CAIRIBU SUMMER STUDENT RESEARCH JAMBOREE

Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

July 29, 2026

1:30 PM Eastern

12:30 PM Central | 10:30 AM Pacific

5th annual summer undergraduate student research jamboree, organized and hosted by the CAIRIBU Interactions Core, featuring presentations by students affiliated with CAIRIBU Urology O'Brien Centers

ABSTRACT BOOKLET

PARTICIPATING PROGRAMS & CENTERS

R25 Summer Program for Undergraduate Urology Research (SPUUR) at UW-Madison

U54 O'Brien Urology Cooperative Research Centers Programs at:

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Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

2026 SUMMER STUDENTS' ABSTRACTS

SLINCR IN ACTION: INVESTIGATING THE ROLE OF A LONG NON-CODING RNA IN BLADDER INFECTION AND REPAIR

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INTRODUCTION AND OBJECTIVE: Mucosal tissues, including the lining of the bladder, are regularly challenged by pathogens. Within minutes of detecting a pathogen, bladder epithelial cells activate a group of genes known as immediate early genes (IEGs). These genes trigger epithelial cells to enter the cell cycle and begin repairing damaged tissue while also stimulating the production of cytokines that recruit leukocytes to fight the infection. How healthy bladder epithelial cells maintain a state of readiness to activate IEGs is not known. The long non-coding RNA 2610035D17Rik (*Slincr*) is highly expressed in mouse mucosal tissues including the bladder. My laboratory recently found that mutating *Slincr* allows the mouse bladder to develop normally but compromises the bladder's ability to clear a uropathogenic *E. coli* (UPEC) infection and repair its epithelium. This study tested the hypothesis that *Slincr* mutant bladders are unable to mount an appropriate IEG response to infection.

METHODS: We collected bladders from uninfected wild type mice, UPEC infected wild type mice, *Slincr* null mice, and UPEC infected *Slincr* null mice (two-to-three mice per group). Bladders were fixed and sectioned and immunostaining was used to visualize phosphorylated ERK1/2 (upstream of IEG activation), phosphorylated JUN (activated form of IEG), FOS (IEG), CD45 (leukocyte marker), and Ki67 (proliferating cell marker). Labeled cells were quantified using QuPath, and the results were analyzed using a two-way ANOVA.

RESULTS: Uninfected *Slincr* null mice bladders have fewer FOS positive epithelial cells than uninfected wild type control bladders. Upon infection with UPEC, *Slincr* null mice bladders have fewer FOS, phosphorylated JUN, and Ki67 labeled basal epithelial cells and fewer epithelial invading CD45+ leukocytes than wild-type control bladders. There is no difference in the quantity of phosphorylated ERK1/2 positive cells between infected wild type and infected *Slincr* mutant bladders.

CONCLUSIONS: *Slincr* acts downstream of MAP kinase signaling and is required to maintain bladder epithelial cells in a state of readiness to activate IEGs after pathogen detection.

HEALTH RELEVANCE: The human *Slincr* gene contains naturally occurring genetic variants, and some of these may reduce *Slincr* activity. Such variants could impair the bladder's ability to maintain a state of readiness for infection, potentially increasing an individual's susceptibility to recurrent or more severe urinary tract infections.

CHARACTERIZATION OF BLADDER AND URETHRAL CHANGES FOLLOWING PROSTATIC RADIATION IN A RAT MODEL

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INTRODUCTION AND OBJECTIVE: Every year in the United States, over 300,000 men are diagnosed with prostate cancer and over 40% of patients are treated with external beam radiotherapy (EBRT). While treatments have contributed to a 10-year 98% survival rate, many survivors have radiation-induced side effects including urinary incontinence and urethral injury that can develop into strictures. Radiation to adjacent tissues can lead to long-term damage to nerves, vasculature, and urogenital organs. Ultimately, these urological adverse effects significantly reduce survivor's quality of life. Currently, there are no preclinical models to study the effects of radiation on urethral injury. This study aims to access prostatic radiation-induced changes to urethra and bladder physiology.

METHODS: Adult male Sprague Dawley rats received a single dose of prostatic EBRT (Sham: 0 Gy, n=4; RT: 25 Gy, n=5). At 10 weeks post-EBRT, uroflowmetry and void spot assays (VSA) measured urinary function. At 12 weeks, the membranous urethra and bladder were collected. Myography was done to measure the contractility of the tissues in response to high potassium chloride (KCl) and electrical field stimulation (EFS). Additionally, contraction to caffeine was measured in the urethra and carbachol in the bladder. Finally, tissues were fixed, stained with picrosirius red, and imaged with a polarized scope to analyze the collagen content.

RESULTS: At 10 weeks post-RT, bladder weight, uroflowmetry and VSA were unchanged. Irradiated bladders had lower contraction to KCl ($p=0.033$) and the muscarinic agonist carbachol (Carb 1x10-6 $p=0.048$). However, there was no change in EFS-induced bladder contraction. The irradiated membranous urethra had no change in contractions to KCl, caffeine, or EFS. Post-RT, bladder had an increase in thick fiber, red collagen content ($p=0.013$). There was no change in the collagen content in the urethras post-radiation.

CONCLUSIONS: At 10 weeks, prostatic radiation led to decreased bladder contractility and increased collagen. Radiation did not have the same impact on the striated muscle of the membranous urethra at this timepoint. Despite these structural and contractile changes to the bladder, the uroflow and VSA did not show voiding changes, which suggests that bladder remodeling may occur before functional urinary changes. Further experiments are required to confirm these findings.

HEALTH RELEVANCE: The establishment of a preclinical model for prostatic radiation induced urethral injury would allow for further understanding of the pathophysiology and to develop novel treatments for urethral disease.

DEAD-BOX RNA HELICASE 3 REGULATION OF ANDROGEN RECEPTOR EXPRESSION IN BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a common age-related condition defined by noncancerous enlargement of the prostate, particularly within the transition zone. This enlargement can compress the urethra and lead to lower urinary tract symptoms such as weak urine flow and incomplete bladder emptying. Androgen receptor (AR) signaling is essential for prostate growth and function. Testosterone is converted into dihydrotestosterone (DHT), which binds to AR and activates genes that support prostate cell growth and survival. Therefore, changes in AR protein expression or activity may contribute to BPH development and progression. The AR mRNA 5′ untranslated region (5′ UTR) contains highly structured regions that may regulate translation efficiency. This project examines whether the DEAD-box RNA helicase DDX3 facilitates translation through these structured regions, potentially increasing AR protein production.

METHODS: I cloned and purified a set of four luciferase reporter RNAs containing 5' UTRs corresponding to wild-type or mutant sequences of AR mRNA and purified human DDX3 containing a maltose-binding protein (MBP) tag from bacterial cells. I performed an in vitro translation assay with the four-luciferase reporter RNAs in the presence or absence of DDX3, and measured translation efficiency by a luciferase activity assay.

RESULTS: Luciferase activity was measured in triplicate for each reporter construct in the presence and absence of DDX3. The DDX3-sensitive AR 5′ UTR construct showed increased luciferase activity when DDX3 was added. In comparison, the no-UTR control showed no increase, while deletion or mutation of structured regions within the AR 5′ UTR reduced or prevented the DDX3-associated increase in translation efficiency.

CONCLUSION: The known DDX3-sensitive 5′ UTR construct showed increased luciferase activity in the presence of DDX3, validating that the experimental system could detect DDX3-dependent changes in translation. These preliminary results are consistent with our hypothesis that DDX3 facilitates translation through structured regions of the AR 5′ UTR.

HEALTH RELEVANCE: Patients with BPH may respond differently to current treatments because the disease can vary in its underlying molecular mechanisms. Understanding whether DDX3 regulates AR mRNA translation may help explain this biological variability and support the development of more personalized BPH treatments.

HEMATURIA SUPPRESSES UTI-INDUCED UROTHELIAL CELL DEFENSE, INFLAMMATION, AND DEATH

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INTRODUCTION AND OBJECTIVE: Uropathogenic Escherichia coli (UPEC) induces exfoliation of superficial urothelial cells, an innate defense mechanism that limits intracellular bacterial community (IBC) formation. Hematuria is a common feature of urinary tract infection (UTI), yet the effects of extracellular heme on urothelial host defense remain poorly understood. We investigated how hemoglobinuria influences urothelial inflammation, epithelial cell death, and innate immune responses during experimental UTI.

METHODS: C57BL/6 mice were inoculated transurethrally with UTI89 to establish cystitis. Hemoglobinuria was induced using phenylhydrazine (PHZ). Urothelial injury, bacterial burden, IBC formation, inflammatory signaling, and pyroptosis were assessed by histology, immunofluorescence, RNAscope, immunoblotting, and RT-qPCR. Urothelium-specific nascent RNA sequencing was performed using Upk2-Cre;ROSA26-UPRT mice following 4-thiouracil labeling to identify early transcriptional programs altered by hematuria.

RESULTS: Hemoglobinuria preserved the superficial urothelial layer despite persistent bacterial infection and increased IBC formation. Heme suppressed pyroptosis-associated signaling, including NLRP3 expression and GSDMD cleavage, and reduced inflammatory cytokine expression and neutrophil recruitment. Urothelium-specific nascent transcriptomics demonstrated broad attenuation of TLR/NF-κB, interferon, cytokine, and cell death pathways. Notably, hematuria suppressed multiple genes associated with epithelial cell-autonomous immunity, including components of the ISGylation pathway, guanylate-binding proteins, autophagy, and intracellular pathogen sensing. Heme-mediated urothelial protection occurred independently of HRG1-mediated heme transport and HMOX1-mediated heme metabolism, suggesting an extracellular mechanism.

CONCLUSION: Hemoglobinuria attenuates early urothelial inflammatory and cell-autonomous immune responses, suppressing epithelial exfoliation while promoting persistence of intracellular bacterial communities. These findings identify extracellular heme as a previously unrecognized regulator of urothelial innate immunity during UTI.

HEALTH RELEVANCE: Hematuria frequently accompanies UTIs and other urologic disorders. Defining how extracellular heme alters epithelial host defense may reveal new therapeutic strategies to enhance bacterial clearance and reduce recurrent infection.

DISSECTING THE INDIVIDUAL CONTRIBUTIONS OF KPC AND NDM TO CARBAPENEM RESISTANCE IN CO-PRODUCING CLINICAL ISOLATES

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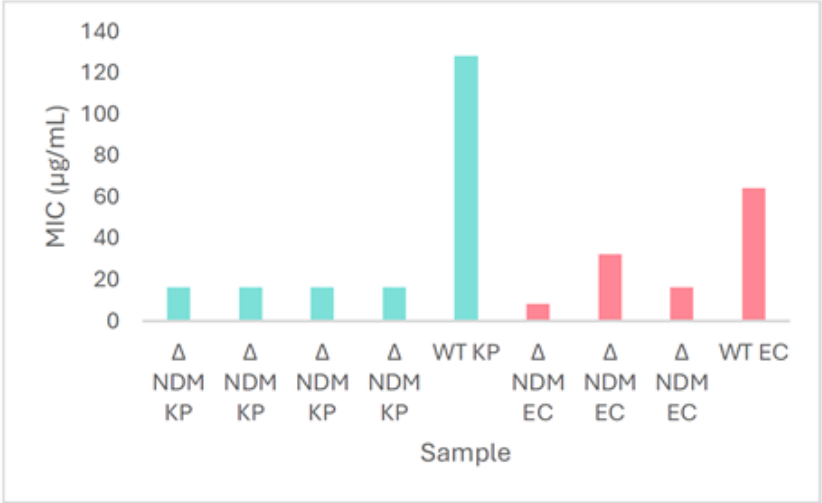
INTRODUCTION AND OBJECTIVE: New Delhi Metallo-β-Lactamase (NDM) and *Klebsiella pneumoniae* Carbapenemase (KPC) are two genes driving carbapenem resistant UTIs, which are currently treatable only with hospitalization and IV antibiotics as no oral treatment options exist. Co-producing isolates carrying both genes have recently emerged, generating highly resistant, difficult to treat infections. There is limited knowledge about how these genes interact to develop resistance. This study aims to determine the individual contributions of NDM and KPC to resistance in clinical Carbapenem resistant co-producers.

METHODS: Clinical isolates of *K. pneumoniae* and *E. cloacae* co-harboring NDM and KPC were used to individually knock out each gene

via an arabinose-inducible CRISPR-Cas9 knock-out system. Isolates were made electrocompetent, transformed via electroporation and selected on nourseothricin. Arabinose induction activated Cas9-mediated knockout, after which cells were cured of the CRISPR plasmid on non-selective LB. Knockouts were confirmed by PCR, and resistance phenotypes quantified by broth microdilution (MIC).

RESULTS: KPC knockouts are currently in progress. NDM knockouts were successful, resulting in the loss of the NDM gene, as confirmed with PCR and gel electrophoresis. Sequencing is currently in progress to determine if additional elements of the NDM plasmid were eliminated conjointly. Loss of the NDM gene reduced meropenem MIC in both *E. cloacae* and *K. pneumoniae* (Figure 1). This magnitude of reduction suggests that the NDM gene provides a large amount of carbapenem resistance to coproducing isolates.

Figure 1. Meropenem MIC values of NDM knockouts in *K. pneumoniae* and *E. cloacae* isolates.



CONCLUSION: Loss of the NDM gene markedly reduced carbapenem resistance in both *E. cloacae* and *K. pneumoniae*, indicating that plasmid-encoded elements and NDM itself contribute meaningfully to the resistance phenotype of co-producing isolates. Ongoing work will further delineate the contribution of the KPC gene to carbapenem resistance in co-producing CRE.

HEALTH RELEVANCE: Carbapenem-resistant UTIs requiring hospitalization and IV therapy present a significant treatment challenge: KPC-producing strains respond to ceftazidime-avibactam or meropenem-vaborbactam, while NDM-producing strains do not. Understanding how these co-occurring resistance mechanisms interact can inform development of targeted therapeutic strategies.

TRANSCRIPTOME ANALYSIS OF THE DORSAL ROOT GANGLIA IN A MOUSE MODEL OF CYSTITIS

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INTRODUCTION AND OBJECTIVE: Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a chronic condition characterized by bladder pain and urinary tract symptoms including voiding pain and urgency. It is believed that short-term bladder inflammation (cystitis) can lead to chronic pain conditions like IC/BPS, yet the exact mechanism is not fully understood. Macrophages residing in the sensory ganglia, including the dorsal root ganglia (DRGs), are known to be critical to chronic pain and consequently may contribute to IC/BPS symptoms. However, the activity of ganglion macrophages in DRGs following short-term bladder inflammation remains unknown. Currently, there are no transcriptomic datasets of bladder-innervating DRGs after cystitis; therefore, this study sought to assess gene expression changes in DRGs in a mouse model of cystitis, with a focus on macrophage markers. We hypothesized that there would be an increase in macrophage marker expression in the cystitis group compared to the control group.

METHODS: Cystitis was induced in adult female C57BL/6J mice through a one-time treatment of acrolein, administered into the bladder through a transurethral catheter. Mice were perfused 48 hours after the instillation of acrolein or saline (vehicle control). RNA was isolated from the left and right L5-S1 DRGs. Bulk RNA-seq was performed on the isolated RNA.

RESULTS: This project will create a transcriptome dataset of bladder-innervating DRGs after cystitis. We anticipate that principal component analysis (PCA) and gene expression heat maps will show clustering of the control and cystitis groups. We plan to visualize differentially expressed genes with volcano and MA plots. Through preliminary analysis and literature review, we generated a list of candidate genes related to inflammation and macrophage activity that may be linked to a possible mechanism of chronic bladder pain following cystitis.

CONCLUSIONS: This study will produce a dataset of gene expression in bladder-innervating DRGs following short-term bladder inflammation to mine for insights and generate further hypotheses related to inflammation and macrophage activity in DRGs. Clustering of the control and cystitis groups will demonstrate that cystitis can cause gene expression changes in the sensory system, potentially linked to the persistence of pain following acute inflammation.

HEALTH RELEVANCE: Understanding macrophage activity through differential expression analysis can improve understanding of the mechanisms that cause chronic pain following short-term bladder inflammation. Macrophage-related signaling can be used as a novel target for the treatment of cystitis-induced chronic pain.

PRECLINICAL TESTING OF AN IGF-1R BLOCKING ANTIBODY USING A PATIENT DERIVED XENOGRAFT MODEL OF BPH AND MAGNETIC RESONANCE IMAGING (MRI)

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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. IGF-1/IGF-1R have been shown to be upregulated in human BPH tissue and are important for prostate development and ductal branching. Animal models enable preclinical testing of therapeutics but are limited by species-specific prostate responses and fundamental differences from human tissue. We utilized our BPH PDX model for preclinical evaluation of an IGF-1R blocking antibody on BPH PDX growth and survival.

METHODS: BPH transition zone prostate tissue cores were collected from a patient undergoing a cystoprostatectomy. The tissue was sliced and implanted under the kidney capsule of male, immunocompromised Rag2-/- mice (n =30) supplemented with a testosterone pellet. After one month of engraftment, mice received teprotumumab, an FDA-approved IGF-1 receptor blocking antibody, at 10 mg/kg or 40 mg/kg or vehicle (1X PBS) via intraperitoneal injection three times weekly for three weeks. An 11.7T Bruker MRI system was used to non-invasively monitor PDX volume starting the day before treatment and once weekly until explant. MRI volumes were calculated using the open-source medical imaging software, Horos. Post-treatment, the tissue grafts were harvested and analyzed via H&E staining and immunohistochemistry for Ki-67 and CC3.

RESULTS: MRI showed that vehicle PDXs grew +31.9 ± 53.8% while low-dose IGF-1R blocking antibody treatment reduced volumes by 18.2 ± 15.2% (p=0.003) and high-dose by 37.4 ± 8.7% (p<0.001). Effects were significant as early as one week of treatment. Explant weights confirmed this dose response (vehicle: +80.8%; low: +6.9%, p=0.006; high: -17.8%, p<0.001). Serum PSA, measured at explant, was also reduced from 50.4 ± 44.8 ng/mL in vehicle to 9.7 ± 8.8 ng/mL in the high-dose group (p=0.034). Furthermore, MRI volumes and explant weights were strongly correlated (R^2=0.908, p<0.001), supporting MRI as an accurate readout of PDX changes over time.

CONCLUSIONS: This BPH PDX model recapitulates features of human prostate tissue and MRI enables noninvasive monitoring of PDX growth in vivo. Treatment with an IGF-1R antibody blockade reduced graft weight, volume, and PSA, supporting its potential as a therapeutic strategy for BPH.

HEALTH RELEVANCE: This clinically relevant PDX model provides a platform for testing therapeutics like IGF1/IGF-1R inhibitors that could reduce BPH growth and improve patients' quality of life. In vivo monitoring improves study design and therapeutic evaluation.

BACTERIAL IMMUNE SYSTEMS IN DRUG-RESISTANT *KLEBSIELLA PNEUMONIAE* FROM URINARY TRACT INFECTIONS

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP) are an urgent threat with few treatment options and no oral regimen. Bacteriophage (phage) therapy is a proposed novel treatment for CRKP. We hypothesized that defense systems and prophages differed by body site, driven by distinct selective pressures.

METHODS: We analyzed 234 non-serial whole genome sequences of confirmed CRKP: 76 urine, 68 blood, 61 respiratory, and 29 other. We identified sequence type (MLST), capsule type (Kaptive), prophages (PHASTEST), and defense systems (Defense Finder). We conducted downstream analysis, statistics, and visualization in Python.

RESULTS: We found that no body site differed significantly in sequence or capsule type. Median prophage count was 6 in urine and 5 across other sites. All sites showed a median of 14 total and 11 unique defense systems per isolate. The diversity of defense systems carried on prophages was stable across sites (14 unique systems for blood and urine, and 11 for respiratory isolates). Of the 65 unique defense systems in the study, 37 (57%) are of unknown mechanism. Common systems such as restriction modification (n=234) and abortive infection (n=192) systems were enriched in urine, while rarer systems such as bacteriophage exclusion (BREX) (n=2) and SanaTA (n=2) were depleted, though neither reached significance. The number of unique defense systems across all urine samples was 46 compared to 65 for blood and 60 for respiratory isolates. Defense systems carried on the chromosome account for the difference in unique defense systems (37 for urine, 62 for blood, and 57 for respiratory isolates).

CONCLUSION: Isolates collected from urine exhibit one notable difference: lower defense system diversity. This yields two directions for further study. First, we can investigate whether a less diverse defense ecosystem for urine CRKP implies that UTIs are more susceptible to phage therapy. Second, we can examine whether other UTIs (i.e., uropathogenic *E. coli*) follow a similar pattern of adaptation through defense system depletion in urine.

HEALTH RELEVANCE: Reliability remains a challenge for phage therapy, as infections can evolve rapid resistance to phage. However, these findings suggest that a larger array of phages may be effective against CRKP UTIs compared to CRKP of other body sites.

COMPARISON OF THE EFFECTS OF RAPID-ACTING ANTIDEPRESSANT KETAMINE TO ITS ACTIVE METABOLITE (2R,6R)-HYDROXYNORKETAMINE ON URINARY HEALTH AND KETAMINE-INDUCED CYSTITIS

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INTRODUCTION AND OBJECTIVE: Ketamine-induced cystitis (KIC) is a progressive bladder disorder marked by lower urinary tract dysfunction, urothelial injury, and fibrosis following chronic ketamine exposure. The major metabolite, (2R,6R)-hydroxynorketamine (HNK), retains antidepressant activity without ketamine's abuse potential or NMDAR-mediated side effects, but its urinary effects remain largely unknown. This study characterized bladder exposure to ketamine and HNK, evaluated urinary dysfunction, and investigated their direct effects on urothelial cells.

METHODS: Adult female C57BL/6 mice received chronic saline, ketamine, or HNK daily for three weeks. Serial urine was analyzed by LC-MS/MS to quantify ketamine, norketamine, and HNK. Urinary function was assessed via Void Spot Assay (VSA) and urine dipstick, with bladder tissue collected for histology (H&E, Masson's trichrome). Ongoing studies expose SV-HUC-1 urothelial cells to ketamine, norketamine, or HNK to assess viability (CCK-8), senescence (β-galactosidase), and inflammatory/profibrotic proteins by Western blot.

RESULTS: LC-MS/MS established distinct bladder exposure profiles: ketamine-treated mice showed sustained urinary ketamine with measurable norketamine and HNK, while HNK-treated mice showed robust urinary HNK with minimal parent ketamine, consistent with HNK's terminal position in ketamine's metabolic pathway. VSA showed increased urinary frequency in both groups (ketamine 36-69, HNK 40-59 voids/week) and increased 0-0.1 mL voids (ketamine 33 to 53, 61%; HNK 34 to 48, 41%). Dipstick showed increased urinary pH and minimal

bilirubin increases in both groups, while other biomarkers remained unchanged. In vitro, ketamine and HNK are both expected to decrease viability, increase senescence, and upregulate COX-2, iNOS, TGF-β1, α-SMA, collagen I/III, and fibronectin.

CONCLUSIONS: Chronic ketamine and HNK produced distinct bladder exposure profiles despite comparable early functional bladder changes, suggesting the terminal metabolite alone may be sufficient to reproduce ketamine's urinary effects. Ongoing studies will determine whether HNK produces urothelial injury and profibrotic signaling comparable to ketamine, informing KIC pathogenesis and bladder-sparing antidepressant development.

HEALTH RELEVANCE: KIC is a debilitating complication of chronic ketamine exposure without disease-modifying therapies. Defining bladder exposure to ketamine and HNK and their urothelial effects may inform KIC pathogenesis and support development of safer antidepressants with reduced urinary toxicity.

GENE-BY-ENVIRONMENT INTERACTIONS IMPACTING BLADDER EPITHELIAL STRUCTURE ACROSS VARYING SHANK3 GENOTYPES

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INTRODUCTION AND OBJECTIVE: Lower urinary tract symptoms (LUTS) are experienced by 77% of people with Autism Spectrum Disorder (ASD). There are numerous genetic risk factors for ASD that play a role in development of LUTS, including mutations in the SHANK3 gene, which encodes for a synaptic protein involved in behavior, learning, and memory. Individuals with mutations in the SHANK3 gene often have an ASD diagnosis and may experience urinary issues. Additionally, environmental contaminants such as polychlorinated biphenyls (PCBs) have been linked to increased severity of ASD and LUTS. The gene-by environment interactions involving SHANK3 mutations, PCBs, and urinary problems are not well understood, which is what this study hopes to clarify. We wanted to determine if bladder dynamics/LUTS are exacerbated by PCBs in SHANK3 mutant mice.

METHODS: Mice with SHANK3 mutations were used in this study to simulate the human pathway, including wildtype (WT), SHANK3 heterozygous (SHANK3+/-), and SHANK3 homozygous (SHANK3-/-) male and female mice. These mice were dosed as juveniles (p21-p42) with peanut butter containing either 0 (control), 0.1, or 1 mg/kg/day PCBs for three weeks starting at weaning.
Bladders were collected from WT, SHANK3+/-, and SHANK3-/- mice and embedded in paraffin wax blocks. Blocks were sectioned and mounted on slides to perform immunohistochemistry (IHC). Antibodies for Keratin 5 (KRT5) and Uroplakin III (UpkIII), two bladder epithelial proteins, were used to assess bladder morphology.
Fluorescence microscopy was used to image the IHC products. Images were analyzed using Image J software, measuring thicknesses of KRT5, UpkIII, and total epithelial thickness in pixels.

RESULTS: KRT5 and total epithelial thickness were significantly greater between control male WT and both SHANK3 mutants. There were no significant differences in UpkIII thickness in any group. Female groups showed no significant changes. PCB doses did not affect bladder epithelium structure.

CONCLUSIONS: There were significant differences in KRT5 across genotypes, increasing in thickness from WT to SHANK3+/- to SHANK3-/-, showing that SHANK3 mutations do affect bladder structure. This is a new finding that must be further investigated to understand how SHANK3 mutations cause this overabundance of basal protein.

HEALTH RELEVANCE: Providing a better understanding of brain and bladder connections in Autism Spectrum Disorder is important for improving the quality of life for individuals with the condition. Determining which risk factors induce LUTS and pathways involved will hopefully improve risk communication and lead to further therapeutic developments for those with ASD.

EXAMINING MECHANISMS USED BY WISCONSIN CLINICS TO IMPLEMENT URINARY INCONTINENCE SCREENING DURING A TRIAL OF PRACTICE FACILITATION STRATEGIES

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INTRODUCTION AND OBJECTIVE: An estimated 40-80% of adult women experience urinary incontinence (UI), with prevalence increasing with age. While this condition impacts quality of life, many patients do not receive care or treatment, possibly due to a lack of screening or accessible effective treatments. However, little is known about specific barriers that providers face when screening or offering treatment to patients with UI. To improve screening and treatment of UI, the WI-INTUIT project aimed to compare two implementation strategy bundles: one strategy focused on tailored support based on the practice's needs and preferences; the other added additional ongoing expert consultations with local and state resources. This work sought to identify practice and provider-level barriers to inform future interventions that improve UI care.

METHODS: Clinics throughout Wisconsin (n=38) were randomly assigned to one of two study arms: streamlined practice facilitation (SPF) or SPF+ partnership building. Each clinic participated in a recorded kick-off meeting with our research team in which they were asked to identify implementation barriers. Recordings from the meetings were transcribed, cleaned, and qualitatively analyzed. The COM-B-TDF model was initially used to code and categorize barriers and facilitators of UI screening and treatment.

RESULTS: A subsample of 10 kick-off meetings with the clinics was analyzed. Identified barriers included time restraints, accessibility of referrals, and general screening and treatment resources for patients. The barrier that was mentioned most frequently on average was time constraints, related to both the perceived time needed for UI patient communication and education and the competing priorities. Among participating clinics, rural clinics reported poor accessibility to referrals due to location, while urban clinics reported difficult time limitations. Providers' perception of the patients' interest in UI during screening varied. Some providers listed a need for more attention and time after

screening to ensure adequate communication with patients. Access to online educational resources for patients was motivating for clinics.

CONCLUSION: This work suggests time constraints and accessibility to resources are barriers to implementing screening for UI in adult women. Future work that aims to improve implementation of screening and treatment of UI at a larger scale and across a variety of settings is needed.

HEALTH RELEVANCE: UI limits quality of life and increases the risk of depression, falls, and institutionalization. Implementing screening and treatment for UI at the provider level is one strategy to link patients to efficacious treatments.

HISTOLOGICAL ANALYSIS OF ACQUIRED URINARY INCONTINENCE IN DOGS SUGGESTS A PRO-FIBROTIC PHENOTYPE

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INTRODUCTION AND OBJECTIVE: Urinary incontinence (UI) is common in humans and dogs. In dogs, ovariohysterectomy (spay) is a risk factor for UI, which is understood to be caused by a combination of genetic predisposition and hormonal alterations affecting tissue integrity. Previous research shows that spayed dogs have more collagen and less muscle fiber in their urethral and bladder tissues compared to intact females; however, this study did not include incontinent spayed dogs. Our objective is to estimate the proportion of collagen relative to muscle in bladder and urethra tissues from incontinent spayed relative to intact female dogs. We hypothesized that incontinent dogs would have a higher proportion of collagen than continent dogs.

METHODS: Archived formalin-fixed paraffin-embedded bladder (N=1 spayed incontinent; N=1 intact) and urethra (N=1 spayed incontinent; N=1 intact) tissue blocks were sectioned and stained using Gomori's Trichrome, which stains collagen green/blue and muscle red/purple. Three images were taken in different places on each slide. QuPath software was used to determine the collagen to muscle fiber ratio by labeling collagen, muscle, and background and obtaining percentages of each.

RESULTS: In bladder, the average proportion of collagen relative to muscle in the incontinent sample was 46.19% compared to the continent sample at 24.72%. Likewise, urethra samples had 85.69% and 77.89% collagen in incontinent and continent samples, respectively.

CONCLUSIONS: Based on the estimated proportions and the visual appearance of the tissues, there appears to be more collagen relative to muscle in spayed incontinent compared to intact dogs. This suggests that incontinent dogs have a profibrotic phenotype in which an excessive production of collagen may cause the urethra to stiffen, making it difficult to remain closed and prevent urine leakage. These findings are limited by the small sample size and variation in breed and neuter status. Tissue collection for this study is ongoing. Further work will include tissues from additional spayed-incontinent and intact female dogs as well as spayed-continent dogs.

HEALTH RELEVANCE: UI in spayed female dogs shares clinical similarities with stress UI in menopausal women. UI can negatively affect daily life and wellbeing for humans and dogs, as well as create a strain in the relationship between dogs and their owners. Defining pathobiological mechanisms that lead to incontinence in dogs may inform human medical advancements and vice versa.

COGNITIVE DEBRIEFING OF THE WORLDWIDE YEARLY SCREENING FOR HYPOSPADIAS (WYSH): EVALUATING ACCESSIBILITY AND ADAPTABILITY ACROSS DIVERSE PATIENT POPULATIONS

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INTRODUCTION AND OBJECTIVE: Hypospadias (HS) is one of the most common congenital anomalies of the male genitalia (1) and occurs when embryonic development of the urethra is disrupted in utero. Untreated or complicated HS may result in functional, reproductive, and psychological complications for up to 50% of patients by adolescence (2) and lacks validated patient-reported outcome measures. (3) Guided by Tourangeau's Four-Stage Cognitive Model, this study evaluated whether demographic factors influenced patients' use of a screening tool and measure for HS, The Worldwide Yearly Screening for Hypospadias (WYSH), to understand its adaptability to diverse patient outcomes.

METHODS: Semi-structured cognitive debriefing interviews were coded using a hybrid thematic analysis approach to develop an operational code book combining Tourangeau's framework with de novo themes by 3 independent codes (HL, DGM, EA). (4) NVivo (Version 15; Lumivero 2025) was used for matrix coding queries analyzing themes across demographic and clinical groups, including developmental stage, race, insurance status, ADI, health literacy metrics, HS severity, and surgical history, to identify patterns in questionnaire interpretation, applicability, and acceptability.

RESULTS: Developmental stage showed the greatest variation. Younger participants (5–10-year-olds) more often discussed child-parent disclosure and age-specific applicability, while pubertal (11–15-year-olds) and post-pubertal (16+ year-olds) commented on the screening topic's age relevance and longitudinal changes following treatment. Participants with more severe HS and more surgeries referred more to the evolution of their condition over time. Comparatively few differences were noted in demographic groups.

CONCLUSIONS: WYSH demonstrated high adaptability with minimal variation across different demographic groups. Developmental stage and clinical experience influenced cognitive response processes more than socioeconomic or

Thematic Domain	Demographic Group of Interest	Sample Quote
Child-Parent Disclosure	5–10-year-olds	P01: PARENT: … Do you ever feel bad because of your hypospadias? P01: Once in a while [I feel bad]… I just don't like it when surgeries don't work.
Age-Specific Applicability	5–10-year-olds	P01: PARENT: … I guess a lot of it, age, yes, age-related. [CHILD would not contact doctors or look up information independently].
Pubertal Relevance	11–15-year-olds	P04: … I'm [AGE]… I don't know. It's just at my age, this section feels pretty redundant because all the questions only apply to people like over 18.
Pos-pubertal Relevance	16+ year-olds	P03: …But like over the course of the last [AGE], it's [HS] definitely something that has… majorly contributed … to my body image, my sense of self and … how I feel about…sexual health…
Longitudinal Experience	Severe HS; >2 surgeries	P03:… And today I would say no. But two years ago, I would have said yes. [Responses changed over time]

demographic factors. These findings support incorporating age-appropriate wording and longitudinal perspectives into the WYSH questionnaire to improve its applicability, acceptability, and content validity across diverse pediatric and adult HS populations.

HEALTH RELEVANCE: An age adjusted HS screening and treatment tool should maintain developmental relevance and should include discussion aids to support youth and parents.

PRECLINICAL TESTING OF AN IGF-1R BLOCKING ANTIBODY FOR BPH TREATMENT USING A PROSTATE SPHEROID PLATFORM

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a condition that obstructs urine outflow and drives lower urinary tract symptoms in aging men, and current therapies have modest response rates and significant side effects. The IGF-1/IGF-1R signaling is elevated in BPH tissue, and mouse knockouts of IGF-1 disrupt prostate development and ductal branching, making the receptor a candidate therapeutic target. We tested teprotumumab, an FDA-approved anti-IGF-1R monoclonal antibody, in 3D spheroids embedded in extracellular matrix, a model that bridges 2D monolayers and *in vivo* studies. We then trained a deep learning segmentation model on whole-well brightfield images to enable morphology quantification at scale.

METHODS: BHPrE1 immortalized prostate epithelial cells were seeded in 3D-embedded, growth-factor-reduced Matrigel in 24-well plates at 1,000 cells per well. Spheroids were cultured in conditioned medium from BHPrS1 prostate stromal fibroblasts and treated with or without teprotumumab at 1 or 50 µg/mL, then imaged at day 10 as whole-well brightfield tiles at 4X on a Keyence BZ-X800. To build the automated segmentation pipeline, 5,207 spheroids were manually annotated in QuPath, and then used to train a nnU-Net 2D segmentation model on AWS GPU instances, with performance evaluated by Dice coefficient on held-out test wells.

RESULTS: On the training set (n=3,712), median Dice was 0.9517 (IQR, 0.9268, 0.9687). The test Dice score, using the rest of the spheroids as testing data (n=1,495) had a median of 0.9608 (IQR, 0.9608, 0.9612). Applying the model, spheroids treated with 1 and 50 µg/mL teprotumumab had significantly smaller per-spheroid mean diameters compared to spheroids grown in only conditioned media (control 264.4 ± 124.8 µm; 1 µg/mL 250.8 ± 114.1 µm ($p<.05$); 50 µg/mL 236.5 ± 110.1 µm ($p<.0001$), Mann-Whitney U with Bonferroni correction). The teprotumumab-treated spheroids also had significantly smaller per-spheroid areas (control 67,144 ± 59,133 µm²; 1 µg/mL 59,606 ± 48,679 µm² ($p<.05$); 50 µg/mL 53,447 ± 44,055 µm², ($p<.0001$)).

CONCLUSION: This deep learning segmentation model is a promising tool to automate the extraction of morphological features from prostate spheroids in a reproducible and highthroughput manner. Additionally, teprotumumab causes decreased growth of BHPrE1 spheroids compared to control spheroids, which may lead to slowed progression of BPH in men.

HEALTH RELEVANCE: Existing medical therapies for BPH leave many patients symptomatic. Our results motivate *in vivo* testing of teprotumumab as a candidate BPH therapy, with the added translational advantage that it is already FDA-approved.

A METABOLITE GENOME-WIDE ASSOCIATION STUDY IDENTIFIES A GLUTAMINE-ASSOCIATED VARIANT IN MINIATURE SCHNAUZERS, A BREED PRONE TO CALCIUM OXALATE STONES

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INTRODUCTION AND OBJECTIVE: Calcium oxalate (CaOx) stones are a common, recurring cause of urinary disease in dogs. Miniature Schnauzers develop stones at higher rates than other breeds, indicating a genetic contribution. A metabolome-wide association analysis for CaOx stones in Miniature Schnauzers found multiple metabolites that were differentially abundant between cases and controls. We aimed to identify genetic variants associated with serum metabolites that may be candidate contributors to CaOx risk.

METHODS: We studied 36 Miniature Schnauzers (13 with CaOx stones, 23 without) with genome-wide genotyping (302,255 SNPs) and blood metabolite measurements. Each of 42 metabolites was tested against every SNP using a linear mixed model accounting for relatedness. Significance was set by a permutation testing procedure. For the strongest signal, multivariate Bayesian analysis jointly modeled the metabolite and stone status. The signal was narrowed by linkage disequilibrium analysis and nearby genes ranked by distance. We then tested whether the candidate gene and its pathway varied by genotype in blood RNA sequencing and urine metabolites.

RESULTS: Serum glutamine showed the strongest association (chr23:31192416; $P = 1.14 \times 10^{-5}$). The variant was common (minor allele frequency 0.40), and mean glutamine decreased with each added copy of the risk allele (+1.26, +0.08, −0.71 standardized units for zero, one, two copies). Joint Bayesian analysis gave a posterior probability near 1.0 for a direct effect on glutamine, with weaker, indirect support for stone status, consistent with mediation through glutamine. Fine-mapping narrowed the signal to a 242,000-base region, where the top variant lay outside any gene, in noncoding sequence conserved across species. The nearest gene, AMOTL2 (17,000 bases), regulates glutaminase via YAP signaling, making it a candidate. However, neither AMOTL2 nor its pathway varied by genotype in blood, so it is not the direct target. Elevated urinary glutamine in affected dogs indicates the glutamine link persists, likely though an indirect or tissue-specific effect.

CONCLUSIONS: We fine-mapped a common variant influencing blood glutamine in a stone-prone breed. Its conserved noncoding location, with no blood expression signal, suggests a regulatory variant acting in the kidney. This suggests a metabolite-intermediate design can localize a signal in a cohort far smaller than a disease GWAS requires, though confirming this variant's role would require replication and kidney expression data.

HEALTH RELEVANCE: Understanding the genetic and metabolic basis of CaOx stones could improve risk prediction and guide dietary or medical prevention in predisposed dogs. Dogs and humans form the same stones, so this may also inform human disease.