

QUANTIFICATION OF MICROTHROMBI IN POORLYPERFUSED AREAS OF PORCINE KIDNEYS

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There is currently a great disparity between the need for kidneys for transplant and the supply of donor kidneys. In order to expand the number of organs available for transplant, optimal preservation conditions need to be described. Flushing organs with preservation fluids after procurement is a common practice in transplant surgery used to maintain organ integrity. The most commonly used preservation fluids in transplant surgery are currently HTK and UW. The principle difference between the two fluids is their viscosity, UW being significantly more viscous than HTK. Here we aim to determine which fluid has the better ability to maintain integrity of organ microvasculature, as measured by the quantity of microthrombi present in the microvasculature. 12 porcine kidneys were subject to 3 different preservation conditions- 4 flushed with HTK immediately after procurement, 4 flushed with HTK 30 minutes after procurement, and 4 flushed with UW 30 minutes after procurement. Biopsies were taken from well and poorly perfused regions of the kidneys, as guided by contrast enhanced CT imaging. 4 μ m paraffin embedded tissue sections were then prepared from these biopsies, which were then stained using Hematoxylin and Eosin. 20 representative 20x images were taken of each tissue section for analysis using an Evos FL Auto 2 microscope. Using custom MATLAB code, microthrombi were quantified. There were significantly ($p < .0001$) more microthrombi present in poorly perfused tissues than well perfused tissues in kidneys flushed with UW 30 minutes after procurement. Similarly, kidneys flushed with HTK immediately after procurement significantly ($p < .05$) more microthrombi in poorly perfused tissues than well perfused tissues. These results help us to optimize preservation conditions for organ transplant in the future.

ACTIVATION OF G PROTEIN-COUPLED ESTROGEN RECEPTOR PREVENTS HIGH SALT-INDUCED HYPERTENSION

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Estrogen protects premenopausal women against cardiovascular and renal diseases. G protein-coupled estrogen receptor (GPER), is a membrane bound protein that prompts protective effects throughout the cardiovascular and renal systems. Data suggests that GPER activation contributes to the maintenance of cardiovascular and renal health in women. In our experiment, female Sprague Dawley (SD) rats were implanted with telemeters, subjected to bilateral ovariectomy (OVX), and treated with vehicle or G1, a GPER agonist. Two weeks later, these rats were placed into metabolic cages to obtain 24-hour urine samples under normal salt (NS = .49% NaCl), and high salt (HS = 4% NaCl) diets. Vehicle treated rats had significantly higher mean arterial blood pressures after OVX and HS challenge compared to their baseline (113 ± 1 vs. 105 ± 1 mmHg, respectively, $n=6$, $p<0.05$). This hypertensive response was prevented by G1 (106 ± 3 vs. 104 ± 2 mmHg, respectively, $n=6$). Similar trends were observed in both diastolic and systolic blood pressures. A separate group of intact female rats were treated with G15, a GPER antagonist. After being challenged with a HS diet, a significant increase in blood pressure was observed compared to baseline (113 ± 1 vs. 109 ± 1 mmHg, respectively, $n=6$, $p<0.05$). GPER activation prevented the significant increase in blood pressure caused by HS in OVX female rats. GPER blockade caused a significant increase in blood pressure in intact female rats when challenged with a HS diet. This data suggests that GPER plays an important role in the maintenance of blood pressure in females fed high salt diet.

BETAINE-DEPENDENT RE-METHYLATION ABNORMALITIES CONTRIBUTES TO HOMOCYSTEINE-MEDIATED ENDOTHELIAL DYSFUNCTION IN POLYCYSTIC KIDNEY DISEASE

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Vascular abnormalities are the most important non-cystic complications in PKD and contribute to renal disease progression. Homocysteine (Hcy)-induced endothelial dysfunction (ED) precedes vascular disease in PKD, but the underlying mechanisms leading to increased Hcy remain unknown. To gain insight into the molecular mechanisms implicated in Hcy-induced ED, we explored the Hcy pathway using targeted metabolomics in a murine model of PKD. Kidneys were harvested, frozen in liquid nitrogen, or preserved in formalin for metabolomics analyses and *ex-vivo* studies from 4-week-old PCK and Sprague-Dawley (SD) control rats (n=12 each). Twenty-four-hour urine and terminal blood samples were collected for metabolite analysis and chemistries. Endothelial NO (eNOS) was assessed by double immunofluorescence staining for CD31/eNOS. Serum creatinine and BUN remained unchanged, yet kidney weight/body weight ratio were increased in PCK. eNOS immunoreactivity was lower in PCK vs SD. Plasma Hcy and urine excretion of its methyl donor betaine were elevated in PCK, and Hcy was positively correlated with urine betaine. Plasma betaine was similar between the groups, but tissue betaine concentration was lower in PCK. Contrarily, tissue glutathione concentration was higher in PCK vs SD, arguing against decreased Hcy transsulfuration. Plasma folate and vitamin B12 were similar, decreasing the probability of a defect in folate-dependent remethylation. Early PKD is associated with elevation in Hcy, likely related to betaine-dependent re-methylation abnormalities. These findings provide novel insights into Hcy-induced ED in PKD and suggest candidate markers that may be useful to assess vascular and renal disease severity early on.

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THE EFFECTS OF ERYTHROPOIETIC DRIVE AND INFLAMMATION ON BMP2-LINKED IRON REGULATION

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Anemia is common in chronic kidney disease (CKD) and affects nearly all patients with end-stage renal disease (ESRD). Current anemia management using erythropoiesis stimulating agents has been linked with negative clinical outcomes, generating interest in other treatment strategies. Heparin excess is a main causal factor contributing to anemia in ESRD by reducing iron availability for red blood cell production. Recently, we identified endothelial cell *Bmp2* as a novel regulator of hepcidin expression. Here, we used mice with an endothelial conditional knockout (CKO) of *Bmp2* to examine how *Bmp2* is regulated and functionally contributes to hepcidin regulation by its major stimuli. Erythropoietin (EPO) did not influence *Bmp2* expression in control mice, and still suppressed hepcidin in *Bmp2* CKO mice. Lipopolysaccharide (LPS) reduced *Bmp2* expression in control mice, but still induced hepcidin in *Bmp2* CKO mice. Thus, *Bmp2* is not required for hepcidin suppression by EPO or hepcidin induction by inflammation; however, *Bmp2* influences hepcidin levels in the context of inflammation by lowering the basal setpoint. Therefore, targeting *Bmp2* may represent a new therapeutic strategy to lower hepcidin and thereby improve iron availability and treat anemia in CKD as well as other inflammatory diseases.

RECOMBINANT EXPRESSION OF COLLAGEN IV FROM *MINISTERIA VIBRANS*: THE ANCESTRAL COLLAGEN OF THE ANIMAL KINGDOM

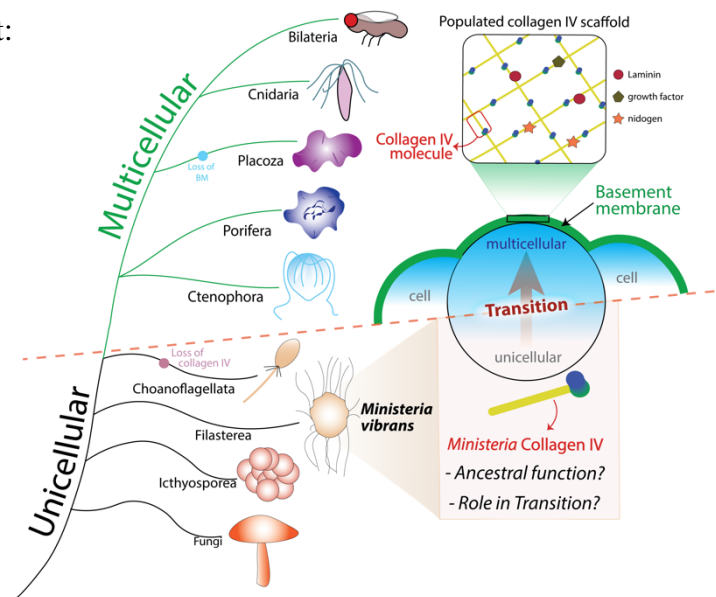
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The transition from unicellular protists to multicellular animals coincided with the appearance of a specialized extracellular matrix (ECM), the basement membrane (BM). BMs are dynamic structures that modulate cell differentiation and behavior during development, and help shape tissue architecture. Collagen IV, a major component of BMs, forms large networks that provide tensile strength to tissues and function as smart scaffolds organizing diverse macromolecules in the BM. Importantly, collagen IV is conserved across all animals and likely played a role in the transition to animals. However, the mechanism in which collagen IV enabled this transition is unknown. The protist, *Ministeria vibrans*, has recently emerged as the first unicellular organism to contain collagen IV based on genomic and transcriptomic evidence. Here, we sought to build a construct with *Ministeria* collagen IV for recombinant expression in Chinese hamster ovary (CHO) cells. We have successfully cloned the *Ministeria* collagen IV gene, however, generation of a construct for transfection into CHO cells has not been successfully completed. Future work will include biochemical characterization of *Ministeria* collagen IV expressed by CHO cells utilizing fast protein liquid chromatography (FPLC) and Western blotting to determine how *Ministeria* collagen IV behaves in a unicellular organism. Together, these will provide insight into the ancestral function of collagen IV, and how collagen IV played a role in the evolutionary transition to multicellular animals.

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Graphical Abstract:



ANTIOXIDANT TREATMENT IMPROVES AFFERENT ARTERIOLE AUTOREGULATORY BEHAVIOR IN ISCHEMIA-REPERFUSION ACUTE KIDNEY INJURY RATS

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Renal ischemia-reperfusion (IR) induced acute kidney injury (IR-AKI) is characterized by reduction in renal blood flow and glomerular filtration rate. Preliminary data suggest excess reactive oxygen species (ROS) contribute to renal autoregulatory dysfunction after IR-AKI. We hypothesized that antioxidant treatment would improve renal autoregulatory behavior in IR-AKI rats through reduction of superoxide and hydrogen peroxide. Renal autoregulation was assessed using the *in vitro* blood-perfused juxtamedullary nephron preparation in sham and IR-AKI rats. IR was induced by 60-minute bilateral renal artery occlusion followed by 24-hours of reperfusion.

Sham control rats exhibited normal autoregulatory responses (n=8). Afferent arteriole (AA) diameter increased to 114±4% of the baseline when perfusion pressure (PP) decreased to 65 mmHg and decreased to 65±3% when PP increased to 170 mmHg. In contrast, autoregulatory responses were attenuated in IR-AKI rats (n=9). AA diameter remained between 90 and 101% of control across 65-170 mmHg.

Acute administration of polyethylene glycol superoxide dismutase (100 units/ml in blood) improved autoregulatory responses in IR-AKI rats (n=4). A decrease in PP to 65 mmHg resulted in a 115±4% increase in diameter, whereas increasing PP to 170 mmHg led to a 71±6% decrease in diameter. Administration of polyethylene glycol catalase (1000 units/ml in blood) had a similar effect on autoregulatory responsiveness (n=3). Diameter averaged 118±5% at 65mmHg and 66±9% at 170mmHg.

Post-treatment with tempol in IR-AKI rats for seven days had no major effect on protein or albumin excretion. In conclusion, excess superoxide and/or hydrogen peroxide contribute to impaired renal autoregulation in IR-AKI rats.

GAINING INSIGHTS THROUGH IMITATION – LEARNING TO ARTIFICIALLY SYNTHESIZE RADIOLOGICAL FINDINGS OF ADPKD

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is an inherited condition that is characterized by fluid-filled cyst development in the kidneys. Studies have shown that truncating mutations in a genotypic variant of ADPKD (PKD1-t) have a different prognosis and phenotype than non-truncating mutations (PKD1-nt). Through a combination of convolutional neural nets (CNNs) and auxiliary classifier generative adversarial networks (AC-GANs), we have created a program that predicts genetic mutation status from MR images.

Our dataset included abdominal T2-weighted MR scans, corresponding kidney segmentations and genetic mutation status for 134 Patients (90 PKD1-t, 44 PKD1-nt). An 18-layer residual CNN was used for image analysis. In certain cases, classical augmentation (CA) was applied to the network. An AC-GAN was designed that generated images that either resembled a PKD1-t or PKD1-nt masked kidney (Figure 1). These generated images were added to the training set for synthetic augmentation (SA). In total, 5 different classes of image inputs were run through the network.

The Single Channel with Mask (CA) performed best with a highest average accuracy of 76.89% after 3 10-fold validations (Figure 2). The synthetic augmentation was an improvement over no augmentation (NA). We created an automated method for predicting underlying genetic mutations from MR images. A larger sample size would likely improve both the accuracy of the CNN and the realism of the synthetic images.

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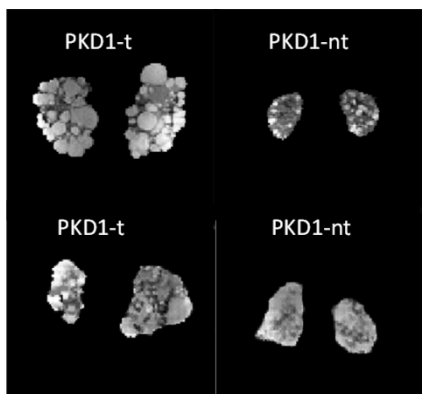


Figure 1. Real (top) and synthetic (bottom) PKD1 masked kidneys

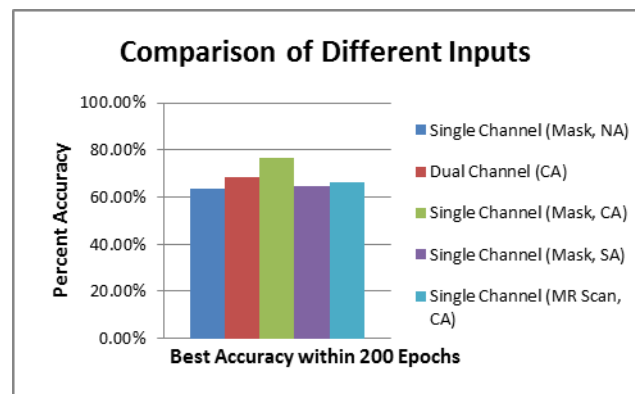


Figure 2. Comparison of CNN accuracies with 5 different image inputs.

UNCOVERING THE MECHANISM OF ZIP10 (SLC39A10) MUTATIONS IN CALCIUM OXALATE NEPHROLITHIASIS

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SLC39A10 (ZIP10) is a Zn²⁺ membrane transporter which was previously implicated in the formation of calcium oxalate (CaOx) urinary stones in dogs through genetic analysis. We have previously used genetics in canines, crystallization in *Drosophila* Malpighian tubules (MT), and function in *Xenopus* oocytes to better understand the role of ZIP10. Here, two human variants (D→N and N→Y) are being investigated for association with human kidney stones. The D in the D→N mutation is highly conserved across species including humans, dogs, and flies. This finding and MT expression (**Table**) implicates *CG10006*, rather than *foi*, as the likely renal-ZIP10 homologue in *Drosophila*. After site-directed mutagenesis, cRNA of the ZIP10 mutations were synthesized and injected into *Xenopus* oocytes. Wild type ZIP10-WT, ZIP10 D→N, ZIP10 N→Y, and non-injected oocytes were sectioned and stained with a ZIP10 antibody four days after injection. Non-injected oocytes had no staining (cell or membrane) indicating no endogenous ZIP10 in oocytes. ZIP10-WT showed staining localized to the membrane consistent with our previous Zn-uptake experiments. ZIP10 D→N displays similar membrane localization as ZIP10-WT, indicating this variant is likely a functional defect to be further investigated. ZIP10 N→Y demonstrated abnormal staining with low amounts of membrane fluorescence and high amounts of intracellular fluorescence indicating this mutation likely is causing a trafficking problem in the protein. These results give insight into functionality of the mutations, and further studies will investigate their role in Zn²⁺ uptake.

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Table. *Drosophila* mRNA signals for Zip10 candidates (flyatlas.org)

Tissue \ gene	<i>CG10006</i>	<i>foi</i>
Adult tubule	902 ± 145	95 ± 1
Larval tubule	3219 ± 48	95 ± 1

EKTACYTOMETRY IN ERYTHROCYTES

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Sickle cell disease (SCD) is an inherited, terminally ill disorder characterized by a point mutation in the HBB gene of hemoglobin causing a distortion of the red blood cell. SCD is estimated to affect 100,000 Americans and occurs in about one in every 265 African American births. Sickle Hemoglobin (HbSS) functions normally when oxygenated but polymerizes when it is deoxygenated, forming the sickle shape and causing rigidity that leads to blood clots and a reduced flow of oxygen to the tissues. These insoluble polymers affect the normal red blood cell (RBC) deformability, ultimately resulting in hemolysis, a shorter life span, organ damage, vaso-occlusive pain crisis, and other destructive effects. RBC deformability can be measured using many techniques, however ektacytometry of RBCs subjected to sheer stress may be used due to its accurate measure of laser diffraction of the cells. Ektacytometry was used in conjunction with the LORCCA machine and Hemox Analyzer to give accurate and observable readings of RBC deformability (Elongation Index-EI max) and hypotonic osmolality (Omin), where 50% of the cells hemolyze in an osmotic fragility assay and EI is at a minimum. In the osmoscan data, HBSS was shifted to the left, suggesting a reduced deformability and hydration status. This also matched the pO₂ data. In the Hemox analysis, p50 was increased in the HBSS RBCs. Our results showed that there was a significant difference in the hemox and ektacytometry images of the sickle RBCs in comparison to healthy RBCs, suggesting that ektacytometry is a useful tool in analyzing the sickle phenotype in SCD.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) CONTROLS KIDNEY FIBROSIS BY REGULATING NUCLEAR LOCALIZATION OF FUSED IN SARCOMA (FUS)

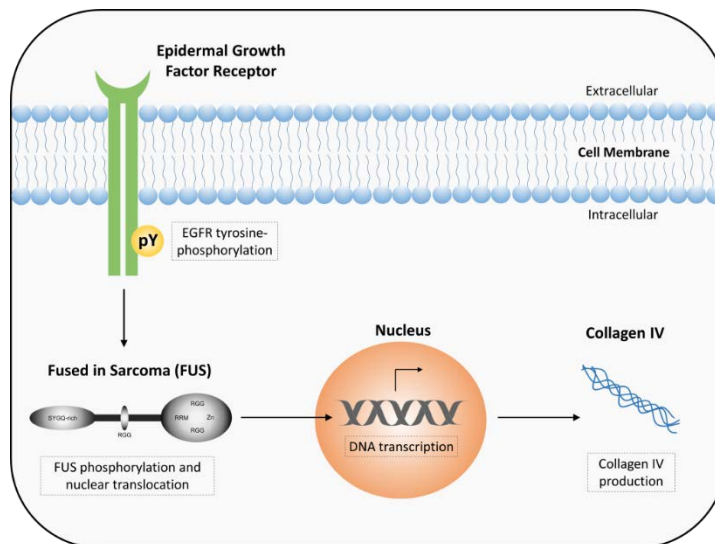
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Kidney fibrosis is a life-threatening consequence of kidney disease. The collagen receptor integrin $\alpha 1 \beta 1$ (Itga1 $\beta 1$) plays an anti-fibrotic role by negatively regulating the activation of the epidermal growth factor receptor (EGFR). In previous work, we showed that Itga1-null cells have increased levels of activated EGFR, produce more collagen, and have higher nuclear levels of Fused in Sarcoma (FUS) than wild-type cells. Our goal was to determine whether nuclear FUS correlates to fibrosis. Indeed, nuclear FUS levels are upregulated in kidneys of mice and humans with kidney fibrosis. Moreover, increased levels of nuclear FUS correlate with increased collagen production in Itga1KO cells. We additionally found that activated EGFR forms a complex with FUS and controls its nuclear translocation. Here, we further determine a link between fibrosis, EGFR, and nuclear FUS in kidney cells using two additional models: DSK-5 mice and waved-2 mice, in which EGFR is either constitutively active or permanently impaired, respectively. We show that glomeruli of DSK-5 mice have higher nuclear FUS levels compared to glomeruli of wild-type mice. Furthermore, we provide evidence that both waved-2 mice and Itga1KO mice treated with EGFR inhibitors show decreased fibrosis, and pharmacological inhibition of EGFR in Itga1KO cells reduces levels of nuclear FUS. Thus, EGFR-mediated FUS nuclear translocation represents a previously undescribed mechanism whereby EGFR controls collagen production and fibrosis. Exploring EGFR/FUS-controlled collagen production in fibrotic diseases and the consequences of its inhibition will offer a novel approach for the treatment and, ideally, prevention of fibrosis.

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Graphical Abstract:



LOSS OF β 1-INTEGRIN DISRUPTS SIGNAL TRANSDUCTION FOR SMOOTH MUSCLE CONTRACTION IN MOUSE BLADDER

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Integrins are membrane proteins which physically link the actin cytoskeleton to the extracellular matrix. Mechanical stress activates integrins, leading to the assembly of multi-protein complexes called focal adhesions (FAs) that facilitate force transmission. To study mechanotransduction in bladder smooth muscle (BSM) we knocked out β 1-integrin using a tissue-conditional, inducible Cre/lox system in mice. Prior work with muscle strips had shown that loss of integrins in BSM led to a loss of contractile force, mediated by acetylcholine and muscarinic receptors. In order to investigate the mechanism, we surveyed known integrin signaling proteins by Western blot and by PCR after six weeks of tamoxifen-mediated Cre induction. RT-PCR confirmed the loss of loxP-flanked exon 3 of the β 1-integrin gene in knockout mice, and Western blotting demonstrated almost complete loss of protein. Consistent with the pharmacology, M2 and M3 muscarinic receptors were downregulated by 39.5% and 50.7% respectively ($P < 0.05$), while ATP receptor P2X1, remained unchanged. Knockout mice also displayed reduced expression of FA-associated proteins, talin 1 and fibronectin by 33% and 81.2% respectively ($P < 0.05$), while vinculin, paxillin, focal adhesion kinase, and integrin linked kinase were unchanged. These results suggest that loss of β 1-integrin leads to downregulation of some FA-adaptor proteins as well as muscarinic receptors and in doing so disrupts force generation through FAs. Future experiments should focus on how integrins interact with and regulate muscarinic receptors in BSM. Disruption to BSM contractility due to injury or disease may result in bladder dysfunctions including overactivity and incontinence.

EXAMINING THE ASSOCIATION OF NEDD4-2 AND NCC

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The sodium chloride cotransporter (NCC) is a transmembrane protein located in both the DCT1 and the DCT2 of the nephron. The function of NCC is to reabsorb NaCl from the tubular filtrate. Aldosterone regulates the activity of NCC. However, the effects of aldosterone on NCC are localized exclusively to the DCT2, due to the co-expression of the mineralocorticoid receptor and 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2). The epithelial sodium channel (ENaC) is another transmembrane protein that reabsorbs sodium and is localized to the DCT2. Previous research has shown that NCC and ENaC associate with each other and appear to directly bind. NEDD4-2 is an aldosterone-regulated protein that ubiquitinates both NCC and ENaC, resulting in degradation. However, while NEDD4-2 binds directly to ENaC it is unclear whether it directly binds NCC. This is because there is no consensus binding site for NEDD4-2 on NCC. This study examines if the interaction between NEDD4-2 and NCC is direct or indirect. To test this, co-transfections (GST-NCC- C-terminus/ NEDD-2, GST-NCC-N-terminus/NEDD4-2) and transfections (GST-NCC-C-terminus and GST-NCC-N-terminus) in COS-7 cells were done. These cells do not natively express NCC, ENaC, or NEDD4-2 permitting us to examine direct interactions. Western Blot analysis was done to verify expression of GST-NCC and NEDD4-2. After confirmation of protein expression, a GST pull-down assay will be done to assess the interaction between NCC and NEDD4-2. Specifically, to test whether the N-terminus or C-terminus of NCC binds to NEDD4-2 in the presence or absence of ENaC. Experiments are ongoing to fully characterize this association.

CROSS TALK OF FGF23 AND IGF1 IN THE REGULATION OF CARDIAC REMODELING

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Patients with chronic kidney disease (CKD) have significantly increased risk for cardiovascular disease and very commonly develop cardiac hypertrophy. The bone derived hormone fibroblast growth factor (FGF)23 regulates renal phosphate excretion by binding to FGF receptor (FGFR)1 in the proximal tubule. In CKD patients, serum FGF23 levels are highly increased, and it has been shown that FGF23 can induce cardiac hypertrophy via binding to FGFR4 in cardiac myocytes. During pregnancy and extensive exercise, the heart undergoes remodeling which doesn't result in cardiac damage and is mediated by insulin growth factor (IGF)1 and IGF receptor signaling. Since IGF1 and FGF23 can cause cardiac hypertrophy, we wanted to determine whether a potential cross talk exists. The cardiac rat myoblast cell line H9c2 as well as neonatal rat ventricular myocytes (NRVM) were treated with recombinant FGF23 protein at time points ranging from 15 minutes to 24 hours. We measured the expression levels of IGF1 by qPCR and IGF receptor activation by studying the phosphorylation of insulin receptor substrate (IRS)1 via immunoblotting. Additionally, we treated NRVMs with established inducers of pathologic cardiac remodeling, i.e. isoproterenol, angiotensin (Ang)2, indoxyl sulfate, and oncostatin M (OSM), followed by qPCR analysis. We detected no significant change in levels of IGF-1 expression or IRS phosphorylation after FGF23 stimulation. Therefore, we conclude that FGF23 does not affect IGF1 production or signaling in cardiac myocytes. However, we found increased expression levels of FGF23, IGF1 and angiotensinogen in cardiac myocytes that were stimulated with isoproterenol, indoxyl sulfate, OSM, and Ang II.

IDENTIFYING FECAL BIOMARKERS OF OBESITY IN MOUSE MODEL USING PROTON NUCLEAR MAGNETIC RESONANCE METABOLOMICS

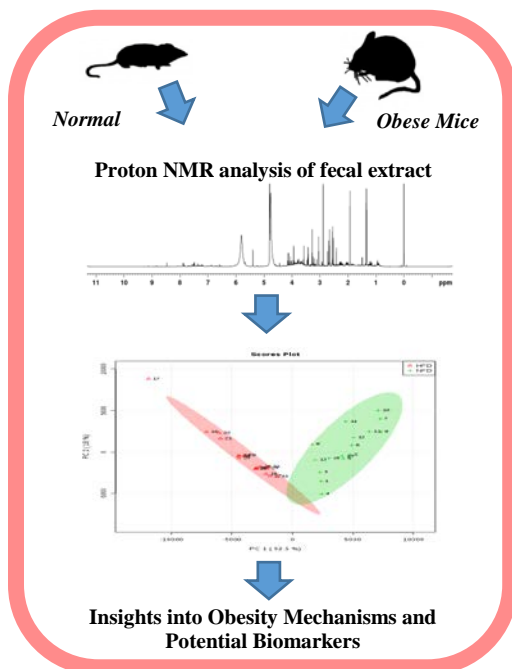
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Obesity is a complex multifactorial disease and a major health concern. It is difficult to treat, and its mechanisms are not well understood. One approach to understanding mechanisms of obesity is metabolomics, which studies small molecules generated during metabolism. These molecules can be biomarkers in the early diagnostics of obesity and/or targets of prospective therapies. We utilized nuclear magnetic resonance (NMR) technology to identify potential biomarkers of obesity in feces of mice receiving high-fat vs. normal diet. Feces collected from obese and normal mice were extracted with buffer, supplemented with the NMR standards and analyzed using a 600 MHz NMR spectrometer. The proton-NMR spectra were analyzed using a principle component analysis and metabolites were identified with Chenomx software. The differences between obese and normal mice were determined by statistical analysis using a Bonferroni (cut-off p-value = 0.083) correction for multiple comparisons. We found 18 fecal metabolites with significantly different levels between obese and normal mice: 13 of these metabolites had lower levels, while 5 metabolites had higher levels in obese vs. normal mice. Six metabolites were present in both groups, while the rest were present in one group but not the other. We conclude that these metabolites may serve as biomarkers of obesity.

Research supported by: Aspirnaut™ Undergraduate Discovery Science Experience in Renal Biology and Disease through the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH), Kidney, Urology, Hematology (KUH) R25DK096999 to Billy G. Hudson; Berea/Aspirnaut™/Hal Moses Summer Research Internships; Vanderbilt University Medical Center.

Graphical Abstract:



INJURY INDUCED KIDNEY FIBROSIS IS REGULATED BY COUP-TFII

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Chronic Kidney Disease (CKD) is characterized by a progressive loss of kidney function associated with fibrosis. Recently, genetic mapping studies demonstrate that myofibroblasts are the major cellular contributors to injury-induced kidney fibrosis. Chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) is an orphan transcription factor that is important for cell fate decisions. We found that COUP-TFII is primarily expressed in pericytes in non-injured kidneys. We hypothesize that COUP-TFII regulates the pericyte-myofibroblast transdifferentiation and contributes to injury-induced kidney fibrosis.

COUP-TFII knockout mice were generated through a tamoxifen-inducible Cre-loxP recombination system by crossing COUP-TFII floxed mice with Rosa26-CreERT2 mice. Unilateral ureteral obstruction (UUO) surgery was performed 10 days after tamoxifen injection. Kidneys were collected 7-days after surgery and analyzed by western blot (WB) and immunofluorescence (IF) for alpha-smooth muscle actin (α SMA) (a marker for myofibroblasts) and Collagen-1 (a marker for fibrosis) expression.

COUP-TFII expression by IF is less in both injured and non-injured kidneys in knockout mice compared to wild-type mice. In wild-type mice, COUP-TFII, α SMA and collagen-1 expression significantly increased in injured kidneys. Furthermore, COUP-TFII and α SMA are co-localized when examined by IF. In knockout mice, α SMA and collagen-1 protein expressions in UUO kidneys were significantly decreased compared to wild-type control littermates.

In conclusion, COUP-TFII regulates the pericyte to myofibroblast transdifferentiation and contributes to injury-induced kidney fibrosis. Genetic absence of COUP-TFII decreases UUO-induced α SMA and collagen-1 upregulation. Targeting COUP-TFII action using inhibitors/antagonists may lead to novel therapies for kidney fibrosis in CKD.

TRANSPLANTATION OF KIDNEYS FROM DECEASED DONOR WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) DID NOT ACCELERATE RENAL CYSTOGENESIS

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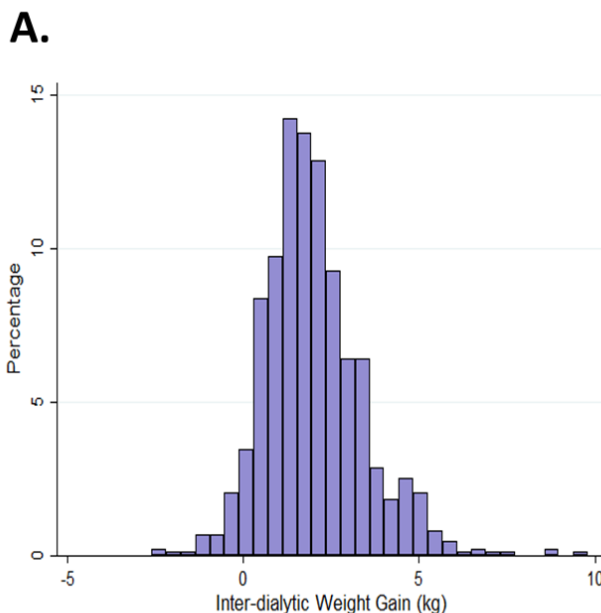
Kidneys from patients with ADPKD may be considered for “marginal” use in renal transplantation if their procurement-related acute kidney injury (AKI) does not trigger cystogenesis that leads to rapid renal function loss and death similarly as it happens after AKI in mice that carry mutation in a single allele of an ADPKD gene (like human ADPKD patients). However, since we did not observe such dramatic AKI-related cystogenic effect in our rat model that also carries an ADPKD mutation in one ADPKD gene allele, we recommended the use of kidneys from a donor mildly affected with ADPKD class 1A. After transplantation of these ADPKD kidneys into two recipients, MRI was performed 1 and 7 months post-transplant. We determined the transplanted kidney and individual cyst volumes and compared these values from the two time-points using ImageJ- and LabVIEW-based tools that we developed for this purpose. In Recipients 1 and 2, we found a 4% and 24% increase in kidney volume, respectively, which is within the expected range for compensatory post-transplant hypertrophy. However, the cyst number was reduced in Recipient 1 (24 to 21) and remained the same (12) in Recipient 2. The volume of individual cysts was reduced in 5 out of 6 well-aligned cysts (on average by 9%) in Recipient 1 and in all 3 well-aligned cysts in Recipient 2 (on average by 63%). These data suggest that ADPKD kidney procurement-related AKI is not a potent trigger of renal cystogenesis, thus mildly affected ADPKD kidneys may be used in renal transplantation.

ULTRAFILTRATION RATE AND POSTURAL HYPOTENSION ARE ASSOCIATED WITH INTER-DIALYTIC WEIGHT GAIN: INSIGHTS FROM THE CARDIS STUDY

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Hemodialysis (HD) is the commonest modality of renal replacement therapy in the United States, with approximately 400,000 prevalent patients. Patients on HD have an unacceptably poor prognosis, with over half dying within three years of initiation. Inter-dialytic weight gain (IDWG) is associated with a higher risk of left ventricular hypertrophy, intradialytic hypotension, cardiovascular disease, and mortality. Given these associations, we aimed to determine modifiable risk factors for IDWG. We performed a prospective cohort study of adult patients initiating HD between February 2016 and January 2018. Session-level data was collected, including demographics, dialysis prescription, and pre- and post-HD vital signs. Hierarchical generalized linear regression models were fit to determine the association of ultrafiltration rate (UFR), sodium gradient, and post-HD postural hypotension with IDWG. A total of 20 patients completed the study with 906 HD sessions available for analysis. Median age was 69.3 years, 35% were female, and 35% were black. The median IDWG was 1.5 kg (Panel A). Each unit increase in UFR was associated with a 40 g increase in IDWG ($p=0.02$, Model 3), and postural hypotension was associated with a 200 g decrease in IDWG ($p=0.04$, Model 3) (Panel B). The sodium gradient was not associated with IDWG. Future studies that reduce UFR and minimize postural hypotension are required to investigate the effects of these interventions on IDWG and hard clinical outcomes.



B.

Predictor	Difference in IDWG per unit increase (β coefficient)	P-value
Ultrafiltration rate		
Model 1	0.04	0.02
Model 2	0.05	0.01
Model 3	0.04	0.02
Model 4	0.04	0.08
Postural hypotension		
Model 1	-0.20	0.04
Model 2	-0.20	0.04
Model 3	-0.20	0.04
Model 4	-0.15	0.20
Sodium gradient		
Model 1	0.01	0.86
Model 2	0.01	0.67
Model 3	0.01	0.63
Model 4	-0.09	0.33

Model 1: unadjusted
Model 2: Model 1 + demographics (age, sex and race)
Model 3: Model 2 + comorbidities (diabetes, heart failure) and access type (catheter versus other)
Model 4: Model 3 + pre-HD laboratories (serum sodium, blood urea nitrogen (BUN) and albumin)

EXAMINATION OF A NOVEL IMMUNOREGULATORY MARKER IN A TRANSPLANT POPULATION

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Autoimmune disease stems from dysfunction of T and NK cell pathways, with the TIGIT/CD226 receptor pathway important for both. CD226 and TIGIT are co-ligands for CD155, with CD226 shown to increase cytotoxic function of NK cells while TIGIT has been suggested to lead to negative signaling and suppression of T cells. Studies show variations in TIGIT expression in NK cells of healthy individuals, suggesting variable potencies of these cells. While the CD226/TIGIT axis has been studied extensively in cancer, little is known of its role in transplantation. Given this discrepancy, differences in the TIGIT/CD226 axis were investigated between healthy controls (HC) and both kidney-transplant and lung-transplant recipients (KTR and LTR). Sample sizes were n=15, n=55, and n=7, respectively. Cell-surface expression of CD226, TIGIT, CD155 and CD112 on peripheral CD4⁺T, CD8⁺T and NK cells was measured by flow cytometer, following peripheral blood mononuclear cells (PBMC) isolation. The data was acquired using a BD Canto II flow cytometer and analyzed using Flowjo and Graphpad Prism. In both T and NK cell populations, transplantation recipients showed a statistically significant decrease in TIGIT and an increase in CD226 expression compared to HC. CD226⁺ NK cells also showed more heterogeneity in KTR populations compared to HC, whereas TIGIT⁺ NK cells showed similar levels to their control counterparts. CD155 and CD112 were not expressed on any of the cells from all groups. In conclusion, transplantation itself may cause a direct immune response of upregulating TIGIT expression and down-regulating CD226 expression, a future application for immunotherapy.

mRNA IS STORED AT STALLED POLYRIBOSOMES IN PODOCYTE FOOT PROCESSES FOR RAPID LOCAL TRANSLATION

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Sustained injury to podocytes can result in irreversible damage and detachment from the glomerular basement membrane, a significant step in the progression to End Stage Renal Disease (ESRD). The effects of injury on the biological pathways that maintain foot process architecture and matrix adhesion are not fully understood. Because of the morphological and cytoskeletal similarities between neurons and podocytes, we suspect that similar to neurons, podocytes utilize mRNA transport and local translation to affect remodeling of the cytoskeleton in response to injury. Using immunogold staining and electron micrograph techniques, we show that both polyribosome complexes and mRNA binding protein Staufen2, known to mediate mRNA transport in neurons, accumulate at podocyte foot processes during injury. Using cell biological techniques to test for various markers of global translation, we found a decrease in translation rate upon injury, and potentially stalling of ribosomes at the elongation step of translation. We propose mRNAs bound to polyribosomes stalled at elongation are stored at podocyte foot processes. By this mechanism, ribosomes bypass the rate-limiting step of translation initiation, allowing for rapid protein synthesis upon signaling. This new model for local translation and storage of mRNA in podocyte foot processes has the possibility to open new therapeutic avenues to treat glomerular disease.

ACTIN CYTOSKELETON BRANCHING IS REQUIRED FOR AQUAPORIN-2 (AQP2) MEMBRANE ACCUMULATION

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Aquaporin-2 (AQP2) trafficking in the kidney principal cells is responsible for water reabsorption and maintaining homeostasis. Vasopressin promotes this process by stimulating AQP2 accumulation in the cellular membrane. In diabetes insipidus, a defect in the AQP2 trafficking mechanism results in an inability to reabsorb water. Previous research has found that depolymerization of actin, a structural protein, plays a role in AQP2 trafficking in principal cells of the collecting duct.

Our research aims to determine an association between actin and aquaporin-2 in LLC-AQP2 cells and rat kidney tissue. First, we focused on confirming co-localization of endocytic vesicles and actin in LLC-AQP2 and LLC-PK1 cells. We observed co-localization in both cell types, indicating that actin is associated with endocytic vesicles that potentially contain AQP2. Next, we explored the role of actin-related protein (ARP) 2/3, which is involved in actin polymerization. Rat kidney tissue was stained with anti-AQP2 and anti-ARP2/3 antibodies. ARP2/3 and AQP2 in principal cells display co-localization in the plasma membrane of the papilla. We then treated LLC-AQP2 cells with CK-666, an ARP2/3 inhibitor; latrunculin, an actin depolymerization agent; and jasplakinolide, an actin polymerization agent. Cells treated with CK-666 display inhibition of VP-stimulated AQP2 accumulation in the membrane. We performed an F-actin assay to determine the efficiency of the previously mentioned drugs and a Western blot to test the effect of the drugs on phosphorylation of AQP2 S256. Interestingly, the combination of CK-66 and jasplakinolide showed less polymerized actin and also decreased phosphorylation of AQP2.

CHARACTERIZATION OF NBCE1B REGULATED BY IRBIT

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The electrogenic Na⁺ bicarbonate cotransporter (NBCe1, SLC4A4) located at the basolateral membrane of the renal proximal tubule (PT) plays a vital role in acid-base homeostasis by moving HCO₃⁻ into the blood. Human or mouse mutations in NBCe1 cause a profound metabolic acidosis. NBCe1A is the dominant isoform expressed in the kidney; however, when NBCe1A is knocked out, the B isoform (NBCe1B) expression in PT increases. Seki and colleagues found that inositol 1,4,5-*tris*-phosphate (IP₃) receptor-binding protein (IRBIT) enhances the activity of NBCe1B to the NBCe1A levels. We have also found that mice with all isoform or NBCe1-A knocked out, are acidotic and develop renal cysts. In this study, NBCe1B mutations were studied by voltage clamp in *Xenopus* oocytes to document their function ±IRBIT. We used immunohistochemistry and western blot analysis of NBCe1A wild-type and knockout kidneys to determine if as NBCe1-B, IRBIT expression was increased. After analysis, it was found that the NBCe1B wild type and mutations G50A and N63S function dramatically increased in the presence of IRBIT. NBCe1B and IRBIT protein were increased in the kidneys of NBCe1-A knockout mice. Imaging also revealed the cystic structures in the kidneys of the knockout mice. Future experiments will evaluate if crossing the NBCe1A knockout with the PKD1-hypomorphic, RC/RC mouse increases the incidence of renal cysts, which would indicate that NBCe1A plays a role in renal cystogenesis.

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RETHINKING TRANSFER LEARNING IN MEDICAL IMAGING – GENERALIZING NEURAL NETWORKS TO NEW MODALITIES

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Segmented kidneys are used to calculate kidney volume, which is an important imaging biomarker for patients affected by polycystic kidney disease (PKD). Automated MRI segmentation of PKD kidneys has been solved at Mayo Clinic. However, many patients receive CT scans instead of MRI scans for a variety of reasons. Using a dataset of 100 CT exams with corresponding gold-standard segmentations, we explored different neural network approaches to segment kidneys in CT images.

To automatically segment CT PKD kidneys, we trained several neural networks on CT scans and their manual segmentations. Learning rate and network complexity were the main parameters we chose to adjust. To synthesize MRI images from CT, we first used a pre-trained neural network to compute style and content features from the input images. Then we used a gradient descent algorithm to adjust the network weights until the output image maintained appropriate content from the CT image while converting the style to an MRI modality.

Automated PKD kidney segmentation resulted in a maximum validation dice coefficient of 79.6%. A neural style transfer result is shown in **Figure 1**.

Automated segmentation of PKD kidneys in CT images shows promising results. Neural synthesis of MRI images from CT retains content information, but tissue texture transfer is suboptimal. While still in the early stages, style transfer may be an important tool to enhance patient care since CT scans are cheaper and much faster to obtain than MRI.

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Figure 1. Left: Style (MRI) image used to create synthesized image. Middle: Content (CT) image used as the image to be stylized. Right: Stylized MRI image.

CLINICAL SYMPTOMS ARE CORRELATED TO PATHOLOGICAL FINDINGS IN CALCIPHYLAXIS PATIENTS

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Calciphylaxis is a rare and life-threatening disease characterized by calcification of microvessels in the subcutaneous adipose tissue that result in vessel blockage and cell death, causing painful skin lesions. It occurs mainly in patients with End-stage renal disease on dialysis. Generally, patients diagnosed with this disease have poor clinical outcomes, and the exact pathogenesis of the disease is not well known. Our research aims to correlate clinical symptoms of skin lesions with pathological findings in biopsies in order to improve our understanding of this disease and establish avenues for future research and potential therapeutic targets. Data from 70 calciphylaxis patients who attended Massachusetts General Hospital between 2014 and 2018 was collected via the EPIC database, including demographics, clinical symptoms of skin lesions, and pathological findings. The majority of patients were between 49 and 69 years old. Most patients reported severe pain at the time of diagnosis with the median pain score being 8/10. There is significant delay in diagnosis with the median time from onset of symptoms to clinical diagnosis being about 9 weeks. The presence of fibrin thrombi in skin biopsies was associated with pain severity ($p=0.04$). This finding opens a potential avenue for a therapeutic target as preventing fibrin deposition could also alleviate pain. Furthermore, the stage of a skin lesion significantly correlated with skin necrosis ($p=0.02$), and the presence of an ulcer was associated with the presence of vascular calcification ($p=0.07$). These findings need further validation in a larger prospective study.

SELECTIVE CD28 BLOCKADE INCREASES IMMUNE SYSTEM SUPPRESSION IN THE SETTING OF VIRAL INFECTION

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Kidney transplantation is a lifesaving therapy for end stage renal disease patients. Immunosuppression is necessary to avoid host rejection but must retain protective immunity. Calcineurin inhibitors have dramatically improved short-term graft survival, but their nephrotoxicity leads to loss of graft function over time. Costimulatory molecules are of interest for immunosuppression as they affect adaptive immune responses. CTLA-4 Ig binds to CD80/CD86, blocking both costimulatory CD28 and coinhibitory CTLA-4 signaling. While drugs using CTLA-4 Ig, abatacept and belatacept, have increased graft function over time, they increase rates of acute rejection and viral reactivation. Recently, anti-CD28 domain antibodies (dAb) that selectively target CD28 and leave CTLA-4 intact are promising new avenues for immunotherapy. This study aims to elucidate the effects of selective CD28 blockade on the anti-viral immune response. Mice were infected with a YFP-labeled recombinant MHV-68, a murine model of EBV, and received either PBS, CTLA-4 Ig, or anti-CD28 dAb and monitored for T and B cell responses. Our results reveal that both dAb and CTLA-4 Ig mice had decreased populations of antigen-specific cells, however the dAb maintained lower populations over time while CTLA-4 Ig approached PBS levels at later time points. Germinal center B cells were decreased in dAb and CTLA-4 Ig treatment compared to no treatment, suggesting that CTLA-4 coinhibitory signals are not involved in antibody producing B cells. The effector T cell populations suggest that dAb is more effective in suppressing the T cells leading to weaker immunity to viral infections.

BETA-CATENIN IN THE RENAL STROMA REGULATES NEPHRON DEVELOPMENT

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The kidney is an essential homeostatic organ vital to maintaining our blood chemistry. Each healthy human kidney is made up of about one million functional units termed nephrons which are completely formed by 36th weeks of gestation. Individual nephron segments are composed of several different cell types, each with unique structural components, that all arise from a single precursor cell population termed nephron progenitor cells. Recently, the stroma has emerged as a critical regulator in nephron progenitor maintenance and differentiation; however, the molecular pathways regulating this critical developmental balance remain incompletely understood. Here, we aim to further define the critical signaling pathways in stromal cells that regulate nephrogenesis *in-vivo*, specifically focusing on the role of Wnt/beta-catenin as it is expressed in the cortical stroma directly adjacent to the nephron progenitor population and has been suggested to modulate cell signaling in these neighboring cells. We used a mouse line with a stromal cell-specific deletion in exon 3 of the beta-catenin gene, CTNNB1, resulting in gain-of-function in beta-catenin. Histological analysis of mutant embryonic kidney tissues showed significantly delayed nephron tubule formation. To further elucidate factors regulating this abnormal development, we performed explant cultures of mutant kidneys and worked to establish a 3D bio-printing technique as a novel method for patterning of the various cell types that make up the complex structure of the nephron tubule. Overall, these studies provide further insight in tubule formation for future advancements in the field of nephron regeneration and tissue engineering.

WHOLE EXOME SEQUENCING IMPLICATES WDR4 AS A NEW, AUTOSOMAL RECESSIVE GENE FOR NEPHROTIC SYNDROME

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Galloway-Mowat syndrome (GAMOS) is a rare, autosomal recessive condition characterized by the combination of nephrotic syndrome and central nervous system anomalies (e.g., microcephaly). Additional features may include intellectual disability, facial dysmorphism, growth retardation, etc. Currently, only four of the KEOPS complex genes (LAGE3, OSGEP, TP53RK, and TPRKB) as well as WDR73 and NUP107 are known to cause GAMOS.

To identify additional monogenic causes of GAMOS, we performed whole exome sequencing, linkage analysis, and homozygosity mapping on three affected siblings from a non-consanguineous family with GAMOS. Applying established criteria for variant filtering, we identified a novel, splice-effect mutation homozygous for all three affected siblings located in WDR4, an essential tRNA modification gene, on chromosome 21. Consistent with previous cases of WDR4 mutations, we observe growth deficiency, microcephaly, and intellectual disability as developmental and neurologic features resulting from WDR4 mutation. The presence of nephrotic syndrome and proteinuria in our patients also marks the first report of renal phenotypes in patients with WDR4 mutations.

Our study expands the previously described phenotypic spectrum of WDR4 mutations by demonstrating that renal phenotypes may present. This discovery suggests that in some cases, GAMOS may occupy a phenotypic spectrum with other microcephalic diseases. WDR4, like KEOPS genes (Braun, *Nat Genet.* 49:1529, 2017), is another example of a gene encoding a tRNA modifying enzyme that can cause GAMOS. Our findings thereby support the recent observation that, like neurons, podocytes of the renal glomerulus are a cell type that is particularly vulnerable to defects resulting from altered tRNA modification.

QUANTITATIVE MAGNETIZATION TRANSFER MAGNETIC RESONANCE IMAGING: A NOVEL NONINVASIVE METHOD FOR DETECTION OF RENAL FIBROSIS

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Renal fibrosis is the final common pathway of various kidney diseases but is hard to detect in the intact kidney. Clinically, a reliable noninvasive imaging biomarker is needed for measurement of renal fibrosis, which is characterized by excessive deposition of collagens. Quantitative magnetic transfer imaging (qMT) can be used to directly measure macromolecule content without limitations related to scan or scanner factors. Here, we tested the hypothesis that qMT could successfully quantify renal fibrosis. Thirteen 11-week-old male 129S1 mice underwent renal artery stenosis (RAS, n=8) or sham (n=5) surgeries. MT scans, B_0 , B_1 , and T_1 maps of a short-axial kidney slice were acquired 4 weeks post-surgery on a vertical 16.4T Bruker scanner. Twenty-four MT-weighted images were acquired with 12 different offset frequencies (1 to 50 kHz) and 2 flip angles (450° and 900°). The bound pool fraction (f), an index of macromolecule content, in renal cortex (CO), outer (OM) and inner (IM) medulla was fitted pixel-wise using a two-pool model. Kidneys were harvested for Masson's Trichrome staining, from which percent renal fibrosis was quantified. The bound pool fractions measured in all 3 regions were significantly elevated in RAS compared to sham kidneys (Fig.1). A good correlation was observed between the MRI-measured bound pool fraction and renal fibrosis by trichrome (Fig. 2). In conclusion, qMT imaging successfully measured renal fibrosis in mouse kidneys with RAS, and may provide a useful imaging biomarker for renal fibrosis. Future studies need to confirm the feasibility of assessing renal fibrosis using qMT in human subjects.

Support: R25-DK101405 and U54-DK100227

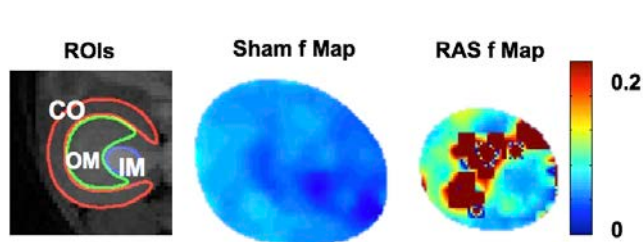


Fig. 1. a) Representative f maps of a normal (left) and RAS kidney (middle), and manually selected ROIs (right) for cortex (CO), outer (OM) and inner medulla (IM). Yellow and red coloring suggests increased macromolecule content. b) Measured f in CO, OM, and IM of RAS and control kidneys.

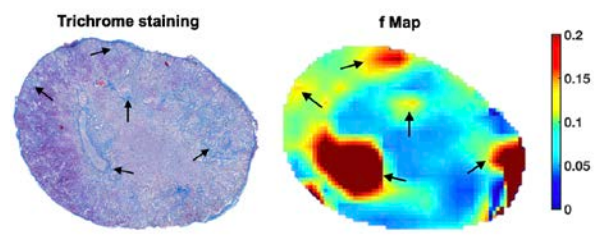


Fig. 2. a) Representative histology micrograph and f map of one RAS kidney. Black arrows highlight fibrosis detected by both methods. b) Pearson correlation of fibrosis measured *ex vivo* and *in vivo* in CO, OM, and IM.

INHIBITION OF TLR4 ACTIVATION PREVENTS RENAL AUTOREGULATORY DYSFUNCTION IN ANG II HYPERTENSIVE RATS

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Autoregulation by afferent arterioles regulates renal blood flow and glomerular filtration rate across widely ranging arterial pressures. Autoregulatory function is impaired in hypertension, perhaps due to release of damage associated molecular patterns that activate renal toll-like receptor (TLR4). Autoregulatory impairment can promote hypertensive kidney injury. We hypothesize that inhibition of TLR4 activation preserves afferent arteriole autoregulatory function in Angiotensin II (AngII) hypertension.

Three groups (n=4/group) of male Sprague-Dawley rats were implanted with osmotic minipumps set to deliver: saline (0.9% NaCl) or AngII (60ng/min). Group three received AngII+anti-TLR4 Ab (3 µg/ml/kg/day, i.p.) for 14 days. Systolic blood pressure (SBP) was assessed (days 0, 3, 7 and 13). Kidneys were harvested on day 14 for juxtamedullary nephron assessment of afferent arteriole autoregulatory function.

SBP increased in the AngII and AngII+Anti-TLR4 Ab groups compared to saline controls over the 14 days (P<0.05), averaging 198±11, 200±8 and 135±3 mmHg, respectively. Baseline arteriole diameters (100 mmHg) averaged 14±0.8, 15.6±1.7, and 13±0.3 microns for control, AngII and AngII+Anti-TLR4 Ab, respectively. Perfusion pressure was decreased to 65 mmHg and increased to 170 mmHg in 15 mmHg increments. At 170 mmHg, arteriole diameters decreased by 31±6% and 33±8% of baseline in the control and Ang II+Anti-TLR4 Ab groups whereas the AngII group had a decline of 11.7±3.7%.

These data establish that autoregulatory function is Impaired in AngII hypertensive rats while it is preserved by anti-TLR4 Ab-treatment. These findings implicate chronic TLR4 activation as causative in the hypertensive decline in autoregulatory capability. Funded by NHLBI; 1R25 HL115473-01 and DK044628-20.

Q-SAS: A QUANTITATIVE KIDNEY STONE ANALYSIS SOFTWARE FOR THE EVALUATION OF URINARY STONES

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X-ray computed tomography (CT) is an essential tool for urinary stone detection and removal planning, providing sub-millimeter information on stone number, diameter and, for dual-energy CT, mineral composition. However, current clinical software and workflow are laborious and lack accuracy in the characterization of advanced stone metrics, including volume and morphological features. The quantitative Stone Analysis Software (q-SAS) has been under development by the McCollough lab at Mayo Clinic, with the goal of providing objective metrics of urinary stones with an efficient workflow.

In this work, we developed and validated an improved workflow for the manual delineation of renal anatomy. In our novel approach, data are down sampled in the longitudinal direction to reduce the number of images that need to be manually outlined. The resulting kidney mask is ultimately interpolated back to the original size of the CT dataset. Our efforts resulted in an 85% reduction in the average time required by the user to identify the kidneys. Moreover, we extended the dual-energy mineral composition assessment to the pixel level. We developed a method that performs a local smoothing, by grouping and averaging pixels, to reduce the impact of noise. Results show the benefit of the smoothing, especially at lower doses and larger patient sizes.

In conclusion, the new iteration of q-SAS features rapid workflow for manual kidney delineation and pixel-level stone composition analysis, which allows assessment of mixed stones while maintaining accuracy for pure stones. This software is soon to be released to Mayo Clinic and the research community.

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MEMBRANE TARGETED YFP SENSOR OF VOLUME REGULATED ANION CHANNELS

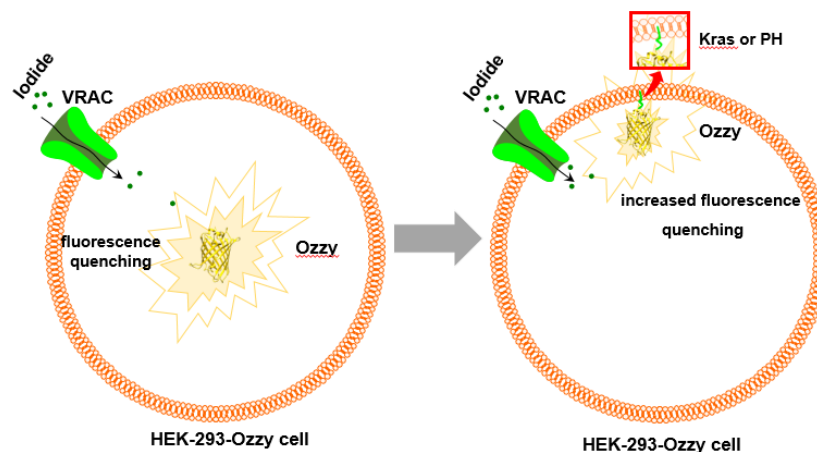
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Cells critically regulate their volume in response to hypotonic cell swelling by transporting chloride and small organic osmolytes out of the cell through volume regulated anion channels (VRACs) encoded by members of the recently defined LRRC8 gene family. VRACs are ubiquitously expressed in mammalian cells and have been implicated in diverse cellular functions in addition to cell volume regulation, including endothelial cell calcium signaling and pancreatic beta cell insulin secretion. The lack of potent and specific pharmacological tool compounds represents a critical barrier to evaluating the integrative physiology and therapeutic potential of VRACs. Therefore, we developed a fluorescence-based assay for high-throughput screening (HTS) to identify novel inhibitors of native VRACs expressed in HEK-293 cells. Briefly, HEK-293 cells express a halide-sensitive YFP mutant (i.e. F46L, H148Q, I152L) termed Ozzy. The assay measures the quenching of intracellular Ozzy by iodide influx through VRACs activated by hypotonic-induced cell swelling. Small-molecule inhibitors should block the iodide-induced quenching of Ozzy. The major goal of this project is to compare the pharmacological sensitivity of assays based on soluble Ozzy and an Ozzy variant targeted to the cell membrane where VRACs reside. Our preliminary results report no difference in Pranlukast-dependent inhibition of VRAC between the soluble and membrane-bound Ozzy quenching assays, having an IC₅₀ of 4.4μM and 4.6μM, respectively.

Research supported by: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH), Kidney, Urology, Hematology (KUH) R25DK096999 to Billy G. Hudson, Aspirnaut™ Undergraduate Discovery Science Experience in Renal Biology and Disease, Aspirnaut™, Vanderbilt University Medical Center, and Vanderbilt Center for Matrix Biology

Graphical Abstract:



MECHANISM OF QUINOLONE RESISTANCE IN *ESCHERICHIA COLI* DNA TOPOISOMERASE II

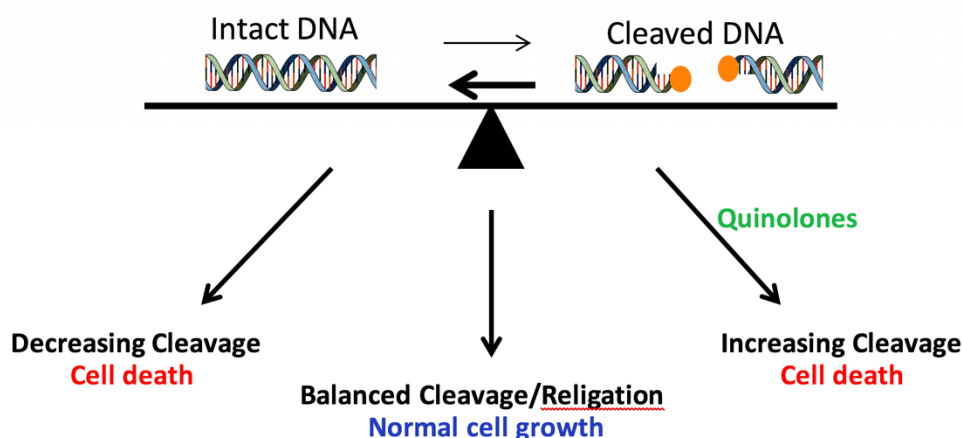
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Quinolones, such as ciprofloxacin, are used to treat bacterial infections that include anthrax, urinary tract diseases, and gonorrhea. The cellular targets of quinolones are the bacterial type II topoisomerase, gyrase, and topoisomerase IV. Quinolones kill cells by stabilizing covalent enzyme-cleaved DNA complexes generated by bacterial type II topoisomerases, inhibiting the overall catalytic activity of these enzymes, or both. Gyrase and topoisomerase IV maintain DNA topology by generating transient breaks in the double helix. Furthermore, gyrase relaxes positive supercoils ahead of the replication fork, while topoisomerase IV separates sister chromatids after replication. Unfortunately, due to overuse, there has been a rise in quinolone resistance since the 1990s. Mutations occur in the amino acid residues that anchor the water-metal ion bridge through which quinolones and type II topoisomerases interact. To better understand how these mutations, cause resistance, we examined how the catalytic cycles of wild-type and mutant quinolone-resistant *Escherichia coli* gyrase and topoisomerase IV are affected by ciprofloxacin. Assays were carried out to assess the impact of the quinolone on DNA cleavage, supercoiling, and relaxation. Ciprofloxacin enhanced DNA cleavage mediated by wild type *E. coli* gyrase and inhibited the introduction of negative supercoils. Moreover, the drug inhibited the relaxation of positively supercoiled DNA by *E. coli* topoisomerase IV. We propose that mutations may cause quinolone resistance in *E. coli* cells by stabilizing DNA cleaved complexes mediated by type II topoisomerases in addition to inhibiting the relaxation of positive supercoils.

Research supported by: Berea/Aspirnaut™/Hal Moses Summer Research Internships, Vanderbilt University Medical Center, Vanderbilt Center for Matrix Biology, and the Aspirnaut™ Program.

Graphical Abstract:



MODELING RENAL CELL CARCINOMA IN MICE: GENETICALLY ENGINEERING BAP1-PBRM1 DEFICIENT MICE

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The aim of this SURF project was to understand the mechanics of generating genetically-engineered mouse models (GEMM) of an aggressive form of clear cell renal cell carcinoma (ccRCC). ccRCC, the most common type of kidney cancer, is characterized by inactivation of the *VHL* gene on chromosome 3p. Our lab previously discovered that 15% of ccRCC also have mutations in the *BAP1* gene, and that for unknown reasons, these tumors tend not to have mutations in *PBRM1* (mutated in 50% of ccRCC). Both the *BAP1* and *PBRM1* genes are also located on chromosome 3p, and one copy of these genes is lost along with *VHL*. We also reported that patients with *BAP1/PBRM1*-deficient tumors have the worst prognosis. We previously generated mice with conditional mutations in *Vhl* and *Bap1* or *Pbrm1*, which develop ccRCC, but were unable to create triple deficient mice because *Bap1* and *Pbrm1* are genetically linked. To overcome this problem, we targeted the *Bap1* allele in homozygous *Pbrm1* floxed embryos using the CRISPR/Cas9 system. To determine the most efficient guide RNA to target the *Bap1* allele, sgRNAs were transfected into mouse embryonic fibroblasts and screened by a mismatch cleavage assay. We have successfully screened the best 5' and 3' guide RNAs, which were then injected into fertilized eggs along with the template oligos. Through this process, we have successfully created mice with conditional alleles for both *Bap1* and *Pbrm1*. This mouse model may help us understand the relationship of BAP1 and PBRM1 and treat patients with aggressive BAP1/PBRM1-deficient kidney cancers.

THE EFFECT OF AUTOPHAGY INHIBITION ON CLEAR CELL RENAL CELL CARCINOMA USING A PATIENT-DERIVED XENOGRAFT MODEL

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Autophagy is a cellular response to stress where the cell degrades damaged organelles to recycle materials in order to prolong survival. During this process, the cell creates double membraned vesicles called autophagosomes that fuse with lysosomes to recycle cellular components. This response is triggered by stressful environments including hypoxia, which is characteristic of many cancers, including renal cell carcinoma (RCC), the most common type of kidney cancer. According to the American Cancer Society, kidney cancer is among the ten most common cancers in both men and women in the United States of America. It is estimated that there will be 63,340 new cases of kidney cancer in the year 2018. The most common subtype is clear cell RCC (ccRCC), accounting for about 70% of all RCC. ccRCC, once it metastasizes remains largely incurable. Our lab has previously developed a large platform of patient-derived RCC tumorgraft lines (over 100) and showed that tumors growing in mice reproduce the features of the corresponding patient tumors. In this study, we used NOD/SCID mice implanted with patient-derived ccRCC tissue to conduct a trial using Cabozantinib, a tyrosine kinase inhibitor FDA-approved for ccRCC treatment, and Hydroxychloroquine (HCQ), an inhibitor of lysosomal function. There were three treatment arms: HCQ (intraperitoneal), Cabozantinib (oral gavage) and HCQ (intraperitoneal), and vehicle (oral gavage) with three mice in each treatment group. By targeting the lysosomes and multiple tyrosine kinase receptors, we predict the tumor volume and cell proliferation will decrease in both the HCQ and combination groups.

IMPACT OF DEEP LEARNING BASED NOISE REDUCTION ON QUANTITATIVE KIDNEY STONE ANALYSIS SOFTWARE

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The objective of this study was to observe the effectiveness of deep learning based noise reduction for Computed Tomography (CT) scans when applied to quantitative kidney stone analysis software. We used a Convolutional Neural Network (CNN) to perform the deep learning. The CNN was trained in noise reduction on images that had been collected in abdominal CT scans at full-dose. The training data had undergone noise insertion to simulate a low radiation dose. The CNN was later fine-tuned on urograms taken with dual-energy CT so that it could denoise images from dual-energy CT scans. The CNN was tested on noise-inserted patient kidney scans. We compared the simulated low-dose and denoised low-dose images to their full-dose counterparts with quantitative analysis software. The software calculated the compositions of the kidney stones and classified them. Processing the simulated low-dose scans resulted in some incorrect kidney stone classifications, but analyzing the denoised versions of the images corrected the inaccurate classifications. Deep learning based noise reduction can be useful when applied to kidney CT scans. This new approach may improve location, segmentation, and composition analysis particularly in low-dose CT scans in kidney stone analysis software.

Support: R25-DK101405; U54-DK100227

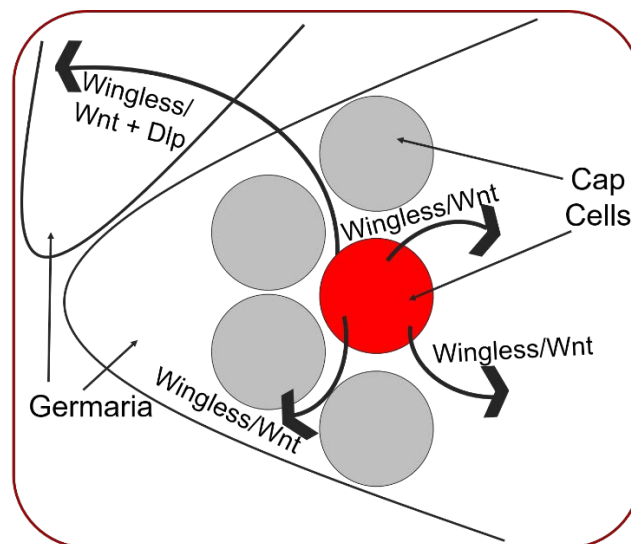
UNDERSTANDING MECHANISMS OF WINGLESS EXTRACELLULAR DISTRIBUTION IN FLY DEVELOPMENT

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Animal cells use extracellular signaling proteins to communicate. Our group is interested in how extracellular signaling proteins spread from cells that produce them to receiving cells, using the fruit fly, *Drosophila*, as the model organism. Here, we analyzed the spread of conserved Wnt/Wingless signaling proteins within the germarium, the part of the fly ovary that contains stem cells. Based on previous research, we know that Wingless protein is made in cap cells, at the tip of the germarium, and can spread up to five cells away. Although all germaria contain Wingless protein and cap cells, 72 percent of germaria lack cap cells that produce Wingless mRNA. The source of Wingless protein in these germaria is unknown. Because ~18 germaria are positioned very closely together at the tip of each ovary, we hypothesize that Wingless protein is shared among germaria within a single ovary. To address this question, we use a sophisticated genetic tool called G-Trace, which labels cells that were producing Wingless at the time of dissection fluorescent red and cells that were producing Wingless in the past fluorescent green, and count the number of each within a single ovary. We are also asking how different Wnts spread in cell culture. We know that Wnts spread by binding to a cell surface protein called Dally-like protein (Dlp). We amplified nine Wnt or Dlp containing plasmids for use in cell culture and confirmed their identities using restriction digests. These plasmids will be used to synthesize protein for future biochemical experiments.

Research supported by: NIH R01 GM117899 to A.P.M., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH), Kidney, Urology, Hematology (KUH) R25DK096999 to Billy G. Hudson; AspirnautTM Undergraduate Discovery Science Experience in Renal Biology and Disease; AspirnautTM, Vanderbilt University Medical Center, and the Vanderbilt Center for Matrix Biology.

Graphical Abstract:



TISSUE-SPECIFIC AND STRESS-SPECIFIC REGULATION OF FIBROBLAST GROWTH FACTOR 21 EXPRESSION

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Fibroblast growth factor 21 (FGF21) is a hormone that plays a regulatory role in energy homeostasis due to its diverse metabolic functions including regulating energy expenditure, glucose metabolism in adipose tissue, and pancreatic exocrine function. In a mouse model of sepsis, we found that plasma levels of FGF21 increase after lipopolysaccharide (LPS) challenge. Interestingly, FGF21 deficient mice are more susceptible to LPS mortality. In order to investigate the source of FGF21, we measured transcriptional levels of *Fgf21* in different organs 4 hours after LPS. At baseline, *Fgf21* is highly expressed in the liver. After LPS challenge, *Fgf21* is downregulated in the liver; by contrast, the expression level of *Fgf21* in the kidney is increased after LPS. In order to identify regulatory mechanisms behind this difference, mouse hepatocyte 1-6 (Hepa 1-6), proximal tubular (MPT), and pancreatic 266-6 acinar cell lines were treated with different stress conditions for 3 hours. We found that treatment with transforming growth factor beta (TGF β), *Fgf21* mRNA expression was increased in MPT cells; similarly, it was also upregulated after treatment with tunicamycin. In pancreatic acinar cells, tunicamycin increased *Fgf21* expression, while TGF β had no effect. The results from Hepa 1-6 cells are unclear. These data suggest that transcriptional regulation of *Fgf21* is likely both tissue- and stress-specific. Future studies are required to determine the transcriptional regulatory pathways, to confirm our results at the translational level, and to determine the role of renal FGF21 in promoting survival in sepsis.

ROLE OF HDX IN CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) is a public health problem that affects 15% of the US population. A large contributing factor to CKD's progression is renal tissue hypoxia. Up-regulation of Hypoxia-inducible factor-2 (HIF-2 α) alleviates renal hypoxia-associated CKD by oxygenating and vascularizing renal tissue. HIF-2 α is a transcription factor that promotes the transcription of genes encoding erythropoietin (EPO), vascular endothelial growth factor (VEGF), and other hypoxia response elements. In kidney nephrons, EPO also counteracts some of the effects of renal anemia. There are 27 known proteins that bind to EPAS1 gene, the gene that encodes HIF-2 α . One of these proteins, the transcription factor Highly Divergent Homeobox (HDX or CXorf43), negatively regulates HIF-2 α expression. The function of HDX is not well understood. We found that mammalian target of rapamycin complex-1 (mTORC1) regulates HDX nuclear translocation through the mTORC1 subunit Raptor. mTORC1 is an amino acid sensing protein complex that is critical in regulating cell growth. Immunoprecipitation and mass spectrometry analysis showed Raptor and HDX interact under both normal and amino acid starved conditions. However, immunofluorescence analysis showed HDX co-localized with mTORC1 only in complete amino acid conditions. Furthermore, western blot analysis showed mTORC1 activity is decreased during hypoxic stress (1% O₂) compared to normoxia (21% O₂). These results suggest that mTORC1 activation is important for HDX function and could be a novel target to suppress the effects of renal hypoxia.

NFkB SIGNALING AND BRANCHING MORPHOGENESIS

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Essential to a vast array of functions including the excretion of wastes, regulation of blood pH, and production of hormones, the kidney serves as a vital organ to keep the body in balance. Although much is known about the developing kidney anatomy, questions remain about the molecular mechanisms that regulate specification, invasion, proliferation, and differentiation. Ongoing experiments in the developing pancreas have tested the step-wise developmental mechanisms regulating endocrine cell fate from progenitors to differentiated beta cells in hopes of generating new beta cells for replacement or regenerative therapies in diabetes treatment, guided by the hypothesis that **Lats1/2 kinases are required to restrict proliferative and inflammatory pathway responses to Yap/Taz and NFkB transcription factor (TF) programs during pancreatic progenitor renewal and differentiation.** We asked if Yap/Taz and NFkB TFs might similarly regulate kidney morphogenesis. Revealing these molecular relationships would be crucial in understanding cancerous kidney tumor formation, since Lats1/2 are known tumor suppressors. With RNA-Seq analysis of Lats1/2DKO pancreas at E11.0 revealing upregulation of novel Yap1/Taz target genes including *Vanin1* and *Lurap1l*, known activators of NFkB signaling, we hypothesized that **NFkB promotes proliferative and inflammatory signaling pathways in the embryonic kidney.** Supporting this idea, we found that when NFkB was inhibited ex vivo, ureteric bud (UB) branching was significantly decreased in WT explanted kidney. Interestingly, we did not observe increased UB branching following treatment with the NFkB activator PMA, suggesting other roles for PMA in kidney. Exploiting our understanding of kidney developmental mechanisms has implications for future kidney disease therapies.

REGULATION OF OXALATE TRANSPORTER SLC26A6 IN HT-29 CELLS

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By secreting oxalate in the intestine, activity of oxalate transporter SLC26A6 reduces urinary oxalate and protects against calcium oxalate urolithiasis. We have used HT-29 cells as an intestinal model to study potential regulators of SLC26A6 and of PDZ scaffolding proteins that associate with SLC26A6, namely PDZK1 and NHERF4. We specifically tested two proposed regulatory factors: estradiol, and activation of PKA by forskolin.

We cultured HT-29 cells in the presence of varying concentrations of estradiol or of forskolin with IBMX. qPCR analyses were performed using primers for SLC26A6, PDZK1, and NHERF4, and expression normalized to HPRT. Western blot analyses with densitometry were used to measure SLC26A6, PDZK1, and NHERF4 protein expression. Cl-oxalate exchange activity was assayed using ¹⁴C-oxalate and liquid scintillation counting.

We found that the presence of estradiol at tested concentrations of 1.00 and 100 nM had no significant effect on SLC26A6 expression or transport activity in HT-29 cells. We found that 100 μ M and 50.0 μ M forskolin decreased oxalate transport in HT-29 cells, as well as PDZK1 and NHERF4 protein expression in a dose-dependent manner, while not altering SLC26A6 protein expression. One potential explanation for this finding may be a PKA effect on surface SLC26A6 expression rather than total SLC26A6 protein expression mediated by down regulation of PDZK1 or NHERF4. Future studies will be directed at testing this possibility.

EARLY DETECTION OF SUBCLINICAL INFLAMMATION IS ASSOCIATED WITH INFERIOR OUTCOMES IN HIGH-RISK RECIPIENTS OF KIDNEY TRANSPLANTS

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Compared to serum-creatinine based monitoring of kidney transplant function, allograft surveillance biopsies can detect early renal inflammation without overt clinical dysfunction. Prior studies in low-risk transplant populations suggest that these biopsies may more accurately guide patient management.

We reviewed all UAB kidney transplant biopsies between May 2015 and December 2017 (n=913). We hypothesized that the presence of subclinical inflammation (SCI) would predict higher rates of late rejection and graft loss in kidney transplant recipients (KTRs).

The primary exposure was SCI, inclusive of subclinical or borderline rejection, as defined by the allograft pathology Banff 2013 criteria. The primary outcome was a composite endpoint of acute rejection or graft loss.

There were 302 6-month surveillance biopsies and 97 had SCI compared to 205 with no major abnormality (NMA) (Table 1). KTR demographics and outcomes are shown in table 1. There were no statistical differences in serum creatinine, HLA mismatch or immunosuppression regimen between groups. The composite endpoint was reached by significantly more recipients with SCI than those with no major abnormalities (18% vs. 5%, $P < 0.001$) and more commonly in African American KTRs than non-black KTRs ($P < .001$).

A high rate of our KTRs demonstrated histological evidence of allograft injury in the absence of functional changes, compared to previously reported, with associated higher rates of rejection and graft loss. This may be reflective of our high immunologic risk population. Further studies are needed to determine the impact of intervention when SCI is identified.

Table 1. KTR demographics and outcomes based on 6-month surveillance biopsy results. * $P < 0.001$

Parameter	SCI (n = 97)	NMA (n = 205)
Age at transplant (yr)	49 ± 1.4	48 ± 0.8
Race (Black/Non-Black)	59/38	107/98
Repeat Transplant	14 (14%)	18 (9%)
Deceased Donor*	68 (70%)	115 (56%)
Serum Creatinine at Biopsy (mg/dL)	1.5 ± 0.06	1.4 ± 0.03
Cause of ESRD	Diabetes	68 (33%)
	Glomerular Disease	65 (32%)
	Hypertension/Other	72 (35%)
Total HLA Mismatch (A/B/DR/DQ)	5.2 ± 0.2	5.1 ± 0.13
BK Viremia*	43 (44%)	43 (21%)
BKV Associated Nephropathy*	25 (26%)	10 (5%)
Urine Protein: Creatinine Ratio*	0.23 ± 0.03	0.15 ± 0.01
1-year estimated GFR (mL/min/1.73 m ²)	57 ± 23	60 ± 19
Type of Rejection after Surveillance*	SC-TCMR	3 (2%)
	SC-ABMR	6 (3%)
	Mixed	0 (0%)
DSA at Biopsy	10 (11%)	9 (5%)
Composite Endpoint*	17 (18%)	10 (5%)
Acute Rejection	15 (16%)	9 (5%)
Graft Failure	7 (7.2%)	1 (1%)

METABOLISM OF GLYCOLATE TO OXALATE IN HUMAN KIDNEY PROXIMAL TUBULE CELLS

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Calcium Oxalate is the most frequent component of kidney stones. Endogenous synthesis of oxalate occurs primarily in the liver and accounts for approximately half of urinary oxalate excretion. Glycolate is one of the sources of oxalate, synthesized through the intermediate glyoxylate, and is well characterized by hepatic pathways. Extra-hepatic metabolism could account for up to 20% of glycolate to oxalate synthesis. Our hypothesis is that glycolate can be metabolized to oxalate by the kidney in proximal tubule cells.

Human Kidney Cells (HK-2, a human proximal tubule cell line) and Inner Medullary Collecting Duct cells (IMCD, a mouse kidney cell line) were incubated with varying doses of ¹³C₂-Glycolate (0-10mM) under increasing time conditions (3-24 hrs). The amount of ¹³C₂- and ¹²C₂-Oxalate in cell culture media was measured using Ion Chromatography coupled with Mass Spectrometry (IC-MS), and results were normalized to total cell protein.

Synthesis of oxalate from glycolate was observed in HK-2 cells for glycolate concentrations as low as 1 mM and as soon as after 12h of incubation. No significant synthesis of oxalate was detected in IMCD cells.

The dose and time-dependent metabolism of glycolate to oxalate seen in HK-2 cells is compatible with the involvement of a proximal tubule-specific enzymatic pathway. Hydroxy acid oxidase 2 is a candidate enzyme related to glycolate oxidase, the liver-specific enzyme known to metabolize glycolate to glyoxylate. Glycolate and oxalate are elevated in primary hyperoxaluria type 1 and understanding the mechanisms underlying renal synthesis of oxalate may help explain the severity of this disease.

PENDRIN AUGMENTS K⁺ SECRETION IN MODELS OF HYPERKALEMIA

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Pendrin is a Cl⁻/HCO₃⁻ exchanger expressed in the apical regions of intercalated cells of the connecting tubule and cortical collecting duct, where it mediates Cl⁻ absorption in tandem with ENaC-mediated Na⁺ absorption across principal cells, which modulates blood pressure. ENaC-mediated Na⁺ absorption creates a lumen-negative transepithelial voltage, thereby augmenting the driving force for the secretion of K⁺. Maxi-K channels are expressed in type B intercalated cells and mediate Na⁺-independent K⁺ secretion. Serum K⁺ is lower in pendrin null mice compared to wildtype littermates, but only in specific treatment models. The purpose of the study was to determine the treatment conditions that unmask this hypokalemic phenotype in the pendrin null mice and to determine its mechanism. We examined ENaC subunit abundance and Maxi K(BK \square) abundance by immunoblots and immunohistochemistry. Following the Na⁺-deficient, K⁺-replete diet or following the Na⁺ and K⁺ deficient diet with the ENaC inhibitor, amiloride, ENaC subunit abundance was much lower in the pendrin null relative to the wild type kidney. However, following a diet deficient in both Na⁺ and K⁺, ENaC abundance was the same or higher in the pendrin null mice and BK \square was higher in pendrin null mice. Conclusion: Pendrin modulates the balance between Na⁺ absorption and K⁺ secretion. In models of hyperkalemia that involve a Na⁺-deficient, diet, pendrin augments ENaC-mediated Na⁺ absorption and K⁺ secretion by stimulating ENaC and Maxi K channels. However, in models of hypokalemia that involve dietary Na⁺ restriction, pendrin does not stimulate ENaC, and blunts K⁺ secretion through Maxi K channels.

ENGINEERING GENE THERAPY VECTORS FOR IMPROVED GENE DELIVERY IN THE KIDNEY

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Many genetic diseases in the kidney could benefit from gene therapy. We tested adenovirus (Ad) and adeno-associated virus (AAV) vectors for their ability to genetically modify kidney cells *in vivo* in mouse models. After intravenous injection, gene delivery was restricted to cells in the glomerulus with only rare tubule cells being modified. In contrast, retroviral injection improved tubule transduction, but not all cells were modified. This project aimed to identify alternate receptors on tubules to be targeted by the vectors to improve transduction.

Lotus Tetragonolobus Lectin (LTA) binds α -linked L-fucose and is a well-known tubule-specific stain in the kidney. To test if α -fucose might serve as a neo-receptor for vectors, mouse kidneys and human Renal Cortical Tubule Epithelial cells (RCTE) were stained with biotinylated LTA and were detected with streptavidin-AlexaFluor594. Confocal imaging demonstrated LTA binding to kidney tubules and also to the surface of RCTE cells. To enable fucose targeting, a biotin acceptor peptide (BAP) and a fucose-binding domain (FBD) were inserted into the genomes of Ad and AAV capsids by cloning. Constructs were validated by sequencing and western blot analysis and efforts are underway to rescue the modified viruses for targeting tests.

Kidney tubule cells appear to weakly express the normal receptors for common AAV and Ad vectors. Targeting these vectors to α -linked L-fucose and other kidney cell-specific receptors may increase gene delivery efficiency to enable gene therapy for a number of genetic and non-genetic conditions affecting the kidney.

Support: R25-DK101405 and U54-DK100227

ENaC ACTIVITY AND REGULATION IN RENAL DISTAL CONVOLUTED TUBULE CELLS

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Previous work has shown that epithelial sodium channel (ENaC) proteins are present in distal convoluted tubule (DCT) cells. The purpose of these experiments was to show whether these proteins were functional. We also wish to show whether the channels were regulated by the chaperone protein MLP-1. We used DCT-15 cells in monolayer culture and to measure transepithelial voltage, resistance, and current. We then examined the effects of amiloride and four MLP FLAG-tagged mutants: wild type (WT), constitutively active (S3A), constitutively inactive (S3D), and myristoylation negative (GA) on DCT-15 current. For comparison, we examined a principal cell line, mpkCCD cells. Our results showed that DCT-15 cells had relatively low resistances and small but measurable amiloride-sensitive currents, indicating that these cells contained ENaC. In untreated DCT-15 cells, median current across all days of measurement was highest in S3A mutant and was lowest in S3D mutant, both constructs which were significantly different from WT. Amiloride lowered the current across all DCT and CCD variants to very low levels. MLP construct expression were also studied in both cell types via Western blotting of lysed mutant protein extraction with FLAG antibody and green fluorescent secondary. In protein constructs with the highest current, expression of ENaC was greatest. Lastly, we calculated based on an average current of 2 mA/cm² and a single channel current of 0.4 pA that there must be approximately 5 million channels per square centimeter.

CARDIOVASCULAR EFFECTS OF ARTERIOVENOUS FISTULAS IN A MOUSE MODEL

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Cardiovascular disease is the leading cause of morbidity and mortality among hemodialysis patients. Arteriovenous fistula (AVF) creation may negatively affect cardiac structure and function further impacting cardiovascular mortality. We hypothesized that AVF creation results in high cardiac workload, inducing structural and functional changes in the left ventricle (LV). AVFs were created in age matched C57BL/6 mice between the carotid artery/jugular vein using an end-to-side anastomosis. Sham AVF mice served as the controls. Echocardiography was performed prior to creation (baseline) and at 7 and 21 days post-surgery. The cardiac output (CO), left ventricular end-diastolic diameter (LVEDD), and end-diastolic volume (EDV) was significantly increased at 7 and 21 days in AVF compared to sham mice. There was also a significant increase in CO, LVEDD, and EDV from baseline to day 21 “within” the AVF group, but not “within” the sham group. A downward trend was observed in ejection fraction in AVF mice from baseline to 21 days which was not present in shams. Collagen staining of the LV using picrosirius red showed an increase surrounding the perivascular areas of AVF mice but not in shams. In conclusion, the creation of an AVF leads to increased cardiac workload which induces changes to several clinically important cardiovascular parameters. Additionally, the presence of an AVF over time shows signs of dilated cardiomyopathy which may further increase risks of cardiovascular mortality. Our future studies will evaluate specific mechanisms leading to changes in AVF-induced cardiovascular structure and function and potential therapies to attenuate and reverse these changes.

ANGIOPOIETIN LIKE 3 AND ANGIOPOIETIN LIKE 8 EXPRESSION IN THE KIDNEY

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Metabolic syndrome represents an increasing threat to the health of populations worldwide. In this syndrome, the trafficking of triglyceride (TG) and cholesterol is altered, resulting in obesity, dyslipidemia, fatty liver disease, and increased risk of cardiovascular disease. Metabolic syndrome also increases the risk of chronic kidney disease (CKD), which affects 15% of all U.S. adults, but the mechanistic basis for this increase remains poorly understood. Previous studies have suggested that two members of the angiotensin-like protein (ANGPTL) family, ANGPTL3 and ANGPTL8, may link CKD and triglyceride metabolism. Genetic ablation of either protein in mice is associated with a striking reduction in plasma levels of TG and with protection from adriamycin- and LPS-induced nephropathy. More recently, ANGPTL3 was shown to act together with an atypical family member (ANGPTL8) to promote TG storage in adipose tissue. The purpose of this study was to evaluate the expression and function of ANGPTL3 and ANGPTL8 in the kidney. We used qRT-PCR to measure the levels of ANGPTL3 and ANGPTL8 mRNAs and immunoblot analysis to detect ANGPTL3 and ANGPTL8 proteins in the kidney. Liver tissue was used as a positive control in these assays. The targeting vector used to generate *Angptl3*^{-/-} and *Angptl8*^{-/-} mice contains a LacZ cassette; therefore we performed β -galactosidase (β -gal) staining to determine the expression profiles of ANGPTL3 and ANGPTL8. β -gal staining and qRT-PCR supported the expression of ANGPTL3 and ANGPTL8 in the liver and kidney. In contrast to the liver, levels of ANGPTL3 and ANGPTL8 in the kidney did not change significantly between fasting and refeeding. Overall, our data supports the hypothesis that ANGPTL3 and ANGPTL8 are expressed in the kidney, but expression of the two genes in this tissue may be under alternative regulation and have a different functional role than in the liver. Further work will be needed to determine the role of ANGPTL3 and ANGPTL8 in the kidney.

EFFECTS OF HIGH FAT VERSUS LOW FAT DIETS ON MURINE CARDIAC METABOLISM

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Background: A contributor to cardiac health is the cardiomyocyte circadian clock which helps to regulate cardiac metabolism. This clock can be disrupted by stresses on the heart such as obesity caused by high fat diets. Disruption of the clock can lead to cardiovascular morbidity and mortality. Time of day restricted (TDR) feeding (Allowing consumption of food only during the awake period) improves circadian rhythms in obese mice. However, the impact of TDR on cardiac function and/or metabolism has not been investigated previously. **Hypothesis:** Impairments in cardiac metabolism in obese mice are normalized by TDR feeding. **Methods and Results:** Four experimental groups were used in this experiment: 1. 20 weeks ad libitum control diet; 2. 20 weeks ad libitum high fat diet; 3. 18 weeks ad libitum control diet followed by 2 weeks TDR; 4. 18 weeks ad libitum high fat diet followed by 2 weeks TDR. Hearts were isolated and perfused ex vivo in the presence of ^{14}C labeled glucose. Coronary effluent was collected at five minute intervals during the heart perfusions followed by ion exchange chromatography for assessment of ^{14}C lactate production (a marker of glucose uptake plus glycolysis). ^{14}C lactate release was greatest in group 1. The high fat diet tended to lower ^{14}C lactate release. Importantly TDR further decreased ^{14}C lactate release. **Summary:** Both dietary composition and time of day of consumption impact cardiac metabolism.

SCREEN FOR LOW TEMPERATURE RESCUED CLCN5 MUTATIONS AS CANDIDATES FOR DENT DISEASE NOVEL THERAPIES

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Dent Disease type 1 (DD1) is an X-linked disorder characterized by low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, kidney stones, progressive chronic kidney disease. However, there is no effective treatment or therapy for Dent disease. DD1 is caused by mutations in the **CLCN5 gene** which encodes **CIC-5**, a Cl⁻/H⁺ exchange-transporter. We previously found that two novel CLCN5 mutations (R345W, Q629X), identified by the Rare Kidney Stone Consortium, cause abnormal protein trafficking (retained in the endoplasmic reticulum) resulting in abnormal function. Intriguingly, the abnormal trafficking of both mutations to the cell surface was reversed by low temperature incubation, suggesting these mutations might be rescued by chemical chaperones. In this study, we aimed to screen known CIC-5 mutations to identify additional candidates for low temperature rescue and novel therapy experiments.

We studied transport function of selective CIC-5 mutations (in the *Xenopus* oocyte overexpression system) using two-electrode voltage clamp experiments. All clones contained a HA-tag in the extracellular loop of the CIC-5 which does not change CIC-5 activity. This HA-tag enabled chemiluminescence detection and quantification of CIC-5 surface expression.

The transport function (current amplitudes at +80mV) of different mutations (S203L, G260V, L469P, and R516W) were reduced by 93, 91, 31, and 66%, respectively, compared to WT. Meanwhile, the surface expressions of the mutants were only 26, 35, 37 and 26% of the WT. Functional defects of these four coding mutations are likely caused by inappropriate CIC-5 trafficking since reduced transport function is highly correlated with the surface expression.

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MITOCHONDRIAL BIOGENESIS IN THE ANUCLEATE PLATELET: A PILOT STUDY

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Platelets are anucleate cells that play key roles in thrombosis and immunoregulation. Platelet mitochondria, through oxidative phosphorylation, provide the energy (ATP) required to maintain platelet health and function. If damaged, as with diabetes mellitus, mitochondria production of toxic ROS, and rupture, can initiate platelet apoptosis (programmed cell death) and increased thrombosis, potentially contributing to life-threatening thrombovascular events (heart attacks and strokes). We have recently reported that diabetic platelets are capable of a protective response (mitophagy) where defective toxic mitochondria are removed (autophagy), sparing functional mitochondria and preventing apoptosis. The primary question of this study was whether platelets are also capable of mitochondrial biogenesis (increasing mitochondria mass and numbers) to recover and restore platelet health and function. This would be particularly intriguing as platelets do not have genomic DNA, and thus transcriptional regulation of many proteins needed for increasing mitochondria mass and numbers. We recruited healthy volunteers from the lab, isolated their platelets and exposed their platelets to mitochondrial oxidative phosphorylation uncoupler CCCP, as well as oxidative stress inducer hydrogen peroxide. We then measured the expression of NRF1 and PGC1a, recognized markers for mitochondrial biogenesis. We identified that both NRF1 and PGC1a are present in the platelets, suggesting that some form of mitochondrial biogenesis is occurring. When we investigated the platelet and plasma of patients with diabetes mellitus, we found the presence of the same markers. Mitochondria biogenesis may reduce thrombovascular risk in diabetic patients and thus serve as a potential therapeutic target for reducing cardiovascular mortality in diabetes mellitus.

TREATMENT WITH CARDIAC GLYCOSIDES REDUCES APOL1 RISK VARIANT INDUCED CELL DEATH

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The G1 and G2 risk variants of the apolipoprotein L1 (APOL1) gene have been linked to higher risk of kidney disease in African Americans and are likely toxic gain-of-function mutations. These risk variants rose to high frequency in sub-Saharan Africa because they protect humans against trypanosome infection. Expression of G1 and G2 in cultured cells leads to greater cell death than expression of the wild type, G0. However, the mechanism of this cell death is not well understood.

Here we show that treatment with two different cardiac glycosides, Ouabain and Digoxin, can rescue APOL1 risk variant mediated toxicity in Human Podocytes and HEK293 cells. Stably transfected HEK293 cells and transiently transfected podocytes were used to overexpress either G0, G1, or G2. Efficacy of drug treatment was measured using a cell death assay. A 100 nM or 1 μ M dose of either Ouabain or Digoxin resulted in a significant ($p < 0.001$) reduction in APOL1-induced cell death. We found that while cardiac glycosides do not affect the transcription of APOL1, they do reduce APOL1 protein levels in G0, G1 and G2 expressing cells. We found that HIF-1 α , a protein known to be inhibited by cardiac glycosides, is upregulated in cells overexpressing either G1 or G2, and is further upregulated in these cells following treatment with cardiac glycosides. Knockdown of HIF-1 α with siRNA did not affect the ability of the drugs to inhibit cell death. Therefore, preliminary data indicate that cardiac glycosides inhibit APOL1-induced cell death, but probably not through the HIF-1 α pathway.

EMPLOYING A NEXT GENERATION SEQUENCING PANEL IN FAMILIES WITH POLYCYSTIC KIDNEY-LIVER DISEASE AND CILIOPATHIES

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Several dominant disorders cause clinically significant polycystic kidney (PKD) and/or polycystic liver disease (PLD). Some are related to defects in the biogenesis and trafficking of membrane proteins. A related renal disease is nephronophthisis (NPHP), with all of these disorders associated with primary cilia defects, ciliopathies. Next generation sequencing (NGS) methods allow genetic screening in a range of related diseases with a single gene panel. Here we screened 486 genetically unresolved PKD/PLD/ciliopathy families employing an NGS panel of up to 137 genes. Evaluation of detected variants highlighted two genes for detailed study, *INVS*, a known NPHP gene, and one encoding a biogenesis protein not previously linked to human disease. Primers were designed to confirm NGS-detected variants by Sanger sequencing and variants evaluated for the likelihood of pathogenicity using *in silico* assessment tools, including multiple sequence alignments, and population databases. Both of the pedigrees with *INVS* variants were complex due to variants also in other genes, *NOTCH2* (associated with Hajdu-Cheney syndrome; a dominant disorder including bone defects and PKD) or *NPHP1* and a PLD gene, *ALG8*. Ultimately, we concluded that *INVS* alleles may be contributing to the phenotype, but that there was complex inheritance. For the biogenesis gene, three pedigrees with PKD had truncating mutations, two nonsense and one frameshifting, and population data supported a dominant disease role. A weakness, was lack of further family members or detailed clinical information to fully evaluate the phenotype and test segregation. Overall, the study showed the value but complications of modern genetic analysis.

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NF-KB MEDIATES ZINC DEFICIENCY-INDUCED NCC UPREGULATION IN MOUSE DISTAL CONVOLUTED TUBULE

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Zn²⁺ deficiency (ZnD) is a common comorbidity with numerous chronic diseases including type II diabetes and chronic kidney disease. Experimental data show that ZnD exacerbates hypertension in these settings. The sodium chloride cotransporter (NCC), located in the distal convoluted tubule (DCT) of the nephron, plays a critical role in blood pressure (BP) regulation and hypertension. Additionally, studies have shown that the transcription factor Nuclear Factor kappaB (NFkB) is activated in settings of ZnD and contributes to numerous essential cellular processes such as gene regulation, cell growth, and inflammatory responses. This study's objective was to determine if NFkB plays a role in ZnD-induced NCC upregulation. We hypothesize that ZnD activates NFkB, which leads to NCC upregulation. To this end, WT mice were fed a diet with 50 ppm Zn²⁺ (Zn²⁺ adequate [ZnA]) or a diet with 1 ppm Zn²⁺ (Zn²⁺ deficient [ZnD]) for 6 weeks. To determine the role of NFkB in ZnD-induced NCC upregulation and subsequent BP increases, WT mice on a ZnD diet (6 weeks) were administered Caffeic acid phenethyl ester (CAPE), a NFkB inhibitor. Systolic BP was monitored via tail-cuff plethysmography. In *in vitro* experiments, WT DCT cells were treated with CAPE for 24 hours prior to inducing ZnD with the intracellular Zn²⁺ chelator TPEN. NCC mRNA and protein expressions were examined *in vivo* and *in vitro* via qRT-PCR, western blot, and immunohistochemistry. NCC activation was assessed by cellular localization via biotinylation and immunofluorescence. Findings show that systolic BP is increased in ZnD mice. This is accompanied by changes in urinary Na⁺ excretion and increased NCC mRNA and protein expression in DCT cells. Notably, ZnD-induced BP increases are reduced by CAPE treatment. Furthermore, CAPE treatment of DCT decreases NCC expression compared to TPEN-treated cells. Taken together, these results indicate that NFkB plays a role in ZnD-induced NCC upregulation and BP.

TIME OF DAY-DEPENDENT REGULATION OF RENAL EXCRETORY FUNCTION IN OBESE MICE

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Obesity can disturb cell autonomous circadian clocks, a molecular mechanism known to impact a variety of biological functions. Disruption of the circadian clock results in numerous physiological impairments including abnormal blood pressure rhythms. However, time-of-day restricted feeding (RF) attenuates many of these effects. Because of the importance of the kidney in obesity-dependent hypertension, we designed experiments to determine the degree to which RF restores the kidney's ability to excrete salt in obesity. C57Bl/6J male mice (8 weeks, n=10-12/group) were fed normal fat (NF) or high fat (HF) diet for 20 weeks. During the last 2 weeks, food was given either *ad libitum* or restricted to the active period (RF). During the last week of the study, the mice were put into metabolic cages where food and water intake, as well as urinary output were monitored. Animals were killed at either ZT0 or ZT12 and blood was drawn via cardiac puncture. There was no significant difference in food intake, water intake, and urinary output among all of the groups. Urinary sodium excretion during active period was not significantly different between the lean and obese mice (1.6 ± 0.6 vs 1.7 ± 0.9 , n=8-9), nor it was affected by RF (2.1 ± 0.9 vs 1.7 ± 0.8 , n=11). Plasma and urine ET-1 were measured (ELISA), but no significant differences were observed. Further, urine aldosterone excretion (ELISA) was not significantly changed by HF diet or RF. These data suggest that high fat diet does not affect diurnal sodium excretion under baseline conditions.

IL-6 ACTIVATES THE MINERALOCORTICOID RECEPTOR VIA THE JAK2/STAT3 PATHWAY, INCREASING SODIUM UPTAKE

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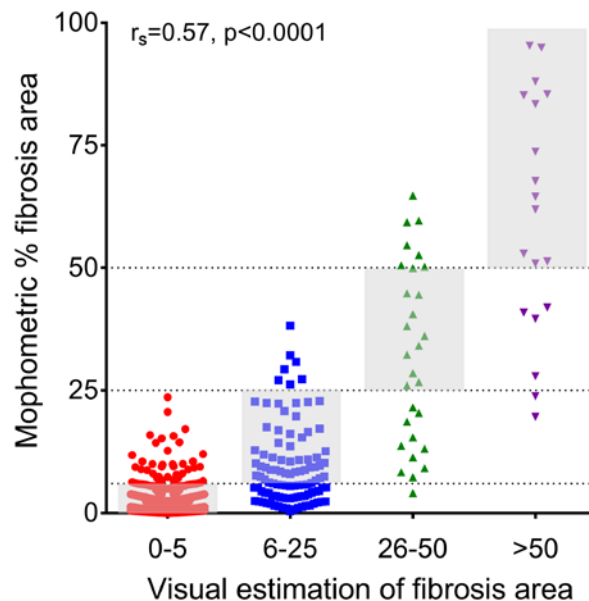
Hypertension is an inflammatory disease that is characterized by increased sodium (Na⁺) reabsorption and is a leading cause of death and disability worldwide. The beneficial effect of mineralocorticoid receptor (MR) antagonists in reducing blood pressure suggests a role for the MR in hypertension. Interestingly, the classical MR ligand, aldosterone (Aldo) is not always increased during hypertension. However, the pro-inflammatory cytokine interleukin 6 (IL-6) is elevated in the serum of hypertensive individuals. Previously we have shown that IL-6 increases MR-dependent Na⁺ uptake *in vitro* and increases expression of the Na⁺ chloride cotransporter (NCC) *in vivo*. Canonical IL-6 signaling occurs via the Jak/STAT intracellular signaling pathway; therefore, we hypothesized that the Jak2/STAT3 pathway is critical for IL-6 mediated MR activation and Na⁺ uptake. Using a luciferase reporter assay and a transiently transfected cell model of distal convoluted tubule cells (mDCT15), we show that IL-6 increases mineralocorticoid response element (MRE) activity via a Jak/STAT3 pathway. To investigate total Na⁺ flux *in vitro*, we used a fluorescent Na⁺ indicator CoroNa Green, and demonstrate that total intracellular Na⁺ levels are increased with IL-6 treatment and reduced following STAT3 inhibition. Furthermore, using a ²²Na-uptake study, we confirm that thiazide-sensitive Na⁺ uptake is reduced in the presence of both Jak2 and STAT3 inhibitors. Together, our data suggest that IL-6 activates the MR and NCC via JAK2/STAT3 in the distal nephron. These data describe an alternative mechanism for increased distal nephron Na⁺ reabsorption in hypertension when aldosterone levels are not increased, but increased levels of cytokines are observed.

COMPARISON OF INTERSTITIAL FIBROSIS IN WEDGE RENAL BIOPSIES EVALUATED BY VISUAL ESTIMATION AND MORPHOMETRIC ANALYSIS

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While a few studies have compared morphometric approaches of studying interstitial fibrosis in a limited number of renal allograft biopsies, there are no such studies in much larger, renal wedge sections. We studied kidney biopsies from patients who underwent a radical nephrectomy (n=845) between 2000 and 2012. From the stored formalin-fixed kidney specimens a large wedge section distal to the tumor was obtained, tissue blocks were cut, slides stained with periodic acid-Schiff, and then scanned into high-resolution digital images. Interstitial fibrosis with tubular atrophy (IFTA) was first estimated by a renal pathologist based upon a visual inspection, as a percentage of the overall affected cortex, and then grouped into 4 categories (0-5%, 6-25%, 26-50% and >50%). Then, masked to pathology readings, we performed manual tracing of cortex areas with IFTA, and calculated the percent fibrosis area. Biopsy measures of nephron size (glomerular volume and tubular area) and arteriosclerosis were also obtained. Two methods of assessing IFTA show general agreement ($r_s=0.57$, $p<0.0001$); however, for each discrete IFTA category (visual estimate), there was a range of continuous morphometric IFTA, with a number of data points outside the discrete range (Figure). We found that morphometric measures of IFTA had stronger associations with clinical characteristics and biopsy pathology than visual estimates of IFTA. Morphometric estimation of IFTA likely outperforms visual estimation of IFTA because it is continuous rather than discrete, and because it is more accurate being based on objective measurements rather than subjective visual inspection (even by experienced pathologists).

Support: R25-DK101405, U54-DK100227 and R01-DK90358



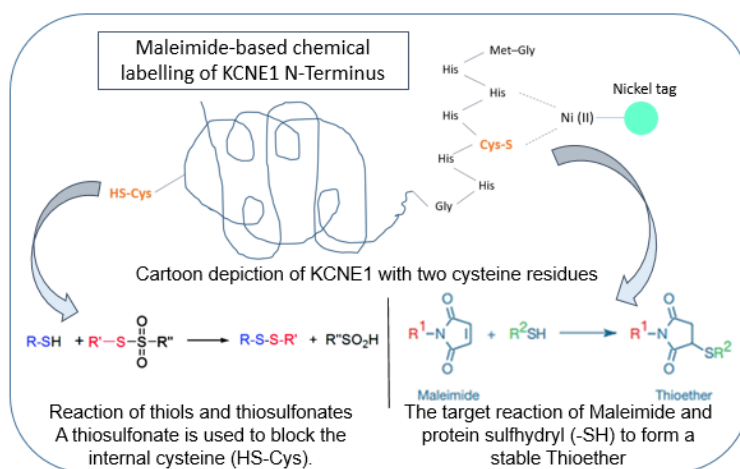
CYSTEINE THIOL DIRECTED CHEMICAL MODIFICATION OF A KCNE1 PROTEIN N-TERMINUS

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The chemical tagging of a protein at or near its N- or C- terminus has remained a challenge when multiple reactive cysteines are present. The development of such a technique would be widely useful in a variety of applications. As a model system, we selected KCNE1, a single span membrane protein that functions in conjunction with KCNQ1 to form a functional potassium channel. The KCNE1 and KCNQ1 complex regulates ion flow during the cardiac action potential. Mutations in the KCNE1 gene causes long QT syndrome (LQTS), which results in cardiac arrhythmia. We added a cysteine residue within the N-terminal hexa-Histidine tag thereby providing a thiol (-SH) reactive site. However, the problem arises when the protein has other cysteine residues with similar affinity towards thiol-reactive reagents. The goal of this project then was to explore potential ways to specifically modify a Cys-SH group located at the protein N-terminus without modifying other reactive Cys-SH groups. While bound to Ni-NTA, the native Cys-SH was modified with a blocking reagent. We hypothesized that the N-terminal Cys residue would be sterically occluded. An Ellman's Reagent (DTNB) assay was conducted to determine the number of reactive cysteines in the protein as well as an electrophoretic mobility shift assay (EMSA). Comparing the data from both assays with that of a wild-type variant revealed an unreactive native cysteine. Unfortunately, the cysteine embedded in the His-tag remained reactive, even when bounded to the Ni-NTA. The results from this study generate many questions about the reactive properties of cysteine residues.

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Graphical Abstract:



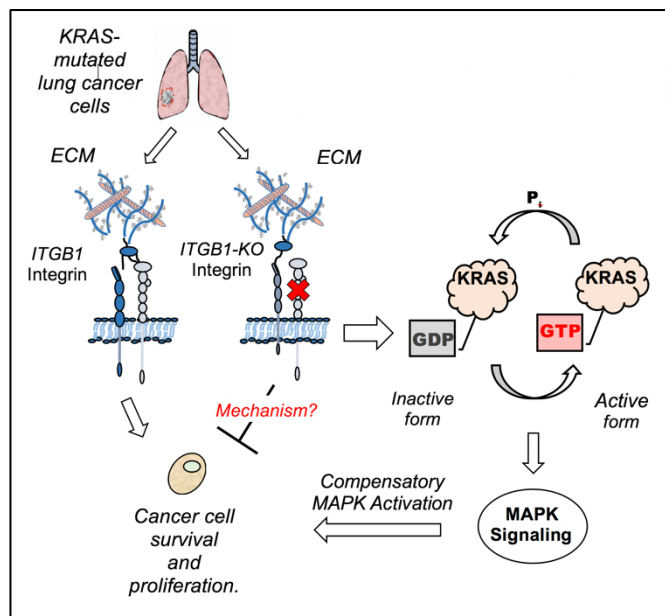
INTEGRIN $\beta 1$ PROMOTES KRAS-MUTATED LUNG CANCER PROLIFERATION VIA MAPK-INDEPENDENT MECHANISM

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Lung cancer is the leading cause of cancer death in the United States. Integrins are transmembrane receptors consisting of 18 α - and 8 β -subunits that combine to form 24 distinct heterodimers that function as the principal extracellular matrix receptors of the cell. *KRAS* is the most commonly mutated oncogene in lung cancer, and studies suggest that *KRAS*-mutated lung cancers require integrins to activate downstream mitogen-activated protein kinase (MAPK) signaling and form tumors. However, preliminary data from our group suggests that *KRAS*-mutated cells are able to activate MAPK signaling irrespective of integrin $\beta 1$ (ITGB1) expression. In order to further understand the impact of $\beta 1$ integrins on growth factor-mediated activation of MAPK signaling in *KRAS*-mutated lung cancer, we directly evaluated *KRAS* activation in human lung adenocarcinoma cell lines with CRISPR-mediated $\beta 1$ knock out (KO). A *KRAS*-activation assay was performed to selectively pull-down GTP-*KRAS* from parental *KRAS*-mutated human lung adenocarcinoma cell lines and CRISPR-mediated integrin ITGB1-KO cells. Cells were either serum starved or spiked with calf serum and GTP-*KRAS* was quantified via immunoblotting. We observed increased GTP-*KRAS* in ITGB1-KO cells relative to wild-type cells, suggesting that *KRAS* is able to activate appropriately in ITGB1-KO cells. Therefore, ITGB1 promotes *KRAS*-mutated lung cancer via a MAPK-independent pathway, and increased *KRAS* signaling may represent a novel mechanism of compensation for loss of ITGB1-dependent signaling.

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Graphical Abstract:



PROXIMAL TUBULE-SPECIFIC HO-1 OVEREXPRESSION MITIGATES ACUTE KIDNEY INJURY (AKI) TO CHRONIC KIDNEY DISEASE (CKD) TRANSITION

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AKI is a pervasive clinical malady causing significant morbidity and mortality, which increases risk for CKD. The mechanism behind this process remains poorly understood. Heme oxygenase-1 (HO-1) is a cytoprotective enzyme that catalyzes the breakdown of free heme in AKI. While this protective role is well documented, the role of HO-1 in the AKI to CKD transition is unknown. Renal proximal tubules (PTs) contain densely packed mitochondria for energy production required for fluid, ion, and nutrient homeostasis, which confers high AKI susceptibility. The PTs produce maximal renal HO-1 expression and are the focus of this study.

Male mice overexpressing HO-1 specifically in the PTs (HO-1^{PT+/+}) and their floxed controls were subjected to unilateral ischemia reperfusion injury with 7-day delayed contralateral nephrectomy (Npx) and mice were harvested at 21 days post-Npx.

While floxed controls experience a significant rise in serum creatinine (SCr) (1.27 ± 0.25 mg/dL), HO-1^{PT+/+} SCr modestly rises 1-day post-Npx (0.7 ± 0.12 mg/dL). Floxed controls exhibit a significant increase in inflammatory markers (*TNF- α* , *MCP-1*) compared to shams while HO-1^{PT+/+} mice did not demonstrate significant differences. Our data suggests that PT HO-1 overexpression does not affect fibrotic remodeling as illustrated by similar fibronectin and α -smooth muscle actin expression levels. Picro Sirius Red staining of kidney sections corroborates similar fibrotic remodeling in HO-1^{PT+/+} mice compared to floxed controls. These findings suggest PT HO-1 overexpression may attenuate uncontrolled inflammation but not fibrotic remodeling 21 days post-Npx. This may provide novel insight into generating therapies to prevent progression of AKI to CKD.

DEEP INTRONIC MUTATIONS IN THE *PKLR* GENE CAUSE ABERRANT SPLICING IN PATIENTS WITH ANEMIA DUE TO PYRUVATE KINASE DEFICIENCY

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Pyruvate kinase (PK) catalyzes the second ATP-forming step in glycolysis. Erythrocytes lack mitochondria and are dependent on glycolysis for energy. Recessively inherited mutations in the pyruvate kinase (*PKLR*) gene lead to hemolytic anemia in affected patients. Symptomatology is variable, ranging from well-compensated anemia to severe disease with lifelong transfusion dependence.

Genetic analyses of PK-deficient patients show most carry missense, frameshift, and nonsense mutations that lead to qualitative or quantitative defects in pyruvate kinase. Studies in a subset of pyruvate kinase-deficient patients identified *PKLR* coding region mutations only on one allele. To search for potential splicing or regulatory mutations on the other allele, whole genome sequencing of genomic DNA from these unique cases (n=5) was performed. This identified deep intronic mutations in the *PKLR* gene in affected members of all 5 kindreds, predicted by bioinformatic analyses to lead to splicing abnormalities.

Minigene assays, performed to examine whether these mutations influenced splicing *in vitro* were performed with patient-specific mutant alleles. Minigenes were transfected into K562 cells, with mRNA harvested after 48 hours and RT/PCR amplified, followed by shotgun subcloning of cDNA into plasmids. After transformation, plasmid DNA extracted from subclones was subjected to Sanger sequencing.

Sequence analysis identified aberrant *PKLR* mRNA isoforms from all 5 minigenes including partial and complete exon skipping and partial intron retention. These isoforms lead to frameshift, with premature chain termination triggering nonsense mediated decay. These results indicate intronic mutations leading to aberrant splicing may cause pyruvate kinase deficiency. This may be an underrecognized mechanism of genetic disease.

PHOSPHORYLATION OF α -ACTININ-4 (ACTN4) MAY PLAY AN IMPORTANT ROLE IN REGULATING FOCAL ADHESION OF PODOCYTES

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Podocytes are important cells in maintaining the kidneys' glomerular filtration barrier. Studies have shown that mutations in α -actinin-4 (ACTN4) lead to podocyte dysfunction and focal segmental glomerular sclerosis (FSGS) in humans. Using mass spectrometry, we detected ACTN4 is phosphorylated at the serine 159 site (S159) in cultured podocytes. Since the ACTN4 protein is of great importance in this disease, we aim to examine whether abnormal phosphorylation of ACTN4 protein could affect podocyte function. ACTN4 crosslinks actin filaments and interacts with proteins in the focal adhesion (FA) complex. Therefore, we hypothesized that abnormal phosphorylation leads to changes in the FA of podocytes. To test our hypothesis, we generated S160D mice (mimics constant phosphorylation) and S160A mice (mimics no phosphorylation) using CRISPR/Cas9 technology. Primary podocyte cells were isolated from WT, homozygous S160D, and homozygous S160A mice. Immunofluorescence staining was performed to examine the distribution of one of the key focal adhesion proteins, zyxin. The number of zyxin in each podocyte was quantified using Image J software. We found that S160D ACTN4 podocytes appeared to have higher numbers of zyxin compared to WT and S160A ACTN4 podocytes. This result suggests that phosphorylation of ACTN4 may play an important role in regulating podocyte adhesion.

GROWTH OF *CLOSTRIDIUM* ISOLATES UNDER ZINC LIMITATION

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Commensal intestinal bacteria from the genus *Clostridium* play a role in resistance to pathogens and host immunity. However, how commensal *Clostridium* respond to changes in the gut environment is unclear. The pathogen *Clostridium difficile* induces intestinal inflammation and subsequent changes in the relative abundances of commensal *Clostridium* bacteria. The mechanisms describing how inflammation alters *Clostridium* abundances is also not well understood. A component of the host's response to *C. difficile* is the release of the metal-binding protein calprotectin. Calprotectin binds and sequesters nutrient metals, including zinc, that are necessary for bacterial growth. While calprotectin is released as a part of the host response to limit pathogen growth, zinc sequestration may also affect the growth of non-pathogenic *Clostridium* species. To determine how *Clostridium* species respond to zinc limitation, *Clostridium* bacteria were isolated by selecting for spores from *C. difficile*-infected mice by heating fecal pellets and plating anaerobically on rich Eggerth-Gagnon media. Three isolates were putatively identified as *C. sulfidigenes*, *C. bifermentans*, and *C. xylanolyticum* by sequencing the 16s rRNA gene. We found that these *Clostridium* isolates grow differently on zinc-replete and zinc-limited media, with *C. sulfidigenes* growing the best in zinc-limited media. Future work will examine Clostridial metabolic changes in response to zinc-limitation and calprotectin. These studies will lead to the future discovery of mechanisms used by commensal *Clostridium* to overcome zinc-limitation.

Research supported by: Berea/Aspirnaut™/Hal Moses Summer Research Internships, Vanderbilt University Medical Center, Vanderbilt Center for Matrix Biology, Aspirnaut™

NOVEL APPROACHES TO CHARACTERIZE PARATHYROID HORMONE (PTH) ISOFORMS IN HEALTH AND CHRONIC KIDNEY DISEASE

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Concentrations of intact PTH (1-84) measured by automated immunoassays fail to accurately predict skeletal outcomes in subjects with chronic kidney disease mineral and bone disorders (CKD-MBD). The purpose of this research endeavor was to develop an accurate and precise high-resolution accurate mass liquid chromatography mass spectrometry method to detect and quantitate intact PTH (1-84) and truncated PTH fragments thought to be involved in the pathophysiology of CKD bone complications.

Prior to analysis, 1 mL of each sample, standard, and quality control is subjected to immunopurification with anti-PTH (44-84) monoclonal antibody coated beads. Samples are washed twice with 1x PBS solution, and PTH species are cleaved from the bead with 1% formic acid. DMSO is added to both mobile phases to enhance peak intensity and coalescence of peptide charge states.

A specific LC-MS method has been developed to quantify 11 unique PTH species in serum at concentrations as low as 50 pg/mL. All PTH variants identified were significantly elevated in subjects with CKD compared to controls.

PTH fragments with unknown bioactivity are present in astronomical concentrations compared to intact PTH (1-84). Subjects with CKD can accumulate such species at levels above 100-times that of normal. The coinciding elevation of PTH fragments along intact PTH in CKD may explain why 3rd generation immunoassays, which strictly measure PTH (1-84), are unable to describe the complex relationship between PTH and bone health. Our findings necessitate further research on the bioactivity of PTH fragments to elucidate the biological mechanisms behind altered bone metabolism in CKD.

Support: R25-DK101405 and U54-DK100227

PATIENT-PROVIDER RACE CONCORDANCE AND ACCESS TO KIDNEY TRANSPLANTATION

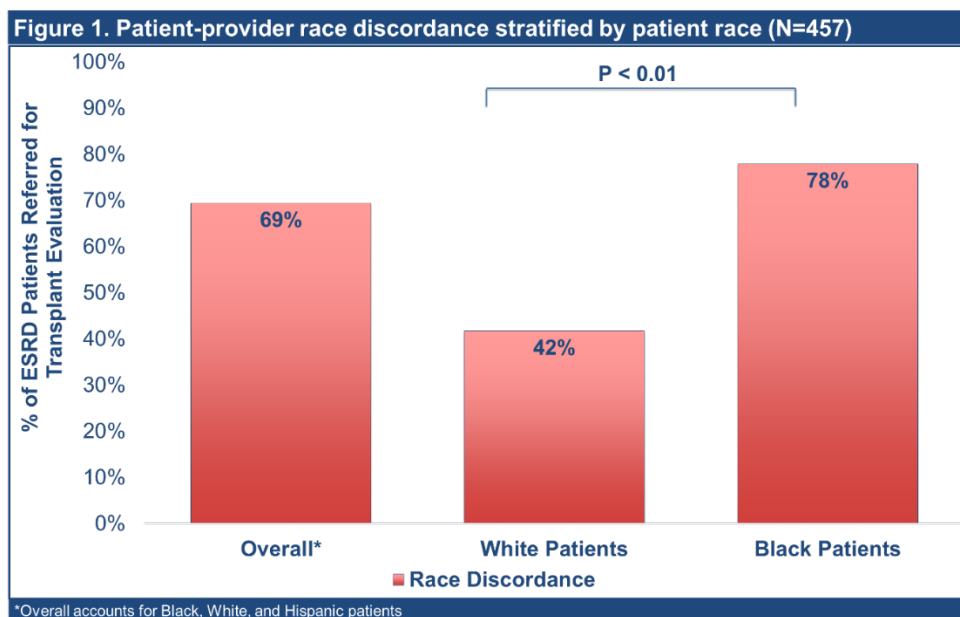
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Although kidney transplantation (KTx) provides significant survival benefits compared to dialysis, many end-stage renal disease (ESRD) patients referred for KTx evaluation at a transplant center do not attend this appointment. Patient-physician race concordant relationships can influence patient satisfaction, adherence and response time in seeking healthcare. Thus, this study investigated the association of patient-provider race concordant relationships with KTx evaluation attendance.

A telephone survey was administered to adult ESRD patients referred for KTx evaluation to three Georgia transplant centers in 2016. During the survey, patients identified their race and the race of their kidney doctor. Patient-provider race discordance was defined as the patient reporting a different race than their provider. Evaluation attendance was abstracted from medical records. Multivariable logistic regression was used to assess the association between patient-provider race discordance and evaluation attendance.

Of the 457 patients, 43.8% were female and 72.2% were black with an average age of 55.7 years. Approximately two-thirds (69.4 %) of patients reported being in race discordant provider relationships, with black patients more frequently reporting discordant relationships compared to whites (77.9% vs. 41.7%; $p < 0.01$) (Figure 1). In multivariable analyses, there was no statistically significant association between patient-provider race discordance and not attending the KTx evaluation (OR 1.31, 95% CI 0.81 2.13). While no statistically significant association was found between race concordance and evaluation attendance, results suggest a lack of racial diversity among ESRD providers. A more diverse provider population may encourage better patient-provider communication and promote culturally sensitive environments to enhance ESRD patient healthcare utilization.



SCREENING FOR DYNAMIN ACTIVATORS

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The disruption of the podocyte actin cytoskeleton plays a pathogenic role in diverse models of chronic kidney disease. We have previously shown that small molecule Bis-T-23 is able to rescue the actin cytoskeleton in injured podocytes through actin-dependent dynamin oligomerization. However, given Bis-T-23's poor bioavailability, solubility and instability, it is necessary to screen and identify alternative dynamin activators.

The purpose of this project was to screen for potential dynamin activators using a confocal microscopy-based assay and develop a HTP compatible plate reader assay to measure actin levels. Intramedullary collecting duct (IMCD) cells were chosen for these assays due their strong actin phenotype and rapid proliferation rate. Cells were treated with compounds under two conditions, control or Latrunculin (LatA) pretreatment which disrupts the actin cytoskeleton.

We demonstrated the disruption of the actin cytoskeleton by LatA using both confocal microscopy and the plate-reader assay. Furthermore, Bis-T-23 rescues the actin structure in IMCD cells challenged with LatA. Subsequently, we evaluated various small molecules to identify potential dynamin activators. We identified SM-1 as a potential lead that was able to rescue the actin phenotype in LatA pretreated IMCD cells. In contrast, a small molecule of similar but not identical structure named SM-2 was unable to rescue the actin phenotype in LatA challenged IMCD cells, thus suggesting a relationship between distinct structure of SM-1 and its phenotype.

In conclusion, this research evaluated and applied microscopy and plate reader-based assay for screening dynamin activators that may serve as novel therapeutics for chronic kidney disease.

REGENERATION OF LATERAL LINE HAIR CELLS IN ZEBRAFISH MODEL OF POLYCYSTIC KIDNEY DISEASE

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most lethal monogenic kidney disease and is characterized by fluid filled cyst growth in the kidneys. Renal tubular epithelial cells share several characteristics with hair cells of the ear and fish lateral line, an established model for regeneration studies. Zebrafish hair cells regenerate after being ablated by chemical exposure or traumatic injury. During this project, hair cells were ablated using an antibiotic, neomycin, then observed as they grew back in *pkd2* mutants and wild type. Neomycin was put on 5 days post fertilization (dpf) zebrafish. Tails were snipped for genotyping using polymerase chain reaction (PCR) and restriction enzyme digest. Fish were then fixed in 4% paraformaldehyde (PFA) at three different time points. Hair cell recovery was monitored using immunofluorescence (IF) labeling. Hair cells and cilia were counted in the posterior lateral line (pLL) neuromasts and compared. We observed structural differences in neuromast regeneration between wild type and mutants, but no differences in the numbers of cilia and hair cells. However, recovery was less robust in these experiments than we have previously observed. Additionally, fish appeared less healthy generally as embryo yields were lower than usual. Previously, preliminary studies suggested that regeneration was inhibited in these *pkd2* mutants. This experiment will be repeated in order to collect data on additional batches of fish and determine whether regeneration is affected in these PKD model zebrafish. If so, this may be a valuable model for studies of regeneration in PKD.

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EFFECT OF REGULAR AEROBIC EXERCISE ON ENDOTHELIAL FUNCTION IN CHRONIC KIDNEY DISEASE

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Cardiovascular disease is the leading cause of death in Chronic Kidney Disease (CKD). In addition to conventional risk factors (e.g. blood pressure), the use of non-conventional risk factors may provide further mechanistic insights into the physiology underlying cardiovascular disease risk in CKD. One non-conventional risk factor that has been underexplored in CKD is endothelial dysfunction. Since aerobic exercise has previously been demonstrated to improve cardiovascular disease risk in cardiac patients, we sought to explore the use of exercise in CKD. We hypothesized that 12 weeks of regular aerobic exercise would improve blood pressure and endothelial function in CKD, supporting the use of this therapy in renal disease. 20 CKD patients (stages 3-4) were randomized to receive either stretching or aerobic exercise (cycling) 3 days a week for the duration of 10-12 weeks. Blood pressure and endothelial function were measured pre- and post-intervention via sphygmomanometry and peripheral arterial tone (PAT) technology respectively. In support of our hypothesis, mean arterial pressure (MAP) decreased with exercise from pre- to post-intervention (Exercise; Pre= 96.3 ± 3.45 mmHg, Post= 88.1 ± 2.31 mmHg, $P=0.004$; Stretching; Pre= 96.9 ± 3.03 mmHg, Post= 98.5 ± 4.09 mmHg, $P=0.523$). While not statistically meaningful, we also observed a trend towards improvements in endothelial function, assessed via reactive hyperemia index (RHI) in the exercise group. Future work should continue to explore the use of exercise in CKD, with a larger sample size and additional indices of endothelial function (e.g. flow mediated dilation, biomarkers). In conclusion, this investigation demonstrates the safety and efficacy of regular aerobic exercise in CKD as a method to improve cardiovascular risk factors.

MAPPING THE BINDING SITES OF FATTY ACID SYNTHASE ON CHIKUNGUNYA VIRAL RNA

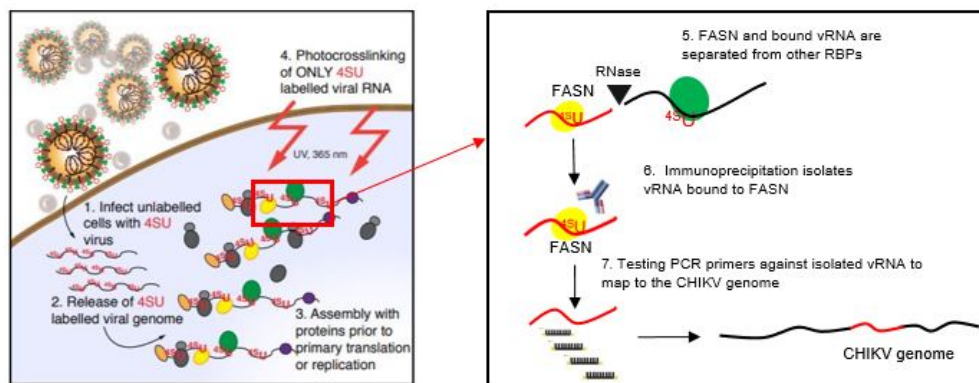
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Chikungunya virus (CHIKV) is a zoonotic arbovirus with a positive-sense single-stranded RNA genome. Despite increasingly high rates of infection worldwide, the early stages of its entry and the host cellular machinery used to promote replication are not well understood. Past research has demonstrated that the absence of the host enzyme FATTY ACID SYNTHASE (FASN) results in a significant decrease in CHIKV replication. However, this work focused solely on the proviral enzymatic activity of FASN in lipid metabolism. Recently, using the novel technique VIR-CLASP, we determined that FASN is a non-canonical candidate RNA binding protein (RBP) that binds directly to the CHIKV RNA genome immediately upon viral entry. The impact of FASN binding to viral RNA genomes – let alone the function of FASN as an RBP – is under investigation. To formally establish that FASN is a *bona fide* RBP, we seek to identify the regions on FASN protein that confer RNA binding and to map the FASN binding sites on the CHIKV genome. I will describe progress towards the localization of the binding site of FASN on CHIKV RNA. Towards this goal, we utilized photochemical biological approaches via RNA-protein crosslinking and immunoprecipitation, followed by reverse-transcription and quantitative PCR. This project serves as an essential first step for a structure-function based analysis towards understanding the mechanism of FASN proviral activity on CHIKV replication and infection.

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Graphical Abstract:



NBCE1 IN THE KIDNEY AND LOWER UROGENITAL TRACT

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NBCE1 is an electrogenic Na⁺ bicarbonate cotransporter expressed as three isoforms. NBCE1A is mainly found in the kidney (100% activity), NBCE1B is a general isoform (~25% activity), and NBCE1C is found in the nervous system (20% activity). This project was to characterize mice with loss of NBCE1 in the kidney and urogenital tract and to test isoform activity ± IRBIT. Previous work has shown that NBCE1-B is activated when IRBIT is present and interacts with the N-terminus (Nt). Even though NBCE1C shares this Nt, the effects of IRBIT on NBCE1C are unknown. Interstitial Cells of Cajal (ICC) in the enteric nervous system (*c-kit*⁺), have altered activity in *nbce1*^{-/-} mice (NBCE1C missing). Similar ICC cells are found in the urogenital tract; however, NBCE1C locations within the kidney and urogenital tract are unknown. Using *c-kit*-copGFP and NBCE1A-knockout mice, kidneys, ureters, prostate, bladder, and urethra were dissected and analyzed via direct imaging, sectioning, and immunohistochemistry. Oocytes injected with NBCE1C ± IRBIT were voltage clamped to determine the cotransporter's activity. "Strings" of *c-kit*⁺ cells were found in the copGFPnbce1A kidneys. In *nbce1*^(+/-)-(Het) mice (**Figure**), prostate ducts appeared enlarged, and in *nbce1A*^{-/-} kidney, *c-kit* protein was broadly expressed, showing cluster colocalization with NBCE1C. NBCE1C is not obviously expressed in WT or copGFP mice. Electrophysiology data showed that IRBIT activates NBCE1C by ~10-fold. As *c-kit* is also a marker of stem cells, NBCE1C/*c-kit* association and *c-kit* increase in *nbce1A*^{-/-} mice, could suggest that NBCE1A loss elicits kidney repair itself (proliferation) and causes expression of other NBCE1-isoforms.

Support: R25-DK101405, U54-DK100227 (Mayo) and U54-DK104310 (UW-Madison)

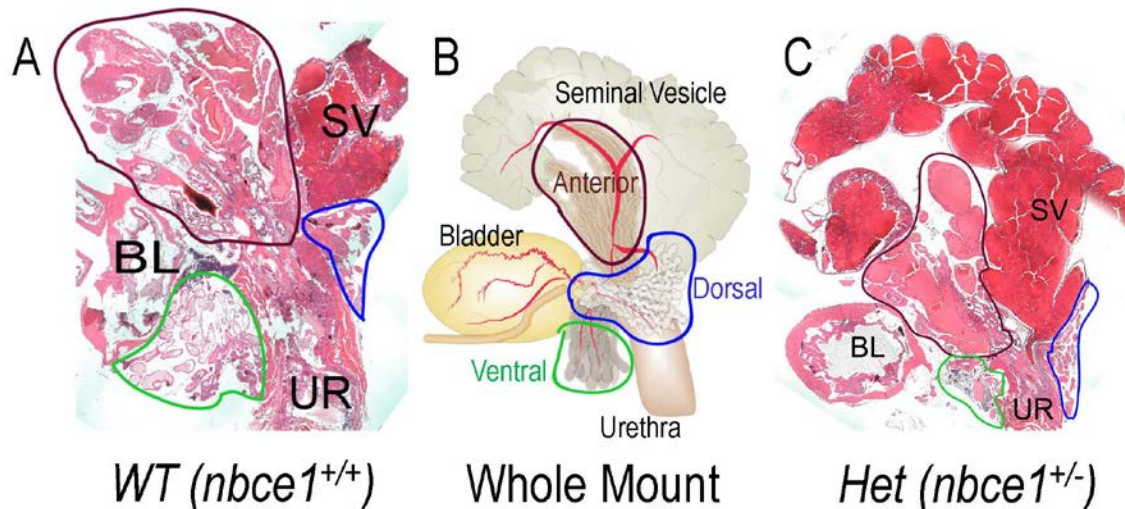


Figure. Effect of NBCE1 gene dose (whole gene) in the Lower Urogenital Tract

CISPLATIN AFFECTS LYSOSOMAL HOMEOSTASIS

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Cisplatin is a chemotherapeutic used to treat a number of cancers, but has many side effects, including kidney damage. It has been shown that cisplatin decreases autophagic flux *in vitro*, pointing to a potential method of toxicity. Renal tubular PGC1 α , a master regulator of mitochondrial biogenesis, protects against acute kidney injury in unrelated conditions including sepsis and ischemia, raising questions of whether PGC1 α modulates the response to tubular toxicity. We proposed that cisplatin decreases autophagic flux by interfering with lysosome homeostasis. We performed quantitative PCR to check for expression of several markers of lysosomal biogenesis, acidification, and trafficking at baseline and after cisplatin treatment. PGC1 α ^{-/-} knockout (KO) cells were generated, via CRISPR-Cas, and analyzed using similar techniques. We show that cisplatin decreases expression of both TFEB and Rab20, but does not affect vacuolar-ATPase (V-ATPase) expression. Additionally, PGC1 α KO cells displayed increased TFEB and V-ATPases, but showed decreased Rab20. Our data raise the possibility that cisplatin affects lysosome homeostasis. The action(s) of cisplatin may be both at the level of biogenesis through the transcription factor TFEB, and at the level of lysosomal trafficking via an effect on Rab20.

IFNAR1 SIGNALING CURBS ALLOIMMUNITY BY INCREASING T_{REG} RECRUITMENT AND DECREASING T CELL MATURATION

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Despite significant advances in attenuating acute transplant rejection, long-term allograft survival remains a critical concern. Unfortunately, most of the available therapies are limited by a deleterious effect on both pro-inflammatory and regulatory T cells (T_{Regs}), affecting lasting graft survival. The latter subset of helper CD4⁺ T cells, however, is one of the most researched cell types with the potential to entrain long-term immune tolerance. In a recent study, we discovered a mechanism that explained worsened disease progression in patients with the plasma cell cancer, multiple myeloma. These patients had increased expansion of T_{Regs} due to enhanced Type 1 Interferon (IFNAR1) signaling. Where more T_{Regs} are detrimental in the context of cancer, however, they are desirable in the case of alloimmunity. We hypothesized that IFNAR1 signaling in a transplant context could induce T_{Regs} and mitigate graft rejection. *In vitro*, we found that CD4⁺ T cells isolated from C57BL/6 mice induced significantly more T_{Regs} in the presence of Type 1 Interferons. Strikingly, using a fully MHC class I/II mismatched murine skin transplant model, we witnessed a two-fold increase in the T_{Reg} induction, as well as a ten-fold decrease in the effector (CD44^{hi}CD62L^{lo}) CD4⁺ and CD8⁺ T cell populations. These findings provide preliminary insights into the therapeutic potential of Type I Interferons in attenuating alloimmunity. Future studies are warranted to further explore the selective effects of Type I Interferons on pro- and anti-inflammatory cells and their safety profile in promoting solid organ acceptance.

ROLE OF P120CTN IN GLOMERULAR FUNCTION AND STRUCTURE

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p120ctn (*Ctnnd1*) is a ubiquitously expressed protein integral to the cadherin-catenin complex in adherens junctions that has been shown to be important in kidney development. p120ctn has pleiotropic effects including trafficking of classical cadherins, regulation of small Rho-GTPases affecting cytoskeletal dynamics, and effects on nuclear transcription. Conditional deletion of *Ctnnd1* from metanephric mesenchyme in mice using a *Pax3-cre* results in abnormal glomerular morphogenesis and hypoplastic cystic kidneys (Marciano *et al.*, 2011). In preliminary studies, we found that loss of *Ctnnd1* from epithelial nephron progenitors alone leads to glomerulosclerosis in adult mice, albeit a much less severe phenotype. We hypothesized that p120ctn plays an essential role in podocytes, serving to mediate cell-cell adhesion and regulate Rho-GTPase function. We examined the localization of p120ctn in the developing and mature mouse kidney, and examined the phenotype in mice with conditional deletion of *Ctnnd1* from podocytes. Our results show that p120ctn is highly expressed in the developing glomeruli, tubules and collecting ducts. Immunofluorescent staining shows that in immature podocytes, p120ctn localizes to cell-cell junctions, while in differentiating and maturing podocytes, p120ctn is predominantly localized to the basolateral side of foot-processes. In mature glomeruli, podocytes contain little/no p120ctn. However, p120ctn localizes to cell-cell junctions of endothelial cells in both developing and mature glomeruli. Mice lacking p120ctn from podocytes (*Nphs2-cre; p120ctn^{ff}*) have no histological abnormalities by 3 months of age. Blood and urine analysis is currently in process. To determine if p120ctn is required for recovery from glomerular injury, we utilized the Adriamycin nephropathy model, injecting 4 week old control and mutant (*Nphs2-cre; p120ctn^{ff}*) mice with 25mg/kg of Adriamycin. The results of these studies are still ongoing.

ELUCIDATING THE ROLE OF PODOCYTE ANGPTL4 AND PODOCYTE GR IN RENAL FIBROSIS

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Renal fibrosis, a manifestation of chronic kidney disease and characterized by extracellular matrix deposition and fibroblast activation, primarily affects the tubulointerstitial space and glomeruli. Angiopoeitin-like 4 (Angptl4) is a secretory protein produced in the adipose tissue, liver, muscle, and kidney. It regulates lipoprotein metabolism but has not been well-studied in the context of renal fibrosis. Recent studies suggest that Angptl4 is secreted from damaged podocytes. Due to large energy needs, renal tubular cells utilize fatty acid oxidation for energy production. During fibrosis, fatty acid levels decrease, which could be associated with higher Angptl4 levels. This study aimed to understand the role of Angptl4 and the glucocorticoid receptor in mediating renal fibrosis. Three mouse models were used to study kidney fibrosis: a diabetic model, a surgical model, and a drug-induced model. Analysis of all three models indicated increased renal fibrosis, demonstrated by Masson Trichome staining and protein levels of fibrotic markers. Renal fibrosis was induced in mouse models with podocyte-specific knockout of both the glucocorticoid receptor (pGR-KO) and Angptl4 gene (pAngptl4-KO). Fibrosis assays are ongoing in the pGR-KO model, though previous analyses demonstrate a protective role of GR in limiting fibrosis. However, the pAngptl4-KO model showed decreased fibrosis. Though this suggests a fibrotic role of Angptl4 in podocytes, further analysis by immunofluorescent staining and qPCR will be performed to confirm this finding. These data demonstrate the opposing, and cell-specific, roles of GR and Angptl4 in podocytes and suggest these genes may be therapeutic targets for renal fibrosis in the future.

THE C-TERMINAL DOMAIN OF CYCLIN G-ASSOCIATED KINASE REGULATES CLATHRIN UNCOATING IN PODOCYTES

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Clathrin mediated endocytosis (CME) plays a fundamental role in podocyte biology. Cyclin G-associated kinase (GAK) is ubiquitously expressed and consists of N-terminal kinase domain, tensin-like (PTEN) domain, a clathrin-binding domain, and a C-terminal J-domain. GAK is known to play an important role in regulating cell cycle and uncoating of clathrin coated vesicles. Global deletion of GAK results in embryonic lethality. However, it remains unclear whether GAK plays a critical role in podocytes and which domain of GAK is essential for podocyte biology. Thus, we generated a germline podocyte specific *Gak* knockout (KO) mice (*Pod-Cre Gak^{fl/fl}*). The KO mice showed massive proteinuria, podocyte foot process effacement, glomerulosclerosis, interstitial fibrosis and developed kidney failure. Clathrin vesicles were accumulated in *Gak* KO podocytes demonstrated by immunofluorescence. Overexpression of C62 GAK, not GAK kinase domain + PTEN domain, in *Gak* KO podocytes resulted in improvement of clathrin accumulation. These results indicate that GAK is indispensable in podocytes, and C62 GAK plays an important role in CME due to its ability to regulate clathrin turnover.

RESTORING NPHP3 RECRUITMENT IN NEK8 AND INVS KNOCKOUT CELLS WITH RCC-1 CHIMERIC PROTEINS

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The inversin compartment (IC) is a structure at the proximal end of primary cilia that contains four critical proteins: inversin (INVS), NEK8, ANKS6, and NPHP3. Nonsense mutations in these IC proteins are associated with multiorgan malformation syndromes, embryonic or perinatal lethality, and cystic kidney disease. While very remarkable disease phenotypes are associated with the IC, little is understood about the role of the IC proteins. It has been demonstrated that the RCC-1 repeat domain of NEK8 kinase interacts with INVS and recruits NPHP3 to the ciliary membrane. To further characterize the structure-function relationship of the RCC-1 repeat domain in the context of the NPHP3 recruitment and NEK8 localization, we generated CRISPR/Cas9 knockout cell lines for INVS and NEK8 and re-introduced a variety of mutant and chimeric NEK8 constructs using lentiviral transduction. RCC-1-CSAP and RCC-1-INVS[555-1065] chimeric proteins were cloned with an N-terminal V5-tag and added back to IMCD *Nek8* and *Invs* knockout cells. Immunofluorescence microscopy revealed that RCC-1-CSAP and RCC-1-INVS successfully localize to the cilia and are able to recruit NPHP3 to the IC. These results indicate that the NEK8 RCC-1 domain alone is sufficient for NPHP3 localization, and that other protein domains (INVS ankyrin-repeat domain, NEK8 kinase domain, ANKS6 protein) are not necessary for NPHP3 recruitment. Thus, the RCC-1 repeat domain represents a critical protein-protein interaction interface, linking microtubule-bound inversin and membrane-bound NPHP3. Future research on disease-causing missense mutations in the RCC-1-repeat domain will help understand the importance of its interactions with INVS and NPHP3.

METFORMIN IMPROVES URINE CONCENTRATING ABILITY IN HUMANIZED SICKLE CELL MICE

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The kidney's ability to concentrate urine is normally dependent on the actions of vasopressin, which promotes phosphorylation of the water channel, aquaporin 2 (AQP2), and water reabsorption. One characteristic of sickle cell pathology is an inability to concentrate urine despite normal vasopressin levels. We studied whether an alternate pathway could improve urine concentration in the humanized sickle cell mice. Metformin activates adenosine monophosphate-activated kinase (AMPK). AMPK phosphorylates AQP2 and the UT-A1 urea transporter. Sickle mice given metformin (800 mg/kg/d) by oral gavage daily for 5 days were compared with sickle mice and control mice that did not receive metformin. Urine osmolality in metformin-treated sickle mice (2413 ± 176 mOsM) was significantly elevated from untreated levels (1903 ± 29 mOsM) ($p < 0.05$; $n = 8-10$ /group). Metformin increased urine osmolality within 24 hours and continued to improve over the next 4 days. This was mimicked by an increase in urinary Na, K and Cl levels. In the inner medulla, the ratio of activated pS256-AQP2 to total AQP2 protein was not significantly changed by metformin but trended toward an increase. The pS261-AQP2/total AQP2 ratio remained unchanged. Inner medullary UT-A1 protein abundance was not changed by AMPK stimulation. These results indicate that AMPK activators, such as metformin, might provide promising treatments for polyuria and dehydration from Sickle Cell disease. Since inner medullary AQP2 and UT-A1 protein abundances were statistically unchanged, future studies are needed to examine additional mice to see whether pSer-AQP2 protein is increased in the IM or altered in the cortex.

THE ROLE OF PLANAR POLARITY IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

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Autosomal Dominant Polycystic Kidney Disease (ADPKD), characterized by bilateral kidney and liver cysts, arises from mutations in the *PKD1* and *PKD2* genes. Various cell signaling pathways have been implicated in ADPKD including the non-canonical Wnt-planar cell polarity (PCP) pathway. PCP regulates the uniform orientation of cells within a tissue plane by way of asymmetric distribution of transmembrane proteins. The core PCP pathway is composed of several proteins including Van Gogh (*Vangl/strabismus*). *Vangl1* and *Vangl2* each contain four transmembrane domains and intracellular N- and C- termini, and their biochemical functions are nearly identical. While PCP has been implicated in ADPKD, its specific role has yet to be elucidated. The primary objective of this study is to investigate the role of PCP in ADPKD. To investigate the role of PCP in ADPKD we examined *Pkd1*^{fllox/fllox}; *Pkhd1*-Cre (SKO) and *Pkd1*^{fllox/fllox}; *Vangl2*^{S464N/+}; *Pkhd1*-Cre (DKO) mouse models. Kidneys were harvested at postnatal day 16 (P16) and 24 (P24). We assessed by if PCP was present in cystic epithelia by examining the presence of *Vangl1* by immunofluorescence microscopy. *Vangl1* was weakly expressed in SKO kidneys at P16. By P24, *Vangl1* protein expression increased and localized at the lateral membrane. Genetic interaction studies enabled us to investigate if elevated PCP in cystic tissue yielded a protective or pro-cystogenic effect. Loss of *Vangl2* with *Pkd1* (DKO) resulted in a worsened cystic phenotype at P16, as shown by higher kidney-to-body weight ratio, cystic index, and BUN by comparison to SKO. Increased proliferation rates were also detected in the DKO by comparison to the SKO at P16. Taken together, these data provide not only evidence of the presence of PCP in ADPKD, but also suggests PCP plays a protective role against disease progression.

LINEAGE TRACING EPITHELIAL-MESENCHYMAL TRANSITION IN SARCOMATOID RENAL CELL CARCINOMA

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Sarcomatoid renal cell carcinoma (sRCC) is a rare but highly lethal form of kidney cancer characterized by tumors with histological and molecular features resembling both epithelial tumors (carcinomas) and mesenchymal tumors (sarcomas). The causes of this disease are unknown. The Hippo/Warts (Mst/Lats) pathway is one of the major signaling pathways regulating tissue growth and organ size during development and mutations in this pathway have been shown to invoke tumorigenesis in multiple organ systems. We ablated the Warts kinases Lats1/2 specifically in adult renal epithelia using a drug inducible Cre-lox system (KspCreERT2). Mutant mice rapidly developed large sarcomatoid tumors in the kidneys that metastasized to the lungs. To provide insight into the mechanism through which loss of Lats1/2 leads to sRCC, I examined the expression of a number of markers of the epithelial and mesenchymal phenotype in mutant tissues using immunohistochemistry. My research reveals that mutant epithelia undergo a full epithelial-mesenchymal transition (EMT) as indicated by the loss of epithelia proteins and gain of mesenchymal proteins leading to the sarcoma phenotype. The existence of EMT in sRCC has been fiercely debated within the scientific community. Subsequent experiments intend to biochemically and functionally identify specific proteins and pathways that may promote EMT within our Lats1/2 loss-of-function mouse model of sRCC.

IDENTIFICATION OF POTENTIAL DRUG TARGETS FOR RESTORING CILIARY POLYCYSTIN LEVEL BY INCREASING AXONEMAL POLYGLUTAMYLATION

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Autosomal Dominant Polycystic Kidney Disease is caused by mutations of either *PKD1* or *PKD2* gene. Decreased functional level of Polycystin-1 or Polycystin-2, which are encoded by *PKD1* or *PKD2* respectively and form a mechanosensory complex on cilia, is thought to be the major mechanism for cystogenesis. Theoretically, ADPKD development will be delayed or even suppressed if functional polycystin level could be restored in cilia context. Importantly, previous study in our lab revealed that increased ciliary polyglutamylation effectively increases ciliary polycystin level, indicating that polyglutamylation modification hold the potential to be targeted in future ADPKD therapeutic applications. However, since there are no drugs available for directly regulating the activity of polyglutamylation enzymes, we intend to identify novel drug targets for manipulating polyglutamylation pathway and subsequent polycystins level. Here, in a small-scale screening searching for potential hits that increased ciliary polyglutamylation, we identified CDK7 as a promising target. By using siRNA and CDK7 selective inhibitors (THZ1 and BS-181), we found that inhibition of CDK7 increased ciliary polyglutamylation level, cilia length, and PC-2 level in RPE cells. Moreover, we found that THZ1 promotes the ciliary entry of tubulin glutamylase TTLL5/6 in a dosage dependent manner. Significantly, *in vitro* cystogenesis of MDCK 3D-cultured cells was suppressed by THZ1. Intriguingly, both exogenous mCherry-tagged and endogenous CDK7 showed exclusive nucleus localization, suggesting its ciliary effector(s) awaiting to be identified. In summary, we identified a CDK7-regulated pathway which is essential for TTLL5/6 mediated ciliary polyglutamylation and could be potentially targeted for restoring Polycystin level and suppressing ADPKD.

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UNDERSTANDING THE ROLE OF m⁶A METHYLTRANSFERASE IN THE KIDNEY

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N⁶-methyl adenosine (m⁶A) is the most common RNA modification. m⁶A modifications on mRNAs regulates mRNA half-life, thereby affecting their translation into proteins. RNA m⁶A methylation is essential for embryonic stem cell differentiation and has been implicated in cancer progression. However, virtually nothing is known about the impact these mRNA chemical modifications have on normal renal physiology or kidney disease pathogenesis. We have found that m⁶A levels are increased on mRNAs from kidneys of humans and mice with autosomal dominant polycystic kidney disease (ADPKD). Thus, the goal of this study is to determine whether modulating the m⁶A pathway affects the pathogenesis of ADPKD. To address this question, we generated and characterized a new conditional *Mettl3^{flox/flox}* mouse model. Methyltransferase like 3 (*Mettl3*) is the enzymatic subunit of the m⁶A writer complex that catalyzes m⁶A methylation. First, we determined whether inhibiting *Mettl3* reduces m⁶A modifications on mRNAs in renal tubules. Using a kidney-specific, doxycycline-inducible cre, we deleted *Mettl3* in renal tubules. Q-PCR analysis and immunofluorescent staining confirmed *Mettl3* knockdown. Importantly, m⁶A levels were also markedly reduced in renal tubules. Next, we studied the effects of chronic m⁶A depletion on renal tubule homeostasis. Analysis of 20-week-old mutant kidneys reveals that these kidneys are identical to their wildtype littermates. Our results suggest that ablating *Mettl3* is safe and m⁶A methylation is not necessary for normal renal tubule homeostasis. These key observations will now allow us to ablate *Mettl3* in mouse models of ADPKD and test the idea that inhibiting the m⁶A pathway may be a novel therapeutic approach for ADPKD.

DROSOPHILA: A ROSETTA STONE OF NEPHROLITHIASIS?

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In the US, 11% of men and 6% of women will have kidney stones (nephrolithiasis) which can cause severe pain, or even kidney failure. No real and concrete therapy exists. Therefore, to mechanistically understand the pathology, translational models become crucial. *Drosophila* renal structures (*Malpighian* tubules “MTs”) are a useful and inexpensive translational model for human kidney diseases. By conducting different experiments with MTs, factors such as calcium oxalate (CaOx) crystallization, fluid-secretion and intracellular pH are quantified. By regulating various genetics and transport proteins, we can identify cause-and-effect relationships leading to kidney stones. Supersaturation is a frequent cause of CaOx stone formation. By controlling aquaporins (AQP) *Drip* and *Prip*, we hypothesized fluid in MT-lumen could be controlled. Using a dog genetic-model, part of our group identified specific genes associated with CaOx kidney stones which we have examined in both fly and humans. One such genes has a *Drosophila* homologue, *Subdued*, with hypothesized lipid-scramblase activity. When aquaporins are knocked down (KD), *Prip-KD* forms crystals spontaneously. Knockdowns of *Subdued* have very large crystals, allowing us to view stone formation in more detail. Fluorescence experiments indicate that previously ignored stellate cells in MTs may be involved in oxalate transport. Proteomic analysis after oxalate feeding shows another Slc26-gene besides dPrestin, *CG9702*, is upregulated. Together, this data allows us to test critical genes in a simple model to understand the paths which can lead to CaOx nephrolithiasis. Future experiments will use qPCR to determine extent of gene-knockdown and localize these proteins in dog and human kidneys.

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REAL-TIME MODEL-DRIVEN DIAGNOSTIC AND THERAPEUTIC EVALUATION OF PATIENTS AT RISK OF ACUTE KIDNEY INJURY: A PILOT STUDY

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Acute Kidney Injury (AKI) is a frequently diagnosed form of kidney disease in hospitalized patients. Although serum creatinine is a primary indicator of kidney function, it is a late marker of AKI because it lags behind decreases in Glomerular Filtration Rate (GFR). This is a major cause of delayed AKI diagnoses. For this reason, we developed a time-updated AKI prognostic model and integrated it into the Electronic Health Record (EHR) of the Yale New-Haven Hospital. The model generated a “Pre-AKI Alert” that notified study personnel when a hospitalized patient exhibited a >30% risk of developing AKI in the near future. Each patient (n=59) was monitored for three days via urine and blood sample collection and EHR examination to check for the development of creatinine-defined AKI. Of the 59 inpatients enrolled, 12 (20%) developed AKI within the next 48 hours post-alert and 7 (12%) died during the hospitalization. 12% received a nephrotoxic medication within 24-hours of the alert. 25% of patients who developed AKI had a MABP < 65 at the time of pre-AKI alert compared to 6% percent of patients who did not develop AKI (p=0.092). 75% of patients who developed AKI had urine hyaline casts at the time of pre-AKI alert compared to 26% of those who did not develop AKI (p=0.002). In conclusion, some patients at high risk of AKI nevertheless receive nephrotoxic medications. The prevalence of hyaline casts among those who developed AKI suggests that IV fluid administration may be a therapeutic option in this population.

PATHOGENIC MUTATIONS IDENTIFIED IN GENETICALLY UNRESOLVED DENT AND PRIMARY HYPEROXALURIA DIAGNOSED PATIENTS USING NEXT GENERATION SEQUENCING

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Primary hyperoxaluria (PH) and Dent are rare monogenic diseases resulting in kidney stones and renal insufficiency. PH is caused by biallelic mutations to *AGXT*, *GRHPR*, or *HOGA1* and manifests as hyperoxaluria and hypercalciuria. Dent disease (X-linked) is caused by single mutations to *CLCN5* or *OCRL* and results in proteinuria. From previous targeted Sanger sequencing in the Mayo Clinic Rare Kidney Stone Consortium, we failed to detect mutations in the known genes in 295 patients: 228 clinically diagnosed as PH (PHN) and 67 as Dent (DN). Here we employed a next generation sequencing (NGS) panel of 90 known or candidate stone disease genes to attempt to genetically explain these unresolved patients. The potential pathogenicity of detected variants was evaluated with *in-silico* tools, including multisequence protein alignments, web-based prediction programs, and population data. For the PHN cases, 11 families were likely resolved with biallelic mutations to the *CLDN16*(x4), *APRT*(x2), *CYP24A1*(x2), *CLDN19*, or *KCNJ1* genes, plus one large deletion. In a further 12 families a single pathogenic variant or two variants of unknown significance in the same gene were detected, but proof of causality was not reached. Of the 67 DN patients, 6 families were likely resolved with biallelic mutations to the *SLC34A3*(x2), *SLC12A1*, *CLDN16* or *KCNJ1* genes, plus one with large duplications. This study shows the value of NGS for providing a firm diagnosis to monogenic stone patients, which can improve their care and treatment. Future research will involve wider genetic analysis to identify pathogenic variants in more of the cohort.

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INCREASED EXPRESSION OF FIBRONECTIN AND PHOSPHORYLATED FOCAL ADHESION KINASE IN A MURINE MODEL OF POLYCYSTIC KIDNEY DISEASE

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Autosomal dominant polycystic kidney disease (ADPKD) is a common monogenic disorder caused by mutations in the genes *PKD1* and *PKD2*. This disease is characterized by the development of multiple cysts in the renal tubules which can lead to chronic renal failure. Few pharmacological treatments exist for ADPKD, nor has a mechanism of cystogenesis been conclusively established; thus, understanding these mechanisms is an important area of research.

Previous proteomic results have shown that fibronectin is one of the most highly overexpressed proteins in an *in vitro* model of ADPKD. We hypothesize that overexpressed fibronectin has a causal role in cystogenesis through an integrin-mediated signaling mechanism, leading to increased activation of focal adhesion kinase (FAK).

We investigated this hypothesis by measuring the expression of fibronectin, integrin $\alpha_5\beta_1$ and phosphorylated FAK Y397 (p-FAK-Y397) in a murine model of ADPKD. Integrin $\alpha_5\beta_1$ is known to bind fibronectin as a ligand and activate FAK at the Y397 autophosphorylation site. Expression was quantified at the mRNA and protein levels, and immunofluorescence imaging was used to assess localization of expression.

Our results demonstrate increased expression of fibronectin; however, integrin $\alpha_5\beta_1$ is not significantly overexpressed in cysts and p-FAK-Y397 expression is mis-localized in cyst-lining cells. These results suggest that mis-polarization of cell growth and adhesion signals, in addition to mis-regulated expression, plays a role in cystogenesis.

GENDER DIFFERENCES IN Na/H-EXCHANGER 2 (NHE2) ACTIVITY AND EXPRESSION IN MOUSE KIDNEYS

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Na/H-exchangers (NHE) in the apical membrane of the kidney tubule are responsible for sodium and bicarbonate absorption. NHE3 is the major isoform that is responsible for 65% of filtered sodium and bicarbonate absorption in the proximal tubule of the kidney. In addition to NHE3, NHE2 is the major isoform that is responsible for absorption of the remaining filtered sodium and bicarbonate in the distal tubule. Previously, we have demonstrated higher NHE3 expression levels in male than in female kidneys.

In this study, we investigated whether there are gender differences in NHE2 activity and expression in the kidney. We examined the effect of a specific NHE2 inhibitor, HOE694, on sodium and potassium transport in WT male and female mice via renal clearance. We also examined NHE2 protein expression levels via western blotting. Urine volume (UV), glomerular filtration rate (GFR), absolute (ENa, EK) and fractional (FENa, FEK) Na and K excretion were measured and compared before and after a bolus IV injection of HOE694 (3mg/kg).

HOE694 did not significantly increase UV, sodium, and potassium excretion in male mice, but did significantly increase UV, ENa, FENa, EK, and FEK in female mice. Western blotting results showed that NHE2 abundance was higher in females than in male mice. We conclude that both NHE2 activity and expression are higher in females than in male mice. The mechanism may be due to lower levels of NHE3 in the proximal tubule, which results in the upregulation of NHE2 in the distal tubule of females.

CHARACTERIZATION OF URINARY EXTRACELLULAR MATRIX VESICLES IN CALCIUM OXALATE STONE FORMERS AND NON-STONE FORMERS

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Matrix vesicles that carry calcium (Ca) and phosphorus (P) play an essential role in biomineralization and pathological calcification. However, the potential roles of kidney-derived extracellular vesicles (EVs) in urinary stone pathogenesis remain unknown. This study quantified specific EV populations in cell-free bio-banked urine samples from calcium stone formers (n=25) and age-/sex-matched controls (n=21) to identify those that have markers for matrix vesicles and may have a role in urinary crystal nucleation. Markers for renal Ca/P pathways regulators (phosphate transporter -1 (Pit-1)/Pit-2, Klotho/fibroblast growth factor 23(FGF23)) and vesicle generation (anoctamin-4 (ANO4) and Huntingtin interacting protein 1(HIP1)) were all quantified by digital flow cytometry to assess the potential role of extracellular matrix vesicles in stone formation. Statistical significance (P<0.05) between groups was evaluated by Wilcoxon rank sum test. The total number of urinary EVs expressing Pit-1 and Pit-2 were significantly (P<0.05) lower in stone formers. EVs expressing Klotho/FGF23 were also lower (P<0.05) in stone formers. The number of EVs expressing Pit-1 and Pit-2 were negatively (ρ -0.35; P<0.05) correlated with urinary phosphate whereas EVs expressing FGF23 were negatively correlated (ρ -0.3; P<0.05) with urinary phosphate and calcium. ANO4 and HIP-1 are 2 genes implicated in stone pathogenesis in Miniature Schnauzers. Excretion of EVs expressing ANO4 (p=0.04) and HIP-1 (p=0.06) were lower in stone formers. These results indicate that urinary EVs expressing matrix vesicle biomarkers may contribute to urinary stone formation and serve as crystal nucleation sites within the kidney. The mechanisms whereby EV-associated biomarkers influence stone pathogenesis warrant further study.

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IN-VIVO NMR MICROSCOPY OF ZEBRAFISH MODELS

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The zebrafish (*Danio rerio*) is a productive model for studying the pathological development of kidney disease. Within this field of research, *in-vivo* imaging methods provide a non-invasive way to quantitatively and qualitatively monitor disease progression in animal models. However, *in-vivo* imaging for zebrafish has not yet been developed for kidney analysis. The goal of this study is to provide researchers with the tools necessary to perform *in-vivo* Nuclear Magnetic Resonance (NMR) imaging for longitudinal studies of zebrafish kidney pathology. To accomplish this task, we created a Vertical Perfusion Chamber (VPC) that restrains the zebrafish and provides a constant perfusion of anesthetic while allowing respiration. The zebrafish remains constrained in the VPC while the NMR scanning protocol is performed. Upon completion, the zebrafish is removed from the VPC and is recovered in a separate tank. The VPC device created for this particular imaging method proved to be an effective system. The zebrafish were successfully sustained in the VPC for scanning protocols ranging from 20 to 45 minutes with a recovery rate of 100 %. Resultant scans had a resolution of 100 microns with a relatively high signal to noise ratio providing a sufficient quality for biomarker identification. The results of this portion of the study have provided us with a baseline protocol from which to expand on. Future studies include the integration of auto-segmentation of zebrafish kidneys and the optimization of scanning programs particular to longitudinal *in-vivo* studies.

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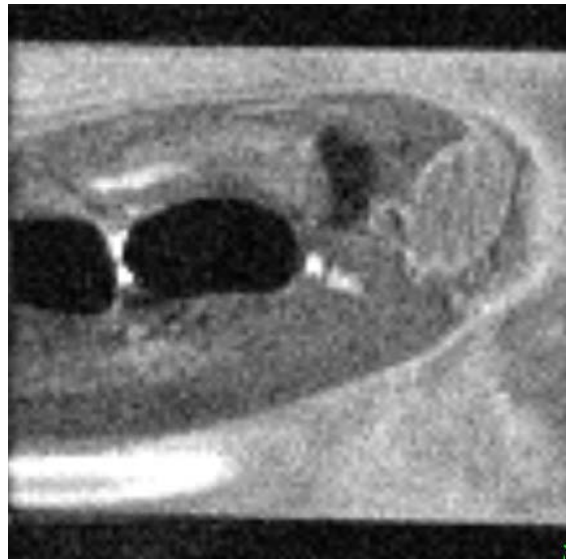


Figure. Saggital zebrafish NMR scan

CHARACTERIZATION OF RENAL INFLAMMATORY CELL POPULATIONS IN NEPHRECTOMY PATIENTS WITH AND WITHOUT A HISTORY OF KIDNEYS STONE DISEASE

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The potential role of renal interstitial inflammation in urinary stone formation is unknown. We analyzed specific inflammatory cell populations in kidney specimens and quantified inflammatory cell-derived urinary extracellular vesicles (EVs) in a second cohort of calcium stone formers and controls.

Bio-banked cell-free urine samples from calcium stone formers (n=24) and matched controls (n=21) were obtained from Mayo Clinic O'Brien Urology Research Center study participants to quantify inflammatory cell-derived urinary EVs by digital flow cytometry. Inflammatory cell populations were identified and quantified by immunocytochemistry in non-cancerous regions of nephrectomy sections from Mayo Clinic Aging Kidney Anatomy Study subjects with (n=45) and without (n=86) a history of stone disease. The expression of active T-lymphocytes (CD3), monocytes/macrophages (CD68), plasma cells (CD138), and mast cells (tryptase) in renal tissue sections were evaluated by renal pathologist, LHH. A subset of age, sex, and inflammation grade matched samples (n=10/group) were digitally scanned and quantified as number of positive cells to confirm the pathologist's readings.

The number of urinary EVs derived from monocytes/macrophages, neutrophils, plasma/epithelial cells, and total leukocytes were significantly lower ($P<0.05$) in stone formers. The total numbers of specific inflammatory cell populations did not statistically differ between groups, but trends pointed to a greater number of total inflammatory cells, monocyte/macrophages, and mast cells in the kidneys of patients with a stone history. These results suggest that lithogenic risk factors and/or crystals may activate inflammatory cells in the renal medulla, potentially reflected in urinary EV populations, and that inflammation may influence urinary stone pathogenesis.

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IMAGING KIDNEYS USING DOPPLER ULTRASOUND AS A METHOD OF DETECTING KIDNEY STONES VIA TWINKLING ARTIFACTS

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Kidney stones affect 7% of women and 17% of men annually in the US according to 2016 data. When a patient is suspected to have a stone, computed tomography (CT) is the primary method for locating the stone. This is followed by a 24 hour urine test for stone characterization. A method to replace CT would be highly beneficial to avoid ionizing radiation exposure. Doppler color ultrasound has been used to produce color mosaic signals termed “Twinkling Artifacts” (TA) on kidney stones in human subjects. In this study, 47 stones with varying size and composition were tested in an isolated system to show the consistency of detecting TAs. The stones were scanned at varying Doppler powers in *ex vivo* porcine kidneys to determine if TAs were visible through tissue and only from the stones. Finally, a randomized stone placement study in *ex vivo* kidney was conducted with varying stone types, sizes, and numbers in each scan to mimic a clinical situation. The isolation study showed no issues in the appearance of TA’s. The *ex vivo* study showed no difficulties in detection and locating the stone can be done at maximum drive voltage. The randomized study detected all 47 stones with only two false signals. These results show that Doppler ultrasound and using TAs might be a viable method for detecting kidney stones, but further testing is required, particularly *in vivo* evaluations.

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ASSOCIATIONS OF AGE AND GENDER WITH DOMAINS OF PHYSICAL FUNCTIONING IN HEMODIALYSIS PATIENTS

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To bridge the gaps in dialysis patient-provider communication, the INFORMED pilot study has collected the physical functioning data of dialysis patients. The end goal of the study is to initiate patient-provider conversations on the individualized physical functioning reports. The present study utilizes the preliminary data to examine associations of age and gender with domains of physical functioning.

Physical functioning was measured across multiple domains in 41 hemodialysis patients at two clinics. Measures included: Short Physical Performance Battery (SPPB; incorporating balance, gait speed, and lower-body strength; range, 0-12; higher scores=better performance); perceived physical functioning (range, 0-100; higher scores=higher perceived functioning); and any impairment in basic and instrumental activities of daily living (ADLs; %). Paired t-tests and Fisher's exact tests were performed to compare functioning by age group (dichotomized <60, ≥60) and gender.

In this population (mean age, 56.9; 53.7% female, 91.9% black), apart from transferring (17.4% vs. 50.0%, ≥60 vs. <60; $p = 0.043$), both age groups reported negligible differences in their ADLs; no statistically significant differences were seen in perceived physical functioning. However, their actual physical performance on the SPPB showed demonstrable age-dependent differences (mean score, 8.08 vs. 5.07, $p = 0.007$). No statistically significant differences by gender were seen for any measure.

Results suggest that, while functioning is generally lower in these dialysis patients than in the general population, there are age (but not gender) differences across domains of physical functioning. Therefore, interventions to improve functioning and gauge physical performance in hemodialysis patients should take age into account.

MATERNAL INFECTION ADVERSELY AFFECTS PLACENTAL HEALTH VIA INDUCTION OF STRUCTURAL AND INFLAMMATORY CHANGES

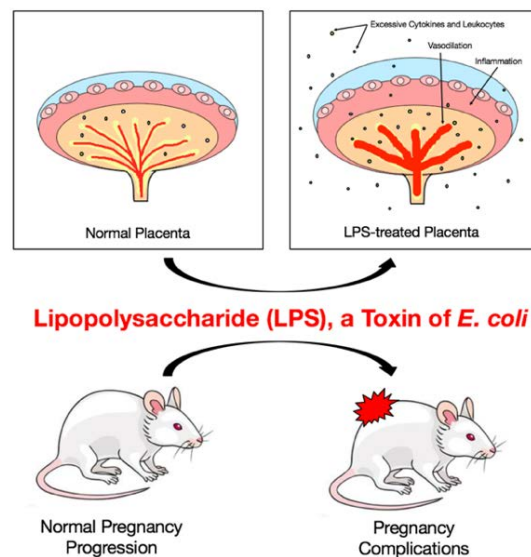
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Preterm birth (PTB) occurs in 5-15% of all pregnancies globally, constituting a major public health, social, and economic issue. PTB has multifactorial causes, with maternal infection accounting for 40% of all cases. *Escherichia coli* (*E. coli*) is one of the most prevalent pathogens responsible for infection during pregnancy, increasing the risk of miscarriage, stillbirth, PTB, and PTB-associated health complications. *E. coli* produces lipopolysaccharide (LPS), a strong proinflammatory endotoxin, that may harm pregnancy via placental impairment. Given that placental health is a key determinant of pregnancy outcomes, placental dysfunction due to infection may contribute to PTB risks. Therefore, this study aimed to examine effects of LPS on placental structure and inflammation. To do so, we utilized an infection-induced mouse model of PTB in which LPS was administered intraperitoneally on day 15 of pregnancy. Quantitative real-time PCR analysis was used to analyze mRNA expression of proinflammatory cytokines interleukin 1 β (IL1- β) and tumor necrosis factor α (TNF- α). Immunofluorescent staining of platelet endothelial cell adhesion molecules (PECAM1), F4/80, and GR1 markers were performed to assess placental vascular changes, and localization of macrophages and neutrophils, respectively. We observed LPS: 1) disturbed placental structure and vasculature, 2) elevated expression of IL1- β and TNF- α , and 3) stimulated leukocyte infiltration. In conclusion, these results clearly demonstrate that LPS exposure during pregnancy triggers placental structural, vascular, and inflammatory changes most likely associated with PTB induction. Understanding LPS-induced placental changes could eventually provide a foundation for developing preventative strategies or therapeutics to reduce the incidence of infection-related PTB.

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Graphical Abstract:



QUANTIFICATION OF TUBULE STONE FORMATION TO DETERMINE THE ROLE OF MFS TRANSPORTER IN DROSOPHILA

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Phosphate homeostasis is regulated by *fibroblast growth factor (FGF)*, *parathyroid hormone (PTH)*, and *1,25-dihydroxy vitamin D (1,25(OH)₂D*) in humans. We used *Drosophila melanogaster* to study the metabolic and homeostatic effects of inorganic phosphate. In previous studies, one of *Major facilitator superfamily (MFS)*, similar to type I sodium-phosphate co-transporters in higher species, is expressed predominantly in the Malpighian tubules (MT) and serves an excretory role.

Using SEM&EDX, we showed that MT stones contain calcium phosphate. To test whether tubule stones can serve as a measure of Pi excretion and regulation of Pi excretion by this MFS transporter affects stone formation we devised an assay to measure calcium in MT. Most calcium is found in the tip of the tubules. While there is no difference between genders, anterior tubules have more calcium than posterior tubules. This corresponds well to the distribution of stones observed microscopically. Also, tubule calcium is increased when flies are cultured on a high Pi diet to stimulate Pi excretion for 7 days and we expect this to worsen after 30-day culture, an experiment which is currently pending. This sensitive assay will permit us to determine whether overexpression of *bnl* and ablation of the MFS transporter increases or decreases tubule stone formation, respectively, in genetically modified flies.