July 2025 Research Jamboree

Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

The CAIRIBU Interactions Core is led by PI, Kristina Penniston, PhD and funded by the NIDDK (U24-DK-127726)

ABSTRACT BOOKLET

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INTRACELLULAR ATP IS A DRIVER OF CATHETER BIOFILM FORMATION IN CHRONICALLY CATHETERIZED PATIENTS

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INTRODUCTION AND OBJECTIVE: Catheter-associated urinary tract infections (CAUTIs) are among the most common healthcare-associated infections, leading to significant morbidity, mortality, and financial burden on the healthcare system. Up to 50% of long-term catheterized individuals experience catheter blockage due to biofilm and encrustation. However, the biological drivers of biofilm formation in catheter isolates remain poorly understood. This project aimed to study the biofilm-forming capacity and key drivers of biofilm formation in catheters from chronically catheterized patients.

METHODS: A total of 38 urinary catheters (34 latex, 4 silicone) were collected from the Palo Alto Veterans Affairs catheter change clinic. Biofilm was removed from the catheters using sequential vortexing and sonication, after which the material was suspended in 10 mL of sterile PBS. Biofilm biomass was quantified using the crystal violet assay. Intracellular ATP levels, turbidity, CFUs, and protein concentration were measured from biofilm eluates. Proteomic analysis was performed on lysed and digested samples to compare protein expression between high and low biofilm formers. A validation experiment was conducted using *Enterococcus faecalis* ATCC 29212 cultured under varying pH conditions (7.5, 6.5, 5.5) to assess changes in intracellular ATP and biofilm formation.

RESULTS: Biofilm quantification revealed three distinct groups: high, moderate, and low biofilm formers. High biofilm formers had significantly elevated intracellular ATP ($p < 0.001$), which strongly correlated with biofilm biomass ($R^2 = 0.76$). Other parameters, including CFUs, protein content, and turbidity, did not distinguish between groups. Proteomic analysis identified increased ATP-generating proteins (e.g., atpA) across multiple uropathogens and reduced proton leakage pathways in high biofilm formers. Given that bacteria can leverage the charge regulation effect to adhere to surfaces, we showed that decreasing the pH resulted in increased intracellular ATP and biofilm production.

CONCLUSIONS: This study demonstrates that intracellular ATP is a significant and reliable marker of biofilm formation in catheter-associated uropathogens. Our findings suggest that increased intracellular ATP production may promote biofilm formation.

HEALTH RELEVANCE: Understanding how intracellular ATP supports bacterial viability and biofilm formation is important, particularly since host environments and urinary pH shift dynamically during infection and treatment. This work may provide novel targets for addressing CAUTIs and blocked catheters, thus reducing the infection burden on these patients.

PELVIC RADIATION DID NOT IMPACT VAGINAL COLLAGEN DEPOSITION OR EPITHELIAL THICKNESS IN AGED RATS

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INTRODUCTION AND OBJECTIVE: In the United States, over 19 million women are pelvic cancer survivors. Pelvic cancers are most commonly treated with radiotherapy (RT). Frequent side effects of RT include vaginal stenosis**,** fibrosis, and dryness, which lead to sexual dysfunction and ultimately a decreased quality of life. Most pelvic cancer survivors are also post-menopausal, which independently causes these symptoms. A previously established model in young rats demonstrated that RT reduced vaginal blood flow, thinned the vaginal epithelium, and increased vaginal contraction. However, it is unknown if the adverse effects of RT are greater in aged rats. Therefore, this study will assess if RT differentially affects the vaginal extracellular matrix and epithelium in young and old rats.

METHODS: Young (12 weeks) and old (15 months) female Sprague-Dawley rats were exposed to a single dose of pelvic radiation (Sham: 0 Gy, n=4-7 or RT: 20 Gy, n=4-7). At 4 and 9 weeks post-RT, vaginas were collected, fixed, sectioned, and stained. Picrosirius red staining and imaging with polarized light assessed collagen content. Masson's trichrome stain was used to evaluate vaginal epithelial thickness, count vaginal layers, and identify vaginal epithelial cells (basal, intermediate, apical). To quantify epithelial thickness and collagen deposition, ImageJ was used. For all parameters assessed, three representative images (40x) were taken, analyzed, and averaged. A two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc tests statistically compared experimental groups.

RESULTS: Total vaginal collagen was increased in old rats compared to young rats, independent of RT ($p<0.05$). RT did not change total vaginal collagen in either young or old rats. Vaginal epithelial thickness was not affected by aging or RT. In young rats 9 weeks post-RT, the total number of epithelial layers and the number of intermediate epithelial cell layers were decreased ($p<0.05$). In all other groups, vaginal epithelial layer number did not change.

CONCLUSIONS: As previously established, aging increased vaginal collagen deposition. However, in aged rats, radiation did not alter vaginal collagen content, epithelial thickness, or epithelial stratification. Collagen remodeling is a lengthy process and 9 weeks post-RT may be insufficient to observe changes. Future studies will assess periods beyond 9 weeks.

HEALTH RELEVANCE: Post-RT, pelvic cancer survivors suffer from sexual dysfunction. To improve lives in this population, increased understanding of the side-effects of cancer therapies is essential.

BONE ARCHITECTURE AND STRENGTH IN PATIENTS WITH SECONDARY HYPERPARATHYROIDISM FOLLOWING PARATHYROIDECTOMY

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INTRODUCTION AND OBJECTIVE: Patients with chronic kidney disease develop over-activity of the parathyroid gland to control calcium levels in the blood and may require removal of the gland (parathyroidectomy) if activity cannot be controlled with medications. Following parathyroidectomy, "hungry bone syndrome" occurs, in which serum calcium levels decrease and aggressive calcium supplementation is required. Improvements in dual-energy X-ray absorptiometry (DXA) areal bone mineral density (aBMD) have been reported in patients undergoing parathyroidectomy but changes in cortical and trabecular microarchitecture, vascular calcification, and muscle strength post-parathyroidectomy are unknown.

METHODS: This one-year prospective study of patients on hemodialysis obtained measurements prior to parathyroidectomy and at 3-month and 12-month follow-up. We obtained bone biomarkers and parathyroid hormone levels, high-resolution peripheral quantitative computed tomography (HRpQCT) to assess bone microarchitecture, isokinetic dynamometry to assess muscle strength, DXA to assess bone density, strength via hip structural analysis, and body composition. Ten patients were enrolled (five men; five women) along with twenty-one matched controls.

RESULTS: At baseline, study participants had significantly lower spine and hip aBMD compared to controls. We observed significant improvement in DXA hip aBMD at 3 and 12 months (+0.087±0.045 in 12 months) and spine aBMD (+0.04±0.073 in 12 months). No improvements were observed in the one-third radius.

CONCLUSIONS: Significant gains in section modulus at the hip were observed, suggesting improved ability of bone to withstand force (+0.27±0.13 at femoral neck in 12 months). Overall improvement in bone density and strength supports possible reversibility of bone losses post parathyroidectomy. Additional analysis will assess bone biomarkers, HRpQCT, and isokinetic dynamometry.

HEALTH RELEVANCE: This study and its findings verify the effectiveness of parathyroidectomies in the treatment of secondary parathyroidectomy, establishing its validity as an effective patient procedure.

INVESTIGATING GENETIC RISK FACTORS FOR KIDNEY DISEASE USING LARGE-SCALE GENOMIC DATASETS

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INTRODUCTION AND OBJECTIVE: Kidney disease impacts over 1 in 7 adults, and many conditions possess genetic factors that impact progression and symptoms. By understanding the genetic variants contributing to kidney disease, it can pave the way for further discovery in diagnostics and treatment. Moreover, genetic risk factors increase likelihood of developing kidney disease, so it is critical in early detection and diagnosis. The objective of this study is to identify and assess emerging renal genetic variants from a case control study using genomic datasets and analysis.

METHODS: We conducted a case control study utilizing two large de-identified health data studies, NIH All of Us and UK BioBank, and a genetic testing database from Natera. With the genomic data from these databases, we highlight key variants to focus deeper investigation on. We further analyze these genes using literature review, variant analysis, and investigation of knockout model findings to narrow our focus on likely genes underlying kidney disease. Variant quality is also assessed using Sherlock and Integrative Genomics Viewer. This multi-step approach enables us to investigate genetic variants underlying kidney disease.

RESULTS: From a set of 131 genes with European ancestry that may underlie kidney disease, we identified 7 genes for further analysis on genetic variants. These genes are Itsn2, Cabin1, Gcdh, Notch3, Mib1, Erbb4, and Manba, which all hold great potential for linkage to kidney disease. With this focused list of genes, we are honing in on the variant analysis, understanding the loss of function effect a variant may have and assessing the quality of the genetic variant.

CONCLUSIONS: By utilizing large datasets and further analyzing to create a focused set of genetic variants, we can uncover novel genes that may be linked to kidney disease. Working through these genes and narrowing it down enables for further discoveries on a genetic variant and holds great significance in early detection and disease progression.

HEALTH RELEVANCE: Kidney genetics play a key role in finding underlying causes, determining treatment options, predicting condition progression, and family planning. Through uncovering novel renal genes, we can expand genetic counseling and the care that patients receive.

THE EMERGING LANDSCAPE OF UROBIOME RESEARCH: AI PREDICTIONS IN COMPARISON TO THE GUT MICROBIOME

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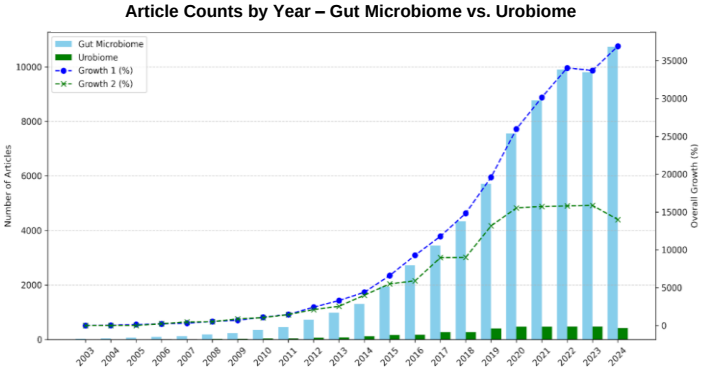
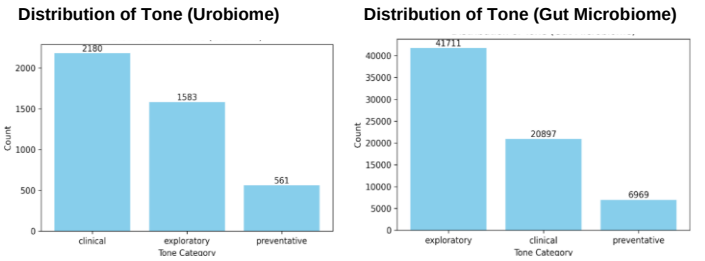
INTRODUCTION AND OBJECTIVE: Despite rising interest in the urinary microbiota (urobiome) over the past decade, a comprehensive understanding of its development remains underexplored. This study conducts a bibliometric analysis of published literature between the urobiome and the more established field of gut microbiome research aiming to evaluate and describe the developmental trajectory of urobiome research.

METHODS: On 7/08/2025, the PubMed API was queried using domain-specific terms for both urobiome and gut microbiome publications. Reviews and duplicates were removed. Each entry contained the title, publication year, author(s), abstract, affiliations, and tone using HuggingFace's Zero-Shot Classification model.

RESULTS: The search yielded 4,324 unique urobiome- and 69,577 gut microbiome-related publications. The earliest gut microbiome reference dated to 1965 vs. 1978 for the urobiome. Gut microbiome publications demonstrated exponential growth, peaking in 2024 with >10,000 publications, whereas urobiome research reached a maximum of just under 500 publications annually, in 2023 (***Fig., panel A***). Geographical analysis revealed that urobiome research is predominantly U.S.-based, while gut microbiome research is largely driven by China. Tonal analysis indicated that urobiome research is largely clinical with an emphasis on diagnostics. In contrast, gut microbiome literature is more exploratory and diversified in tone (***Fig., panel B***).



Figure, panel A. Gut microbiome research demonstrates both earlier and more rapid expansion vs. urobiome research, which has grown at a much slower pace, highlighting a substantial disparity in field development and publication volume.



Figure, panel B. The stark contrast in tone distribution displays the differences in research focus between the two fields: gut microbiome literature exhibits a broader exploratory base while urobiome research leans more toward clinical contexts.

CONCLUSIONS: The urobiome field lags behind the gut microbiome in both volume and research depth. A significant gap exists in preventative studies within urobiome research, possibly affecting translatability within the field. This lag may be influenced by limited funding or technical constraints. Additionally, the gut microbiome benefits from a larger biomass and established model systems compared to the urobiome; this may favor mechanistic and translational research in the gut. Finally, slower growth of the urobiome field could be influenced by social stigmas and hidden disease burden that are not as prevalent in gut microbiome research. Addressing these barriers is essential for developing a broader research agenda.

HEALTH RELEVANCE: A possible primary shift toward exploratory and preventative inquiry could enhance the clinical relevance and growth of the urobiome field.

NON-INVASIVE ASSESSMENT OF BLADDER DIVERTICULUM BIOMECHANICS USING DYNAMIC MAGNETIC RESONANCE IMAGING

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INTRODUCTION AND OBJECTIVE: Bladder diverticula (BD) are pouches extending outside the bladder lumen from herniation of the bladder mucosa through the muscularis propria often in the setting of chronic bladder outlet obstruction (BOO). These may result in lower urinary tract symptoms (LUTS) or be asymptomatic and are uncommon but still notable in urologic patients. The clinical relevance of BD in LUTS treatment decisions is unclear, and the biomechanical effects of BD treatment versus surveillance are similarly unstudied. Dynamic magnetic resonance imaging (MRI) is a non-invasive method used to study BD biomechanics to determine the effects on voiding dynamics, post-void residual (PVR), and to guide treatment for patients.

METHODS: Three male patients (ages 30, 69, and 55) underwent voiding-phase MRI analysis. Images of the voiding bladder were obtained by imaging the subjects with a 3D Differential Subsampling and Cartesian Ordering acquisition sequence and segmented using MIMICS. Each bladder and diverticula were segmented, including the urine inside the bladder, to determine PVR, volumes, and biomechanics of the BD.

RESULTS:

Asymptomatic BD (30): During voiding, the BD protrudes from the bladder during the contraction of the detrusor muscle resulting in urine reuptake into the BD. At the peak urinary flow rate, the BD reaches its max volume (65 cc). After the void, the BD empties the urine back into the bladder, resulting in the bladder increasing by 64 cc.

LUTS BD (69): At the beginning of the void; the diverticulum expands outward, filling it with urine. During voiding, the BD decreases in volume while the bladder stays relatively the same. The subject empties the BD before the bladder, showing that there was an increased pressure in the BD before voiding. This shows differences between asymptomatic and LUTS patients with BD.

Healthy (55): The healthy bladder voids at a near constant rate at an average of 6.5 cc/s with coordinated detrusor contraction resulting in a normal void.

CONCLUSION: To our knowledge, this is the first study investigating the biomechanics of BD using dynamic MRI. MRI offers a valuable tool for understanding BD biomechanics and how these interact with bladder function. Going forward, the observed differences in BD urodynamics can inform surgical management decisions.

HEALTH RELEVANCE: BD can occur in patients of all ages and can vary from debilitating to asymptomatic. LUTS from BD and BD caused by BOO both decrease the quality of life (QOL) of patients. By studying the biomechanics of BD, clinical interventions can be advanced to improve QOL. This methodology allows for the study of the role of BD in LUTS diagnosis and treatment decision making.

IMPACT OF CHITOSAN PHYSICOCHEMICAL PROPERTIES ON ANTIMICROBIAL ACTIVITY AND UROTHELIAL INTERACTIONS

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) are among the most common bacterial infections, particularly in patients with kidney stones where biofilms shield bacteria from antibiotics. Chitosan, a cationic biopolymer, has demonstrated promise in biofilm disruption but can also exfoliate the urothelium, the specialized epithelial lining of the urinary tract. This study aims to evaluate how chitosan's physicochemical properties, specifically degree of deacetylation (DDA) and molecular weight (MW), affect the antimicrobial activity, exfoliative capacity, and cytotoxicity of different derivatives to better characterize their efficacy and biocompatibility.

METHODS: Chitosan and salt derivatives varying in DDA (70–95%), MW (100–1000 kDa), and concentration (0.0005–0.005%) were assessed. Antimicrobial activity was quantified via OD600 absorbance of *E. coli* and *S. aureus* after 4-hour exposure. Cytotoxicity will be evaluated in T24 and UMUC3 bladder cancer cells using lactate dehydrogenase (LDH) release assays. Exfoliative effects will be examined via histology of *ex vivo* pig ureters, with select formulations retested in fresh tissue. *In vivo* bladder imaging with fluorescently labeled chitosan will be conducted in catheterized animal models. Immunofluorescent staining (DAPI, Keratin 5, Keratin 20) will be used to visualize urothelial exfoliation over time.

RESULTS: Preliminary results indicate that most chitosan derivatives exhibit antimicrobial activity, with significant bacterial killing in both *E. coli* and *S. aureus* cultures. Ongoing and future analyses will quantify cytotoxicity through LDH absorbance and assess exfoliation histologically. Fluorescence imaging is expected to show chitosan-urothelium interactions and selective exfoliation of umbrella cells.

CONCLUSIONS: This study will define how chitosan's DDA and MW influence its antimicrobial, cytotoxic, and exfoliative properties. The findings aim to inform rational design of chitosan formulations tailored for safe and effective intraluminal applications.

HEALTH RELEVANCE: Optimized chitosan formulations have the potential to improve UTI treatment and prevention, reduce biofilm-related complications, and enhance diagnostic accuracy by safely increasing cellular yield in urine cytology and facilitating urothelial tumor clearance.

PSA VARIABILITY TO DIAGNOSE CLINICALLY SIGNIFICANT PROSTATE CANCER AND PREDICT PROGRESSION DURING ACTIVE SURVEILLANCE

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INTRODUCTION AND OBJECTIVE: Prostate-specific antigen (PSA) is a widely used biomarker for prostate cancer screening, but its diagnostic accuracy is limited. PSA levels can fluctuate due to benign conditions, making it challenging to distinguish clinically significant cancer. This study investigates whether PSA variability can be used to identify clinically significant prostate cancer at diagnosis and predict disease progression in patients on active surveillance for low-grade disease.

METHODS: We initially identified 48,623 men diagnosed with localized prostate cancer and a modestly elevated PSA within a large, nationally distributed integrated healthcare system. We extracted all PSA measurements from the 5 years prior to diagnosis and calculated PSA variability using multiple metrics and tested for associations between PSA average real variability (ARV) and the presence of clinically significant prostate cancer at diagnosis. We are also extending the analysis to the Prostate Active Surveillance Study (PASS) cohort to determine whether PSA variability predicts progression from surveillance to treatment.

RESULTS: Lower PSA variability over the five years prior to diagnosis was associated with a higher likelihood of clinically significant prostate cancer at diagnosis, even after adjusting for baseline PSA and PSA velocity. These findings support PSA variability, particularly ARV, as a potential diagnostic marker. Ongoing work will assess whether PSA variability can also predict disease progression and the transition to active treatment among men under active surveillance.

CONCLUSIONS: Lower PSA variability was significantly associated with the presence of clinically significant prostate cancer, supporting PSA ARV's potential as a low-cost, accessible tool to improve diagnostic precision and risk stratification. These findings lay the groundwork for ongoing work evaluating PSA variability's role in guiding treatment decisions for patients on active surveillance.

HEALTH RELEVANCE: Incorporating PSA variability into routine screening may improve early detection of clinically significant prostate cancer, reduce unnecessary biopsies, and enhance monitoring during active surveillance—ultimately supporting more precise diagnosis, management, and better patient outcomes.

A CLINICALLY RELEVANT BPH PATIENT DERIVED XENOGRAFT MODEL FOR EVALUATING BMP5 SIGNALING AND NON-INVASIVE MONITORING VIA ULTRASOUND

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is the non-cancerous enlargement of the prostate gland that impairs urinary function in older men. Current medical therapies show modest benefits, and surgeries can have significant side effects. Animal models enable preclinical testing of therapeutics but are limited by species- specific prostate responses and fundamental differences to human tissue. We utilized our BPH PDX model to determine the effects of BMP5 (which has been shown to be upregulated 50-fold in human BPH tissues) and BMP inhibitors on paired BPH or N PDX growth and survival.

METHODS: Paired BPH and normal (N) peripheral zone prostate tissue cores were collected from a patient undergoing cystoprostatectomy. The tissue was sliced and implanted under the kidney capsule of 30 male, immunocompromised Rag2$^{-/-}$ mice (n =15 BPH; n=15 N) supplemented with a testosterone pellet. After one month of grafting, mice were assigned to one of three treatment groups (vehicle, BMP5, or BMP inhibitor (BMPi)) and treated for 3 weeks. The small molecule inhibitor, K02288, was used as the BMPi for its specificity in targeting BMP receptor 1A, a known binding partner of BMP5. BMP5 was administered once weekly at 0.2mg/kg, while BMPi was administered three times weekly at 0.4 mg/kg. High-frequency ultrasound imaging was used to non-invasively monitor PDX volume, and transverse images were compared to paired *ex vivo* scans of harvested PDXs in ultrasound gel. Post treatment, the tissue grafts were harvested and analyzed via H&E and immunohistochemistry for Ki-67.

RESULTS: The paired BPH and N PDXs treated with BMPi showed significant reductions ($p< 0.001$) in weight compared to the vehicle. N PDXs treated with BMP5 significantly ($p<0.001$) increased in weight and proliferation whereas BPH PDXs treated with BMP5 did not significantly increase in weight or proliferation compared to the vehicle. H&E imaging showed mostly normal acini and secretory differentiation in both BPH and N PDXs even after grafting and treatment (total 59 days); however, some grafts did show minor metaplasia. Ultrasound tracked PDX volume (RMSE 0.072mm^3, n=3), providing a non-invasive method for *in vivo* monitoring.

CONCLUSIONS: This BPH PDX model recapitulates histologic and proliferative features of human prostate tissue and enables evaluation of BMP5 pathway modulation *in vivo*. BMPi treatment reduced graft weight and proliferation, supporting its potential as a therapeutic strategy for BPH. Ultrasound imaging enables non-invasive monitoring of PDX growth.

HEALTH RELEVANCE: This PDX model provides a platform for testing therapeutics like BMP inhibitors that could reduce BPH growth and improve patients' quality of life.

NEPHROCALCINOSIS IN THE KIDNEY ALLOGRAFT: A CASE SERIES FROM A SINGLE-CENTER

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INTRODUCTION AND OBJECTIVE: Kidney transplantation is the preferred treatment for end stage kidney disease. However, due to the limited availability of donor kidneys in California, the median wait time is approximately 5.5 years and can exceed more than 10 years. Long dialysis vintages due to prolonged wait times are associated with metabolic abnormalities and mineral disorders such as elevated blood serum phosphorous, parathyroid hormone, FGF23, and plasma oxalate. These disorders (e.g. tertiary hyperparathyroidism) are linked to higher rates of graft loss. The objective of the study is to identify clinical characteristics and risk factors for nephrocalcinosis that were diagnosed histologically on kidney transplant biopsy. The objective of the study is to identify clinical characteristics and risk factors for nephrocalcinosis that were diagnosed histologically on kidney transplant biopsy.

METHODS: We searched for all adult kidney transplant recipients who underwent a kidney transplant biopsy between 2006-2022 and excluded patients with a non-functional allograft. Biopsy indications were elevated creatinine, proteinuria, or surveillance.

RESULTS: We included clinical characteristics and the laboratory data from 20 patients with a histological diagnosis of nephrocalcinosis. Of those, 17 of those patients received a deceased donor kidney. The mean dialysis vintage was 6.88 years,

and 16 patients were on hemodialysis prior to transplant. Mean PTH before transplant is 1237.59 pg/mL, while the peak serum calcium was 11.38 mg/dL. Mean nadir creatinine was 1.15 mg/dL and was 1.73 at time of biopsy. For all 7 patients included in the 24-hour urine collection, hypocitraturia (defined as citrate of < 400 mg/day) was present.

CONCLUSION: Patients at risk for graft dysfunction from nephrocalcinosis may be those with long dialysis vintage and secondary or tertiary hyperparathyroidism. Quantifying calcium phosphate deposition on biopsy may identify patients who may benefit from an earlier parathyroidectomy for hyperparathyroidism treatment and healthier allograft outcomes.

Health application regarding this research is to advocate for kidney allograft preservation initiatives and find early interventions that could be beneficial.

INVESTIGATING THE RELATIONSHIP BETWEEN P2X3 AND CGRP EXPRESSION AND REFERRED PAIN IN INTERSTITIAL CYSTITIS

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INTRODUCTION AND OBJECTIVE: A 2019 cohort study reported that ~75% of enrolled patients with Urinary Chronic Pelvic Pain Syndrome (UCPPS) experienced pain outside the pelvic area (Clemens et al. 2019), though it is unclear exactly how this pain could originate from bladder disease itself. Interstitial cystitis (IC), a bladder disease under UCCPS, is characterized by increased urinary urgency and frequency and pelvic pain. Effective treatments do not exist for treating IC or associated referred pain. The Crawford Lab seeks to understand the molecular mechanisms of pain in IC by examining the expression of nociceptive markers, P2X3 and CGRP, in the L3-S1 dorsal root ganglia (DRG) in a cystitis mouse model. This study examines the L3-L5 DRG in particular, which contain varying abundances of paw and bladder afferents. I hypothesized that there would be an increase in P2X3 and CGRP expression in the L3-L5 DRG in the cystitis group.

METHODS: Adult female C57BL/6 mice had FastBlue tracer injected into the sural region of their hindpaws (where cystitis mice had previously shown hypersensitivity) and were randomly split into control and treatment groups. Mice were euthanized and perfused for tissue collection 48 hours after instillation of acrolein (cystitis group) or saline (vehicle control group) into their bladders via urinary catheter. L3-L5 DRG were isolated, paraffin-embedded, and sectioned onto slides for immunohistochemistry (IHC). A Thunder 3D Tissue Imaging Microscope DM6B-Z was used to acquire images of the stained slides. Regions of interest highlighting neurons to be analyzed within the images were generated using CellPose, converted to PNGs via ImageJ, and refined in NIS Elements-AR software. Data were extracted from NIS Elements into Excel, and comparisons were made in GraphPad using mixed-effects analysis and Kolmogorov-Smirnov tests where significance was determined at $p < 0.05$.

RESULTS: A significant difference in mean intensity of CGRP+ neurons was found between treatments in the L3 DRG specifically ($p=0.0171$). Cystitis did not significantly increase the number of total neurons or paw afferents expressing P2X3 or CGRP in the L3-L5 DRG. Measures of P2X3 mean intensity expression were also not significantly different.

CONCLUSIONS: The L3 DRG appears to be the most promising clinical target for treating referred pain in cystitis as it showed a significant increase in intensity of CGRP expression. Further molecular changes in the acute cystitis model may be detectable at the RNA level.

HEALTH RELEVANCE: By characterizing the molecular changes that occur in the L3-L5 DRG in a mouse model of cystitis, we hope to identify clinical targets for relieving referred pain in human patients with IC.

INVESTIGATING THE LINK BETWEEN ENVIRONMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCB) AND DEVELOPMENT OF ACQUIRED URINARY INCONTINENCE IN DOGS

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INTRODUCTION AND OBJECTIVE: Urethral sphincter mechanism incompetence (USMI) is the most common acquired urinary incontinence (UI) in female pet dogs, with clinical signs similar to stress or mixed incontinence in women. Loss of hormones (e.g. estrogen) after sterilization (removal of ovaries) is considered a major risk factor for USMI. Polychlorinated biphenyls (PCBs) are environmental pollutants with neurodevelopmental and endocrine-disrupting effects, both of which may impact UI risk. Previous research has shown that mice exposed to PCBs during development exhibit UI-like signs as adults. Our objective for this research study was to measure PCB concentrations in blood from dogs with USMI compared to healthy controls. Our hypothesis was that dogs with USMI would have a higher concentration of PCBs than controls.

METHODS: We used biobanked serum samples from the Golden Retriever Lifetime Study, a longitudinal health study conducted by the Morris Animal Foundation. Cases had a USMI diagnosis. Samples were taken from the year the dog was diagnosed with USMI. Controls were dogs of the same breed, age, sex, and geographic location as case dogs. A liquid-liquid extraction method was used to simultaneously extract and analyze PCBs using a gas chromatography-tandem mass spectrometry (GC-MS/MS) system. All 209 PCB congeners were measured. We analyzed the total concentration of PCBs in cases compared to controls using a logistic regression model. We separately analyzed subsets of PCB congeners with estrogen receptor (ER) agonistic and antagonistic properties.

RESULTS: In general, controls had higher PCB concentrations than cases, however this result was not statistically significant ($P=0.40$). While it appeared that concentrations of E2-antagonist PCBs may have been higher in cases than controls, this result was also not statistically significant ($P=0.52$). Concentrations of E2-agonist PCBs were also not statistically different between cases and controls ($P=0.23$).

CONCLUSIONS: We were not able to detect a difference in PCB concentrations from dogs with and without USMI. This survey is valuable information to the community, as PCB concentrations have not been measured in dogs located in the US since 1987.

HEALTH RELEVANCE: PCB exposure may impact a variety of health conditions in dogs and humans beyond the urinary tract. Future work could explore additional associations with various phenotypes.

OLEIC ACID SUPPLEMENTATION IN AGED MICE WITH LOWER URINARY TRACT DYSFUNCTION (LUTD)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH), which affects 90% of men over age 80, frequently leads to lower urinary tract symptoms (LUTS) which can reduce quality of life. Fibrosis, proliferation, and smooth muscle contraction are hallmarks of LUTS/BPH; more recently, mitochondrial dysfunction has been implicated. In previous studies, oleic acid (OA), a mitochondrial modulator, mitigated LUTD in aged mice; however, the mechanism of action remains unclear. This study aims to elucidate the mechanism of OA in LUTD. We hypothesize that OA supplementation will reduce fibrillar collagen in the prostate and bladder. We also hypothesize that the fatty acid transporter CPT1A will decrease with age and increase with OA supplementation.

METHODS: C57BL/6J aged mice (24-25 month old) were treated with 40 mg/kg/day OA (voluntary feeding) over eight weeks (6x weekly). Void spot assays were performed weekly, then mice were euthanized, and tissues were harvested. Prostate and bladder tissues were stained using picrosirius red (PSR). PSR images were captured under circular polarized light and quantified using ImageJ. Tissues were also stained using immunofluorescence for CPT1A (Abcam, 1:1000). Using Inform, we quantified CPT1A percent positivity.

RESULTS: CPT1A transporters are significantly increased in aged mouse anterior prostate (AP) compared to young ($p=0.0001$) but are not significantly altered in aged mice treated with OA. There was no significant difference in fibrillar collagen in OA treated bladder or prostate lobes when compared to untreated tissues.

CONCLUSIONS: This study shows that OA does not alter collagen content in the prostate or bladder of aged mice. Surprisingly, CPT1A expression increased with age, which may explain why fatty acid supplementation caused no further increase in expression. Given LUTD improves with OA treatment, more experiments examining fatty acid oxidation and other mechanisms are required to understand OA in the prostate.

HEALTH RELEVANCE: This research uses a preclinical model of LUTD to evaluate a treatment option targeting mitochondrial dysfunction. As the aging population continues to grow and patients fail current medications, new interventions are necessary. Mitochondrial dysfunction is a novel treatment target for LUTS/BPH.

LINEAGE TRACING REVEALS UNEXPECTED EXPRESSION OF OSR2-CRE IN THE EFFERENT DUCT EPITHELIUM

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INTRODUCTION AND OBJECTIVE: Infertility affects around 18% of couples globally; males contribute to half of these cases. A major contributor to male infertility is developmental defects in the efferent ducts, the tissue that connects the testis and epididymis and aids in sperm concentration and transport. It is known that cell types and contents in the efferent duct epithelium are distinct between proximal and distal regions (near the testis and epididymis, respectively). However, it is unclear how these regional differences arise. To address this, we need to identify genetic tools that target the efferent duct epithelium in a region-specific manner. Osr2 is a transcription factor known to be expressed in the fetal stage and drives development of multiple tissues including the male reproductive tract. We previously found Osr2 expression in the efferent duct during embryogenesis, but it is still unknown whether that expression is restricted to the mesenchyme or the epithelium and whether it persists postnatally. Based on our compelling results from previous genetic labeling studies using Osr2-Cre, we aim to determine whether Osr2-Cre targets the efferent duct epithelium and whether Osr2+ epithelial cells are localized in any specific region of the efferent duct.

METHODS: Osr2-Cre/+ male and Rosa-nTnG female mice were set up for breeding. Male pups were collected at post-natal day (PND) 21 and euthanized with CO_2 gas. Mice were dissected, and the testis, efferent ducts, and epididymis were removed and collected. Tissues were de-fatted and embedded in OCT/sucrose to preserve cellular fluorescence. Frozen tissues were sectioned using a cryostat machine and mounted onto slides. Slides with frozen tissue sections were stained with DAPI and imaged with a Leica Thunder microscope.

RESULTS: Osr2+ cells were discovered in the mesenchyme of the initial segment, efferent ducts, epididymis, and in the Leydig cells of the testis. Mesenchymal Osr2+ cells exist in the efferent ducts as well, but we also observed Osr2+ cells within the epithelium of the proximal efferent ducts.

CONCLUSIONS: The presence of Osr2+ cells in the epithelium of the proximal efferent ducts at PND 21 is unique when compared to mesenchymal expression in the rest of the postnatal male tract. We now believe that Osr2-Cre can be used as a tool to study gene function in the efferent duct epithelium and gain insight into the detailed process that constitutes male tract development.

HEALTH RELEVANCE: With a better understanding of the target tissues and cell types of Osr2-Cre in the efferent ducts, we can now confidently utilize Osr2-Cre to study functions of infertility-associated genes in an attempt to develop better treatments for male infertility.

THE DEVELOPMENTAL EFFECTS OF POLYCHLORINATED BIPHENYLS AND HIGH-FAT DIETS ON URINARY FUNCTION

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INTRODUCTION AND OBJECTIVE: Polychlorinated biphenyls (PCBs), the once-popular chemicals for various industrial uses, have been linked to decreased bladder functional capacity in mice. Previous studies have shown PCBs and other exogenous factors, such as a high-fat diet, can increase immune cells in the bladder, leading to inflammation and voiding dysfunction. We hypothesize the effect of PCBs on urinary function will be worsened when another stressor, a high-fat diet, is introduced through a convergent mechanism involving bladder immune cells.

METHODS: We used $Ccr2^{RFP}$ mice (Jackson Laboratories, 017586t, n=9-25), which allow for the visualization of monocyte recruitment into the bladder via the Ccr2 pathway ($Ccr2^{RFP/+}$) or mice that lack this recruitment ability ($Ccr2^{RFP/RFP}$). Dams were dosed with vehicle or PCBs at 0.1 and 1 mg/kg, before being mated and continued dosing through gestation and lactation. Once the offspring reached six weeks of age, they were placed on control or high-fat diets. Voiding function was assessed monthly via void spot assays. After 16 weeks, the mice were euthanized and bladders processed for immunohistochemistry.

RESULTS: PCB and diet effects on urine spot count occur later in female mice than males. Homozygotes have a greater number of larger void spots than heterozygotes at baseline, though this difference reduces with time. 1 mg/kg PCB doses increased spot

number versus the vehicle in male heterozygous mice, which was prevented in homozygous mice at baseline, though this PCB effect diminished over time. A high-fat diet greatly altered PCB responses in female mice at 16 weeks, with 0.1 mg/kg PCB only increasing urine spots in mice on the high-fat diet of both genotypes.

CONCLUSIONS: We found several sex, genotype, and diet effects on voiding function, and in some cases, indications that recruitment of Ccr2 immune cells underlies effects of PCBs or diet on voiding. We also have evidence that sex differences exist in response to PCB or diet changes related to voiding function, and diet can alter PCBs' effects on voiding. Because the data processing is still ongoing, the differences in genotype that led to some findings are yet to be seen. Immunohistochemistry to quantify the immune cells present in the bladder will help to confirm the requirement of recruited immune cells in mediating each phenotype. Completing the study should provide avenues for further testing.

HEALTH RELEVANCE: PCBs have already been detected in humans. The high-fat diet commonly found in the US makes the environment proposed in this study relevant to US residents. Further research on the effects of PCBs on urinary function could lead to a better understanding of the pathology of lower urinary tract symptoms.

PATERNAL TRANSGENERATIONAL EFFECTS OF PRENATAL ESTROGEN EXPOSURE INCREASES SUSCEPTIBILITY TO URINARY FREQUENCY IN MOUSE MODELS OF BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) affect 90% of men by age 80. BPH progression is driven by hormone imbalances in the prostate, which are dependent on both androgens and estrogens. This study investigates the paternal transgenerational effect of prenatal estrogenic compound exposure on symptomatic and histological expression of BPH.

METHODS: Pregnant CD-1 mice were implanted with silastic capsules containing corn oil (CO), 25 µg 17 beta-estradiol (E2), or 60 µg bisphenol S (BPS). After the first litter (F1), a subset of mature F1 males was mated with untreated virgin females, resulting in the second generation (F2). This process was repeated for the third generation (F3). Each generation was separated into two groups: a sham surgery control (n=8-15) or a group implanted with 25 mg testosterone and 2.5 mg E2 (T+E2) for 4 weeks to model BPH (n=15-20). Void spot assays (VSAs) for each mouse group were performed weekly to assess urinary frequency. Androgen receptor (AR) protein expression and localization in the anterior prostate were examined by immunohistochemistry (IHC). AR positivity was calculated using InForm via H-score and four bin percent positivity (0, 1+, 2+, 3+). Data were analyzed using two-way ANOVA with Tukey's multiple comparisons test for VSAs and Sidak's multiple comparisons test for IHC.

RESULTS: T+E2 mice prenatally exposed to BPS had no change in voiding from F1 to F3. This group increased in H-score from F2 to F3 ($p<0.01$) and F1 to F3 ($p<0.001$) and the 2+ ($p<0.01$) and 3+ ($p<0.001$) AR intensity bins from F1 to F3. T+E2 mice prenatally exposed to E2 showed increased voiding from F2 to F3 ($p<0.05$) and F1 to F3 ($p<0.0001$). This group decreased in H-score and 2+ bin intensity ($p<0.05$) from F1 to F2 but increased in H-score ($p<0.01$) and 2+/3+ bins ($p<0.05$ and 0.01) from F2 to F3 (***Fig. 1***).

CONCLUSIONS: Prenatal exposure to E2 increases urinary frequency which may then increase AR expression. Prenatal exposure to BPS immediately increases AR, suggesting higher expression that is not impacting urinary frequency. More work includes investigating the molecular mechanisms of AR regulation and their downstream consequences.

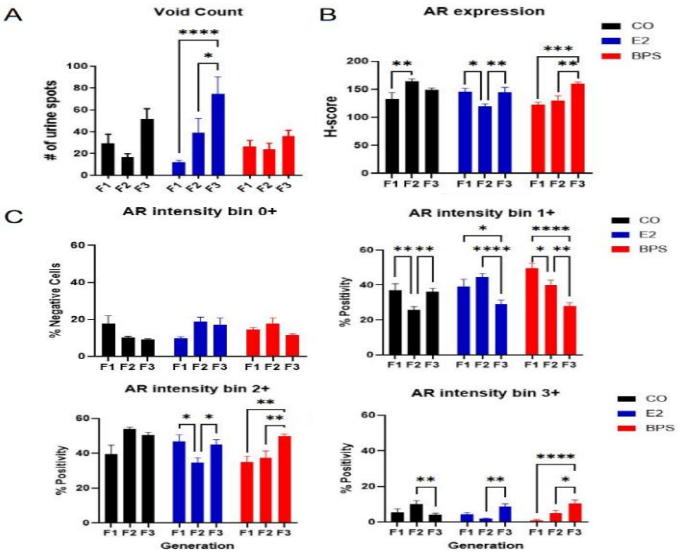

Figure 1. Paternal transgenerational impact of BPS and E2 on urinary frequency and AR expression in three generations of CD-1 mice. Urinary frequency was measured via void spot assays (VSAs) on the fourth week of T+E2 treatment (A). AR expression in the anterior prostate measured by H-score (B). AR expression in the anterior prostate binned by intensity (C). Data were analyzed using two-way ANOVA with Tukey's multiple comparisons test for VSAs and Sidak's multiple comparisons test for IHC. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

HEALTH RELEVANCE: This work identifies epigenetic differences underlying BPH progression, allowing communities to find potential biomarkers, making treatment more effective in populations where current drug therapies are less effective.

IDENTIFYING UNIQUE TRANSCRIPTOMIC AND MOLECULAR SIGNATURES IN BLADDER MACROPHAGE POPULATIONS

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INTRODUCTION AND OBJECTIVE: Macrophages (Mϕs) are a subset of the myeloid cell population and exhibit tissue-specific functions essential for immune surveillance and homeostasis. In the bladder, there have been two populations of macrophages described, MacL (in the lamina propria) and MacM (in the muscle layer), and have been shown to be transcriptionally divergent and have shared and unique functions. Bladder macrophages are less well studied in comparison to macrophages in other tissues, such as the spleen or lung. Evaluating the unique and shared transcriptomic and molecular signatures of the two bladder macrophage populations will elucidate their specialized roles in tissue immune regulation and homeostasis.

METHODS: Publicly available RNA sequencing datasets were obtained from the NIH Gene Expression Omnibus, the Immunological Genome Project, and a literature search from PubMed. The data used in the analysis was generated on the same platform and assembled on the same reference genome. Mϕ populations used in the analysis were derived from different tissues, such as the bladder and kidney. To identify the differentially expressed genes (DEGs) between each of the bladder Mϕ populations, the DESeq2 package in R was used. DEGs were defined by a log2fc $\leq$ -1 or $\geq$ 1 and an adjusted p-value < 0.01. Pathway analysis was conducted using the Gene Set Enrichment Analysis tool and STRINGdb to identify biological pathways and protein-protein interactions.

RESULTS: Comparing MacM and MacL Mϕ populations to other tissue Mϕs in the steady state, we identified 138 unique differentially upregulated genes in the MacL Mϕ, and 46 unique differentially upregulated genes in the MacM Mϕ. Pathway analysis of the unique genes of the MacL Mϕ in the steady state were

associated with genes switched off during inflammation. Pathway analysis of the unique genes of the MacM Mϕ in the steady state revealed genes associated with pathways involved in both innate and adaptive immunity and the OSM/LIF response. We are evaluating additional tissue Mϕ populations and single-cell RNA sequencing datasets.

CONCLUSIONS: RNA-seq analysis demonstrated unique sets of upregulated genes expressed in the two bladder Mϕ populations in the steady state. However, we need to explore if there are DEGs unique to each bladder Mϕ population that are not differentially expressed in macrophages derived from other tissues during an immunological challenge.

HEALTH RELEVANCE: Mϕs are involved in multiple roles in the tissue including homeostasis, organogenesis, and immunity. Understanding the functional heterogeneity of Mϕs will improve our understanding of how these cells maintain the health of a tissue.

TNFα AUGMENTS SEROTONIN-INDUCED CONTRACTIONS OF THE MALE MOUSE URETHRA

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INTRODUCTION AND OBJECTIVE: Lower Urinary Tract Symptoms (LUTS) commonly occur in aging men and reduce quality of life. LUTS has been linked to prostate inflammation and is sometimes treated with smooth muscle alpha-adrenergic receptor blockers, but with mixed efficacy (Roehrborn, 2009). We recently showed that serotonin (5-HT) causes female mouse urethral contractions (Ambrogi *et al,* 2025). We tested the hypothesis that 5-HT drives male urethral contractions and that TNFα, a pro-inflammatory cytokine, will augment urethral serotonergic smooth muscle contractions.

METHODS: Prostatic urethras were dissected from eight-week-old C57BL/6J mice (n = 3). Tissues were mounted onto wire myograph pins and maintained in individual chambers with 37°C Krebs Buffer solution (NaCl 130 mM, KCl 4.7 mM, KH_2PO_4 1.18 mM, $MgSO_4$ 1.18 mM, $NaHCO_3$ 14.9 mM, Glucose 5.6 mM, $CaCl_2$ 1.56 mM. pH 7.4), respirated with 95% O_2 and 5% CO_2. Chambers were washed with Krebs buffer every 15-20 minutes and between treatments. 60 mM KCl in Krebs was administered to chambers to verify vitality and obtain maximum contraction response. Tissues were incubated in TNFα (50 pg/mL) for 1hr to model inflammation. Then, a graded dose response curve was obtained for 5-HT (100 nM-3 mM) and the alpha- adrenoreceptor agonist Phenylephrine (0.1-300 μM). Reported contraction values were normalized against 60 mM KCl response. A two-way ANOVA test using GraphPad Prism was performed against non-TNFα incubated values from a prior experiment (n = 5) with a significance threshold of $p < 0.05$.

RESULTS: TNFα pretreatment significantly augmented 5-HT mediated urethral contractions at 5-HT concentrations of 3 μM ($p = 0.0463$), 10 μM ($p = 0.0463$), 100 μM ($p=0.0031$), and 3 mM ($p < 0.0001$). TNFα pretreatment did not significantly change Phenylephrine induced contractions ($p > 0.05$).

CONCLUSIONS: TNFα augments serotonergic but not alpha-adrenergic contractions in adult male mouse urethras. These results reveal a new way that serotonin and inflammation interact to drive urethral contraction unaddressed by alpha-blocker treatments. Future studies should investigate potential cell receptors and cell types involved in the 5-HT and TNFα pathway.

HEALTH RELEVANCE: To improve treatment for LUTS, future treatments for aging men may target 5-HT-induced muscle contractions.