Conclusions: Our study shows that Namp plays a critical role to maintain podocyte homeostasis with a deleterious effect in the podocyte causing severe proteinuria and renal damage. This effect is dependent on NAD+ as its supplementation rapidly corrects the increase in proteinuria, supporting a reversible mechanism through NAD+ metabolism instead of impairment during development.

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Ezetimibe Restores the Communication Between Lipid Droplets and Mitochondria via Modulation of PLIN5

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Background: Alport Syndrome (AS) is a hereditary disease caused by mutations in collagen type IV. Pathogenic renal lipid droplets (LD) and triglyceride (TG) accumulation have been demonstrated in experimental AS (Col4a3KO mice). FFA catabolism resulting from excessive lipolysis of TG is a major contributor to cell lipotoxicity. Peroilipin 5 (PLIN5) is an LD-related protein that plays a critical role in regulating TG lipase activity and the interactions between LD and mitochondria, where it protects mitochondria from excessive exposure to FFA. Here we test the hypothesis that PLIN5 expresses in podocytes and that PLIN5 deficiency in AS causes excessive TG breakdown and the loss of LD-mitochondrial contact, thus contributing to kidney failure.

Methods: In vitro, Immortalized AS podocytes and WT podocytes were established and characterized in our laboratory by breeding the Col4a3KO mice (Jackson Laboratory) to H-2k-haA58 transgenic mice (Charles River). PLIN5 expression was determined by RT-PCR and western blotting in podocytes from Col4a3KO mice when compared to controls. TG lipolysis and FFA quantification were determined and normalized to protein content. LD-Mitochondrial contact was determined by TEM analysis. PLIN5 expression was studied in kidney cortexes, and the effect of Ezetimibe on PLIN5 modulation, on LD-Mitochondrial contact and on podocyte injury was studied in vitro and in vivo.

Results: We demonstrate that PLIN5 is expressed in podocytes, and the expression of PLIN5 is significantly decreased in AS podocytes compared to WT podocytes (p<0.01). AS podocytes also showed significantly increased rates of TG lipolysis (p<0.05), intracellular free fatty acids (p<0.05) and apoptosis (p<0.01) when compared to WT podocytes. AS podocytes had reduced number of LD-mitochondrial contacts (p<0.05), implying that and apoptosis. Moreover, Ezetimibe, which restored LD-Mitochondrial contact in vitro (p<0.05) and improved kidney function in vivo, was found to restore PLIN5 expression in vitro (p<0.05).

Conclusions: PLIN5 deficiency in AS podocytes causes excessive TG lipolysis and inefficient FA transfer from LD to Mitochondria, leading to mitochondrial dysfunction. Ezetimibe improves LD-mitochondria communication via restoring PLIN5 expression.

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Adriamycin-Induced Nephropathy Is Robust in N and Modest in J Substrain of C57BL/6 Mice

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Background: Adriamycin (ADR)-induced nephropathy remains the leading model to study human primary focal segmental glomerulosclerosis (FSGS), a common pathway for podocyte damage and glomerular loss of function that leads to chronic kidney disease. However, the use of this model for reverse genetics is limited by historical categorization of C57BL/6 mice as an ADR-resistant strain, which is also the most commonly genetically modified strain. Additionally, conflicting reports exist utilizing C57BL/6 for ADR-nephropathy due to lack of understanding of strain differences (J/N) and variability in ADR dosage, timing, and frequency to induce damage. We have undertaken a systematic approach to elucidate the specifics of ADR-nephropathy in C57BL/6 N and J substrains.

Methods: We induced nephropathy with 2 doses of ADR, and measured albuminuria for 6 weeks. Additional serum chemistry was performed along with histological evaluations. Podocyte injury markers were evaluated to assess the damage of glomerular filtration barrier.

Results: Our findings revealed induction of robust and modest proteinuria in N and J substrains, respectively. The serum creatinine levels were elevated in N, but not J substrain. Both the substrains showed reduction in body weight with N greater than J, although mortality remained at 0% in both substrains. Histological analysis showed worse glomerular lesions in the N than the J substrain. Podocyte markers synaptopodin, nephrin, podocin, and WT1 were reduced to a greater extent in the N than the J substrain.

Conclusions: In summary, we provide the nephrology community with a reproducible mouse model for FSGS, in a strain otherwise assumed to be ADR-resistant and highlight the differences between J and N substrains. This enables future studies, especially concerning genetically manipulated animal models in C57BL/6.

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Development of a Treatment Response Prediction Strategy for Sparsentan in Glomerular Disease

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Background: Sparsentan is a dual endothelin receptor A inhibitor and angiotensin blocker that demonstrated reduced proteinuria in patients with FSGS in Phase II studies (DUET). Gene expression data from Adriamycin (ADR) treated rats with sparsentan were used to develop a response profile.

Methods: An interventional dose-response study was performed in rats challenged with Adriamycin (ADR) to induce an FSGS phenotype. UPCR randomization was performed at day 7 post-challenge, and daily dosing of sparsentan across a dose range was carried out for 35 days. Kidneys were harvested, stored for FFPE, and kidney cortex RNAseq profiles were generated. Differentially expressed gene (DEG) analysis followed by enrichment analysis was performed. A sparsentan response profile from the model was mapped onto human kidney RNAseq profiles from the NEPTUNE cohort and interrogated against demographic and clinical features of the cohort.

Results: DEG profiles were generated in ADR+vehicle vs. Sham animals (4271; q<0.05) and from ADR+sparsentan relative to ADR + vehicle. A high dose group (60mg/kg) generated the largest number of DEGs compared to ADR+vehicle (583; q<0.05); consistent with attenuation of proteinuria (p<0.05) and glomerulosclerosis (p<0.05) in this group at Day 33. Sparsentan reversed directionality 388 DEGs from the model. The 388 genes were enriched for genes in the endothelin pathway (p<0.01) and for genes consistent with the active EDN1 (p<0.01). Based on gene expression profiles, activities of IL1B, IFNγ, TNF, and TLR4 were predicted to be attenuated by sparsentan. Mapping the signature onto human data revealed elevated signaling in glomerular and tubule profiles of patients with FSGS (p<0.05). The signature was negatively correlated with eGFR (p=0.01) and positively correlated with UPCR (p=0.05). Changes in intrarenal transcriptional profiles were correlated with glomerular and tubule pathology.

Conclusions: Sparsentan treatment attenuated gene expression and activity of disease-related networks in a rat model of FSGS. The sparsentan response signature from rats was elevated in human FSGS kidney tissue, associated with disease severity. Candidate non-invasive biomarkers were identified and are being developed for the NEPTUNE Match clinical trial (NCT04571658).

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Secreted Frizzled Related Protein 2: A New Therapeutic Target for Glomerular Disease?

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Background: Wnt/b-catenin signaling, critical during development but silenced in adult kidneys, is re-activated in podocytes following injury and plays a detrimental role in glomerular injury. Secreted frizzled-related proteins (sFRPs) modulate Wnt signalling and are involved in conditions such as myocardial infarction and cancer but their role in glomerular pathology is unknown. Here, we examined the role of sFRP2 in podocyte injury in vivo and in vitro approaches.

Methods: Glomeruli from patients with minimal change disease, focal and segmental glomerulosclerosis, Type 2 Diabetes Mellitus and healthy donor kidneys were isolated from biopsy specimens and gene expression examined on Affymetrix gene chip arrays. Adriamycin (ADR) nephropathy was induced in Balb/c mice with 10mg/kg IV ADR. FRP2 neutralising antibody or IgG was injected at day 0, and every 3 days, until day 14. Urine and serum samples were collected at 7 and 14 days. In a separate experiment, mRNA changes were examined in isolated glomeruli 2 days after ADR. An immobilised mouse podocyte cell line was used for in vitro experiments.

Results: sFRP2 mRNA levels were significantly upregulated (6 to 24-fold) versus live donor controls, in all patient cohorts. In mice, ADR injury increased sFRP2 mRNA expression (2-fold, p<0.05) 48hrs after induction, prior to the appearance of proteinuria. In vitro, ADR induced podocyte sFRP2 mRNA expression (10-fold, p<0.01). Increased sFRP2 staining was observed in podocytes following ADR injury. Exposure of podocytes in vitro to sFRP2 led to longer processes (53a 4.6 vs 76 ± 9.1), arbitrary units, p<0.05) and more processes/cell (8 ± 0.96 vs 11 ± 1.01, p<0.05). Next, we examined the effect of sFRP2 blockade on ADR-induced glomerular injury. ADR significantly increased albuminuria compared to baseline (38323 ± 0.96mg/24hr, p<0.001; n=10 mice/group) and blood urea nitrogen (BUN) levels (38.14 ± 21.57mg/dL, p<0.05, n=10) at 14 days. sFRP2 inhibition significantly attenuated both albuminuria