# KUH Summer Undergraduate Research Conference

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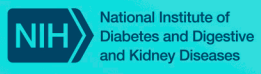
Dobbs University Center (DUC)
Atlanta, GA

# Abstracts

July 30, 2015 – August 1, 2015



# IDENTIFICATION OF LEAD COMPOUNDS FIBROSIS THAT DIFFERENTIALLY TARGET INTEGRIN α1β1 AND α2β1 I-DOMAINS FOR THE TREATMENT OF KIDNEY

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Kidney fibrosis is the accumulation of extra cellular matrix (ECM) – proteins, primary collagen, often resulting in organ failure. Integrins are transmembrane receptors that interact with ECM and regulate collagen production and turnover. Collagen binding to integrin α2β1 initiates collagen production while collagen binding to integrin α1β1 initiates collagen turnover. This study specifically focuses on subdomains of integrins called I-domains that are responsible for ligand binding and initiation of signal transduction. Ligand adhesion to the I-domain is contingent on a metal ion dependent adhesion site (MIDAS). This study investigates if small molecules have agonistic or antagonistic effects on the binding of Calcium and Magnesium ions to integrin α1β1 and α2β1 I-domains in turn inhibiting collagen binding. Three classes of compounds: inorganic, antibiotic, and vasodilators were screened to test differential inhibition of collagen binding. Competitive solid phase binding ELIZA assays were used to measure the effect of compounds on binding. We observed that Diltiazem, a vasodilator, produces a 4-fold increase in collagen binding to the α1 I-domain, but not the α2 I-domain. We anticipate the identification of lead compounds that differentially effect integrin α1β1 vs α2β1 I-domains collagen binding will aide in the development of therapies in the treatment of kidney fibrosis.

**DEFINING THE ROLE OF NUMB AND NUMB-LIKE IN KIDNEY DEVELOPMENT**

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With the stark rise in obesity, one of today's silent killers is Chronic Kidney Disease (CKD), an end result of diabetes and hypertension. In CKD, nephrons are replaced with fibrotic tissue and since there is no *de novo* nephron formation in adults, this leads to significant decrease in kidney function. We believe that greater understanding of nephrogenesis will allow us to engineer new nephron structures that ultimately can be used to treat or prevent CKD. Final nephron number is attained through a balance in the renewal and differentiation of an embryonic stem cell population known as Nephron Progenitor Cells (NPCs). In several model systems, whether a stem cell renews vs. differentiates is determined by whether the cell undergoes symmetric vs. asymmetric cell division. To determine whether such a mechanism regulates NPC fate, we ablated Numb and Numb-like from the NPCs using a Six2 Cre, as these factors have been shown to regulate asymmetric cell division and stem cell fate in other model systems. Data from antibody stains and H&E analysis demonstrates both that Numb and Numb-like are necessary for asymmetric cell division, and that the absence of Numb and Numb-like results in a phenotype of decreased glomeruli. This finding leaves open the question of whether the distortion of NPC division is related specifically to glomerular formation. More studies need to be conducted to fully determine the processes that Numb regulates in kidney development to assist the proper development of nephrons.

**INACTIVATION OF TOLL-LIKE RECPTOR SIGNALING MAY BE PROTECTIVE OF PROGRESSIVE KIDNEY FIBROSIS IN A MOUSE MODEL OF NEPHRONOPTHISIS**

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Nephronophthisis (NPHP), one of the most frequent genetic causes of chronic renal failure in children, is a group of autosomal recessive diseases characterized by progressive kidney cystic degeneration and fibrosis. Mutations in the gene *GLIS2*, which encodes a transcription factor, cause NPHP type 7. *Glis2* knockout mice (*Glis2*$^{mut/mut}$) overexpress toll-like receptor Tlr2 in kidney tubular cells. Toll-like receptors (TLRs) are important mediators of cellular response to stress and tissue damage and repair. TLR2 and TLR4 are the two TLRs expressed in renal tubular cells. Both TLRs signal through the common intermediate protein Myd88, stimulating the pro-inflammatory pathway NF-kB and inducing premature cell senescence. We hypothesize that activation of TLRs/NF-kB axis in *Glis2*$^{mut/mut}$ kidneys induces overproduction of inflammatory cytokines, progressive interstitial fibrosis and senescence of kidney tubular cells. To inhibit TLRs/NF-kB axis in *Glis2*$^{mut/mut}$ mice, we generated *Glis2*$^{mut/mut}$; *Tlr2*$^{-/-}$ and *Glis2*$^{mut/mut}$; *Myd88*$^{f/f}$ tubule-specific double knockouts. We quantified the area of interstitial collagen and the number of senescent tubular cells (lysine 9 tri-methylated histone 3) by immunofluorescence microscopy in kidneys of *Glis2*$^{mut/mut}$, *Glis2*$^{mut/mut}$; *Tlr2*$^{-/-}$ and *Glis2*$^{mut/mut}$; *Myd88*$^{f/f}$ mice at 3 and 6 months of age. Preliminary results suggest that tubule-specific inactivation of *Myd88* but not constitutive inactivation of *Tlr2* in *Glis2* knockout mice is protective of interstitial fibrosis and tubular senescence. More experimental mice will be needed to confirm these conclusions.

# MUTATION IN *XPO5* IS A NOVEL GENETIC CAUSE OF NEPHROTIC SYNDROME

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Nephrotic Syndrome (NS) is a kidney disorder characterized by proteinuria, high blood pressure, edema, hypoalbuminemia and hypercholesterolemia. To date, it is estimated that 70% of Steroid Resistant Nephrotic Syndrome (SRNS) cases are due to unknown causative genes. In this study, we examined by Whole Exome Sequencing (WES) a consanguine Turkish family with an affected child with Focal Segmental Glomerulosclerosis (FSGS). We hypothesized that the patient had a homozygous recessive mutation since: i) the parents are related, which increased the probability of the patient being homozygous by descent for the disease allele, and ii) statistically, the majority of pediatric diseases are recessive monogenic disorders. To identify the monogenic cause of the disease, we performed WES and homozygosity mapping in the affected child. We detected a homozygous missense mutation (c.1654G>A, p.Val552Ile) in *XPO5* (Exportin-5) that segregated heterozygously in the parents. Immunofluorescence microscopy of adult rat glomeruli revealed that *XPO5* is highly expressed in adult rat glomerular podocytes.

Taken together, our data suggest that mutations in *XPO5* are a novel cause of Steroid Resistant Nephrotic Syndrome (SRNS).

**ASSESSING BACKBONE INTEGRATION IN THE *PIGGYBAC* TRANSPOSON SYSTEM**

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The *piggyBac* (PB) transposon system has been studied extensively for use in pre-clinical gene transfer. Although PB is effective for gene delivery, past studies have observed plasmid backbone (outside of the transposon) integration into the genome *in vitro.* We used a detector transposon encoding GFP on the backbone to assess the rate of plasmid backbone integration *in vivo*. Hydrodynamic tail vein injection was performed on 6 FVB mice. One mouse received buffer, two mice were administered a combination of detector transposon and a luciferase-encoding transposon, and three other mice were administered a combination of detector transposon, a luciferase-encoding transposon, and transposase. Bioluminescence imaging techniques were utilized to assure gene delivery. After one week, the livers and kidneys were harvested. Frozen sections of mouse livers and kidneys were fixed and stained with DAPI. Fluorescence microscopy was used to image the livers and kidneys from each mouse. We observed increased levels of GFP fluorescence in the livers of mice in the group receiving transposase, signaling increased expression from the transposon plasmid backbone consistent with increased integration. Additionally, kidney and liver samples were analyzed using qPCR to detect transposon backbone integration. Genomic DNA was isolated and treated with the restriction enzyme *DpnI* to eliminate bacterially methylated plasmid DNA. qPCR probes to the neomycin resistance gene located on the transposon, to the GFP gene located on the transposon backbone, and to the genomic murine ITK gene were performed. In conclusion, this is the first study to find evidence of transposon backbone integration *in vivo.*

**GENOME EDITING OF A NOVEL SPLICE ACCEPTOR SITE IN THE MIR-17-92 ONCOGENIC MICRORNA CLUSTER**

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MicroRNAs (miRNAs) are small, noncoding RNAs (~22 nucleotides) that posttranscriptionally regulate gene expression in numerous biological pathways. In particular, the oncogenic miRNA cluster miR-17-92 has been demonstrated to promote tumorigenesis in multiple types of human cancer as well as provide essential functions during mammalian development. As such, mechanisms that regulate expression of these miRNAs are of considerable interest. Previously, using transcriptome analysis of HEK293T cells expressing a dominant negative form of DROSHA that allows stabilization of miRNA primary transcripts, our laboratory identified a novel, conserved, alternatively-utilized splice acceptor (SA) site in the miR-17-92 cluster. Although the vast majority of miRNA primary transcripts are spliced, it is currently unknown whether alternative splicing can influence miRNA biogenesis. Targeted mutation of the novel SA in HEK293T cells to block splicing dramatically reduces expression of mature miRNAs of the cluster, concomitant with a slow growth phenotype. To further confirm that this effect on mature miRNA expression was indeed a result of loss of splicing, Transcription Activator-like Effector Nucleases, or TALENs, were engineered to disrupt the branch point consensus sequence (BPS). Following confirmation of TALEN activity, 160 single cell clones were picked and screened by PCR for deletion of the BPS consensus regions. Surveyor assay using T7 endonuclease confirmed the presence of indels in 26 clones. To fine map the indel mutations, a 506bp fragment spanning the BPS consensus was TOPO cloned and sequenced. Multiple clones with 5-10bp deletion of the BPS were identified. Reduced miRNA expression in these mutant cell lines would substantiate the role of splicing in regulation of the miR-17-92 cluster.

**EXPRESSION AND PURIFICATION OF TRANSFORMING GROWTH FACTOR RECEPTOR B-II CYTOPLASMIC TAIL FROM ECOLI.**

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Transforming growth factor-β (TGF-β) mediates both the development and the progression of fibrosis via activation of Type II TGF-β receptor (TβRII). The laboratory of Dr. Pozzi has recently identified tyrosine residues within the cytoplasmic tail of TβRII able to activate downstream signaling and essential for SMAD-dependent fibrotic signaling cascade. However, it is unclear how these tyrosine residues are phosphorylated, since TβRII lacks tyrosine kinase activity.To screen for tyrosine kinases that can directly phosphorylate the TβRII cytoplasmic tail (TβRIICT), we sublconed, expresed and purifiied TβRIICT protein from E. Coli. To do this, we subcloned TβRIICT cDNA in PGEX-6P-1 and correct and 'in frame' insertion was verified by partial DNA sequencing and restriction enzyme digestion. Then, we transformed the newly generated constructs into the E. Coli line BL21. Following an overnight culture, we induced TβRIICT protein expression by treating the culture with Isopropyl β-D-1-thiogalactopyranoside (IPTG) at 18ºC. GST-tagged-TβRIICT was then purified through Glutathione-Agarose column and GST was removed by TβRIICT by by site specific HRV3C protase cleavage. We verified succesful cleavage by Coomassie and Western blot analysis using western bloting, and the purifed protein is now ready for Kisase screening throught the Kinase Fiber Service. Finding the tyrosine kinases involved in TβRIICT phosphorylation will significantly contribute to our studies aimed to understand how TβRIICT tyrosine phosphorylation controls fibrosis.

**EFFICACY OF SODIUM THIOSULFATE TREATMENT FOR CALCIPHYLAXIS-ASSOCIATED PAIN**

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Calciphylaxis is a rare dermal arteriolar calcification disorder with high mortality and morbidity. It is characterized by severely painful skin lesions that ulcerate and get infected. Calciphylaxis-associated pain is frequently resistant to opiate analgesics. Anecdotal evidence suggests that intravenous sodium thiosulfate (STS) may improve calciphylaxis-associated pain by two weeks, but systematic evaluation is lacking.

In this retrospective cohort study, we identified 22 calciphylaxis patients who were treated with STS as inpatients at Massachusetts General Hospital between May 2013 and April 2015. Data on pain intensity (0-10 visual analog scale), clinical characteristics, medications, and laboratory parameters were retrieved from medical records. Responders were defined as ≥ 3 point reduction in pain intensity between baseline and 2-week follow-up. Responders and non-responders were compared in univariate analyses.

Of the cohort studied, 59% were male, 95% were Caucasian, and 55% had end-stage renal disease (ESRD). The mean age was 59 ± 16 years. Forty-one percent of the patients were responders. Opiate doses were similar among the responders and non-responders. The following clinically relevant differences were observed but none reached statistical significance: non-ESRD patients were more likely to respond than ESRD patients (50% vs. 33%), patients requiring surgical treatment were less likely to respond than those who did not require surgery (31% vs. 67%), patients with proximal lesions were more likely to respond than patients with non-proximal lesions (50% vs. 30%).

Potential analgesic effects of STS on calciphylaxis-associated pain observed in this study warrant evaluation in a randomized clinical trial.

**STUDY OF MUCIN AND PROTEOGLYCAN GENES IN MOUSE BLADDER**

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The bladder is one of the least permeable organs in the body, but the method behind this is not fully understood. One hypothesis is that the bladder has a mucus layer on the urothelium, which was the focus of this project.

Mucus layers are present on wet surfaces of the body such as the gastrointestinal and respiratory tracts and are composed of different molecules including two distinct types of proteins called mucins and proteoglycans. The expression of different mucin and proteoglycan genes in mouse bladder samples from the urothelium and the muscle layer was studied as an attempt to demonstrate the existence of a mucus layer.

Primers for 8 mucin genes and 16 proteoglycan genes were designed through the Integrated DNA Technologies website. PCR was performed on a sample containing cDNA from mouse bladder urothelium and muscle, with lung and colon used as controls. Luminosity of PCR bands were ranked on a 0 to 3 scale, with 0 signaling a lack of bands and 3 referring to very bright bands.

Between the mucins and proteoglycans, the proteoglycan genes were present to a larger extent, with few of the mucin genes showing expression in the bladder samples. Further work will need to be done to document the presence of these genes within the bladder in order to fully understand the manner in which the bladder gains its low permeability.

**ABSENCE OF PKCα ALTERS ANGIOTENSION II-TRIGGERED IMMUNE RESPONSES IN THE HYPERTENSIVE KIDNEY**

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Angiotensin II (ANG II) stimulation of T-cell proliferation and accumulation plays an important role in the development of hypertension and end-stage kidney damage. Unfortunately, the signaling mechanisms behind the immune response within the kidney remains underexplored. One pathway that is important to both ANG II and T-cell response is mediated by PKCα. To determine the role of PKCα in facilitating T-cell infiltration in the kidney during ANG II-dependent hypertension, PKCα null (KO) mice were treated with either saline or ANG II and compared to PKCα control (WT) mice treated similarly. After 14 days, systolic blood pressure, water intake and urine output were increased in all ANG II-treated mice. Kidney damage and collagen deposition is elevated with ANG II treatment and appears more extensive in KO mice. ANG II-treatment significantly increased spleen weight in WT mice only; suggesting that the absence of PKCα attenuates increased immune cell production. Histological examination of immune cell infiltration of the renal cortex revealed that ANG II-treatment increased macrophages, CD4+ and CD8+ T-cells in both WT and KO mice and suggests that CD4+ T-cell recruitment is higher in ANG II-treated WT compared to treated KO mice. Immune cell infiltration and cytokine production was also measured via flow cytometry and results suggest that PKCα is important for ANG II-meditated cytokine release in renal tissues. Collectively, these data suggest that the absence of PKCα decreases inflammatory response to ANG II-induced hypertension in the kidney.

**A RAPID COLORIMETRIC ASSAY TO ACCURATELY MEASURE URINARY CYSTINE**

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Cystinuria is a heritable form of kidney stone disease. Thiol drugs are efficacious in reducing stone formation in cystinuric patients; however, there are various adverse side effects to such drugs. An accurate measurement of free cystine is necessary to monitor the effectiveness of drug therapy as well as provide an indication of the minimal effective dose. Quantitative cystine measurement by liquid chromatography-tandem mass spectrometry (LC-MS/MS) will accurately reflect intact cystine in a patient's urine, but this test is relatively labor intensive and costly. A colorimetric assay is relatively fast and inexpensive; however, publications suggest issues related to creatinine interference. A modified colorimetric assay that corrects for this creatinine interference could provide a high-throughput screening process to clinically monitor cystinuria patients.

A published cyanide-nitroprusside colorimetric assay was modified to run using microplate settings. Sodium cyanide was added to reduce cystine to cysteine, followed by the addition of sodium nitroprusside to induce the formation of a cysteine-nitroprusside colored complex. Creatinine interference was corrected for using a formula derived from the slope of multiple creatinine standard curves.

Conditions of the microplate colorimetric assay for urinary cystine were developed and verified using cystinuric patients and patients without the disease. The assay performed well as a high-throughput screen for urinary cystine. Accuracy of cystine quantification was good by comparison to the gold standard LC-MS/MS amino acid assay.

Using modified reaction conditions and an equation to subtract creatinine interference, a rapid nitroprusside assay is suitable for identifying and monitoring patients with cystinuria.

R25-DK101405

**CHARACTERIZATION OF $CL^-/H^+$ COUPLING PROPERTIES OF CLC-5 MUTANTS AND TRANSPORT STOICHIOMETRY**

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There have been a lack of studies exploring the effects of mutations on electrogenic $Cl^-/H^+$ exchanger activity of CLC-5. Therefore, we will focus our study on CLC-5 $Cl^-/H^+$ coupling properties of wild-type and mutant CLC-5.

Using ion selective microelectrodes, the intracellular pH ($pH_i$) and intracellular $Cl^-$ concentration ($[Cl_i]$) were monitored while clamping at +40 mV *Xenopus* oocytes expressing WT and mutant CLC-5. The apparent $H^+$: $Cl^-$ coupling ratio through the CLC-5 protein can be calculated using the Gibbs-free energy equation.

In the presence of 104 mM $Cl^-$ (ND96), the $pH_i$ and $[Cl^-]_i$ of the CLC-5 WT increased linearly at a rate of 45.8 ± 11.5 $x10^{-5}$ pH units•$s^{-1}$ and 0.15 ± 0.016 mM•$s^{-1}$. T657S increased at similar rate while there was a significant decrease in the rate of $pH_i$ and $[Cl^-]_i$ change for S244L, R345W, and Q629X compared to WT. The stoichiometry of CLC-5 WT was calculated to be 2.0 ± 0.8 ($n$$Cl^-$:$H^+$) in ND96.

The study showed that the $Cl^-/H^+$ exchange activity decreased for S244L, R345W, and Q629X mutations, i.e., altering the transport stoichiometry. The T657S mutation showed similar trends to the CLC-5 WT and seems to be a benign mutation.

*R25-DK101405*

**USING NMR TO DEFINE THE STRUCTURE OF O6METHYLlGUANINE WITHIN CODON 13 OF THE HUMAN KRAS GENE**

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$O^6$-methylguanine is a common adduct caused by methylating agents from known exogenous carcinogens, endogenous bodily functions, and chemotherapeutic agents $O^{6-}$MeG is considered to be both cytotoxic and mutagenic. The sequence under analysis is the human K-ras gene, a gene important in cell differentiation and cell growth. The codon 13 of this gene is a "hotspot" for $O^6$methylguanine adducts. In this study, we used NMR (Nuclear Magnetic Resonance) spectroscopy to analyze this sequence, more specifically: 5'-CTGGTGGCGTAGG-3'. It was found that the sequence produces a clean and readable NMR spectrum. This information will be important in the future to analyze $O^{6-}$methylguanine within the codon 13 of the Kras gene.

**PENDRIN GENE ABLATION REDUCES BIRTH WEIGHT BUT NOT LITTER SIZE POSSIBLY THROUGH VASCULAR VOLUME CONTRACTION**

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Pendrin (*Slc26a4*) is a $HCO_3^-/Cl^-$ exchanger localized to the apical membrane of type B and non-A, non-B intercalated cells in the aldosterone-sensitive region of the kidney. Aldosterone stimulates pendrin-mediated $Cl^-$ absorption, which then stimulates ENaC-mediated $Na^+$ absorption. Due to the critical effect of pendrin on the renal regulation of NaCl absorption, pendrin null mice are vascular volume depleted. Volume expansion, however, is observed during pregnancy to facilitate nutrient delivery to the pups. Since pendrin is greatly upregulated during pregnancy, pendrin may participate in the vascular volume expansion observed during pregnancy. Thus, the purpose of this study was to explore the effect of pendrin gene ablation on litter size and body weight. To do so, we compared litter size and body weight of age- and sex-matched pendrin null and wild type mice. A two-way ANOVA demonstrated that pendrin null and wild type females statistically differed in weights until 11 weeks of age. For females greater than 11 weeks of age, the differences in weights did not reach statistical significance. Pendrin null and wild type males statistically differed in weights at nearly all ages between 4 and 14 weeks, although the magnitude of this difference appeared to decline with age. A one-way ANOVA revealed that there was not a statistically significant difference in litter sizes between any combination of pendrin null and wild type parent mice, suggesting that pendrin gene ablation does not affect the reproductive capabilities of mice. Conclusions: In young mice, pendrin gene ablation reduces body weight, although the difference between wild type and pendrin null body weights declines with age. The reduced body weight observed in young pendrin null mice may reflect reduced apparent vascular volume in pendrin null mothers. However, pendrin gene ablation does not change litter size.

**THE EFFECTS OF TNF-ALPHA ON THE EXPRESSION OF ANDROGEN RECEPTOR-BOUND ENHANCER RNAS (AR-ERNAS) IN BPH1 AND LNCAP PROSTATIC CELLS**

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The androgen receptor (AR) signaling axis is known to perform an essential role in the development of Benign Prostatic Hyperplasia (BPH), a nonmalignant enlargement of the prostate gland that is associated with a host of lower urinary tract symptoms. Blockage of AR receptor signaling has been shown to reduce BPH volume and help alleviate its symptoms. It has further been demonstrated that AR binds to the enhancers of its target genes and mediates expression of enhancer RNAs (eRNAs). These AR-eRNAs can then enhance gene transcription by participating in the AR-dependent looping complex that helps facilitate communication between target promoters and distal enhancers. Besides androgens, it is also thought that prostatic inflammation may represent a significant factor in the pathogenesis of BPH. In this study, we examined the effects of the proinflammatory cytokine TNF-alpha on the expression of AR-eRNAs. BPH1 and LNCaP cells were treated with TNF-alpha in time-dependent and dosage-dependent manners, and expression of AR-eRNAs was determined using RT-qPCR. While TNF-alpha seemed to induce minimal change in LNCaP cells, BPH1 cells showed a significant increase in AR-eRNA expression, in both a time-dependent and dosage-dependent fashion. Our results help to confirm the idea that TNF-alpha may play a key role in the regulation of AR expression in both BPH and prostate cancer.

*R25-DK101405*

**RENAL NBCE1 KNOCKOUT MOUSE HAS OCULAR PHENOTYPES**

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Patient mutations in electrogenic $Na^+$ bicarbonate cotransporter-1 (NBCe1) cause proximal renal tubular acidosis, cataracts, and glaucoma. To distinguish whether these phenotypes are due to a systemic disturbance or are tissue specific, both whole gene and A-isoform *nbce1*-knockout mice were followed.

Blood electrolytes, pH, renin-angiotensin-aldosterone (RAAS) components, corneal thickness, and anterior chamber thickness were measured. Ocular coherence tomography (OCT) was used to collect *in-vivo* images of the cornea, anterior chamber and retina of wild type (WT), heterozygous (Het), and knockout (KO) genotypes for both whole gene and A-isoform knockout mice. InSight software was used to make measurements of these regions of interest.

While all nbce1-knockout mice are acidotic, and RAAS components elevated, we sought to use genotype and aging to determine if ocular manifestations are due to systemic responses or transport changes in ocular tissues. Comparing genotypes, we found that KO mice have thicker corneas, and smaller anterior chambers and retinas then WT and Het mice. There was no significance in any of the ocular parameters between WT and Het mice. However, Nbce1A-knockout ($KO^A$) mice only show smaller anterior chambers and retinas than $WT^A$ and $Het^A$ mice. Preliminary experiments indicate that IP $NaHCO_3$ injections can normalize some of the ocular pathology.

While other variables are being investigated, evidence suggests that NBCe1 is important for ocular function and morphology. Whether the phenotypes are caused by systemic disturbance or tissue specific gene dysregulation needs further investigation.

*R25-GM075148*

**THINKING WITH THE KIDNEY: THE DUAL ROLE OF NARP AND REELIN AT NEURONAL SYNAPSES AND PODOCYTES**

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Neuronal Activity-Regulated Pentraxin (Narp) can bind to and induce clustering of synaptic glutamate receptors and has been shown to participate in homeostatic scaling of excitatory synapses, particularly on Parvalbumin-expressing interneurons, which is relevant for excitatory/inhibitory network balance. Reelin is expressed in a subset of cortical/hippocampal interneurons and also contributes to synaptic plasticity by supporting synaptic expression of glutamate receptors and other Long Term Potentiation (a mechanism of memory formation) relevant receptors. Kidney glomeruli and neuronal synapses in the brain share a common molecular make up: notably, neuromodulators such as Reelin and Narp are found to regulate glutamate receptor function in both glomerular podocytes and postsynaptic densities to control glomerular filtration and synaptic activity, respectively. Dysregulation of podocyte glutamate receptors occurs in carcinogenesis. Interestingly, overexpression of both proteins leads to increased tumor cell proliferation and migration in metastatic cancers in tissues including brain and kidney.

To investigate the molecular relationship between Narp and Reelin we decided to focus on the CNS where much is known about the regulation of these proteins. Our study sought to determine if Reelin contributes to the activity-dependent regulation of synapse proteins, specifically Narp. Cultured primary neurons of conditional Reelin knockout mice were treated with a panel of drugs that alter neuronal activity. Using immunocytochemical detection methods, we found that the presence of Reelin induced an increase in Narp surface levels. This suggests that Reelin participates in activity-dependent regulation of Narp to rebalance excitatory and inhibitory network tone. Determining the mechanism of this interaction in neurons may aid in understanding how glutamate receptors become dysregulated to promote tumor cell migration and invasion.

**WHOLE GENOME SEQUENCING AND MINIGENE FUNCTIONAL ANALYSES OF SPLICING VARIANTS IN RECESSIVE HEREDITARY SPHEROCYTOSIS**

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Hereditary spherocytosis is an inherited hemolytic anemia affecting ~1:2000 individuals of Northern-European ancestry, making it the most common cause of inherited anemia in this population. Inheritance is autosomal dominant in ~two-third of cases with the remaining cases representing autosomal recessive inheritance. Clinically, patients with recessively inherited HS (rHS) are more severely affected. Erythrocytes from most rHS patients are spectrin deficient and this deficiency is thought to play a pivotal role in the destabilization of the lipid bilayer. Previously, using whole exome sequencing of the SPTA1 locus, we identified numerous deleterious alleles in a cohort of 19 patients. However, the causative variant was not identified in 7 patients. Two of these patients, heterozygous for damaging alpha-spectrin mutations, were from well characterized rHS, alpha-spectrin linked kindreds. We subjected these two patients to whole genome sequencing using the Illumina genome sequencing (WGS) platform. Analyses of WGS data at the SPTA1 locus revealed a shared non-coding variant (C>T) on intron 30, previously identified in literature as LEPRA. This mutation is thought to produce six times less correctly-spliced alpha-spectrin transcript than the wild type allele, but detailed functional studies are lacking. To investigate the specific splicing patterns of the LEPRA variant, we are performing functional minigene assays. These assays will allow us to quantify wild type and mutant LEPRA mRNA transcripts with RT-PCR in transfected cells. Together, these studies will provide insight into the influence of the spectrin LEPRA variant on erythrocyte membrane structure and function in rHS.

**EVALUATION OF *ARTEMIA SALINA* AS A NON-MAMMALIAN MODEL FOR THE STUDY OF DIABETIC RENAL DISEASE.**

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Diabetic nephropathy is associated with the augmentation of collagen IV deposition in the basement membrane of mesangial tissues of the kidney. Recent studies suggest that altered epigenetic mechanisms and potential posttranslational modifications of histones by the glycolysis-derived alpha-oxoaldehyde, methylglyoxal (MG) may contribute to dysregulation of normal gene expression leading to the observed alterations in collagen IV deposition. We hypothesized that exposure to elevated glucose levels in a simple model organism *Artemia salina* would reflect similar epigenetic and post-translational alterations as well as elevated levels of collagen IV protein. To explore this possibility, we exposed *Artemia salina* to 30 mM glucose for 24 hours and compared the protein levels of collagen IV in treated and untreated samples using Western blot analysis. We also determined the expression levels of peroxidasin as this enzyme is essential to form suflimine bonds between the collagen IV fibers in the basement membrane. Our results show that the collagen IV and peroxidasin levels increase in Artemia exposed to 30 mM glucose for 24 hours, suggesting that elevated glucose impact the synthesis of these basement membrane components. Our findings demonstrate that Artemia is a simple animal model for investigating the fundamental mechanisms that can be perturbed, contributing to the pathogenesis of human diabetic nephropathy.

**A NOVEL DOPAMINE TRANSPORTER (DAT) VARIANT (ΔN336) ASSOCIATED WITH AUTISM ABLATES DOPAMINE TRANSPORT FUNCTION**

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Autism spectrum disorder (ASD) is a neuropsychiatric condition affecting approximately 1% of the population and is characterized by a spectrum of impairments in social interactions and communication, and patterns of rigid-compulsive behaviors. ASD risk is predominantly determined by genetic factors; however, this etiological architecture is highly complex. Rare, functional genetic variation is accepted as a significant contributor to susceptibility, and multiple alleles within a particular gene can harbor coding variants predisposing an individual to risk.

Our recent discoveries on the functional consequences of a *de novo* missense mutation (T356M) within the dopamine (DA) transporter (DAT) in an ASD proband have strongly implicated disruption of DA transport as a potential biological risk factor in ASD. Here we report a novel, DAT variant, also identified in a proband from whole exome sequencing. It is an in-frame deletion of three nucleotides resulting in a deletion of amino acid N336. Located in the third intracellular loop, N336 is conserved from human to *Drosophila*, and *in silico* algorithms predict a functionally damaging effect. Expression of ΔN336 DAT in Chinese hamster ovary cells revealed an absence of DAT-dependent DA uptake relative to wildtype DAT, yet surface expression was not affected.

These results, with prior association of abnormal DAT function with attention deficit-hyperactivity disorder (ADHD), may inform the link between ASD and ADHD, which co-occurs in ~40% of people with ASD. Most importantly, this work adds to the growing body of literature implicating altered regulation of DA homeostasis/transport as a potential biological mechanism underlying liability to ASD.

**LYSYL OXIDASE HOMOLOG EXTRACELLULAR PROCESSING**

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Glomerulosclerosis, the final common pathway for all glomerular diseases including diabetic nephropathy, is characterized by an accumulation of excessive amounts of cross-linked collagen IV and other extracellular matrix (ECM) proteins in the glomerulus leading to a sustained and progressive decrease of kidney function. Our previous research suggests that a lysyl oxidase homolog (LOH) promotes crosslinking of collagen IV. Although LOH function is important for maintaining ECM integrity, its deregulation may lead to excessive crosslinking and fibrosis. LOH is found as a full-length form (~100kDa) and processed form (~65 kDa), indicating that the enzyme undergoes proteolytic processing that may be important for LOH-mediated crosslinking of collagen IV. Because the enzyme responsible for processing the homolog has not been identified, the purpose of this study was to test an in-silico predicted subtilisin-like cleavage site that may drive LOH processing. Using site directed mutagenesis PCR, we introduced specific mutations on the predicted subtilisine-like cleavage site designed to modulate processing of the LOH. Vectors containing the mutated cDNAs were transfected into a human cell culture model to express the proteins. Immunoblotting detection of LOH in the cell culture media demonstrated that the predicted cleavage site is responsible for the generation of the ~65 kDa form. However, further work is required to characterize the role of proteolytic processing in LOH activation and collagen IV crosslinking.

**DOXORUBICIN INDUCES GLOMERULAR ENDOTHELIAL INJURY FOLLOWED BY EFFACEMENT OF GLOMERULAR PODOCYTES AND PROTEINURIA**

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Doxorubicin (Dox, commonly known as Adriamycin) is used to treat a wide variety of cancers. The effects of Dox-induced cardiac and renal toxicity have previously been studied, although the mechanisms of Dox's effects on the kidney are not as well known. Dox-induced proteinuria is thought to be associated with glomerular damage, but whether Dox first causes podocyte or endothelial injury is still up for debate. Klotho is a renoprotective protein shown in ischemia and cisplatin induced kidney injury models, although whether it protects glomeruli remains to be confirmed. Dox can affect autophagic activity of cells by disrupting the balance of normal autophagy necessary for healthy tissue and organ function, thus inducing cardiotoxicity. Autophagy's role in Dox-induced proteinuria is not clear. The goal of this project was to determine whether Dox upregulates or downregulates autophagy and whether Klotho can protect the kidney against Dox nephrotoxicity.

*In vivo* and *in vitro* models were used to conduct our studies. *In vivo* models were studied by measuring Dox-injected mouse kidney function based on histology, western blots, IHC staining, and urinalysis. *In vitro* models were studied with LDH assays and western blots using MSI and HEK 293 cell lines.

Our studies show that autophagy flux seems to be upregulated by Dox and that autophagic damage in the endothelium of the glomeruli precedes damage to the podocytes or tubules. Additionally, proteinuria is reduced in Klotho-transgenic mice in comparison to wild-type and Klotho deficient mice, suggesting that Klotho may reduce initial endothelial damage caused by Dox-induced autophagy.

**INHIBITION OF HEDGEHOG (Hh) SIGNALING USING HEDGEHOG PATHWAY INHBITOR-4 (HPI-4) REDUCES SOFT TISSUE INVASION**

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Oral cancer is the 8th most common cancer in the U.S. Tumor cell invasion into the lymph nodes and mandible results in poor prognoses and a five year overall survival of 20-50%. Oral tumors demonstrate an upregulation of Hh signaling components, both in human cell lines and patient samples. The purpose of this study was to determine if using HPI-4 to prevent ciliogenesis will reduce invasion of the oral cancer cell line CAL27 due to loss of Hh signaling.

Viability assays were used to measure the metabolic activity of CAL27 cells. Cell counts were performed to determine proliferation differences over six days. Transwells were used to measure cell motility and invasion assays, using Matrigel to model soft tissue were used to measure cell invasion. Quantitative PCR (qPCR) was used to detect gene expression changes. Immunofluorescence was used to detect primary cilium.

CAL27 cells treated with HPI-4 had lower viability five days post-treatment. Additionally, HPI-4 caused an initial decrease in cell proliferation. While there was no significant difference in cell motility, HPI-4 prevented cell invasion through Matrigel. qPCR confirmed lower levels of Hh target genes, while the immunofluorescence staining confirmed lower levels of primary cilia in HPI-4 treated cells.

Collectively, primary cilium inhibition decreased Hedgehog signaling as well as cell viability and proliferation. Also, HPI-4 treated cells show a dramatic loss of cell invasion, supporting the potential of HPI-4 as a targeted therapeutic. We anticipate that if HPI-4 can reduce soft tissue invasion clinically, improved prognoses in patients should be expected.

**NEPHRONECTIN MAY CO-OPERATE WITH α8-INTEGRIN TO REGULATE THE DEVELOPMENT OF THE GLOMERULUS**

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Nephronectin is an extracellular matrix protein that plays a critical role in adhesion during kidney development; it is a ligand for several integrins, as shown by in vitro testing. Nephronectin is expressed by podocytes and localizes to the glomerular basement membrane. In preliminary data, we have shown nephronectin co-localizes with α8-integrin, which is produced in the glomerulus by mesangial cells. Our ongoing studies have shown a conditional deletion of nephronectin leads to mesangial expansion. The goal of this investigation is to identify nephronectin ligands within the glomerulus. We hypothesize that nephronectin interacts with α8-integrin and tethers the capillary loops, preventing mesangial expansion and excessive matrix deposition. The experimental plan is to use a CRISPR/Cas9 to generate mesangial cell knockouts for several α-integrins. We will use these knockouts to test mesangial cell adhesion to nephronectin, along with mesangial cell proliferation and matrix deposition. In this poster, we present our methodology for generating the knockout mesangial cell lines.

**STABILIZATION OF BETA-CATENIN IN RENAL STROMA MAY LEAD TO WILMS' TUMOR PATHOGENESIS**

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Recent studies have shown that opposing signals from the ureteric bud and the stroma play a major role to balance nephron progenitor cell renewal and differentiation. Accumulation of undifferentiated nephron progenitor cells makes them susceptible to genetic mutations leading to the formation of tumors. Wilms' Tumor is one such pathological condition. 12-16% of Wilms' tumors have a gain of function mutation in the gene encoding transcription factor beta-catenin, downstream effector of Wnt signaling. We hypothesized that activation of beta-catenin in the stromal cells contributed to Wilms' tumor pathogenesis. To test this hypothesis we stabilized beta-catenin only within the stromal cells of the kidney. The phenotype displayed a surprising similarity to the tumor pathology of Wilms' tumors. A comparison of the mRNA profiles of wild type and beta-catenin mutant kidneys identified secreted factors that could be acting non-autonomously on the nephron progenitor cells. Extensive localization analysis using in-situ hybridization led us to compartmentalize our results into three distinctive categories: genes that were significantly upregulated in the nephron progenitor cells, the stroma, and the vasculature. We hypothesize that the altered expression and misregulation of these genes in the mutant kidneys promotes nephron progenitor renewal at the cost of differentiation. Further research will determine whether misexpression of any of these candidates is sufficient to cause a Wilms' tumor like phenotype in embryonic mouse kidneys. This will bestow insight into which genes play causal roles in Wilms' tumor pathogenesis, and hopefully allow for targeted gene therapy for Wilms' tumor patients in the near future.

**SALT-SENSITIVE HYPERTENSION AND GLOMERULAR ATROPHY IN MICE WITH A GENETIC DEFECT IN MITOCHONDRIAL CALCIUM UPTAKE.**

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Aside from their role in ATP production, mitochondria play other important roles in the cell including intracellular calcium sequestration. Patients with a genetic defect in mitochondrial calcium uptake present with a hypertensive phenotype, though no mechanism has been hypothesized. The renal epithelial channel (ENaC) is expressed in the distal nephron and functions as a regulated step in salt and water homeostasis and therefore blood volume and pressure. We hypothesize that mitochondrial calcium uptake is important in ENaC regulation and that elimination of this pathway influences the body's ability to regulate blood pressure. Using several different microscopy techniques, we found that mitochondria in cells in which ENaC are concentrated in regions near the apical and basal membranes. This localization may reflect the mitochondria's ability to sequester calcium from certain regions of the cell and create calcium signaling microdomains. We used electrophysiology to show that ENaC is regulated by intracellular calcium in a polarized fashion. We also assessed the phenotype of mice who lack the ability to take up calcium into their mitochondria. We used two different strains of mice and found one to be hypertensive at baseline and the other to be salt-sensitive hypertensive. ENaC can be regulated by membrane abundance, transcription, or probability of opening. The salt-sensitive hypertensive mice showed a decrease in percent of ENaC at the membrane and an interesting phenotype of glomerular atrophy. The phenotype of these mice and ENaC transcription and probability of opening will be investigated in the near future.

**HIGHER MORTALITY RATE AND CARDIAC MYOCYTE NECROSIS IN SMAD3 DEFICIENT MICE IN 2K1C HYPERTENSION**

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Smad3 is a potential therapeutic target for renal artery stenosis due to its protective effect on the kidneys from atrophy and interstitial fibrosis in 2K1C (2-kidney, 1-clip) hypertension model. The goal of this study is to investigate whether there is a difference in mortality rate and heart phenotype between Smad3 deficient mice (KO) and 129S wild type (WT) mice in 2K1C hypertension.

2K1C hypertension model was used to induce renal artery stenosis (RAS). 151 WT RAS mice, 45 WT shams, 146 KO RAS mice, and 40 KO shams were studied. Mortality rates, heart weights, blood pressure, and plasma renin were compared at 4, 7, 14, and 42 days post-surgery. Fibrosis, necrosis, and macrophage infiltration of the heart were analyzed from histology stains. Inflammation, TGF-B, and hypertension arrays and gene expressions were performed to identify potential causes of differences in cardiac phenotype and mortality rate.

36 deaths were found in 146 KO RAS while no deaths were found in 151 WT RAS. 24.7% of the KO RAS developed necrotic hearts compared to only 6.6% in WT RAS. KO RAS hearts were significantly ($p < 0.05$) heavier than WT RAS hearts at all time-points and had significantly more fibrosis ($p < 0.05$) than WT RAS at 4 days. KO RAS also found to have more M2 macrophage infiltration than WT RAS at 4 days.

Higher mortality in KO RAS could be correlated to greater cardiac hypertrophy, necrosis, fibrosis and M2 macrophage infiltration at early time points.

*R25-DK101405*

## *PKD1A/1B* DOUBLE MUTANT WORSENS THE PHENOTYPE OF *PKD1A* MUTANT IN A ZEBRAFISH MODEL

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Polycystic kidney disease (PKD), one of the most common heritable human diseases, is categorized into Autosomal Dominant PKD (ADPKD), Autosomal Recessive PKD (ARPKD), and other syndromic forms such as Meckel-Gruber syndrome. Mutations in PKD1 account for 85% of ADPKD. We leveraged the Transcription Activator-Like Effects Nucleases (TALENs) technology in zebrafish to generate stable mutants for PKD1 homologues. Because zebrafish contains two PKD1 homologues, *pkd1a* and *pkd1b*, TALENs pairs targeting both homologues were made. Exons corresponding to exon 17 of the Pkd1 gene of the mouse, whose mutations has been proven to result in PKD pathogenesis, were targeted. Previous studies have shown that the phenotypes in *pkd1a*$^{-/-}$ mutant fish was embryonic lethal, which manifests kidney cyst on day 3, and developed edema on day 5, while the *pkd1b*$^{-/-}$ mutant didn't show any phenotypes. However, the phenotype of *pkd1a*$^{-/-}$*; pkd1b*$^{-/-}$ double mutant is still unknown.

Here, we generated *pkd1a*$^{-/-}$*; pkd1b*$^{-/-}$ double mutant fish. We found the phenotype of this double homo fish is similar as *pkd1a*$^{-/-}$ mutant only but showed edema earlier. We continued to test the kidney cyst on day 2, one day earlier than the test on *pkd1a*$^{-/-}$ mutant only. *pkd1a*$^{-/-}$*; pkd1b*$^{-/-}$ double mutants showed more kidney cysts than *pkd1a*$^{-/-}$ mutant only, although *pkd1b*$^{-/-}$ mutant only didn't show any kidney cyst.

In summary, our data demonstrated that the TALENs technology enables the generation of gene-based zebrafish models for human diseases. These models can be used for further pathway analysis and future studies to develop novel therapeutics.

*P30-DK090728*

**PREGNANCY ACCELERATES PROGRESSION OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE IN THE *MUS MUSCULUS* LABORATORY MODEL**

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Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder characterized by the accumulation of kidney cysts that eventually results in renal failure. Current research suggests that the deterioration of renal function occurs through a pregnancy-associated plasma protein A (PAPP-A) metabolic pathway. PAPP-A is a secreted metalloproteinase which cleaves insulin-like growth factor binding proteins (IGFBPs) and is involved in proliferative processes such as bone remodeling and wound healing. It is also used as a biochemical marker during pregnancies to identify aneuploid fetuses. Preliminary data indicates that naturally higher levels of circulating PAPP-A during pregnancy may aggravate pre-existing ADPKD and engender more cyst proliferation and fibrosis in the kidneys. Previous studies indicate that the frequency of pregnancy-unrelated hypertension and the frequency of preeclampsia and eclampsia were higher in women with polycystic kidney disease. However there is no evidence that pregnancy had an adverse effect on the natural course of PKD. We show that kidneys harvested from multiparous mouse model of ADPKD (*Pkd1*$^{RC/RC}$) were visibly enlarged, discolored and severely fibrotic and yielded higher cyst index values than recorded in nulliparous specimens. Additionally, relative mRNA levels measured through real time PCR confirmed that kidney injury, inflammatory and fibrotic markers and PAPP-A levels are higher in multiparous *Pkd*1$^{RC/RC}$ mice when compared to those of nulliparous PKD and control C57/BL6 mice. While preliminary data strongly indicate a significant correlation between pregnancy and the accelerated progression of cystic disease in ADPKD mice, more data must be collected to establish a steadfast correlation between pregnancy and ADPKD.

*P30-DK090728*

**PURIFICATION AND REFOLDING OF TREM2 FOR STRUCTURAL STUDIES**

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The **T**riggering **R**eceptor **E**xpressed on **M**yeloid Cells 2 (TREM2) protein is a 230 residue single span membrane protein that is a key immunoreceptor of Microglial cells in the central nervous system (CNS). Microglia cells are the major neuroimmune cells of the CNS and are responsible for the recognition and clearance of foreign entities as well as self molecular assemblies. TREM2, a cell surface receptor on microglial cells recognizes potentially neurotoxic entities and assemblies promoting phagocytosis and degradation of these neurotoxins. Variants of the protein TREM2 have been shown to increase the risk of Alzheimer's disease. The most common of these variants rs75932628 which results in an R47H substitution, in the extracellular immunoglobulin (IG) like domain. In this study we hoped to purify and refold TREM2 *in vitro* to perform structural studies using NMR spectroscopy. TREM2 was expressed in bacterial *E.coli* BL21 cells and isolated as aggregates from inclusion bodies. From there, a denaturant was added to solubilize the inclusion bodies. Refolding was preformed via rapid dilution into a refolding buffer. After this we hoped to elute the refolded protein into bicelles for structural studies using NMR, however we were not successful in obtaining refolded TREM2 as visible by gel electrophoresis of the precipitate found in refolding experiment. Using the information we gathered from refolding experiments we are now developing a protocol that will yield refolded and pure TREM2 for structural studies.

**ENDOTHELIAL CELL DEPENDENT INFLAMMATION IN ACUTE KIDNEY INJURY.**

David A. Heron and David Briscoe, MD.

Acute Kidney Injury (AKI) is the term used to describe the rapid decrease in glomerular filtration rate (GFR) occurring within hours to days, resulting in inability of the kidney to filter blood and electrolytes, regulate blood pressure, and produce urine. Sometimes AKI resolves on its own but generally it leads to some degree of chronic renal injury. During AKI, peritubular endothelial cells (ECs) are injured which is well established to be a critical factor in the evolution of chronic kidney disease. Injured ECs become activated, undergo apoptosis and are sloughed off leaving bare basement membrane and an absence of local blood flow. Thus, repair of the ECs must occur in order for the nephrons of the kidney to recover fully from an injury/insult. Microvasculature repair may originate from the local ECs or from the circulating endothelial progenitor cells (EPCs). Circulating EPCs replace existing ECs along the now exposed basement membrane. Once ECs are injured or replaced, several intracellular kinases support their survival and proliferation. Among these, the Protein Kinase B (AKT)/mammalian Target of Rapamycin (mTOR) kinase pathway is critical because this pathway is responsible for angiogenesis, cell growth, survival, and metabolism. Published data from the Sabatini Lab indicates that DEP domain containing mTOR interacting protein (DEPTOR) is a negative regulator of AKT/mTOR in ECs. It would be interesting to look at DEPTOR expression during injury because it may affect phosphorylation of downstream pro-survival kinases. To assess this, mouse ECs were isolated, cultured *in vitro*, subjected to *in vitro* tests for mTOR kinase activation, and protein expression was verified through Western blot. My studies verified the expression and regulation of DEPTOR in EC.

**RENAL-ASSOCIATED PHENOTYPES CAUSED BY NBCE1 (SLC4A4) -KNOCKOUT**

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Human mutations in the $Na^+$ bicarbonate cotransporter NBCe1 (Slc4a4) cause proximal renal tubular acidosis (pRTA), cataracts and glaucoma. Loss of bone mineral density can result from metabolic acidosis. We reported that whole-gene *NBCe1* knockouts (KO) and A-isoform knockouts (*NBCe1A*, $KO^A$) are acidotic [EB-2015]. This work sought to characterize renal-associated phenotypes of these NBCe1knockout mice and to develop a novel method to assess renal function.

WT, HET and KO mice were generated by crossing HET mice. Kidneys and eyes were fixed, embedded and sectioned for histology. Bone mineral density and content were measured using a Lunar PIXImus 2. Ocular fluorescence was used to measure the fluorescent decay in ophthalmic vessels.

KO mice are acidotic (7.08±0.004), have low blood [$HCO_3^-$], and have lower bone mineral density and bone mineral content than their HET and WT counterparts (BMD – KO: 0.0343±0.0056). Aged HET and WT mice appear to have similar bone mineral density and bone mineral content (BMD – WT: 0.1222±0.0255; HET: 0.1079±0.0183). With renal histology, KOs show cortical cysts. FITC-Sinistrin can be detected, and its decay seems to track GFR.

KO mice have lower bone mineral density and content illustrating that pRTA causes bone density loss. Why nbce1-KO's have cortical cysts which worsen with $HCO_3^-$ treatment is unexpected and unclear. Ophthalmic fluorescent tracking may be a convenient and direct method to assess GFR in mice. These KO mice and their renal assessments should enable a better understanding of NBCe1's role in renal and systemic disorders.

*R25-DK101405*

**EVALUATING *ARTEMIA SALINA* AS A NON-MAMMALIAN ANIMAL MODEL FOR THE STUDY OF THE INNATE IMMUNE SYSTEM.**

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Stimulator of INF genes (STING), functions as an innate immune response protein by detecting foreign DNA in an organism. Activation of STING signals downstream adaptive immune responses that promote immunological mechanisms in the organism. Innate and adaptive immune responses have been studied in *Artemia* because of their importance to aquatic industry. *Artemia* are susceptible to infection by two strains of the bacteria, *Vibrio*, and it has been shown that non-lethal heat shock prior to *Vibrio* exposure can protect *Artemia franciscana* from infection. These studies suggested that Artemia can be used to study other innate immune responses. We hypothesized that the innate immune surveillance system protein STING would be elevated in response to non-lethal heat shock. To this end, we cultured *Artemia salina* for 24 hours and subsequently subjected cultures to heat shock for thirty minutes and allowed recovery for six hours. Non-lethal heat shock is accompanied by the elevation of a variety of Heat Shock Proteins (HSP) including HSP70 in *Artemia*. HSP are known to be active participants in innate immune responses. Utilizing Western blot analysis we determined if heat shock altered the expression levels of STING and HSP70 in *Artemia*. We found alterations in both HSP70 and STING and conclude that the innate immune response protein STING is present and active in *Artemia salina,* and responsive to heat shock in these organisms. Our studies demonstrate that *Artemia salina* can be used as an animal model to study immune surveillance by STING.

**DIRECTED DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO FUNCTIONAL HUMAN PODOCYTES**

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Currently, podocytes for research are obtained through glomeruli isolation which is cumbersome and invasive. Therefore, differentiation of pluripotent stem cells into podocytes would address the growing demand for podocytes. The Bonventre lab recently developed a chemically-defined protocol to differentiate induced pluripotent stem cells (iPSCs) into nephrons. In this study, we improved efficiency of the protocol and induced functional podocytes.

Previously, CHIR treatment of human dermal fibroblast iPSCs, a necessary step in the protocol, was shown to induce bone morphogenetic protein 4 (BMP4) production which inhibits differentiation into the posterior intermediate mesoderm. We tested BMP4 inhibitors, noggin and dorsomorphin to suppress excessive BMP4 signaling. We obtained SIX2+ nephron progenitor cells (NPCs) at efficiencies of 97.12% and 62.90%% respectively.

From NPCs, we induced nephron-like structures expressing markers for the glomerulus (Podocalyxin, Nephrin), proximal tubule (LTL, N-cadherin), and distal tubule (E-cadherin). We co-cultured NPCs with human umbilical vein endothelial cells (HUVECs) starting from day 9 and day 16 of differentiation, assuming formation of glomerular basement membrane with endothelial cells facilitates maturation of podocytes. Endomucin staining showed successful incorporation of HUVECs when co-cultured from day 16. Flow analysis showed that 15.37% of cells were positive for Podocalyxin.

In our work, we obtained efficient NPC induction from iPSCs treated with BMP4 inhibitors. Subsequently, the NPCs were differentiated into structures with all three segments of the nephron. We also successfully incorporated endothelial cells which may be beneficial for the maturity of podocytes. Lastly, flow analysis confirmed that our method is viable for obtaining podocytes.

**CO-REGISTRATION OF REAL-TIME 2D ULTRASOUND AND PREOPERATIVE 3D-CT SCAN FOR MORE ACCURATE IMAGE GUIDANCE DURING LAPAROSCOPIC PROCEDURES**

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Laparoscopic surgery presents various challenges such as restricted view inside the body cavity. Therefore, accurate image guidance is necessary to facilitate such procedures. Ultrasound provides real time images of organs, but limitations include attenuation shadowing and low image resolution. CT scanning on the other hand provides high resolution images; however, it cannot be performed in real time. Hence, the purpose of this study was to combine pre-operative CT and real-time ultrasound imaging modalities to provide better visualizations of the internal anatomy during image-guided procedures, with specific reference to nephrectomy and Kidney stone removal. Therefore, an anthropomorphic phantom was created to model a diseased kidney. To register the ultrasound and CT images, a 3D ultrasound volume was first generated from the 2D ultrasound images that were acquired. This involved calibration of the ultrasound images to a real world coordinate system through a series of rigid transformations computed using the linear least squares method. This was done using a Z string phantom, MATLAB and the Aurora Magnetic tracker. The 3D ultrasound and 3D CT volumes were then registered using the Analyze software. It was demonstrated that the calibration from frame space (Z string phantom) to global space (magnetic tracker) was accurate to within 0.5mm. Thus, combining CT and ultrasound has the potential for offering better visualization of the internal anatomy during image-guided procedures.
*R25-DK101405*

**ROLE OF EPSIN IN PODOCOYTE BIOLOGY**

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The purpose of this study is to elucidate the role of epsin in podocytes, or the cells that line the glomerular filtration barrier. Because global *Epn1*, *2*, and *3* triple knockout (KO) mice are embryonic lethal, and *Epn2* and *Epn3* double KO mice have no obvious phenotype, a *Podocin Cre Epn1*$^{fl/fl}$; *Epn2*$^{-/-}$; *Epn3*$^{-/-}$ mouse (herein referred to as Epsin KO) was generated in this study. This mouse was viable and born in the normal Mendelian frequency, but was devoid of an overt phenotype. However, when the Epsin KO mouse and littermate controls were injured with lipopolysaccharide (LPS) or nephrotoxic serum (NTS), the Epsin KO mouse had more severe glomerular damage than the littermate controls, indicated by greater foot process effacement and urine albumin/creatinine ratio (45.03 (ug/mg), 67.78, 362.78, 1331.93, 251.12, and 405.56 in Wild type (WT), Epsin KO, WT+LPS, Epsin KO+LPS, WT+NTS, and Epsin KO+NTS, respectively).

We next studied the interaction of epsin with clathrin and actin in isolated control podocytes. Live cell imaging revealed epsin at endocytic pits with clathrin and arrival of actin prior to epsin internalization, showing that epsin is involved in clathrin-mediated endocytosis in podocytes. However, there was no overt difference in the rate of clathrin-coated pit turnover between Epsin KO podocytes and control podocytes.

These findings suggest that epsin is dispensable for normal podocyte development, but may play an important role in sustaining podocyte function following glomerular injury.

**THE EXPRESSION OF COLLAGEN IV AND LAMININ V IN MAMMARY EPITHELIUM**

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Breast cancer is the second leading cause of death of women in the U.S., with HER2 positive breast cancer being one of the most aggressive forms of breast cancer. The HER2 oncogene is an Epidermal Growth Factor Receptor, which is normally overexpressed in patients diagnosed with breast cancer. In these experiments, the expression of Collagen IV and Laminin V were evaluated in MCF-10A (normal) and MCF-10A-HER2 (oncogenic) cells. The cells were grown in a three-dimensional organoid culture using Growth Factor Reduced Matrigel, allowing us to recapitulate the growth of normal mammary epithelial cells and oncogenic mammary epithelial cells in vitro. The expression of Collagen IV in cells overexpressing HER2 was significantly less than the expression of Collagen IV in the MCF-10A cells. The expression of Laminin V was significantly greater in the MCF-10A HER2 cells compared to the MCF-10A cells. This data suggests that the presence of the HER2 oncogene transforms the deposition of Collagen IV and Laminin V in mammary epithelium. Patient data from The Cancer Genome Atlas was also analyzed for RNA Expression of the genes associated with Collagen IV and Laminin V.

**ISOLATION AND GENERATION OF KIDNEY SPECIFIC EBF1 KO CELLS BY FLUORESCENT ACTIVATED CELL SORTING**

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Early B cell factor 1 (Ebf1) is a transcription factor that is expressed during late kidney organogenesis and is crucial for proper morphology and function. In its absence, glomerular maturation and glomerular filtration rate are reduced, vascularization is abnormal, and podocytes are severely effaced. Ebf1 is expressed in select tubular segments, glomerular mesangium, interstitial pericytes, and podocytes. Interestingly, ablation of Ebf1 only in the glomerular mesangium and interstitial pericytes, utilizing the Foxd1-specific cre, recapitulates the majority of the phenotypic effects observed in the global knockout mouse. To specifically enrich for mesangial and interstitial pericytes, we have crossed the mT/mG-reporter cre mouse with our Foxd1-cre Ebf1 KO mouse allowing for isolation of cells expressing green fluorescent protein (GFP). The goal of this project was to develop a protocol to easily identify kidney specific cell populations lacking Ebf1 that co-express GFP. Kidneys were digested with various concentrations of collagenase/dispase and trypsin solutions at both 37$^{o}$C and room temperature. Our results indicate that a solution of 1µg/mL of collagenase and dispase along with 0.025% trypsin incubated at 37$^{0}$C for 30 minutes completely digests the kidney and allows for successful fluorescence activated cell sorting (FACS) which separates specific populations of cells. Utilizing this technique to enrich in a cell specific manner will enable us to determine Ebf1 interaction and regulatory partners by high-throughput sequencing and Chromatin Immunoprecipitation (ChIP) assays, thus unlocking clues in identifying Ebf1's role during kidney development and maintenance.

**EVALUATING TEMPERATURE DEPENDENT RENAL VISCOELASTIC PROPERTIES**

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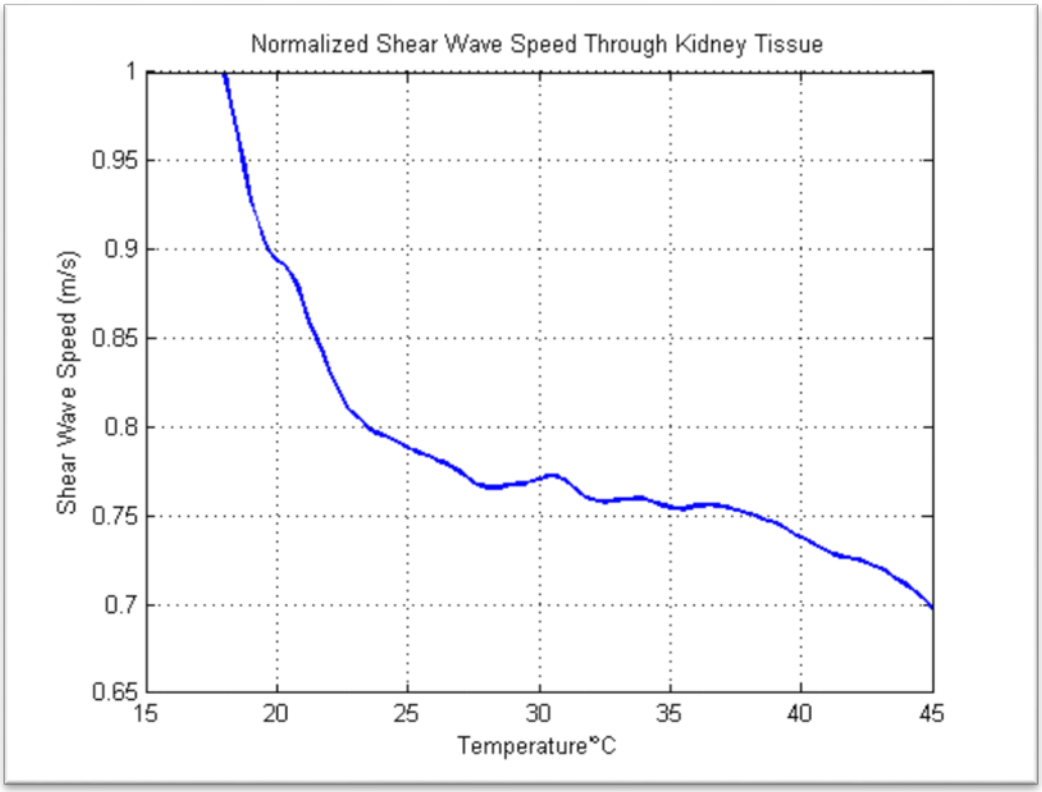
This study intended to utilize ultrasound techniques in order to characterize the viscoelastic properties of renal tissue as it relates to temperature variation. We used a method called shear wave elastography (SWE), which uses focused ultrasound to induce propagating shear waves in soft tissue. We measured the speed and attenuation of the shear waves at various frequencies, which are related to the viscoelastic properties.

In the experiments, *ex vivo* porcine kidneys acquired from euthanized pigs were used for the SWE measurements. The kidneys were submerged in a saline solution and shear waves were induced and measured with ultrasound. The temperature in the kidney was taken at two different locations within the sample as the solution was heated from room temperature (20 °C) to 45 °C, with measurements being taken every 2 minutes.

Analysis of the shear waves indicated an inverse relationship between temperature and shear wave speed in the tissue over a temperature range of 20 to 45 °C. In addition, the shear wave attenuation was observed to be directly proportional to frequency changes, over a range of 100 to 200 Hz. Shear wave speed and attenuation can then be used to calculate renal stiffness and viscosity. This relationship is fundamental to the understanding of how SWE measurements may contrast between room and body temperature, which will help improve translational application of the technique from the laboratory to the patient in the clinic.

*R25-DK101405*



**DF508 CFTR AND ENDOPLASMIC RETICULUM STRESS**

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When misfolded proteins accumulate in the Endoplasmic Reticulum (ER), and the amount of misfolded protein overpowers the degradation machinery, a state of Endoplasmic Reticulum Stress is induced. This ER Stress elicits the Unfolded Protein Response (UPR), which is characterized by an elevated production of ER-Stress Markers such as BiP and CHOP. The very prevalent dF508 mutation of CFTR is a class II mutation, meaning that it causes CFTR protein to not be properly exported from the Endoplasmic Reticulum due to folding issues; therefore, because of the protein misfolding issues, we hypothesized that the dF508 CFTR mutation will induce ER Stress. Understanding how this mutation affects the body can provide insight into novel treatment methods for Cystic Fibrosis patients containing this dF508 mutation on their CFTR gene.

In order to test this hypothesis, HeLa cells were injected with a plasmid vector that contained a tetracycline-dependent promoter as well as either WT or dF508 CFTR, so when tetracycline was added, the CFTR would start to be produced, proposedly inducing ER-stress. We used Western Blotting to determine relative protein levels of ER-Stress markers after the induction of CFTR as well as qRT-PCR in order to determine relative mRNA transcript levels for the same ER-Stress markers. Our data suggests that the sxBP1 ER-Stress pathway is activated when mutant CFTR is overproduced, whereas the BiP and CHOP pathways are not. The sxBP1 pathway works to correct the protein misfolding issue, so this suggests that in cells containing dF508 mutant CFTR, the main remedial method is to rescue the cell by improving CFTR folding instead of sending it straight to apoptosis.

## ROLE OF BRAIN DERIVED NEUROTROPHIC FACTOR AND TYROSINE RECEPTOR KINASE B IN THE PATHOGENESIS OF PREECLAMPSIA

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Preeclampsia (PE) is a major hypertensive disorder of pregnancy that can lead to maternal and fetal illness or death. PE may also progress to eclampsia with a sudden onset of seizures and other neurological complications. To identify novel pathways that mediate the neurological complications of eclampsia/preeclampsia, we characterized a novel tyrosine receptor kinase B (trkB) that was found to be elevated in a placental mRNA microarray studies. TrkB is a high affinity receptor to brain derived neurotrophic factor (BDNF), and upon binding, activates downstream signaling cascades related to neuronal differentiation and survival, and synaptic plasticity. Some studies suggest that the trkB/BDNF signaling system plays a role in the feto-placental unit development as a nutrient sensor and angiogenesis via upregulation of vascular endothelial growth factor A. We confirmed by quantitative PCR studies that placental expression of trkb was markedly elevated in patients with preeclampsia compared to non-hypertensive controls (RQ for trkB was 11.14 +/-11.17 in preeclampsia (n=7) versus 1.22 +/- 1.18). TrkB mRNA levels in the placenta correlated modestly with sFlt1 (a previously characterized target) expression (r=0.2). We then confirmed that plasma BDNF levels were also elevated in preeclampsia (plasma levels of BDNF were 8.73 +/-4.47 ng/mL in preeclampsia (N=15) versus 6.98 +/-4.16ng/mL)in controls n=12). This pilot study suggests that aberrant expression of trkB and BDNF may contribute to the pathogenesis of preeclampsia. Further studies with larger sample sizes and in vivo functional studies to evaluate the role of trkB/BDNF signaling axis in preeclampsia are in progress.

**RESCUE OF MISSENSE MUTATIONS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE SHOWS IMPLICATIONS FOR PERSONALIZED MEDICINE**

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Autosomal Dominant Polycystic Kidney Disease (ADPKD), a late-onset genetic disease resulting in end stage renal disease, is caused by mutations to *PKD1* (85% of cases) or *PKD2* (15% of cases). Polycystin-2 (PC2, encoded by *PKD2*) complexes with Polycystin-1 (PC1, encoded by *PKD1*) and assists trafficking of PC1 to the primary cilia and plasma membrane, where it has a mechanosensory function. Mutation to *PKD2* can result in misfolded PC2 proteins, leading to reduced surface localization of PC1. This study analyzes the effect of 16 missense *PKD2* mutations on surface localization of PC1. Plasmids were mutagenized to mimic these possibly pathogenic PKD2 variants, then electroporated into *PKD2*$^{-/-}$ human renal epithelial cells and incubated at 37 or 30°C. PC1 surface localization was detected by flow cytometry and fluorescence microscopy. Six variants (37.5%) resembled the wild-type at both temperatures, so we concluded they were not pathogenic due to a trafficking defect. The level of surface PC1 was at a low level for the other 10 variants (62.5%), but it increased to the wild-type level in six (60% of trafficking mutants) when incubated at the lower temperature. We concluded that the mutants with low surface localization at any temperature had folding defects. In the six where rescue was possible at lower temperatures, trafficking under appropriate conditions may allow proper complexing and localization of PC1. These findings indicate that chaperone treatment could be utilized to revert ~60% of mutant PC2 to the wild-type conformation, potentially slowing or halting the progression of the ADPKD.

*R25-DK101405*

## *DROSOPHILA MELANOGASTER* AS A MODEL OF POLYCYSTIC KIDNEY DISEASE USING BICAUDAL-C?

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a leading cause of end-stage renal disease in adults affecting 1 in 800 people in the US. Knocking down bicaudal-C (Bic-C) in zebrafish and *Xenopus* has been reported as a method used to model the cystic changes in ADPKD. Gamberi and colleagues [ASN-2015] recently presented a similar knockdown in *Drosophila melanogaster* resulting in cystic Malpighian Tubules (MTs) homologous to the human kidney.

Our group has investigated the PKD phenotype in *Drosophila* using a Gal4/UAS-RNAi system to knockdown the expression of Bic-C in MTs. Penetrance of Uro and CapaR drivers in the MTs were compared using UAS-YFP, resulting in Uro::Bic-C expression in ~50% of cells with CapaR::Bic-C approaching 100%. MT morphology was analyzed: controls [WT (0%); parental (18%)] occurrence v Bic-C knockdowns [Uro::Bic-C (25%); CapaR::Bic-C (15%)]. Functional tests using Ramsay secretion assays reveal a slower secretion rate in CapaR::Bic-C knockdowns compared to WT MTs, even after stimulation with *Drosophila*-kinin. Since there is an increased risk for ADPKD-related kidney stones, MT calcium oxalate (CaOx) crystallization experiments were performed to quantify crystal formation in Bic-C knockdown vs WT MTs. While crystals are present in all oxalate exposed MTs, Bic-C knockdowns using Uro::Bic-C have more CaOx crystals than CapaR::Bic-C. The same pattern was observed when comparing total crystal area and average area/individual crystal.

These results suggest that Bic-C knockdown parallels the kidney stone increases associated with PKD, nevertheless, Bic-C does not yet consistently mimic morphologic ADPKD-induced cystic phenotypes usually observed in PKD models.

*P30-DK90728*

**INDUCING BONE MARROW DERIVED MACROPHAGES TO IMPROVE ACUTE KIDNEY INJURY OUTCOMES**

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Arginase-1 (Arg-1) and Chitinase-3-Like-1 (Chi3L1) are two proteins expressed by macrophages previously shown to have therapeutic benefits in renal ischemia/ reperfusion (I/R) injury. This study presents the first step to directly test if Arg-1 and Chi3L1 expressing macrophages can be used therapeutically for *in vivo* injection after I/R injury. Using harvest of mouse bone marrow cells and stem cell culture differentiation/expansion, two distinct bone marrow derived macrophage (BMDM) culture methods were designed. The first method is a 7 day culture in 10% L929 media, with a 16hr stimulation of 5ng/mL GM-CSF. Attached cells are harvested for RNA extraction, reverse transcription, and quantitative PCR (qPCR). The second method is a 4 day culture in 5 ng/mL GM-CSF media. Non-attached cells are harvested in the same manner as the L929 culture. Fluorescent-assorted cell sorting (FACS) shows the cultured cells are F4/80$^{+}$, which is a biomarker for macrophages. The L929 culture condition shows a high Arg-1 and a low Chi3L1 mRNA expression via qPCR. The GM-CSF culture condition exhibits low Arg-1 and a high Chi3L1 mRNA expression. Both of these culture conditions express low TNF-α mRNA. Thus, these two conditions provide a novel approach for isolating macrophages that selectively express one of the two therapeutic proteins. The therapeutic benefits of Arg-1 or Chi3L1 expressing macrophages can now be directly compared after induction of I/R injury in mouse models and intravenous injection of the cultured macrophages.

# CENTRAL CONTROL MECHANISMS ASSOCIATED WITH URINARY INCONTINENCE IN PARKINSON'S DISEASE

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To evaluate baseline factors and central control mechanisms associated with urinary incontinence (UI) in persons with early Parkinson's disease (PD). Secondary analysis of baseline resting state MRI data from the Parkinson Progression Markers Initiative (PPMI) using cortical and subcortical regions of interest (ROI) with functional connectivity analysis (fcMRI). Explore ROIs of micturition and UI from selected literature to determine their association with UI in PD. Subjects were men and women ≥30 years of age diagnosed with PD within 2 years of the study. The PPMI study included n=423 PD subjects, with n=80 useable resting state MRI. Of the 80 subjects, 20 had UI. Those with UI reported involuntary loss of urine at least 'sometimes in the past month' based upon the Scale for Outcomes in Parkinson's disease for Autonomic Symptoms. Descriptive statistics compared baseline factors of PD subjects with and without UI. The UI population was older and reported more constipation, but there were no differences in gender, medical conditions, depression, cognitive function, or motor symptom severity. Measures of functional connectivity as assessed by fcMRI showed lower network coherence in the limbic monitoring circuit (bilateral insula, putamen, amygdala, and medial frontal region). In early PD, presence of UI is not associated with motor symptom severity or cognitive function. Resting state fcMRI data suggest PD patients with UI have disrupted limbic monitoring possibly resulting in urinary urgency and subsequent incontinence. These data support the introduction of behavioral therapy in this population for rehabilitative intervention.

**Table I.** Baseline characteristics of early Parkinson's disease subjects with and without incontinence

| Variable | **No UI (n=60)** | **UI (n=20)** | **P-value‡** |
|---|---|---|---|
| **Age *** | 57.1 ± 10.5 (37-77) | 64.6 ± 9.1 (40-75) | 0.006 |
| **Gender (men)†** | 42 (70 %) | 13 (65%) | 0.7 |
| **Nocturia†** | 52 (87%) | 20 (100%) | 0.08 |
| **Constipation†** | 12 (20%) | 9(45%) | 0.03 |
| **BPE†** | 5/42 (12%) | 1/13 (8%) | 0.7 |
| **Cardiovascular disease†** | 23 (38%) | 9 (45%) | 0.6 |
| **Diabetes†** | 2 (3%) | 1 (5%) | 0.7 |
| **Depression (GDS)**** | 20 (33%) | 7 (35%) | 0.9 |
| **MoCA *** | 28.1 ± 1.9 | 27.9 ± 1.8 | 0.7 |
| **UPDRS Part III*** | 18.9 ±8.2 | 19.4 ± 9.8 | 0.8 |

* Mean ± SD (range)
† n (%), BPE – Benign Prostatic Enlargement only among men
‡ p-value represents chi-square (categorical variables) or t-test (continuous variables) analysis
** GDS – Geriatric Depression Scale, depression defined as GDS score > 5 out of 15 total points.
MoCA-Montreal Cognitive Assessment (range 0-30, < 26 suggests cognitive impairment)
UPDRS Part III-Unified Parkinson Disease Rating Scale Part III (total 108, higher score = more severe motor symptoms)

**MOLECULAR STUDY OF INTRAGENIC DELETIONS AT THE *PKLR* LOCUS IN PYRUVATE KINASE DEFICIENT PATIENTS WITH HEMOLYTIC ANEMIA**

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Mutations in the *PKLR* gene result in pyruvate kinase (PK) deficiency, the second most common inborn error of red blood cell (RBC) metabolism associated with hemolytic anemia (HA). PK controls one of the three regulatory steps in glycolysis, and is critical in the maintenance of RBCs, which rely on glycolysis for energy due to absence of mitochondria. Consequently, PK deficiency causes congenital chronic HA. The purpose of this study was to identify and characterize intragenic deletions at the PKLR locus in PK-deficient patients. DNA from anemic PK-deficient patients was screened by long-range PCR. Intragenic deletions were identified in seven patients. Primer sequences were designed to amplify deletion junctions. Target sequences were PCR-amplified, subcloned, and sequenced for analysis by alignment using DNASTAR's Seqman software and The University of California Santa Cruz (UCSC) Genome Browser. Breakpoints were identified for three different deletions, including the previously reported "PK Gypsy," "del 5006bp" and one novel mutation. Sequences flanking the breakpoints exhibited high similarities, indicating that deletions might have originated from misalignments during genetic recombination. These findings are consistent with rearrangements reported in other inherited RBC defects and may provide new insights in understanding the mechanisms of this disease.

**IMPAIRED FUNCTIONAL SYMPATHOLYSIS IN END-STAGE RENAL DISEASE**

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End-stage renal disease (ESRD) patients have an exaggerated increase in blood pressure during both static and rhythmic handgrip exercise, contributing to exercise intolerance in these patients. The increase in blood pressure during exercise is largely due to an increase in reflex sympathetic nervous system (SNS) activation; however, vasoconstriction within the exercising skeletal muscle is prevented due to local vasodilatory forces in order to preserve blood flow, termed functional sympatholysis. We hypothesized that ESRD patients have impaired functional sympatholysis, which contributes to the exaggerated exercise pressor response and exercise dysfunction. We tested this hypothesis by measuring change in muscle oxygenation (tissue saturation index, TSI) using near infrared spectroscopy during sympathetic activation induced by lower body negative pressure (LBNP) alone, and during LBNP with concomitant handgrip exercise. The difference in muscle oxygenation (TSI) during LBNP alone to LBNP with concomitant handgrip exercise represents functional sympatholysis. There was a significant difference in the change in TSI during LBNP alone and during LBNP with concomitant handgrip exercise between the ESRD and control groups ($p=0.025$). While control subjects have a significant attenuation in the decrease in muscle TSI from LBNP alone to LBNP with concomitant handgrip exercise ($p=0.017$), ESRD patients have no significant improvement in muscle oxygenation during LBNP with concomitant handgrip exercise compared to LBNP alone ($p=0.210$). These results suggest that ESRD patients have impaired functional sympatholysis, which could contribute to the augmented exercise pressor response and exercise intolerance in these patients.

**THE CORRELATION BETWEEN HIGH β-CATENIN EXPRESSION AND CYSTOGENESIS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE**

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Autosomal Dominant Polycystic kidney disease (ADPDK) is a monogenic disorder that causes multiple cyst formations due to mutations in *Pkd1* and *Pkd2*. This debilitating disease leads to chronic kidney failure, yet no conclusive mechanism of cyst formation has been established. Elevated expression of β-Catenin is shown to correlate with cyst formation by possible mechanisms of increased Wnt7a and -7b pathway signaling modulated by hyperactivated c-Met expression. We hypothesize that upregulated β-Catenin is correlated with manifestation of disease progress.

In this experiment, we performed quantitative Real time-polymerase chain reaction (qRT-PCR) to determine mRNA transcription levels of β-Catenin and its downstream targets, Tcf-1, Lef-1, Tcf-3, and Tcf-4 *in vitro* in a *Pkd1* knockout cell line and then, *in vivo* in *Hoxb7-Cre* deletion of *Pkd1*$^{flox/flox}$. Non-phosphorylated β-Catenin and two phosphorylated β-Catenins (S552 and S675) were observed first *in vitro* in a *Pkd1* knockout cell line and then, *in vivo* in *Hoxb7-Cre* deletion of *Pkd1*$^{flox/flox}$ and human kidney samples by Western blot. Fluorescent activated cell sorting (FACS) plots sorted cyst lining cells to show specific β-Catenin levels at relevant subcellular locations via qRT-PCR.

In *Pkd1* knockout samples, both *in vitro* and *in vivo,* β-Catenin expression levels were higher than controls, corresponding to the increased cyst formation seen from extracted kidneys from *Hoxb7-Cre-IRES-eGFP;Pkd1*$^{flox/flox}$. The results suggest that the elevated levels of β-Catenin may be causal to cystogenesis, opening up the potential for β-Catenin to be a possible target for a novel molecular approach to treating ADPKD.

**ENGINEERED ASCORBATE PEROXIDASE AND THE CILIARY PROTEIN ARL13B AS GENETIC TOOLS TO STUDY THE ROLE OF THE PRIMARY CILIUM IN CYST FORMATION**

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of the most commonly occurring genetic disorders in the world with limited treatment modalities, resulting in many ADPKD patients progressing to end stage renal disease. Thus, to understand the fundamental biology of ADPKD is an important area of research. Several recent studies suggest a critical role of the primary cilium, an immotile signaling organelle with various sensory functions, in the development and manifestation of cysts in ADPKD. Notably, *PKD2*, one of the two ADPKD genes, encodes a cation channel targeted to cilia. This study focuses on understanding the role of *PKD2* within the primary cilium by using an engineered cilia targeted ascorbate peroxidase (APEX) tag. The APEX tag can catalyze the biotinylation of nearby proteins within a 20 nm radius. To target APEX to the cilia, we genetically fused the peroxidase reporter to the ciliary protein Arl13b using Gibson assembly. Renal epithelial (RCTE) cells were transfected with lipofectamine and expression of our APEX tag was observed using immunofluorescence. Successful localization of Arl13b-APEX will allow us to isolate and identify ciliary specific proteins to generate a differential proteomics map of the ciliary proteins in wild-type cells and *PKD2* mutant cell lines using mass spectrometry. Results will provide novel insight into the function of *PKD2* within cilia and the role of the primary cilium in ADPKD, including other cystic disorders.

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## A ROLE FOR PKHD1 IN METABOLISM

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Autosomal recessive polycystic kidney disease (ARPKD) is a genetic disorder that affects approximately 1 in 20,000 people, and is often fatal in the first month of life. ARPKD is caused by mutation of the gene encoding the PKHD1 protein. Expression of many mitochondrial proteins is altered in the kidneys of ARPKD patients, suggesting that mitochondria may play a role in ARPKD pathogenesis. The c terminal tail of PKHD1 is known to be cleaved, and a bioinformatics analysis showed that it contains a potential mitochondrial targeting sequence. We transiently transfected HEK cells with a GFP-tagged PKHD1 c-terminal tail construct and saw co-localization of the c-terminal tail with mitochondrial markers. To investigate whether a full length construct would yield a cleaved c terminal tail that also translocated to the mitochondria, we established a stable LLCPK cell line expressing a PKHD1-GFP full length construct, in which the GFP tag is at the c-terminus. We incubated cells under normoxic, hypoxic, or hyperoxic conditions and analyzed these cells by immunofluorescence. We also performed Western Blot analysis on control cells and cells treated with the hypoxia mimicking compounds DMOG and $CoCl_2$ to investigate the cleavage pattern of the protein. A difference in expression was seen with DMOG treatment but not with $CoCl_2$ treatment. Localization to the mitochondria was seen by IF. From these preliminary results we conclude that the c-terminal tail of PKHD1 may play a direct role in metabolism. Further research is required to determine the specifics and confirm the presence of that role.

**NADPH OXIDASES MEDIATE ZINC DEFICIENCY-INDUCED OXIDATIVE STRESS IN MOUSE KIDNEYS**

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$Zn^{2+}$ deficiency (**ZnD**) is common in diabetes. Experimental studies show that ZnD worsens diabetic complications, including diabetic kidney disease. Oxidative stress, caused by increased reactive oxygen species (**ROS**), plays a role in the harmful effects of ZnD. However, the sources of oxidative stress continue to be identified. In diabetes, NADPH oxidases (**Nox**) are enzymes that promote oxidative stress in the kidneys. This study seeks to determine the role of Nox enzymes in ZnD-induced oxidative stress in mouse kidneys. WT mice were pair-fed a ZnD or control diet for 6 weeks. After collecting kidneys and urine, kidney damage (BCA Protein Assay), ROS levels (Amplex Red) and Nox enzymes expression (qRT-PCR and Western blot) were examined. To further investigate the effect of $Zn^{2+}$ bioavailability on Nox enzymes, mouse distal convoluted tubule (mDCT) cells were exposed to a $Zn^{2+}$ chelator (TPEN) or vehicle (DMSO) for 48 hours with or without $Zn^{2+}$ supplementation for the last 24 hours. Findings show that a $Zn^{2+}$ restricted diet induces urinary protein that is accompanied by elevated Nox2 expression and ROS levels in mice kidneys. In mDCT cells, inhibition of Nox enzymes with DPI blocks $Zn^{2+}$ depletion-induced ROS levels. Consistent with this finding, $Zn^{2+}$ depletion increases Nox2 and Nox4 expression. Furthermore, $Zn^{2+}$ supplementation reduces $Zn^{2+}$depletion-induced Nox2 increases. These findings suggest that Nox enzymes mediate ZnD-induced oxidative stress and kidney damage. Understanding how ZnD causes kidney injury can have an important impact on the treatment of diabetic kidney disease.

**Funding:** R25DK101390 (MSL), VA-MERIT (JLG), R01DK085097 (RSH), T32-DK00756 (CRW)

**EVALUATING *In Situ* TRYPTIC DIGESTION METHODS FOR MALDI IMAGING MASS SPECTROMETRY USING A MOUSE KIDNEY MODEL**

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Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) is an analytical technology that allows for the molecular surface analysis of thin tissue sections. Mass spectra are collected at varying coordinates over the surface of the tissue and intensity-based heat maps are generated by selecting specific *m/z* windows across the mass spectra, revealing spatial localizations of analytes within the tissue. Peptides can be analyzed by applying enzymes to tissue prior to MALDI IMS analysis. This approach extends the effective mass range of observed proteins by analyzing the digested peptides.

Current methodologies for analysis include the use of electroacoustic spotters to deposit enzymes and matrix onto tissue. This approach can be both time-consuming and limited in its spatial resolution capabilities. Such spatial resolution limitations only allow for the differentiation of major structures in the kidney such as the cortex or medulla, and this methodology has not yet been proven for investigating smaller kidney substructures such as the glomerulus.

By incorporating an automatic sprayer into the enzyme and matrix application workflow, the coatings are applied homogenously, allowing for higher spatial resolution imaging, as well as significantly decreasing application time. For method development, the electroacoustic and automated spotting methods were evaluated at 350 µm resolution. Comparisons of signal intensities and localization patterns were performed.

The automated spray method suggested an increase in image clarity, ion signal, and digestion efficiency. Further experiments will explore the magnitude of peptide delocalization and the potential use of such methods with formalin-fixed paraffin-embedded (FFPE) tissues.

**INTERACTION OF SLC26A6 WITH COPI SUBUNITS IN HUMAN KIDNEY CELL LINE HK-2**

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SLC26A6 is a chloride-oxalate exchanger, and mice with Slc26a6 deleted develop calcium oxalate urinary stones. Previously, co-immunoprecipitation from mouse duodenum identified COPI proteins, specifically beta and delta COP, as interacting with SLC26A6. Our goal is to characterize the Human Kidney-2 (HK-2) cell line, a human epithelial cell culture model that expresses SLC26A6 and the COPI proteins and to study the interaction. We characterized the chloride gradient dependent oxalate uptake in a cell culture in HK-2 cells by measuring influx of [14C] oxalate. An outward chloride gradient stimulated oxalate uptake by 5.9-fold compared to the absence of a chloride gradient. Western blots showed high levels of SLC26A6 expression. We will use siRNA knockdown of SLC26A6 to confirm SLC26A6 is the primary means of chloride dependent oxalate transport. Co-immunoprecipitation of SLC26A6 with beta and delta COP will be verified in HK-2 cells. The effect of siRNA knockdown of one of the COPI proteins on SLC26A6 trafficking will be studied to understand the functional significance of the interaction. In summary, the HK-2 cell line has a high level of chloride stimulated oxalate transport and SLC26A6 expression, and should be a useful model to study the interaction of SLC26A6 with COPI proteins.

**A COMPARISON OF ELISA AND MESOSCALE METHODOLOGIES FOR QUANTIFYING ANGIOTENSINOGEN AS A BIOMARKER FOR DIABETIC KIDNEY DISEASE**

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Angiotensinogen, a protein component of the RAS system, is a urinary biomarker used to measure inter-renal RAS activity. RAS system is activated with the deterioration of renal function, and is particularly important in diabetic kidney disease, which is the most common cause of end stage renal disease. Therefore, an efficient, cost effective, accurate and sensitive test is needed to quantify angiotensinogen in patient urine.

The aim of this project was to optimize a custom MesoScale assay (MSD) and compare the R&D angiotensinogen sandwich ELISA with a MSD in order to determine the best method to quantify angiotensinogen. A MSD is preferred as it minimizes the amount of sample needed, improves the dynamic range of detection (LLOD pg/mL) and reduces variation due to handling (ability to multiplex versus single-plex). The first step was to optimize the preferred diluent. It was found that diluent 100 had a greater working range, higher sensitivity (measured by LLOD) and high precision. Thus, diluent 100 was chosen as the diluent for angiotensinogen. Then, 40 urine samples were used to compare the MSD and ELISA. Although the trend was the same, the absolute concentrations of angiotensinogen were different. From this data, it can be concluded that angiotensinogen can be reliably measured on MSD assay and potentially be combined with other assays utilizing diluent 100 while yielding high quality results, thus conserving sample volume, time and resources.

**ANALYSIS OF CLC-5 PROTEIN TRAFFICKING AND LOCALIZATION IN DENT DISEASE**

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Dent disease is a rare, X-linked disorder characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and progressive renal failure. Approximately 60% of patients have mutations to *CLCN5* (Dent disease 1), encoding ClC-5, a chloride/proton exchanger essential for receptor-mediated endocytosis in renal proximal tubular cells. We examined the effect of 3 mutations (Q629X, R345W, S244L) on ClC-5 protein trafficking and localization to better understand pathogenesis and explore genotype-phenotype correlations. Renal epithelial cells were transfected with wild-type or mutant ClC-5 constructs in pEGFP-c2 and incubated at 37 or 30°C. Protein trafficking and co-localization with subcellular compartments were analyzed by immunofluorescence microscopy and surface expression quantified by flow cytometry using an extracellular HA tag. Q629X was largely absent at the plasma membrane and retained in the ER and Golgi at both temperatures. Surface expression of R345W was 60% reduced at 37°C but reverted to wild-type levels at 30°C. S244L, consistent with previous studies, appeared to have normal trafficking and membrane localization. Thus, we hypothesize that for the R345W and Q629X mutants the Dent phenotype is due to defective ClC-5 trafficking to the plasma membrane. Since the S244L mutant had normal cell surface expression, pathogenesis might instead be due to a loss of exchanger function, leading to defective endocytosis and plasma membrane accumulation of ClC-5. Differences in these molecular effects of specific mutations may contribute to phenotypic variability of Dent patients and highlights the potential importance of assessing the genetic profile to design individualized therapies.

*R25-DK101405*

**TISSUE, PLASMA AND URINE METABOLITE PROFILING OF THE PCK RAT: OPTIMIZATION OF HARVESTING TECHNIQUES, EXTRACTION STRATEGIES AND SAMPLE PREPARATION FOR $^{1}$H-NMR**

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Recent evidence suggests that Polycystic Kidney Disease (PKD) cells undergo metabolic reprograming. Thus, elucidating metabolic changes may help in the understanding of the pathogenesis as well as lead to new pathways for therapies. Careful tissue sampling/extraction is very important in metabolomics studies as active enzymes may cause rapid changes in metabolite levels. The aim of this study was to optimize tissue harvesting/extraction techniques for rapid termination of enzyme activity to mimic *in vivo* metabolite concentrations.

Urine, plasma and left/right kidneys (LK/RK) from PCK rats were collected and $^{1}$H-NMR-based metabolomics analysis was performed on a Bruker 600MHz spectrometer. Two harvesting techniques; clamp-freezing before exsanguination (LK), and snap-freezing after (RK), were timed and metabolite concentrations compared. LK and RK thicknesses were measured to calculate freeze-time. Three metabolite extraction protocols (perchloric acid, methanol/chloroform and methanol/acetone) were performed by three operators to assess reproducibility, and tested by HPLC. Quantitative analysis of NMR spectra was done with Chenomx software.

The three metabolite extraction protocols performed well; however, the perchloric acid protocol was the most reproducible. Clamp-freezing vs snap-freezing resulted in significantly lower ischemia/freezing times, ultimately causing changes in the metabolite profile (Fig. &Table1). For example, ATP concentrations were significantly higher while those of AMP and lactate were significantly lower in LK vs. RK respectively.

Clamp-freezing before exsanguination significantly reduced ischemia/freezing times resulting in metabolite changes, particularly in compounds known to be oxygen-dependent. Similar analysis of other metabolites in PCK and wild-type rats (in progress) may help elucidate the metabolic changes in PKD.

*P30-DK090728*

**Figure 1.** Lactate, AMP and ATP concentrations (*mM*) LK and RK from left to right

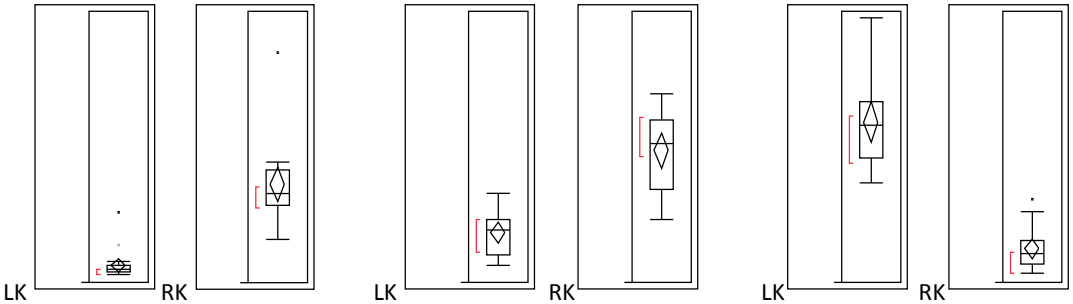


**Table 1** Baseline characteristics

| General Characteristics | Mean | Stand. Dev. | | | |
|---|---|---|---|---|---|
| **Age (days)** | 55.4 | 4.4 | | | |
| **Gender (M/F)** | 10M/10F | N/A | | | |
| **Animal weight (g)** | 263.4 | 43.7 | | | |
| **24 hour urine vol. (mL)** | 11.4 | 4.0 | | | |
| **LK and RK Characteristics** | **LK Mean** | **LK St. Dev.** | **RK Mean** | **RK St. Dev.** | **p-value** |
| **Weight (g)** | 1.8 | 0.7 | 1.7 | 0.6 | 0.729 |
| **Thickness (mm)** | 3.8 | 1.1 | 9.6 | 1.0 | <0.001 |
| **Ischemia time* (s)** | 7.1 | 1.0 | 551 | 89.9 | <0.001 |
| **Freeze time** (s)** | 9.4 | 5.0 | 54.9 | 12 | <0.001 |
| **Total time (s)** | 16.5 | 5.0 | 609.9 | 93.1 | <0.001 |
| **[ATP] (mM)** | 0.885 | 0.239 | 0.187 | 0.114 | <0.001 |
| **[AMP] (mM)** | 0.273 | 0.122 | 0.73 | 0.207 | <0.001 |
| **[Lactate] (mM)** | 1.3 | 1.4 | 5.419 | 2.007 | <0.001 |

*Ischemia time: time from clamping of vessels to clamp-freezing **Freeze time: calculated with equation; time = 2.36 * $x^2$, x is half of the tissue thickness

**ANALYSIS OF PHOSPHODIESTERASE MUTANT ZEBRAFISH**

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Phosphodiesterases (PDEs) hydrolyze cyclic adenosine monophosphate (cAMP), and decreased activity of PDE1A is thought to contribute to elevated levels of cAMP causing cystogenesis in Autosomal Dominant Polycystic Kidney Disease (ADPKD). The effects of PDE1A loss-of function were studied by using Transcription activator-like effector nuclease (TALEN) generated mutant zebrafish. The purpose of this study was to further evaluate the importance of PDE1A in cyst formation previously shown using morpholinos (MOs) as well as validate the PDE1A functional deficit in the mutant lines. F2 heterozygous mutant fish were in-crossed, and the embryos were examined for phenotypes. To amplify phenotypes, some embryos were treated with forskolin or injected with MO targeting *pkd2* (transcript of one of two genes mutated in ADPKD). Embryos were phenotyped and collected for genotyping using restriction fragment length polymorphism (RFLP). Phenotypes observed after treatment with forskolin included spinal curvature, wrinkled fins, and edema. Neither curvature nor wrinkled fins correlated with genotype; however, embryos developing edema were more frequently homozygous than non-edematous embryos (41% vs. 29%). When embryos were injected with *pkd2* MO, they developed a range of curvature from mild (>90˚ tail-body angle), moderate (<90˚), to severe (tail curved back on the body). Preliminary data showed greater incidence of severe curvature in homozygous embryos than in wild type. These studies are ongoing. Overall, data suggest that edema at 6 dpf and increased response to PKD2 depletion occur in *pde1a* mutant zebrafish, consistent with a PDE1A functional deficit, and with effects of *pde1a* MOs.
*P30-DK090728*

**REGULATION OF GLYCOSYLATION OF THE POTASSIUM CHANNEL Kir7.1 BY ADRENERGIC RECEPTORS**

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Kir7.1 is an inwardly rectifying potassium channel expressed in sites such as the brain, retina, and uterine smooth muscle. Kir7.1 is known to interact with the melanocortin 4 (MC4R) G-protein coupled receptor (GPCR), which plays a role in energy homeostasis. Surprisingly, while characterizing this interaction in control studies, we found that another GPCR, the β2 adrenergic receptor (β2AR), leads to inhibition of mature glycosylated forms of Kir7.1 when co-expressed with the channel in HEK293T cells. Therefore, the aim of this project was to determine whether the regulation of glycosylation of Kir7.1 is specific to β2AR, by studying the effect of other adrenergic receptors on glycosylation.

First, a tunicamycin treatment assay was performed to determine the glycosylated forms of the receptors (previously performed for Kir7.1). Next, the β1, β2, or β3 adrenergic receptors were co-expressed with Kir7.1 in HEK293T cells and analyzed by western blots. Preliminary data revealed the glycosylated forms of the β2 and β3 adrenergic receptors. Additionally, when β2AR and Kir7.1 were co-expressed, no changes in β2AR glycosylation were observed. Our next set of experiments will be to determine whether these and other GPCRs across the entire adrenergic receptor family do indeed interact with Kir7.1.

The unique interaction between Kir7.1 and GPCRs is still quite novel, and defining these different relationships could lead to new pharmacological therapeutics. As GPCRs are targets of forty-percent of all current drugs on the market, it is therefore important that we understand how they work.

**THE EFFECT OF GROWTH FACTORS ON CELL CYCLE IN PROXIMAL TUBULE EPITHELIAL CELLS**

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Proximal tubule epithelial cells are kidney cells and are the main target of renal diseases. TGF- β is a ligand that is involved in renal development and the development of fibrosis. TGF-β binds to the TβRII receptor located on proximal tubule cells. Proximal tubule cells with and without this receptor were treated with a growth factor, Wnt3a, in order to determine their response. Cells were also exposed to conditioned media (CM) containing Wnt3a from L-Cells that overexpress the Wnt3a gene, as well as wild type L-Cell media. Flow cytometry was used as a primary method to determine the distribution of the cells across cell cycle. The cells were divided into three categories: $G_0/G_1$, S, and $G_2$/M.

Cells with the TGF-β receptor, TβRII flox/flox, entered cell cycle arrest when exposed to Wnt3a CM, while cells without the receptor, TβRII -/-, did not enter cell cycle arrest. Both cell lines entered cell cycle arrest in the Wild Type L-Cell CM. When exposed to exogenous Wnt3a ligand in proximal tubule media, both cell lines saw a rise in the number of cells in $G_2$/M, and when exposed to exogenous Wnt3a in Wild Type L-Cell CM, the TβRII flox/flox cells moved towards cell cycle arrest, while the TβRII -/- cells did not experience cell cycle arrest. These results suggest that the inhibition of TBRII prevents cells from going into cell cycle arrest, which can exacerbate the development of kidney fibrosis.

**METABOLIC SYNDROME MODULATES THE CARGO PACKED WITHIN MESENCHYMAL STEM CELL-DERIVED PARACRINE EXTRACELLULAR VESICLES**

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Effective strategies to alleviate the deleterious effects of metabolic syndrome (MetS) are yet unavailable. Mesenchymal stem cells (MSCs) are a promising regenerative medicinal tool, but might be damaged by comorbidities, hindering their potential use for autologous transplantation. This study tested the hypothesis that MetS impairs the integrity of swine adipose tissue-derived MSCs, thus altering the cargo packed in extracellular vesicles (EVs), the vectors of their paracrine effects.

Autologous MSCs were collected from abdominal fat of domestic pigs after 16 weeks of normal (Lean) or high cholesterol/carbohydrate (MetS) diet (n=3 each), and EVs subsequently collected by ultracentrifugation. Next-generation mRNA sequencing was performed to identify genes selectively enriched in Lean- and MetS-EVs compared to their parent MSCs. Functional annotation clustering analysis was performed to identify cellular pathways regulated by genes enriched in EVs.

RNA-seq generated reads for over 15,000 annotated mRNAs, but only 68 genes were enriched in Lean-EVs compared to Lean-MSCs and 77 in MetS-EVs compared to MetS-MSCs. mRNAs from Lean-EVs were found to target genes that participate in several cellular pathways, including angiogenesis, cellular transport, apoptosis, and proteolysis, whereas mRNAs from MetS-EVs target transcription factors and genes that regulate inflammation, as well as bone, urogenital, and nervous system development.

MetS MSC-derived EVs show distinct genetic material content compared to their lean counterparts, with decreases in angiogenic and increases in inflammatory mRNA. These observations could greatly advance our understanding of the vulnerability of this repair system as a ‘target tissue’ for MetS and identify therapeutic targets to improve MSC quality.

*R25-DK101405*

**UTILITY OF CT SCANNING TO DETERMINE MORPHOLOGICAL FEATURES THAT AFFECT KIDNEY STONE FRAGILITY**

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Ultrasonic percutaneous lithotripsy is a minimally invasive method of removing kidney stones. In this study, we investigate the relationship between morphological factors and time to comminution of kidney stones via ultrasonic lithotripsy, and the utility of CT scanning in predicting the fragility of stones.

35 urinary stones of known composition were scanned with a microCT and on a third-generation dual-source CT scanner (Somatom Force, Siemens Healthcare) using ultra-high resolution (UHR) and standard resolution modes. MATLAB was used to determine the volume and porosity of each stone, and a 3D textural analysis technique was used to calculate Haralick textural features of all CT data sets. The stones were then disintegrated using an Olympus LUS Lithotriptor and the time to comminution was recorded. Spearman analyses were performed using JMP software to interrogate the data for statistically significant relationships ($P<0.05$).

Volume showed a moderate positive correlation to comminution time by microCT, and porosity showed a moderate negative correlation with time. The UHR clinical CT data demonstrated 7 significant relationships: a moderate positive correlation of volume, contrast/inertia, and sum mean to time; a moderate negative correlation of “Haralick textural correlation” to time, a weak positive correlation of entropy, variance to time, and a weak negative correlation of energy to time. Pure calcium oxalate monohydrate stones had a median time to breakage twice that of stones with mixed compositions.

Quantitative analysis of clinical CT images demonstrated multiple correlations with time to comminution, indicating the potential to use patient CT data to predict stone fragility.

*R25-DK101405*

**IDENTIFICATION OF FACTORS STABILIZING THE 3D STRUCTURE OF THE GOODPASTURE AUTOANTIGEN OF GLOMERULAR BASEMENT MEMBRANE.**

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In Goodpasture disease, autoantibodies bind to α3 and α5 NC1 domains of collagen IV in glomerular basement membrane (GBM), leading to rapidly developing glomerulonephritis and renal failure. These domains exist in GBM as a hexameric complex, termed the Goodpasture autoantigen, which is composed of α3, α4 and α5 NC1 monomers. Significant amounts of these monomers are crosslinked with sulfilimine bonds, which might be the reason why the native α345 hexamers do not bind Goodpasture autoantibodies. Here, we investigated the factors that contribute to the hexamer stability and inertness toward binding of autoantibodies. For this study we have purified human recombinant α3, α4, and α5 NC1 monomers from mammalian cell culture media using FLAG affinity chromatography. Through FPLC size exclusion chromatography on Superdex S200 column, we demonstrated that α345 hexamer assembly requires the presence of physiological concentration of NaCl. Furthermore, we showed that sulfilimine bond introduced through the oxidation of methionine residues with hypobromous acid stabilizes hexamer and prevents dissociation into monomers in the chloride-free environment. We conclude that both $Cl^-$ ions and sulfilimine crosslinks stabilize the α345 hexamer and that perturbation of either may induce pathological conformational changes initiating Goodpasture disease.

**LIPOTOXIC ENDOPLASMIC RETICULUM STRESS IN RENAL PROXIMAL TUBULE CELLS**

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Conditions such as obesity and diabetes are associated with ectopic accumulation of lipids in nonadipose tissues, which in turn may contribute to organ injury and dysfunction through a process termed lipotoxicity. The mechanism and consequences of lipotoxicity vary according to cell type and are incompletely understood in the kidney. We seek to explore if lipid accumulation in renal cells can induce endoplasmic reticulum (ER) damage and ER stress, and to investigate whether Klotho, a multifunctional protein expressed in the kidney and involved in renoprotection, can mitigate these deleterious effects. To test this hypothesis, we used an established model of excess lipid delivery to opossum kidney proximal tubule-like (OKP) cells by incubation with 1.5 mM albumin-bound palmitate (a representative saturated fatty acid) at time points ranging from 1 to 48 hours. Control cells where incubated with albumin alone at equivalent concentration and time points. Oil Red O staining showed significant intracellular lipid accumulation in palmitate-incubated cells. ER stress markers were measured by immunoblotting, with results indicative of moderate activation of the Unfolded Protein Response (UPR). Transmission electron microscopy was used to analyze possible changes in cellular and organelle morphology. Finally, the effect of Klotho is examined in ongoing experiments by incubating cells with or without recombinant Klotho protein in the presence or absence of palmitate.

**THE EFFECT OF ZIRAM ON CORTICAL NEURONS THROUGH E1 INHIBITION.**

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Dysfunction of the ubiquitin-proteasome pathway has been suggested to play a role in Parkinson's disease pathogenesis. The ubiquitin-activating enzyme E1 plays a critical role in starting the cascade of events for ubiquitin activation. Ubiquitin is vital for several neuronal mechanisms such as cellular viability, proliferation, synaptic plasticity, synapse development, and neuronal transmission. Here we show that Ziram, one of the pesticides believed to be associated with increased risk for Parkinson's disease, causes neurotoxicity and inhibits the activity of the ubiquitin-activating enzyme E1 in cortical glutamatergic and midbrain dopaminergic neurons differentiated from human-induced pluripotent stem cells derived from control subjects and Parkinson's disease patients.

**EFFECT OF VITAMIN D SUPPLEMENTATION ON INSULIN RESISTANCE AND BETA-CELL FUNCTION IN OBESE NON-DIABETIC ADULTS**

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Higher vitamin D levels are associated with reduced insulin resistance and improved beta-cell function. However, it is unclear whether vitamin D supplementation can improve insulin resistance or beta-cell function in obese nondiabetic individuals. The purpose of this study was to determine the effect of vitamin D supplementation on insulin resistance and beta-cell function in obese nondiabetic adults. Obese nondiabetic adults (n=93, age=39.3, BMI= 33.5) were randomly assigned to receive Ergocalciferol (50,000 units/week) or placebo for 8 weeks. Vitamin D levels, HOMA-IR, HOMA- β, adiponectin, fasting glucose, and fasting insulin were measured at baseline, and then again at an 8-week follow-up. Analysis of variance (ANOVA) results show a significant difference in the change in vitamin D (mean=15.4, SD=6.7 vs. mean=3.0, SD=17.0, $p<.0001$), HOMA-IR (mean=0.27, SD=0.83 vs. mean=-0.12, SD=0.78, $p=.03$), and fasting insulin (mean=1.1, SD=3.3 vs. mean=-0.5, SD=3.4, $p=.04$) between the vitamin D and placebo groups. There was no significant difference in the change in HOMA-β, fasting glucose, or adiponectin between treatment groups. The significantly greater increase in HOMA-IR in the vitamin D group suggests that vitamin D may worsen insulin resistance in obese nondiabetic adults. However, due to differences in baseline HOMA-IR between treatment and placebo groups, this effect may have resulted from regression to the mean. There was no effect of vitamin D supplementation on HOMA- β. Our findings do not support a beneficial effect of vitamin D supplementation on insulin resistance or beta-cell function in obese nondiabetic individuals.

**ROLE OF ENDOTHELIAL-DERIVED CUES IN KIDNEY DEVELOPMENT**

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Endothelial cells play an important role in organogenesis, independent of blood circulation. Importantly, development of different organs is affected differently by perturbation of resident endothelial cells, suggesting that there may be organ-specific endothelial-derived cues governing organogenesis. In order to study the role of organ-resident endothelial cells during development, we performed expression profiling on Vascular Endothelial Growth Factor Receptor 2- eGFP endothelial cells of three developing organs, kidney, lung and pancreas, at three different embryonic stages. Our data analysis showed that gene pathways important for cell-cell communication are regulated differently in endothelial cells of different organs. Of particular interest to our lab, in the larger context of an NIH consortium effort to engineer kidneys in vitro, we found distinct novel genes to be enriched in endothelial cells of the developing kidney. Using a candidate gene approach, we identified secreted and transmembrane factors that have the potential to play key roles in kidney development and nephron progenitor cell behavior. Here, we confirm expression of our candidates in the kidney endothelial cells by *in situ* hybridization and demonstrate differing expression patterns for these genes within the organ. We aim to pursue candidates expressed in the cortex, where nephron progenitor cells are located and may require signals for their self-renewal. Future work will be aimed at carrying out functional studies, *in vitro* and *in vivo*. We hope that our study will unravel novel mechanisms whereby endothelial cells impact nephron progenitor cells, and contribute to constructing kidneys *in vitro*.

**COMPARING ATROPHY-INDUCING SIGNALS ON ER STRESS IN C2C12 MUSCLE CELLS**

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Muscle wasting (atrophy) is a consequence of chronic diseases, such as kidney disease or diabetes, and reduces the patient's overall quality of life and functional independence. Previous studies in this lab show that muscle cells treated with palmitate (Palm, a saturated fatty acid) activates ER stress and contributes to the atrophy process. The purpose of our experiments is to determine whether ER stress is a common feature of atrophy signaling by comparing the effects of two known inducers of muscle atrophy, glucocorticoids and Palm, on ER stress markers. Glucocorticoids are elevated during physiological stress like chronic diseases and are permissive for muscle wasting. Therefore, we tested the hypothesis that glucocorticoids increase markers of ER stress. $C_2C_{12}$ muscle cells were treated with 1µM Dexamethasone (DEX, a glucocorticoid) and/or 500µM of Palm. The mRNA levels of the ER stress markers ATF4 and CHOP were measured by qPCR methods. ATF4 represents one of three arms of the ER stress response and CHOP represents an integrated response of all three arms. Treating myotubes with DEX had little effect on ATF4 or CHOP mRNA levels compared to the control. Palm did not change ATF4 mRNA but, similar to previous work, Palm increased CHOP mRNA. Co-treatment of Palm and Dex synergistically increased both ATF4 and CHOP. These results suggest that in the presence of other atrophy signals, glucocorticoids act synergistically to enhance ER stress signaling. Future studies will evaluate whether activation of ER stress in co-treated cells prolongs cell survival.

**Funding**: R25DK101390 (ERN), VA-MERIT X01BX001456 (SRP), R01 DK 95610 (SRP), T32 DK 007656 (JAR)

**ANTIBODIES AGAINST SECRETED RENALASE AS THERAPUETIC AGENTS FOR PANCREATIC DUCTAL ADENOCARCINOMA**

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Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-related mortality. Current therapeutic strategies are only marginally effective, extending life expectancy by several months. We hypothesize that renalase (RNLS), a recently discovered survival factor that is upregulated in PDAC and involved in tumor cell proliferation, may serve as a potential therapeutic target for PDAC.

In this study, we sought to compare the inhibitory efficacy of RNLS monoclonal antibodies against the human pancreatic carcinoma cell line (PANC-1). We evaluated the effect of 2 RNLS antibodies (1 against the secreted isoform RNLS-1, and one against the non-secreted isoform RNLS-2) on PANC-1 cells in-vitro, varying the dose between 5 to 50μg/ml. Cell metabolism was evaluated using the WST1 method and the percentage of cell death was estimated using the trypan blue exclusion assay. We found that the antibody (m26-RNLS), directed against the carboxy terminus of RNLS-1, increased apoptosis of PANC-1 cells and inhibited cell growth by 62% ($P < 0.01$). In contrast, m3A-RNLS, which is specific for the non-secreted isoform RNLS-2, had no effect on PANC-1 cells.

We conclude that inhibition of RNLS-1 signaling may have therapeutic value in PDAC, and that m26-RNLS and should be evaluated further using xenograft and genetic mouse models of PDAC.

## PLASMA URIC ACID LEVELS AND HISTOPATHOLOGICAL FINDINGS ON KIDNEY BIOPSY

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26 million adults in the United States have Chronic Kidney Disease (CKD). Thus, there has been a search for biomarkers that can assist with the care of patients with or at risk of the disease. Recent studies have shown that elevated levels of plasma uric acid (PUA) may be correlated with the development of CKD as it has been associated with hypertension, gout, and diabetes. In our cross-sectional study, we hypothesize that uric acid also contributes to fibrosis, inflammation, and vascular sclerosis.

We measured PUA in blood samples from 235 patients undergoing native kidney biopsy. PUA levels were measured through enzymatic assays and compared with clinical, demographic, and biopsy characteristics.

Preliminary analysis reveals a correlation between PUA and increasing levels of interstitial fibrosis and tubular atrophy (IFTA) ($P < 0.001$) Analysis also found that PUA and SCr are significantly correlated ($R^2=0.1607$, $P < 0.001$). Thus, the association between PUA and IFTA appeared to be confounded by SCr: whereas SCr remained associated with greater IFTA when adjusting for PUA ($P < 0.001$), PUA was not ($P = 0.157$), suggesting no association between PUA and IFTA in consideration of kidney function.

Generally, we did not observe an independent association between PUA and hitopathological findings in our cohort. Given the strong pathophysiological rationale for an association between PUA and CKD progression, however, we still believe that prospective studies are warranted. We intend to follow the patients over time to determine whether higher PUA at the time of biopsy predicts subsequent kidney function decline.

**THE ROLE OF Dgcr8 IN POST-NATAL KIDNEY TUBULE MAINTENANCE**

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MicroRNAs (miRNA) are small, non-coding RNAs (~22 nucleotides) that function in sequence-specific RNA silencing and post-transcriptional regulation of gene expression. miRNA biogenesis is a multi-step pathway, beginning with the transcription of independent genes to form primary miRNA (pri-miRs) transcripts, which are further processed to ~70 nucleotide long precursor miRNAs (pre-miRNA) by the Drosha-DiGeorge Syndrome Critical Region 8 (Dgcr8) protein complex. The pre-miRNAs are cut by the cytoplasmic protein Dicer to form a ~22 nucleotide long miRNA/*miRNA duplex. Argonaute (Ago) proteins facilitate complementary base pairing between the seed sequence of a mature miRNA and target mRNA on the RNA induced silencing complex (RISC), leading to mRNA degradation or inhibiting translation. miRNAs play a major role in numerous cellular and developmental processes. The aim of this study was to characterize the role of the miRNA biogenesis enzyme, Dgcr8 in kidney tubule maintenance. Immunofluorescent (IF) staining reveals that Dgcr8 is expressed in the nuclei of tubular cells of the cortical and medullary regions of adult kidneys. Cre/LoxP recombination, a site-specific recombinase technology, was used to knock out the expression of Dgcr8 specifically from the collecting ducts of the mouse kidneys. Western blot analyses confirmed a decrease in Dgcr8 expression in adult mouse kidneys. IF analyses showed that Dgcr8 expression is absent specifically from nuclei of collecting duct cells. Histological analyses at post-natal day 35 (P35) using Hematoxylin and eosin (H&E) and trichrome staining indicated that the kidneys from Dgcr8 knockout mice developed fibrosis. Thus, Dgcr8 plays a role in post-natal tubule maintenance in mice kidneys.

**THE ROLE OF PALMITOYLATION IN THE BIOGENESIS OF NONMOTILE CILIA**

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Nonmotile cilia are microtubule-based protrusions of the cell membrane. Defects of the cilia have been linked to polycystic kidney disease and various other human diseases. Arl13b is a small ciliary G protein that localizes to nonmotile cilia and plays an important role in its biogenesis and signaling. Palmitoylation, the reversible covalent post-translational attachment of a saturated lipid palmitate to cysteine residues, is integral to maintaining the regulatory function of Arl13b. We are interested in understanding the regulation of Arl13b palmitoyltion and the mechanisms by which it affects Arl13b functions. A pair of cysteine residues in the N-terminus of Arl13b forms the putative palmitoylation site. It is unclear if a single of these cysteines is sufficient or if both are required for palmitoylation to occur. Using site-directed mutagenesis via PCR, we substituted the cysteines for serines at positions 8 (C8S), 9 (C9S) or both (C8/9S). Metabolic incorporation of a palmitic acid analogue (17-ODYA) followed by Arl13b immunoprecipitation and bioorthogonal click-chemistry allowed for detection and quantification of Arl13b palmitoylation via western blotting. The absence of 17-ODYA in the C8/9S Arl13b mutant corroborated findings that these specific cysteine residues are required for palmitoylation to occur. Additional studies are underway on the single cysteine mutants C8S and C9S. Furthermore, microscopy studies are planned to study the localization of these Arl13b mutants and how they affect cilia biogenesis.

**METABOLIC EFFECTS OF ADIPONECTIN SIGNALING IN THE KIDNEY**

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Diabetes is the leading cause CKD and is thus on the rise with the obesity epidemic. Expanding adipose tissue alters adipocyte secretion of adipokines: hormonal factors that impact systemic fatty acid oxidation and glucose sensitivity. These generally negative signaling events lead to lipotoxic stress and insulin resistance. Adiponectin, however, demonstrates positive effects in an array of in vivo and in vitro studies, but its levels are reduced in obesity. Systemically elevated adiponectin levels are renoprotective in models of acute kidney injury. To uncouple the positive systemic benefits of adiponectin from those locally in the kidney we generated a novel mouse model of inducible adiponectin expression in renal tubular epithelium. This "KSP-APN" mouse utilizes a doxycycline-inducible TRE-Adiponectin (APN) transgene that permits tight control over adiponectin expression. After doxycycline 600 mg/kg diet for 10 days gene array analysis identified two noteworthy metabolic pathways increased by adiponectin: fatty acid oxidation and gluconeogenesis. We probed these pathways by qPCR, protein expression, and functional assays to confirm adiponectin's effects. Through qPCR, we found significant increases in the gluconeogenic enzyme PEPCK1 and mitochondrial biogenesis marker PGC1α mRNA. We confirmed PEPCK1 upregulation in the KSP-APN kidney by Western blot. Tissue fatty acid oxidation was measured by $^{3}$H-triolein infusion in vivo; we found a significant increase in fatty acid oxidation in the kidney (but not other tissues). Renal adiponectin signaling thus has profound metabolic effects that should protect kidney function from the negative consequences of the metabolic syndrome and provides a target to potentially ameliorate diabetes-induced CKD.

**CAMP SIGNALING REGULATES POLYCYSTIN SIGNALING AND PROMOTES CILIARY ENTRY OF PKD-2 IN *C. ELEGANS***

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Autosomal dominant polycystic disease (ADPKD) is the most common human genetic disease that occurs because of defects in cilia, sensory organelles on the surface of most eukaryotic cells. *C. elegans* is used to study ciliopathies like ADPKD because of conserved pathways regulating cilia formation and function, and no cure for ADPKD is yet available. Increased levels of cAMP are thought to further promote cyst development; thus, we proposed study of potential crosstalk between polycystin signaling and cAMP signaling as well as strategies for future therapeutic treatment. Transgenic animals were generated to analyze protein expression and localization of phosphodiesterases (PDEs). Compound mutants were generated and genotyped, and mating behavior assays were used to assess the polycystin-mediated ciliary mechanosensory function in different mutants. Humanized worms expressing human PDE1A were generated. We found PDE-1, but not PDE-3, are the major ciliary calcium-activated PDEs in *C. elegans*. *Pde-1* knockout worms showed significant polycystin signaling defects with an additive defect when combined with *pkd-2* deletion. Surprisingly, PKD-2 protein abnormally accumulates in PDE-1-depleted cilia due to increased ciliary entry but not disrupted ciliary removal. We also found that PKA and PDE-1 co-localize inside cilia. Further study is needed in the cAMP-regulated portion of the polycystin signaling. If conserved, the cAMP-promoted cilia entry of polycystins could be explored as an avenue to increase effective cilia dosage of polycystins to delay or revert cystogenesis in PKD. Humanized PDE worm models and our proposed suppressor screening in *C. elegans* have great potential to reveal new therapeutic compounds/targets for ADPKD.

*P30-DK090728*

**CRISPR-CAS9 FAILURES REVEAL THE ROLE OF NONSENSE-MEDIATED DECAY IN EPIGENOMIC DISTORTION OF GENETIC INFORMATION**

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Clustered regularly interspaced short palindromic repeats (CRISPR)-mediated genome editing technology has revolutionized modern molecular biology by enabling gene analysis by directly introducing mutations in DNA. CRISPR technology relies on the expression of the RNA-guided DNA nuclease Cas9 combined with a gene-targeting single guide RNA (sgRNA) to induce double stranded breaks in the host genome. As part of an effort to leverage CRISPR technology to study the renal tumor suppressor Lkb1/Stk11, we uncovered several limitations of this technology for achieving gene deletion. Our efforts to understand these failings revealed the importance of mRNA nonsense-mediated decay (NMD) in predicting the ability of CRISPR to achieve gene deletion. Our findings delineate rules for improving the design of CRISPR reagents for gene editing studies and regenerative medicine goals, and reveal the importance of NMD biology when leveraging cancer genomic information for personalized medicine.

**USING STRESS ASSAYS TO STUDY DROSOPHILA IONO- AND OSMOREGULATION**

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Renal epithelial ion transport is essential to ion homeostasis at the organismal level, and it is dysregulated in several diseases, such as electrolyte disorders and hypertension. Our laboratory uses *Drosophila melanogaster* as a model to study ion transport processes relevant to human renal physiology. Two genes of interest are the Na(+)-K(+)-2Cl(-) (NKCC) cotransporter and the swell-activated chloride channel Bestrophin 1 (dBest1). Here, we develop *in vivo* stress assays to study organismal outputs of epithelial ion transport processes. Regulation of ion homeostasis is a key physiological mechanism during recovery from cold stress in flies. In the chill coma assay, comas were induced in flies by placing them on ice slurry for four hours. An activity monitoring device was used to measure the time of recovery. For the salt sensitivity assay, flies were placed on 0.2-0.6 M NaCl or KCl containing foods for 7 days to study the effects of varying salt concentration on lethality. The 50% recovery time was 150 minutes for control flies, 200 minutes for $NKCC^{null}$ flies, and 85 minutes for $dBest1^{null}$ flies. High salt resulted in 80% lethality in $dBest1^{null}$, 50% lethality in $NKCC^{null}$, and 10% lethality in the control. Stress assays prove to be useful methods to study fly osmoregulation and epithelial ion transport *in vivo.*

**IMPAIRED FIBROCYSTIN IN POLYCYSTIC KIDNEY DISEASE IS LINKED TO ABNORMAL VASCULAR FUNCTION**

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Autosomal Recessive Polycystic Kidney Disease (ARPKD) is caused by abnormal fibrocystin protein. We have shown that ARPKD (PCK model) has reduced vascularity and endothelial function, even prior to kidney dysfunction. The aim of this study is to determine whether these changes are linked to abnormal fibrocystin.

In order to link fibrocystin (point mutation in *Pkhd1*) to vascular dysfunction rat aortic endothelial cells (RAECs) were isolated from Sprague-Dawley rats and were transfected using either fibrocystin-targeting siRNA or scrambled siRNA (negative control). Real time RT-PCR was used to determine the knockdown efficiency of the siRNA. Matrigel assays were used to compare angiogenic capabilities of the two transfected groups. Expression of proteins involved in endothelial function were measured in siRNA and compared to control and PCK.

The real-time RT-PCR showed a 67% decrease in fibrocystin transcripts after using the fibrocystin-targeting siRNA. The matrigel assay showed reduced tube formation by the RAECs transfected with fibrocystin-targeting siRNA (17,777 µm total) compared to the negative control (30,288 µm, $p=0.025$). Western blots of RAECs transfected with fibrocystin-targeting siRNA showed a shift in some protein expression to appear more similar to PCK cells.

Abnormal fibrocystin linked to lack of vascularization and endothelial function, suggesting a direct involvement of this protein in the abnormal vascular structure and functions seen in PKD.

*P30-DK090728*

**PH INDUCED COMPACTION OF THE N-TERMINAL DOMAIN OF LIPIDATED CAVEOLIN-3.**

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Caveolae are 50-100 nm omega-shaped invaginations in the plasma membrane that are involved in cellular signaling and trafficking. Caveolar biosynthesis and stabilization are dependent upon caveolins, which are 21-24 kDa scaffolding proteins in the plasma membrane. Caveolin-3 (Cav3), the member of the caveolin family that is expressed in muscle tissue, has been implicated in the pathology of muscular and cardiac disorders. Structural characterization of Cav3 is necessary to determine the molecular basis of these diseases. Cav3 is characterized by its intramembrane horseshoe turn which facilitates cytoplasmic localization of both the N and C terminal domains. Our initial research has indicated that at low pH, the N-terminus of Cav3 weakly binds to the membrane interface, which may enhance oligomerization. The purpose of this study was to continue to determine how pH influences the structural and topological characteristics of Cav3. Cav3 was expressed in *E. coli*, and purified with affinity chromatography. Solution NMR was used to probe the structural characteristics of Cav3 at varying pH. Transverse relaxation-optimized spectroscopy (TROSY) and nuclear Overhauser effect spectroscopy (NOESY) experiments were conducted at two different pHs. Results indicate that the N-terminal domain adheres to the membrane at low pH, perhaps providing protection from lysosomal degradation. Further characterization of the structure of Cav3 will provide critical information on its role in various muscular diseases.

**TYPE 1 INTERFERON EXPRESSION FOLLOWING SMALL MOLECULE INHIBITION OF CYCLIC GMP-AMP SYNTHASE**

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The innate immune pathway consisting of cyclic GMP-AMP synthase (cGAS), its second messenger product cyclic GMP-AMP, and Stimulator of Interferon Genes (STING) plays a key role in cytosolic DNA sensing by initiating a type 1 interferon response. This pathway acts as a cellular anti-viral and anti-bacterial defense mechanism. However, self-DNA can also trigger this pathway; cGAS has been implicated in autoimmune disorders such as Aicardi-Goutières Syndrome. The development of small molecule antagonists of cGAS has potential utility as an autoimmune therapy. The lead compounds of a diverse high-throughput small molecule drug screen are being evaluated by various *in vitro* and cellular assays to characterize their inhibition of cGAS activity. The goal of this project was to establish quantitative PCR (qPCR) as a primary assay to assess the effectiveness of these potential immunomodulators in a murine primary cell culture of bone marrow-derived macrophages. Following stimulation with immunoreactive double-stranded DNA and treatment with candidate cGAS antagonists, beta interferon expression was measured by qPCR. Conditions were established and optimized for this qPCR testing. Work is ongoing to fully assess the efficacy of various small molecule antagonists in inhibiting cGAS and to determine their subsequent effects on interferon expression.

**PARALOG SPECIFIC FUNCTION OF *sec23a* AND *sec23b* IN COLLAGEN SECRETION**

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Zebrafish are an ideal model system for studies of human disease and congenital syndromes. Cartilage and bone development can be easily studied in zebrafish embryos due to tractable genetics and rapid development. Our laboratory previously identified a mutation in COPII vesicle component *sec23a/crusher* that disrupts cartilage development due to a defect in transporting collagen from the rough endoplasmic reticulum to the Golgi apparatus. Similar mutations in human patients manifested craniofacial dysmorphisms and skeletal defects. However, mutations in its closest paralog, *sec23b*, leads to anemia, which suggests that *sec23a* and *sec23b* paralogs may carry out distinct functions. We performed pairwise sequence alignment and identified a divergent 18-amino acid loop between primary sequences of *sec23a* and *sec23b.* Using mosaic gene replacement assays in *sec23a* mutant background, I tested the rescue efficiency of collagen transport by overexpression of *sec23a, sec23b, sec23a with b-loop, and sec23b with a-loop.* Overexpression of *sec23a* and *sec23b with a-loop* were able to restore collagen secretion in *sec23a* mutant chondrocytes whereas *sec23b* and *sec23a with b-loop* failed to clear intracellular accumulation of collagen. These results suggest that *sec23a* and *sec23b* paralogs carry out distinct functions that is mediated by the divergent 18-amino acid loop structure. This novel mechanism can potentially explain why mutations in *SEC23* paralogs result in different disorders. There is functional distinction between *sec23a* and *sec23b* mediated by a divergent 18-amino acid loop.

**SEARCH FOR METHYLGLYOXAL INDUCED DNA-PROTEIN CROSSLINK**

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Abstract: Methylglyoxal (MGO), is a byproduct of glycolysis that is capable of modifying proteins and DNA, often disrupting function. Recently, MGO has been reported to generate a DNA-protein crosslink between lysine and guanine *in vitro*. We sought to determine whether this crosslink is present in a relevant model for hyperglycemia. We treated HEK293 cells and *Artemia salina* with high glucose levels (25mM) to increase cellular MGO. Chromatin was then extracted and the proteins and DNA were digested into amino acids and nucleosides, respectively. Mass spectrometry was then used to analyze protein and DNA adducts in addition to the crosslinked species. The product of the reaction between MGO and deoxyguanosine, CE-dG, was found; however, the crosslink was not. We hypothesize that the crosslinked species is being repaired by nucleotide excision repair. In the future, to test this hypothesis we will do the same protocol in repair enzyme deficient cells in order to see if the crosslink can be found.

**THE ROLE OF NITRIC OXIDE IN ACUTE KIDNEY INJURY**

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Ischemic-reperfusion injuries (IRI) of the kidney can lead to permanent loss of function (End Stage Renal Disease, ESRD). Understanding the progression toward ESRD is a critically important challenge as preventive treatment could be more effective and economical than dialysis or transplant.

Depending on IRI severity, epithelial kidney cells lose function transiently or die. Monocytes/macrophages infiltrating the injured kidney initially display a pro-inflammatory (M1) phenotype characterized by expression of the inducible nitric oxide synthase enzyme (iNOS). M1 macrophages accelerate tubular cell injury (Lee et al., 2011). As the repair phase takes place, macrophages transition to a reparative (M2) phenotype where iNOS expression is absent. We hypothesized that nitric oxide (NO) generated by M1 macrophages plays a role in worsening kidney injury by inactivating normal protein function via tyrosine nitration.

*In vivo*, we show that unilateral IRI leads to increased levels of cleaved caspase-3 in the injured kidney but not in the contralateral control kidney. In vitro, we show that addition of DETA-NO (chemical nitric oxide donor) to cultured mouse proximal tubule (MPT) cells induces apoptosis as determined by decreased viable cell percentages and increased levels of cleaved caspase-3. The observed apoptosis correlates with the accumulation of nitrated proteins in the same cells. Therefore we suggest that NO contributes to IRI through the nitration of tyrosine residues which may induce tubular cell apoptosis via the caspase-3 pathway.

**REGULATION OF MICRORNA-218 IN CELLULAR MODELS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE**

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Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disease that results from mutations in the genes encoding polycystin-1 (pc1) and polycystin-2 (pc2). ADPKD leads to end-stage renal disease in approximately 70% of patients. Previous data from our lab have suggested potential upregulation of microRNA218 in human embryonic kidney (HEK) cells overexpressing pc1 and pc2, as well as in HEK cells overexpressing the 200-residue C-terminal tail of pc1. The C-terminal tail of pc1 is cleaved from full-length pc1 and trafficked to the nucleus, where it influences gene transcription. MicroRNA-218 is a key regulator of the Slit-Robo signaling pathway, which is critical in renal development, renal injury, and angiogenesis. We investigated the expression of mir218 and its downstream genes in cellular models, including HEK cells overexpressing pc1 and pc2, HEK cells overexpressing the C-terminal tail of pc1 (termed "p200"), and HEK cells overexpressing the C-terminal tail of pc1 with a deletion in the nuclear localization sequence that prevents it from trafficking to the nucleus (termed "p200delta"). We used **SYBR green** qRT-PCR to analyze mRNA expression patterns of Slit2, Robo1, and Robo2 in these cells, quantified by the delta-delta-Ct method. In our first biologic replicate the results suggested that the fold change of the genes Slit2, Robo1, and Robo2 decreased in the three overexpressing cell models. Unfortunately, the results were not consistent when the experiment was repeated, so further investigation of these findings will be necessary to determine whether there are biologically significant changes in Slit-Robo signaling in ADPKD.

Fold Change:

| **Slit2** | Replicate1 | Replicate2 | Replicate3 |
|---|---|---|---|
| WT | 1 | 1 | 1 |
| PC1+2 | .5 +/- .1 | .65 +/- .1 | 2.5 +/-.1 |
| p200 | .3 +/- .1 | 5.5 +/- .2 | 1.9 +/- .2 |
| p200delta | .8 +/- .2 | .5 +/- .5 | 1.4 +/- .2 |
| **Robo1** | | | |
| WT | 1 | 1 | 1 |
| PC1+2 | .75 +/- .1 | .7 +/- .1 | 2.0 +/- .05 |
| p200 | .6 +/- .1 | 3.0 +/- .2 | 1.5 +/- .05 |
| p200delta | 1.05 +/- .2 | 1.1 +/- .2 | 1.3 +/- .15 |
| **Robo2** | | | |
| WT | 1 | 1 | 1 |
| PC1+2 | .3 +/- .05 | .7 +/- .2 | 3.9 +/- .2 |
| p200 | .35 +/- .1 | 3.1 +/- 1.5 | 1.5 +/- .15 |
| p200delta | .25 +/- .05 | 1.2 +/- 1.0 | .7 +/- .15 |

# TUBULE AND INTERSTITIAL CELL DERIVED FIBRONECTIN PROMOTES CYST GROWTH IN ADPKD MODEL

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We have shown that inactivation of tubule epithelial Fibronectin (Fn1) reduces cyst growth in multiple genetic models of autosomal dominant polycystic kidney disease (ADPKD). As Fn1 is a secreted extracellular matrix protein, we speculate that Fn1 generated from interstitial cells might also contribute to cyst formation. We combined a collecting duct specific Cre recombinase transgenic line, *Pkhd1-Cre*, and an interstitial cell specific Cre recombinase, *Foxd1-Cre*, knockin mouse line. This allowed us to inactivate *Pkd1* conditional alleles along with one *Fibronectin* conditional allele to reduce the dosage of Fn1 from both type of cells. Western blotting confirmed further reduction in Fn1 protein expression in cystic kidneys with tubule and interstitial cell inactivation of one copy of Fn1 compared to tubule cell-only Fn1 inactivation. We found that reduced dosage of Fn1 in the collecting duct-only knockout ameliorates cystic burden and the cystic phenotype is reduced further when interstitial cell Fibronectin dosage is additionally decreased in double Cre *Pkhd1-Cre;Foxd1-Cre* mice. Therefore, Fn1 from both tubule epithelial cells and surrounding interstitial cells contributes to the progression of cyst formation in ADPKD models, and interstitial cells contribute to cyst formation through a paracrine mechanism.

**DETERMINING A MOLECULAR BASIS FOR GOODPASTURE'S DISEASE**

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Goodpasture's disease is an organ-specific autoimmune disease that causes acute renal failure and lung hemorrhaging. While little is understood regarding the etiology, patients display pathogenic autoantibodies that target collagen IV α345 protomers located in the kidneys and lungs. Novel sulfilimine crosslinks have been identified in collagen IV, and their presence is suspected of preventing autoantibody binding to collagen IV. Thus, the loss or absence of sulfilimine bonds appears to be a risk factor for the disease. Interestingly, the heme enzyme peroxidasin has been shown to catalyze sulfilimine crosslinking in α112 collagen IV protomers while evidence for a similar reaction in the α345 protomer has been elusive. We hypothesize that peroxidasin forms sulfilimine crosslinks in the a345 protomer, representing a key step in preventing Goodpasture's Disease. To test our hypothesis, we have utilized a human cell culture model of collagen IV scaffolds, as a new system for examining endogenous expression of α345 collagen IV and peroxidasin.

**REGULATION OF OXALATE TRANSPORTER SLC26A6 BY ASSOCIATED PDZ-SCAFFOLDING PROTEINS**

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Previous studies have demonstrated that SLC26A6-null mice develop hyperoxalemia, hyperoxaluria, and a high rate of calcium oxalate urolithiasis. SLC26A6 is an oxalate transporter located on the apical cell membrane of epithelial tissues, including kidney, intestine, and pancreas. Therefore, this transporter maintains oxalate homeostasis and reduces the risk of developing urolithiasis. Aim of the present study was to examine and compare the possible effects of PDZ-domain scaffolding proteins PDZK1 and PDZK2 on SLC26A6, as these proteins were found to associate with SLC26A6 by co-immunoprecipitation. *Xenopus laevis* oocytes were used to test whether co-expression of PDZK1 or PDZK2 would affect SLC26A6 activity. We compared wild-type and mutant SLC26A6, where the C-terminal 3-amino acid PDZ-binding motif was deleted. Oocytes were micro-injected with PDZK1 or PDZK2 cRNA and 48 hours later SLC26A6 function was assayed by measuring uptake of [14C] oxalate, and its expression was assayed by Western blotting. Injection of SLC26A6 cRNA induced far higher oxalate uptake compared to un-injected oocytes, confirming successful functional expression of SLC26A6. Expression of SLC26A6 was also confirmed by Western blotting. Effects of co-expression of PDZ scaffolding proteins on SLC26A6 function and expression have not been consistent and entail additional experimentation. In conclusion, successful functional expression of SLC26A6 in *Xenopus* oocytes has been demonstrated, and this experimental system will be used to evaluate the effects of PDZK1 and PDZK2 on SLC26A6 function and expression. It is important to understand what regulates SLC26A6 since this exchanger has a critical role in preventing hyperoxaluria and reducing the risk of developing urolithiasis.

**MUSCLE ENERGETICS IN CHRONIC KIDNEY DISEASE: A PILOT STUDY**

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The purpose of this study is to assess muscle quality in patients with chronic kidney disease (CKD) and healthy controls. Epidemiologic studies have shown that, despite similar muscle mass measurements, muscle strength is reduced as CKD progresses. Our hypothesis is that this decrement in muscle quality is due to decreased concentrations of phosphate compounds in the myocyte. Eighteen participants older than 45 with an estimated glomerular filtration rate (eGFR) of 15-60 ml/min/1.73m$^2$ and 6 healthy controls will be studied in a cross-sectional framework. All participants undergo physical function testing, anthropometry, bio-electrical impedance analysis, dual-beam x-ray absorptiometry, 24-hr urine collection for creatinine and $^{31}$P Magnetic Resonance Spectroscopy to assess myocellular energetic phosphates. Correlations between these factors, eGFR and other clinical parameters will be assessed with Spearman correlation coefficients. Subject recruitment is ongoing. To date, we have evaluated 8 participants. The mean age is 60 and the mean eGFR is 48.5 ml/min/1.73m$^2$. We have noted a striking variability in body composition, with body fat percentage ranging from 26.3% to 44.3%, and in physical function with performance on the short physical performance battery ranging from a score of 1-4 on balance, 2-4 on the 4m walk, and 1-3 on the chair stand. Given this variability, we are confident that significant differences in myocellular phosphate content will be shown when full recruitment is achieved. If our hypothesis is supported, the data can be used for larger interventional studies evaluating ways for improving creatine metabolism in CKD patients.

**SIGNIFICANCE AND BIAS OF UNQUANTIFIED STONE BURDEN DATA IN RADIOLOGY REPORTS FOR COMPUTERIZED TOMOGRAPHY SCANS**

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To determine the significance and bias of stone burden data omitted from radiological reports on computerized tomography (CT) scans.

A sample of patients whom received a CT evaluation during a symptomatic kidney stone episode was split into two groups: those with radiological reports stating stone sizes as “tiny,” and those with reports missing data on largest stone diameter. The corresponding CT’s were then re-examined to quantify maximum diameter of the largest stone in each kidney along two axes.

Of 182 “tiny” stones from 131 patients, 167 (92%) had maximal diameters <3 mm. Of 1,352 stones reported as “missing” from 676 patients, there was no stone found on re-assessment in 58% of cases. However, when a stone was present on re-assessment, the mean diameter was 2.7 mm (SD 1.6). Finally, patients who received radiologic reports with any missing stone burden data showed a greater risk of stone recurrence than those with complete reports over the 10 years following their episode (p=0.016).

Radiographic reports with omitted stone burden data included a combination of data entry error for a value of zero stones and data entry error with an identifiable stone diameter, which may bias results based upon stone diameter in opposite directions. Despite this, patients with missing stone diameters experienced greater risk of recurrence, suggesting the bias from identifiable stones may be quite marked.

*R25-DK101405*

**DIFFERENTIAL LOCALIZATION OF NOVEL V-ATPASE INTERACTING PROTEINS IN THE KIDNEY**

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The multi-subunit proton pump V-ATPase functions to regulate luminal pH in several body regions. Various V-ATPase subunit isoforms are differentially expressed throughout the kidney, with the highest level of expression of the B1 isoform in collecting duct intercalated cells (IC). Our recent co-immunoprecipitation studies using B1 V-ATPase subunit antibodies revealed several interacting proteins potentially involved in regulating V-ATPase activity. The proteins ARHGEF7, RPS6KA3, and OXR1 were of particular interest due to their high affinity for V-ATPase and their seemingly unrelated documented functions. Wild type rat and mouse kidneys were examined using immunofluorescence staining to identify possible co-expression and co-localization patterns of these proteins with V-ATPase. Specificity of the antibodies used for staining was verified by Western Blotting with mouse kidney and brain lysates. ARHGEF7 was shown to co-localize with V-ATPase primarily in the proximal tubules; RPS6KA3, in the proximal tubules and the cortical collecting duct IC; and OXR1, in the distal tubules and collecting duct IC. These results suggest region-specific V-ATPase interactions with all of these proteins, revealing new targets for regulatory pathways of this critical enzyme on a region-specific basis.

**INTEGRIN SUBUNITS α3 AND α6 MEDIATE FGF10-DEPENDENT SIGNALING EVENTS IN COLLECTING DUCT CELLS BOUND TO LM511**

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The collecting system of the kidney develops from the ureteric bud (UB), which undergoes branching morphogenesis, a process regulated by multiple factors, including cell–extracellular matrix (ECM) interactions. Integrins are the principal cell receptors for ECM and the laminin (LM)-binding integrins (Itg) α3b1, a6b1 and a6b4 are most important integrin's in this process. In addition growth factors, like fibroblast growth factors (FGFs) are involved in cell signaling pathways in this developmental process. The role of laminin-binding integrins, α3β1, α6β1 and α6β4, in FGF10-dependent signaling events that regulate renal branching morphogenesis has not been investigated. In this study, we used collecting duct cells (CD) which lack specific integrin subunits to identify these interactions. Itg$\alpha3^{f/f}\alpha6^{f/f}$ and Itg$\alpha3^{-/-}\alpha6^{-/-}$ CD cells were plated on LM-511, treated with FGF10 and after which they were assessed for spreading. In addition, CD cell lysates were immunoblotted to determine Akt activation. . Itg$\alpha3^{-/-}\alpha6^{-/-}$ CD cells showed reduced spreading and Akt activity. Akt activity was also reduced in Itg$\alpha3^{f/f}\alpha6^{f/f}$ CD cells treated with Akt Inhibitor IV. These data suggest that that integrin α3b1 and α6 containing integrins interactions with LMs regulate FGF10-dependent activation of Akt signaling which mediates CD cell spreading .Thus, the interaction of FGF10 with LM binding integrins α3β1, α6β1 and α6β4 may play a role in UB branching morphogenesis.

**SPECIFIC RENAL PARENCHYMAL-DERIVED URINARY VESICLES IDENTIFY AGE-ASSOCIATED STRUCTURAL PATHOLOGY IN KIDNEYS FROM LIVING KIDNEY DONORS**

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Clinically relevant anatomical pathology associated with aging in the kidney is not detectable without invasive procedures. This study assessed whether specific populations of urinary extracellular vehicles (EVs) reflect the macro- and microstructure of kidneys derived from adult living kidney donors in whom glomerular filtration rate and urinary albumin are within the normative range.

Computer tomography (CT) angiogram and random urine samples were obtained from age-stratified (18-70 years) living kidney donors (n= 69 men and 69 women) prior to kidney donation. A kidney biopsy was obtained during transplantation. Macro-structural CT analyses included indices of nephron hypertrophy (increased cortical volume) and nephrosclerosis (decreased cortical volume and surface roughness). Micro-structural biopsy analyses included indices of nephron hypertrophy (glomerular volume, cortical volume per glomerulus, and mean profile tubular area) and nephrosclerosis (% fibrosis, % glomerulosclerosis, arteriosclerosis, and arterial hyalinosis). EVs (>0.2 micron) in cell-free urine were analyzed by digital flow cytometry using fluorophore conjugated renal cell-specific antibodies with or without annexin-V, and normalized to urine creatinine.

Nephron hypertrophy was negatively associated ($P<0.05$) with urinary EVs positive for phosphatidylserine (PS), monocyte chemoattractant protein-1 (MCP-1), and those derived from mesangial cells and parietal cells of Bowman's capsule. Nephrosclerosis positively associated ($P<0.05$) with urinary EVs positive for PS and MCP-1, and negatively ($P<0.05$) with EVs derived from podocytes and parietal cells.

Specific renal cell marker positive populations of urinary EVs may non-invasively identify the underlying structural pathology of the kidney.

*R25-DK101405*

**INHIBITION OF CALCIUM-MEDIATED PODOCYTE INJURY AS A STRATEGY TO PREVENT KIDNEY FAILURE**

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The human filter barrier is responsible for filtering more than 180 liters of plasma per day. Damage to the filter barrier results in protein spilling into the urinary space, a condition called proteinuria. It has been previously shown that a calcium influx through transient receptor potential canonical 5 (TRPC5) channel results in cytoskeletal rearrangements in podocytes and treatment with a TRPC5 specific inhibitor, ML204, prevents these changes in mice. However, it is unclear whether inhibition of TRPC5 can prevent or halt proteinuria in chronic kidney disease. Transgenic rats with podocyte-specific over-expression of Angiotensin II Type 1 Receptors (AT1R) serve as an ideal model of chronic kidney disease as these rats develop proteinuria and die of kidney disease at 40 to 50 weeks. Using AT1R transgenic rats, we tested whether TRPC5 inhibition can prevent or halt the progression of chronic kidney disease. AT1R transgenic animals were treated with either PBS or ML204 every 12 hours for 2 weeks and urine samples were collected 3 days prior to treatment and every 7 days during treatment. Protein quantification of albumin showed that inhibition of TRCP5 channel by ML204 arrests chronic proteinuria in AT1R transgenic rats.

## CIRCULATING CELL-FREE DNA: A DIAGNOSTIC BIOMARKER FOR RENAL CELL CARCINOMA

**Purpose**: Rapid and noninvasive biomarkers are critical in identifying renal cell carcinomas (RCCs) at an early stage. Given that circulating cell-free DNA (cfDNA) has been shown to be a promising diagnostic biomarker in multiple biofluids in other cancers and The Cancer Genome Atlas (TCGA) has identified 14 genes associated with RCC, this study aims to correlate cfDNA levels with clinical data and identify somatic mutations in plasma that could play a role in the early diagnosis, monitoring, and prognosis for RCC.

**Methods**: Plasma and whole blood samples were extracted from 9 clear cell RCC patients. Leukocyte and plasma DNA were isolated using Qiagen DNA Mini and Blood Mini kit, and plasma cfDNA levels were quantified using Qubit Fluorometer. 14 genes associated with RCC were selected from TCGA, DNA regions to be sequenced were amplified using Fluidgm Access Array, and whole exome sequencing was performed on amplified samples using Illumina MiSeq. FASTQ files were analyzed in Galaxy workflow, mutations were manually inspected using human hg19 in IGV, and somatic mutations called were evaluated in COSMIC and cBioPortal. Associations between cfDNA levels and tumor stage and grade were evaluated, and somatic mutations called for the patient cohort were reported.

**Results**: There was a nonsignificant trend toward higher cfDNA levels in tumors with higher stage ($p=0.105$) and grade ($p=0.202$). Moreover, one somatic mutation for one patient was reported in COSMIC and cBioPortal and other somatic mutations called were not previously documented.

**Conclusions**: Plasma ctDNA levels could be useful for monitoring patients during follow-up or for active surveillance. Previously undocumented somatic mutations could serve as potential tumor-specific genetic alterations, which could play a role in the early diagnosis, monitoring, and prognosis for RCC.

Abstract without subheadings:

The development of noninvasive biomarkers that can be used clinically to detect and monitor renal tumors continues to be a major challenge. Recent studies have shown highly fragmented DNA released from tumor cells, called circulating cell-free DNA (cfDNA), can serve as an accurate biomarker for the diagnosis of multiple cancers. For clear cell renal cell carcinoma (ccRCC), while some studies have demonstrated cell-free DNA levels in plasma are higher in metastatic patients than in patients with localized ccRCCs and others have shown mutations in select histone/chromatin regulator genes and genes that activate the mTOR pathway are associated with worse prognosis in serum and tumor tissue, little work has been done that associate these findings in plasma with clinical data. To investigate these associations, plasma samples from nine ccRCC patients were extracted and their cfDNA levels were quantified. Deep sequencing was performed, somatic mutations called from the Galaxy workflow were evaluated in COSMIC and cBioPortal, and mutational frequencies for each gene were compared with The Cancer Genome Atlas (TCGA). Results show a nonsignificant trend toward higher cfDNA levels with higher tumor stage and grade, suggesting plasma cfDNA levels could be useful for monitoring patients during follow-up or for active surveillance. Previously undocumented somatic mutations have also been discovered, suggesting these mutations could serve as potential tumor-specific genetic alterations. Further investigation is needed to determine whether these mutations are present in tumor tissue and could be associated with clinical data, which could play a role in the early diagnosis, monitoring, and prognosis for RCC.

**RECOMBINANT PROTEIN EXPRESSION OF DENGUE VIRUS-SPECIFIC HUMAN MONOCLONAL ANTIBODY 5J7**

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The objective of my Summer Internship was to learn methods of characterizing antiviral human monoclonal antibodies produced using hybridoma technology. For this purpose, I used a recombinant anti-dengue antibody, 5J7, as a candidate molecule for expression and purification. Bacterial clones containing the plasmids encoding the heavy and light chains of the antibody variable genes were cultured for 16 h and DNA was extracted by the alkaline lysis method. HEK293 Freestyle cells were co-transfected transiently with purified DNA encoding the antibody heavy and light chains and cultured for 7 days. The secreted antibody was purified on Protein G affinity columns and quantified. The purified antibody preparation was resolved on an SDS-PAGE gel and stained with Coomassie Blue reagent to reveal a single ~150 kDa band, suggesting a properly assembled IgG molecule. 5J7 IgG then was tested for binding to antigen in an enzyme-linked immunosorbent assay (ELISA). As expected, the recombinant form of this type-specific antibody bound to dengue serotype 3 virus but not to the other three virus serotypes. Recombinant antibody expression is one of the several steps in characterizing antibodies, and I have performed this process successfully with 5J7 IgG.

**IDENTIFYING STIMULI RESPONSIBLE FOR ABNORMAL α-ACTININ-4 PHOSPHORYLATION IN HUMAN PODOCYTES**

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Protein phosphorylation is a critical post-translational modification necessary for regulating protein function and basic cellular processes. Point mutations in the gene ACTN4, which encodes for the α-actinin-4 protein responsible for bundling actin filaments, have been associated with the development of focal segmental glomerulersclerosis (FSGS). Recent data suggests that abnormally phosphorylated ACTN4 behaves similarly to mutant ACTN4. We hypothesize that abnormal α-actinin-4 phosphorylation could potentially lead to FSGS. However, little is known about the regulation of ACTN4 phosphorylation. Thus, we aim to identify a stimulus or stimuli that could change the level of ACTN4 phosphorylation in human podocytes. Phos-tag gels were used to detect ACTN4 phosphorylation after stimuli treatment. We found that the human vascular endothelial growth factor (hVEGF165) stimulated an increase in ACTN4 phosphorylation.

Future directions include testing whether the phosphorylation effect in response to the hVEGF165 treatment is time or dose-dependent and determining at which ACTN4 site this phosphorylation is occurring by mass spectrometry.

**VINCULIN IS REQUIRED FOR ZONULA OCCLUDENS-1 (ZO-1) STABILIZATION FOLLOWING INJURY**

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The purpose of our study is to explore the role vinculin plays in maintaining the integrity of the glomerular filtration barrier. Recent evidence suggests the importance of focal adhesion proteins in podocytes, which regulate the cell matrix interactions with the glomerular basement membrane. Vinculin is one such protein and participates in linking actin filaments to integrins as well as intercellular tight junction proteins. To determine the potential role of vinculin in podocytes, we used the Cre-Lox system based on *Podocin-Cre* to generate podocyte-specific *Vcl*-KO mice. These mice are viable and born in the normal Mendelian frequency without evidence of glomerular damage. However, the ablation of vinculin in podocytes was found to exacerbate albuminuria in mice treated with either lipopolysaccharide (LPS) or nephrotoxic serum (NTS) when compared to control (284±40 vs 411±31 µg/mg, and 205±28 vs. 314±38 µg/mg, $P<0.05$). Furthermore, ultrastructural evidence suggests worsened podocyte foot process effacement in the mutant mouse following LPS or NTS treatment when compared to control. *In vitro*, LPS or protamine sulfate treated primary podocytes harvested from control and Pod-*Vcl*-KO mice revealed no significant difference in focal adhesion number, localization, or size. However, these conditions induced a mislocalization of ZO-1 to the cytoplasm in the Pod-*Vcl*-KO podocytes as observed by immunofluorescence. Our results suggest that podocyte specific loss of vinculin worsens proteinuria and leads to the mislocalization of tight junction protein ZO-1 following glomerular injury.