthy zebrafish larvae neither migrate along the GBM nor show motility of their processes (Endlich et al. 2014), remaining static over up to 23 h. In this study, we wanted to clarify whether podocytes exhibit a migratory phenotype after the induction of injury.

To study this in vivo, we used the NTR/MTZ zebrafish model expressing mCherry and nitroreductase (NTR) which converts the prodrug metronidazole (MTZ) to a cytotoxin specifically in podocytes (Zhou et al. 2012). For *in vivo* observation by 2-photon microscopy (2-PM) this zebrafish strain was crossed with the translucent zebrafish *Casper* and named *Cherry*. The application of MTZ (5 mM) to *Cherry* larvae (3 days post fertilization) for 20 h induced apoptosis which was demonstrated by TUNEL assay. We found decreased levels of nephrin and podocin mRNA measured by qRT-PCR. Moreover, we observed foot process effacement of the remaining podocytes and a widespread denudation of the GBM shown by immunohistochemistry and electron microscopy.

Since podocin was downregulated after MTZ application, we crossed *Cherry* with *ET* (Endlich et al. 2014) resulting in a zebrafish strain (*Chet*) with podocytes expressing additionally eGFP. Currently, we can follow the dynamics of podocytes of up to 31 larvae over 24 h simultaneously by 2-PM. In a time-dependent 3-D reconstruction of z-stacks of the glomerulus, we found that the remaining podocytes retracted their major processes and that Bowman's space dilated significantly. However, no *podocyte walking* was observed during 24 h in zebrafish larvae (n = 61).

Taken together, we show that podocytes do not migrate along naked GBM in our zebrafish injury model during the early time period of 24 h.

64

CIN85 Deficiency Prevents Nephrin Endocytosis and Proteinuria in Diabetes

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Background: Podocytes are important for the maintenance of the glomerular filter in the kidney. Podocyte damage is associated with ultrastructural changes and decreased expression of components of the slit diaphragm in many glomerular diseases including diabetic nephropathy. Nephrin, a podocyte specific protein is crucial for the intact filtration barrier. Loss of nephrin has been observed in rodent models of experimental diabetes as well as in human diabetic kidney disease. CIN85, a homologue of CD2AP, was identified as a binding partner of nephrin and mediates the nephrin endocytosis via ubiquitination in podocytes.

Methods: Using low-dose streptozotocin injection, we induced a type I diabetes in C57BL/6J wild type and CIN85Dex2 mice to examine diabetes induced dysregulation of glomerular filtration barrier and alteration of extracellular matrix. We then generated immortalized cell lines of podocytes for the CIN85Dex2 deficiency and the CD2AP knockout to examine nephrin endocytosis in both cell types on the molecular level. To inquire the impact of CIN85 and CD2AP on filtration barrier integrity in zebrafish, we examined proteinuria in zebrafish injected with capped CIN85 and CD2AP RNA.

Results: We can demonstrate that the loss of nephrin expression and onset of the proteinuria in diabetic mice correlates with an increased accumulation of ubiquitinated proteins and expression of CIN85 in podocytes. The CIN85Dex2 deficiency leads to preserved nephrin surface expression, reduced proteinuria, reduced mesangial matrix expansion and Collagen-IV deposition in glomeruli under diabetic conditions.

High glucose levels induced an increased CIN85 expression in contrast to a significantly reduced expression of CD2AP and nephrin in both murine and human podocytes. Furthermore, nephrin endocytosis by short term high glucose stimulation was examined. The CD2AP knockout podocytes, which express more full-length CIN85, showed an increased nephrin endocytosis compared to the CIN85 knockout podocytes. Moreover, overexpression of full-length CIN85 in human podocytes increased endocytosis of nephrin after high glucose stimulation. In addition, injection of capped CIN85 RNA induced a servere edema and proteinuria in zebrafish embryos which could be rescued by co-injection of CD2AP mRNA.

Conclusion: Our findings suggest that CIN85 is involved in the endocytosis of nephrin in podocytes under diabetic conditions promoting the development of glomerulopathy. Therefore CIN85 might be a novel treatment target to prevent diabetic nephropathy.

65

The Role of AMPK-Dependent Pathways in the Regulation of Filtration Barrier Permeability

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Hyperglycemia is a primary factor that disturbs podocyte function in the glomerular filtration process; this disturbance leads to the development of diabetic nephropathy, and ultimately, renal failure. The podocytes with their foot processes are important cellular layer of glomerular barrier involved in regulation of glomerular permeability. Retraction of the podocyte foot processes forming slit diaphragm is a common feature of proteinuria. At present, the correlation of retraction with the development of proteinuria in not well understood. The AMP-activated protein kinase (AMPK) is the central regulator of cellular and organismal metabolism in eukaryotes which is activated when intracellular ATP levels decrease. The activity of AMPK is suppressed in disorders associated with insulin resistance; however, its pathophysiological role in podocytes is not well recognized. In this study, we investigated the role of AMPK-dependent signaling on podocytes cytoskeleton and filtration barrier permeability.

We measured glomerular permeability to albumin (Palb) in a single isolated rat glomerulus based on the video-microscopy method, vasoconstriction of capillary network of the glomerulus, permeability to albumin across the podocytes monolayer. AMPK and upstream proteins was confirmed in the podocytes using Western blotting and immunofluorescence.

Activation the AMPK – energy sensing pathways, crucial for metabolic control – caused a decrease in permeability to albumin. The transmembrane flux for albumin decrease in the present of metformin and AICAR (AMPK activators) in the present of normal and high glucose concentration (5 days). Metformin and AICAR decrease albumin permeability in podocytes from 88.9 ± 5.0 to 53.1 ± 5.7 and to 43.9 ± 2.4 μg/ml ($P < 0.05$) in normal glucose concentration, and from 136.9 ± 12.1 to 65.3 ± 6.3 and to 95.9 ± 7.7 ($P < 0.05$) in high glucose concentration. Moreover activation of AMPK-dependent pathways led to subcortical actin reorganization in cultured rat podocytes. The experimental results suggest a molecular mechanism that could explain podocyte injury.

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66

Whole Exome Sequencing Identifies Mutations in TUBAL3 as a Novel Cause for Proteinuria Associated with Congenital Abnormalities of the Kidneys and Urinary Tract (CAKUT)

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Introduction: Proteinuria and congenital anomalies of the kidney and urinary tract (CAKUT) represent a relatively rare clinical combination. The morphogenesis of the kidney and the urinary tract is governed by genes that if mutated may lead to CAKUT, usually without accompanying proteinuria. Identifying the genetic basis of this unique condition provides a deeper insight on disease mechanisms and diagnosis.

Methods: We performed homozygosity mapping and whole exome sequencing (WES) in 20 consanguineous families. Homozygous recessive mutations within the homozygous region were further evaluated as the strongest candidates. All identified mutations were confirmed by Sanger sequencing.

Results: We identified two different homozygous mutations in the gene TUBAL3, encoding the protein tubulin, alpha-like 3, in two unrelated families. In family A3838, originally from India, we detected a homozygous truncating mutation (c.537C>A; p.Tyr179*), which results in a premature stop codon. In addition we detected a highly conserved homozygous missense mutation (c.28G>A; p.Gly10Ser) in a second family (A1347) of Kurdish descent. Both identified mutations are located in a highly conserved Tubulin domain. Tubulin, alpha-like 3 is a major constituent of microtubules and is highly conserved across different species.

Conclusions: We identified recessive mutations in TUBAL3 as a novel single-gene cause for the association of proteinuria with CAKUT. Further genetic and functional studies will help determine underline disease mechanisms.

67

Endolysosomal Disturbances as Novel Patho-Mechanisms in Kidney Disease

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The low abundance lipid PI (3,5)P2 is a crucial factor in determining membrane identity of endolysosomal structures. The interconversion of PI(3)P to PI(3,5)P2 is regulated by the Vac14 complex, represented by the scaffold protein Vac14, the associated kinase PIKfyve and the Phosphatase Fig4. Previous in vitro studies showed that lack or overexpression of Vac14 complex components result in massive vacuolization of cells. Here, we characterize a novel mouse line overexpressing Vac14 in the nephron, which subsequently develops a mild phenotype.

We generated transgenic mice (TgVac14 L156R) capable of tissue-specific overexpression of human PIKfyve binding deficient Vac14 mutant. These mice were crossed with a Six2-Cre driver line to determine the effect of increased Vac14 levels during development and maintenance of the nephron. After 4–6 months mice (Six2-Cre x TgVac14 L156R) developed a mild albuminuria accompanied by a glomerular expansion and alteration of podocyte structures. The localization of the slit diaphragm proteins Nephrin and Podocin was changed without altering its total protein expression level.

Overexpression of Vac14 in immortalized podocytes led to the formation of huge intracellular vacuoles, accompanied by an accumulation of late endosomal and autophagy markers proteins (e. g. Rab7, Lamp2, LC3-II). Surprisingly, this phenotype was weakened by Bafilomycin A1 (100 nM), indicating that inhibition of the v-ATPase, which is responsible for the acidification of endolysosomal structures, is able to modulate this effect.

Our in vitro and in vivo data indicate, that disturbances of endolysosomal trafficking or maturation are potential patho-mechanisms in kidney injury, affecting proper localization and recycling of slit diaphragm proteins. Deciphering the interconnections between acidification, endolysosomal trafficking and the understanding of the related signaling pathways could help finding new molecular targets or therapeutic approaches for the treatment of kidney diseases.

68

ADCK4 Mutations Lead to 6.25% of Cases of Proteinuric Nephropathy

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Recessive mutations in ADCK4 cause steroid-resistant nephrotic syndrome (SRNS) and experimental treatment with coenzyme Q10 may be effective. To date, about 20 families with ADCK4 nephropathy have been reported. However, there is a scarcity of data for Chinese patients with this disease. Using a multigene next generation sequencing panel of nephrotic syndrome-related 28 genes, we performed genetic analysis of 80 patients, from our single center, with SRNS or hereditary proteinuria manifested before 18 years of age. Five different ADCK4 mutations were detected in 5 patients from 5 unrelated families, with a mutation detection rate of 6.25%. We investigated the clinical and genetic aspects of these patients. Onset of disease in 1 patient was 10 days of age, ranged from 8 to 11 years of age in 3 patients, and 17 years of age in 1 patient. Two of three patients presented with SRNS, one patient with nephrotic-level proteinuria, and the remaining one with mild proteinuria. Of 4 patients undergoing renal biopsy, 3 showed focal segmental glomerulosclerosis, and 1 showed mild mesangioproliferative glomerulonephritis. Of 3 patients with follow-up information, 2 had normal renal function with follow-up of 12 to 18 months, and 1 progressed to end-stage renal disease with follow-up of 28 months. Four patients had a positive family history of proteinuria with or without end-stage renal disease. All of 5 families were non consanguineous. Mutational analysis revealed two nonsense mutations (namely p.E81* and p.R150*) and three missense mutations (namely p.S246N, p.D250H and p.R490C) in 5 patients. These five mutations were novel. Three patients had homozygous missense mutation, and two compound heterozygous mutations. DNA samples from 10 first degree relatives were available, and segregation analysis revealed 9 healthy parents a heterozygous ADCK4 mutation, and the elder brother of a patient, presented with proteinuria and end-stage renal disease, was affected with the ADCK4 homozygous mutation (p.S246N). In conclusion, congenital nephrotic syndrome can be caused by mutations in ADCK4, and ADCK4 was a frequent causative gene in Chinese childhood proteinuric nephropathy.

69

Epigenetic Regulation of Nephron Progenitor Cell Multipotency by BET Bromodomain Family Proteins

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Nephron number is determined during embryonic development and influenced, among others, by genes and nutrition. Individuals with a low nephron endowment are of greater risk to develop chronic kidney disease and ESRD. However, the role of epigenetic regulation for the determination of nephron number is still mostly unclear. To increase our understanding of the regulatory role of epigenetic modifications during renal development, we established an in vitro kidney culture screening system. Using transgenic Six2.TGCtg;Tomato/EGFP reporter mice, we screened epigenetic inhibitors for their potential to regulate in vitro kidney development evaluating parameters such as ureteric bud branching, cap mesenchyme and nephron induction. Inhibition of BET Bromodomain family proteins could be shown to lead to severe growth retardation and depletion of the Six2-positive progenitor cell pool. Furthermore, there was an accumulation of dilated nephron precursors. RNA-Seq analysis revealed the downregulation of several key molecules of the cap mesenchyme and verified increased markers of differentiating nephrons. We therefore conclude that inhibition of BET bromodomain family proteins affects the potential of nephron progenitor cells to self-renew and drives abnormal differentiation.

In summary, epigenetic regulation of renal progenitor cells is crucial for the balance of self-renewal vs differentiation of the nephron progenitor cells. In the future, further investigation of epigenetic regulation of kidney development will contribute to our knowledge on nephron induction and might help identify risk factors leading to decreased nephron endowment.

70

DNA Methylation Regulates Nephron Progenitor Cell Fate in Early Kidney Development

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The number of nephrons is a major determinant for the development of chronic kidney disease and hypertension. Although studies show that maternal factors during pregnancy cause low nephron numbers in the offspring, the underlying molecular mechanisms remain unknown. We hypothesize a link between environmental factors, epigenetic modification and nephron formation. Expression of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b could be shown to localize specifically to the nephrogenic zone of the developing kidneys. Deletion of Dnmt1 but not Dnmt3a/b in nephron progenitor cells resulted in a marked reduction of renal mass and nephron numbers at birth. Analysis of nephron progenitor cells indicates a reduced expression of key markers and changed cap mesenchyme morphology over time. Global demethylation could be shown to influence gene expression and lead to reactivation of retrotransposons. In summary, these findings highlight a key role of DNA methylation in the formation of nephrons and kidney development.

71

Small Molecule Screening to Detect Potential Therapeutic Targets in Human Podocytes

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* Eugen Widmeier and Weizhen Tan contributed equally to this work

Introduction: Steroid resistant nephrotic syndrome (SRNS) inevitably progresses to end-stage renal disease, requiring dialysis or transplantation. However, treatment modalities and drug discovery remain limited. Mutations in over 30 genes have been discovered as monogenic causes of SRNS. Most of these genes are expressed in the podocyte, placing it at the center of the pathogenesis of SRNS. Podocyte migration rate (PMR) represents a relevant intermediate phenotype of disease in monogenic causes of SRNS (Gee et al. JCI 123:3243, 2013 and Gee et al. AJHG 94:884, 2014). We therefore adapted PMR in a high-throughput manner to screen small molecules as potential therapeutic targets for SRNS.

Methods: We performed a high-throughput drug screening of an NIH Clinical Collection (NCC) library (n = 725 compounds) measuring PMR by continuous video microscopy. We used the Woundmaker™ to perform a 96-well scratch wounds and screened compounds using a quantitative kinetic live cell imaging migration assay using IncuCyte ZOOM® technology.

Results: Using a normal distribution of the average PMR of wild type podocytes with a vehicle control (DMSO), we applied a 90% confidence interval to define 'outliers' (5% faster/slower PMR) and found that 61 of 725 compounds (10 μM) altered the PMR. Clusters of drugs that alter PMR include actin/tubulin modulators and cell cycle regulating drugs such as azoles/vinca alkaloids and topoisomerase/DNA-methyltransferase inhibitors, respectively.

Conclusion: We have identified compounds that alter PMR. These compounds reveal potential novel therapeutic targets for glomerular diseases such as SRNS.

72

AT2R Deficiency Impairs Podocyte Function via the Alteration of Hedgehog Interacting Protein (HHIP) Gene Expression

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Introduction: Angiotensin type 2 receptor (AT2R) deficient mice (AT2RKO) exhibit a spectrum of congenital abnormalities of the kidney and urinary tract (renal hypoplasia/dysplasia and renal pelvis/ureteral abnormalities); however, the mechanisms by which these abnormalities occur are incompletely delineated. The present study aimed to study whether AT2R deficiency impairs glomerulogenesis (e.g., loss of podocytes) in vivo and in vitro.

Methods: Suckling pups – neonate to 3 weeks old – of both wild-type (WT) and AT2RKO (C57BL/6) mice were used. Physiological parameters, podocyte morphology and gene expression were assessed. Immortalized mouse podocyte cells (mPODs, obtained from Dr. Shankland SJ (U Washington)) were also used for in vitro studies.

Result: As compared with WT, pups lacking AT2R had retardation of podocyte maturation, resulting in renal dysplasia with decreased podocyte number as revealed by WT1 staining and decreased slit diaphragm protein expression – e.g., nephrin and synaptopodin. Moreover, lack of AT2R was associated with significantly elevated reactive oxygen species (ROS) in glomeruli, increased Nox4 and/or vascular endothelial growth factor (VEGF) and Hhip gene expression. In vitro, the AT2R agonist CGP42112A supported actin cytoskeleton integrity, with increased synaptopodin expression and maintained the integrity of podocyte foot process. In contrast, loss of AT2R (siRNA or PD123319) collapsed actin cytoskeleton integrity, resulting in foot process effacement. These changes were associated with elevation of Hhip targets, such as TGFβ1 signaling, promoting the fibrotic process occurring in mPODs.

Conclusions: AT2R deficiency is associated with retardation of podocyte maturation, resulting in renal dysplasia via up-regulation of Hhip (This project is supported by Canadian Diabetes Association).

73

Arctigenin (ATG) Attenuates Podocyte Injury in Diabetic Kidney through Inhibition of PP2A/NFKB/NOX4 Pathway

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Arctigenin (ATG) has been used frequently in Chinese Medicine formulae to treat patients with diabetic kidney disease (DKD). However, it remains unknown whether ATG alone has renal protective effect. To determine this, we treated streptozotocin (STZ)-induced diabetic eNOS–/– mice (STZ-eNOS–/–) with either ATG or vehicle from 10 weeks post-STZ injection to 18 weeks. We found that ATG significantly attenuated proteinuria, glomerular hypertrophy, mesangial expansion, foot process effacement and podocyte loss in STZ-eNOS–/– mice as compared to mice treated with vehicle. To understand the underlined mechanism, we performed RNA sequencing of glomeruli isolated from these diabetic mice with or without ATG treatment. We found that ATG significantly affects focal adhesion, actin cytoskeleton, and ROS pathways in diabetic glomeruli. Among ROS-related genes, NOX4 expression was significantly suppressed in diabetic glomeruli by ATG, which was further confirmed by western blot and RT-PCR analysis. To understand the mechanism of NOX4 regulation by ATG, we performed mass spectrometry analysis of ATG-bound proteins in cultured podocytes and identified protein phosphatase 2A (PP2A) was on the top of the list. This interaction was further confirmed by in vitro binding assay. Since PP2A is known to interact with NF-kB pathway, we examined and found that ATG reduced p65 phosphorylation in podocytes induced by TNF-a through increased interaction between PP2A and p65. Finally, we confirmed that ATG suppressed NOX4 expression through inhibition of PP2A/NF-kB pathway in podocytes. In conclusion, we demonstrated a renal protective effect of ATG in DKD and its underlined mechanism of action in podocytes. Our studies suggest that ATG could be potentially developed as a new drug to treat podocyte injury in DKD.

Author Index

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