



December 3-5

Madison, WI

CAIRIBU MEETING ABSTRACTS

The number of abstracts submitted this year broke all previous CAIRIBU meeting records. This meeting features 98 abstracts submitted by 91 individuals. Only trainees and early-stage investigators (those within 10 years of their terminal degree) may submit abstracts for CAIRIBU meetings. This year, the 3 abstract below were selected as “best abstracts:”

1. **Ashika Goel**, graduate student – Michigan State University (mentor: Nathan Tykocki, PhD) [Abstract #27, poster session 1]
- *Neurokinin A increases bladder mechanical compliance and augments transient pressure events*
2. **Ananya Pinnamaneni**, undergraduate student – Duke University (mentor: Michael Odom, PhD) [Abstract #63, poster session 2]
- *Estrogen-dependent mechanisms elevate urothelial c-fiber density and drive overactive bladder pathogenesis in female type 1 diabetic Akita mice*
3. **Hannah Anderson**, graduate student – Medical College of Wisconsin (mentor: Aaron Mickle, PhD) [Abstract #5, poster session 1]
- *Angiotensin II type 2 receptor agonism elicits sex differences in murine bladder contractile responses ex vivo*

This year’s CAIRIBU meeting abstracts are listed in alphabetical order by the presenting author. In order to help you find each abstract’s corresponding poster in the poster sessions on Wednesday and Thursday, the poster number is listed after each title. Posters are distributed in 2 sessions – see below. Within 12 poster categories, a “best poster” will be awarded at the end of the CAIRIBU meeting on Friday.

5:45 PM WEDNESDAY, DECEMBER 3 - POSTER SESSION 1 (with cash bar reception until 7:30 PM)

1. Adrian – ECM, mitochondria, metabolism	18. Clevidence – Non-invasive imaging	35. Heye – Evaluating pro-inflammatory agents
2. Allen – Impact of age and sex on bladder	19. Cornelius – T cell exhaustion in bladder	36. Horne – Validating mouse models of BPH
3. Alom – Modulation of bladder function	20. Costa Lopes – Beyond the bladder	37. Huffman – The relevance of age
4. Ambrogi – Serotonin signaling in UTI	21. Crouch – Establishing In vitro/vivo platforms	38. Jensen-Wachspress – Sleep fragmentation
5. Anderson – Angiotensin II receptor agonism	22. Dalvi – Fatty acid auxotrophy	39. Jordan – Effects of developmental exposure
6. Barrett – Aged rats pelvic radiation	23. Dobihal – Crosstalk in signaling systems	40. Kasturi – Sex-specific proteostasis
7. Barteld – Prepubertal leuprolide treatment	24. Forsythe – Development of xenium spatial	41. Katz-James – Relationship P2X3 and CGRP
8. Bautista-Ruiz – Prenatal exposure	25. Gadhvi – Comparative analysis prostate	42. Kercsmar – Potential role for retinoic acid
9. Bergemann – Development of a 3D bladder	26. Garcia – Selective elimination of cells	43. Khanmammadova – Preop pelvic floor PT
10. Beserra – Estrogen-mediated protection	27. Goel – Neurokinin A increases compliance	44. Khosla – Cystitis molecular changes in L3
11. Bhandari – Effects of prophage induction	28. Gourdine – Survival of the sweetest	45. Kim – Development of humanized mouse
12. Bicki – Using generative AI to characterize	29. Hammel – Characterizing radiation damage	46. Lafin – Lessons in multimodal cell LUT atlas
13. Bogdan – Notch signaling in urothelium	30. Haramaty – Accelerating Delphi consensus	47. Lange – Utilizing system usability screening
14. Boron – Purinergic receptor activation	31. Harrie – Characterization of normal bladder	48. Lewis – Co-culturing urinary lactobacilli
15. Boyer – Multiomic integration	32. Harrison – Comparing sequencing approaches	49. Li – PCR-based testing for urinary symptoms
16. Chaudhary – Mapping bladder, tibial nerve	33. Hassan – Role of membrane transporters	50. Sun – Data linkage from PKIDS study
17. Chiu – CAIRIBU mobile phone app	34. He – Development of loow-cost catheter	51. Yarger – Delayed or forgone care for UTI

5:45 PM THURSDAY, DECEMBER 4 - POSTER SESSION 2 (with cash bar reception until 7:30 PM)

52. Limkar – Urinary incontinence in Alzheimers	67. Ridlon – Nerve-evoked bladder muscle	82. Tran – Calcium imaging in cystitis
53. Makori – Oleic acid supplementation	68. Robilotto – Chronic losartan delivery	83. Valdes-Arciniega – Investigating the link
54. Masino – Transient receptor potential	69. Rodriguez-Tirado – PIEZ01 and urothelium	84. Wattier – Age-stratified clinical presentation
55. McGlynn – How variable is urine microbiota	70. Rundquist – Ascending UTIs	85. West – Kidney stone fatigue in lithotripsy
56. Moore – Exploring demographic and clinical	71. Salaudeen – Defining metabolic pathway	86. White – Investigating use of oxygen
57. Myklebust – Murine models of BPH	72. Samadifam – Functional MRI analysis	87. Wilkins – Burst-patterned sacral nerve stim
58. Nair – Serotonin-induced contractions	73. Seifert – Effect of estrogen signaling	88. Wu – Glycoproteomic analysis of BPH
59. Neugent – Urinary biochemical ecology	74. Spanier – Interplay of meal-timing	89. Yarger – Telehealth vs. in-person visits
60. Ng – Building collaborative research	75. Stuedemann – Investigating changes	90. Zaher – Rare variant association study
61. Patel – Low-rank adaptation model for CT	76. Su – Clinical correlates and prognosticators	91. Zhang – Identification of G quadruplex
62. Pelissero – Single-cell antimicrobial	77. Sun – Post-op trajectories of pain	92. Zhu – Phosphodiesterase-5 inhibitor
63. Pinnamaneni – Estrogen mechanisms	78. Talluri – Spatial transcriptomics mapping	93. Allen – Spoti-find void spot assay
64. Polasko – Intracellular ATP production	79. Tanaka – Neuro-uro mechanisms in LUTD	94. Haramaty – Defining what to say and when
65. Rengo – Intrinsic G-protein receptor	80. Tello – Paternal transgenerational effects	95. Horne – GRP78-mediated endoplasmic
66. Reske – Lineage tracing in efferent duct	81. Tlapa – PCB exposure and urethra	96. Ippolito – Longitudinal study of urobiome

THE EXTRACELLULAR MATRIX, MITOCHONDRIA, AND METABOLISM: A PROTEOMICS INVESTIGATION OF BPH TISSUE (poster #1)

Alexis E. Adrian^{1,2}, Feixuan Wu³, Allen Zhao⁴, Han Zhang¹, Peng-Kai Liu⁵, Yajing Lu⁶, Douglas W. Strand⁷, Lingjun Li³, William A. Ricke¹

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INTRODUCTION AND OBJECTIVES: Benign prostatic hyperplasia (BPH) often causes lower urinary tract symptoms (LUTS) as men age. 90% of men develop histological BPH by their eighties and LUTS double for men in their seventies compared to fifties. These often-bothersome symptoms can reduce quality of life as men age. The pathophysiology of LUTS/BPH is not fully understood and limited data sets exist comparing the proteome of BPH tissue to healthy controls. The goal of this study is to use untargeted proteomics to identify pathways of interest with differential protein expression in BPH.

METHODS: Prostate specimens from young organ donors (n=7) and aged BPH patients (n=7) were acquired from the UTSW tissue repository. Frozen tissue was homogenized, and proteins were extracted, quantified, reduced, alkylated, and digested with trypsin. Peptides were then labeled with DiLeu isobaric tags and pooled. Samples were analyzed using reversed-phase liquid chromatography on a Dionex Ultimate 3000 UPLC system coupled to a Thermo Scientific QE-HF mass spectrometer. Data were processed using Proteome Discover against the UniProt *Homo sapiens* reviewed database with a 1% false discovery rate. Statistical significance was defined as a p-value <0.05. Gene ontology (GO) analyses were performed for molecular function pathways and cellular compartment.

RESULTS: A total of 253 proteins were significantly different between BPH and donor tissues; of these, 206 also exhibited a fold change greater than ± 1.2 . GO analysis of molecular functions revealed alterations in pathways such as extracellular matrix structural constituents and fatty acid binding. Proteins related to mitochondrial function, fibrosis, glycolysis, and other metabolic processes were also differentially expressed.

CONCLUSIONS: The proteome is significantly altered in BPH. Findings support changes in the extracellular matrix, cellular adhesion, cytoskeleton, and fatty acid metabolism. Given recent work investigating the role of metabolic dysfunction and fatty acid metabolism in BPH, this data provides clinical relevance to these findings.

HEALTH RELEVANCE: This study used clinical samples to identify protein changes between donor and BPH tissues, potentially revealing disease-relevant alterations that can be targeted in preclinical models.

IMPACT OF AGE AND SEX ON BLADDER FUNCTION AND STUM GENE EXPRESSION IN C57BL/6J MICE (poster #2)

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The Jackson Laboratory, Bar Harbor, Maine

INTRODUCTION AND OBJECTIVE: Bladder dysfunction lacks known genetic targets. Recent transcriptomic studies identified *Stum* as a novel gene expressed in detrusor smooth muscle (DSM) of the mouse bladder. *Stum* is known to regulate proprioceptive neuron function in fruit flies, and similar cells in the bladder help sense pressure and control urination. Prior experiments showed sex-specific effects of *Stum* knockout on bladder function, prompting further investigation into its expression across age and sex in C57BL/6J mice.

METHODS: Bladder function was assessed using a void spot assay in young and old male and female mice. Spatial expression of *Stum* was analyzed using RNAscope on bladder tissue, and gene expression levels were quantified via qPCR.

RESULTS: Older males exhibited significantly reduced bladder function, indicated by increased voids, compared to younger males ($p = 0.05$) and age-matched females ($p = 0.02$). Female mice showed no significant age-related changes in bladder function. RNAscope revealed *Stum* expression was more prevalent in the mucosa of females ($p = 0.0005$) and in the muscularis of males ($p = 0.0003$). qPCR showed lower *Stum* expression in old males compared to age-matched females ($p = 0.02$).

CONCLUSION: The study demonstrates sex- and age-dependent differences in bladder function and *Stum* gene expression, suggesting *Stum* may play a role in bladder regulation.

HEALTH RELEVANCE: These findings position *Stum* as a potential genetic target for bladder dysfunction therapy, offering new avenues for sex-specific treatment strategies.

SPOT-FIND: A NOVEL, OPEN-SOURCE VOID SPOT ASSAY IMAGE ANALYSIS TOOL (poster #93)

Maya Allen¹, Cara C. Hardy¹, Susan Sheehan¹, Seamus Mawe¹, Nick Sebasco¹, Will Ricke², Zaenab Dhari³, Stephen J. Crocker³, Matt Mahoney¹, and Ron Korstanje¹

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INTRODUCTION AND OBJECTIVE: The void spot assay (VSA) is a widely used, non-invasive method for evaluating urinary behavior in rodents. However, current analysis tools are limited in scope, throughput, or accessibility. To address these limitations, the authors developed Spoti-find, a stand-alone, open-source VSA image analysis application aimed at enhancing the interpretive power of VSA through novel metrics and improved usability.

METHODS: Spoti-find introduces biologically meaningful metrics such as void circularity, distance to paper edge, and volume-based binning into primary, micro-, and nanovoids. The tool includes a graphical interface that supports manual or semi-automated spot identification, adjustable thresholds, and streamlined data export. Importantly, it is designed to be accessible to users without coding expertise.

RESULTS: Validation of Spoti-find was performed across diverse datasets. The tool demonstrated:

- Strong inter-user consistency
- Sensitivity to known phenotypes in aging and disease models
- Accuracy in capturing novel parameters
- High agreement with existing analysis tools

CONCLUSION: Spoti-find enhances the analysis of voiding patterns by incorporating behavioral context and spatial morphology. It offers a flexible, user-friendly platform for phenotyping urinary dysfunction in preclinical studies.

HEALTH RELEVANCE: By improving the resolution and interpretive capacity of urinary behavior assays, Spoti-find supports more precise phenotyping in models of aging and disease. This contributes to better understanding and potential therapeutic targeting of urinary dysfunction in translational research.

MODULATION OF BLADDER FUNCTION BY LOCAL INJECTION OF ADENO-ASSOCIATED VIRUS (poster #3)

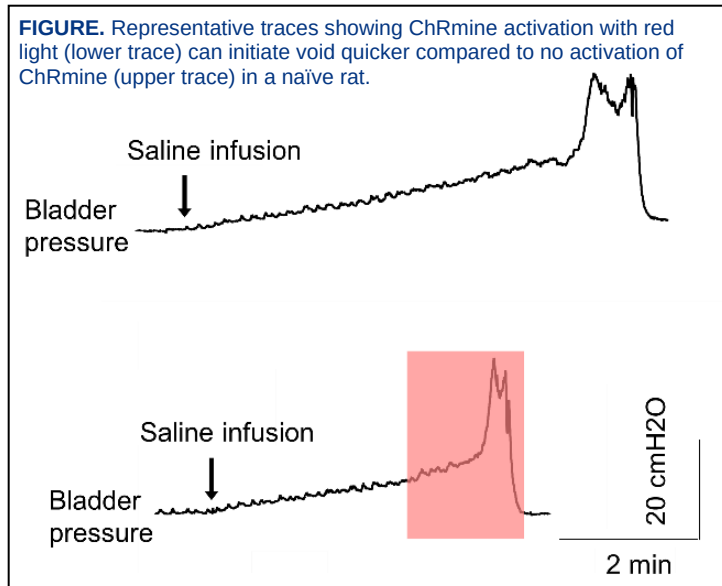
Firoj Alom^{1,2}, Kyle Ratcliff², Zach Smith¹, Bhavana Talluri¹, Aaron D Mickle^{1,2,3}

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INTRODUCTION AND OBJECTIVES: Incomplete functional recovery of the bladder frequently occurs in spinal cord injury (SCI) patients. Electrical neuromodulation of the lower urinary tract remains an attractive therapeutic target, but it has yet to be fully deployed clinically. Optogenetic techniques can be useful in modulating the function of the bladder to initiate voiding successfully in SCI patients.

METHODS: We injected AAVretro-CAG-mScarlet (vector control) and AAVretro-CAG-ChRmine-oScarlet directly into the bladder wall. ChRmine is light-gated cation conducting channelrhodopsin, which can depolarize the neuron after its expression when activated by red light. We then performed cystometry after 5-7 weeks of virus injection, to evaluate the modulation of bladder contraction by application of red light, which activates ChRmine. In a sub set of rats, we performed SCI at T9 with 100 kilodyne impact force after 5 weeks of viral injection into female rats, followed by cystometry 5 days post-SCI to evaluate whether ChRmine activation with red light can improve bladder function. Following cystometry, we perfused the rats and collected tissues to evaluate ChRmine transduction into the peripheral neurons.

RESULTS: Single fill cystometry showed that the bladder injected with ChRmine took significantly less time to initiate void and accordingly less void volume when stimulated with red light in naïve rats. Bladder injected with vector control showed no significant difference in time for initiating void and voided volume with red light stimulation. In the SCI rat, ChRmine activation largely decreased post-void residual volume by increasing voided volume compared to no activation of ChRmine in the same animal. We confirmed the retrograde expression of mScarlet and ChRmine-oScarlet by histology.



CONCLUSIONS: Our preliminary data suggest that local injection of AAVretro-CAG-ChRmine could be effective in transducing neurons, and its activation improves bladder function after SCI. Further studies are needed to conduct this research on a group of SCI animals with different extents of injury levels to evaluate more precisely the effects of ChRmine activation on bladder function after SCI.

HEALTH RELEVANCE: We hope that the translation of these results will help to improve neuromodulation techniques targeting lower urinary tract function.

SEROTONIN SIGNALING IN URINARY TRACT INFECTION: INSIGHTS FROM MOUSE MODELS AND CHILDREN WITH BLADDER BOWEL DYSFUNCTION (poster #4)

Marcela Ambroggi^{1,2}, **Laura Hernandez**³, **Taylor Garvey**², **Sarah Reiels**², **Jennika Finup**², **Vinaya Bhatia**², **Kristin Ebert**², **Shannon Cannon**², **Chad Vezina**¹, **Walid Farhat**²

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INTRODUCTION AND OBJECTIVE: Gastrointestinal pathologies such as bladder bowel dysfunction (BBD) can increase urinary tract infection (UTI) risk by altering gut and urinary tract function. Serotonin (5-HT), produced mainly by enterochromaffin cells in the gut, regulates gut motility and immune responses. Altered 5-HT signaling can contribute to constipation and may also influence local inflammation in the urinary tract. The serotonin transporter (SERT) controls 5-HT activity, while neutrophil gelatinase-associated lipocalin (NGAL) serves as a marker of epithelial inflammation. In this study, we investigated how 5-HT and SERT affect urethral inflammation and susceptibility to uropathogenic *E. coli* (UPEC) in mice, and explored associations between urinary 5-hydroxyindoleacetic acid (5-HIAA), symptom scores, and UTI history in a pilot cohort of children with BBD.

METHODS: Female *Sert null* mice (6–8 weeks old) with reduced SERT and elevated extracellular 5-HT, mimicking gastrointestinal dysregulation, were compared to wild-type (WT) controls. Urethral NGAL was assessed by immunohistochemistry. UPEC was instilled into the distal urethra (n=6/group), and bladder bacterial burden measured 4 hours later. In 24 children with BBD (10 males, 14 females), urinary 5 HIAA was measured by tandem mass spectrometry and correlated with Dysfunctional Voiding Symptom Score (DVSS), sex, and UTI history.

RESULTS: *Sert null* mice showed elevated basal urethral NGAL, indicating ongoing inflammation. Following UPEC instillation, *Sert null* mice had higher bladder bacterial loads than WT (p=0.0108). In children, urinary 5-HIAA levels varied widely. Average DVSS scores were similar between females (8.3 ± 5.2) and males (8.95 ± 4.41), however a history of UTIs was more frequent in females (50%) than in males (10%). Mean urinary 5-HIAA was higher in males (7.3 ± 3.5 mg/g creatinine) than in females (5.4 ± 3.1 mg/g creatinine), though not statistically significant (p=0.19).

CONCLUSIONS: These findings implicate 5-HT signaling and SERT function in urethral inflammation and UPEC susceptibility in mice, and they highlight potential sex differences in urinary 5-HT metabolism and UTI risk among children with BBD. The human data, while limited by sample size, are exploratory and hypothesis-generating, warranting validation in larger cohorts.

HEALTH RELEVANCE: Targeting 5 HT pathways may reduce recurrent UTIs in bladder and bowel dysfunction. Urinary 5 HIAA could serve as a noninvasive biomarker, pending validation in larger cohorts.

ANGIOTENSIN II TYPE 2 RECEPTOR AGONISM ELICITS SEX DIFFERENCES IN MURINE BLADDER CONTRACTILE RESPONSES EX VIVO (poster #5)

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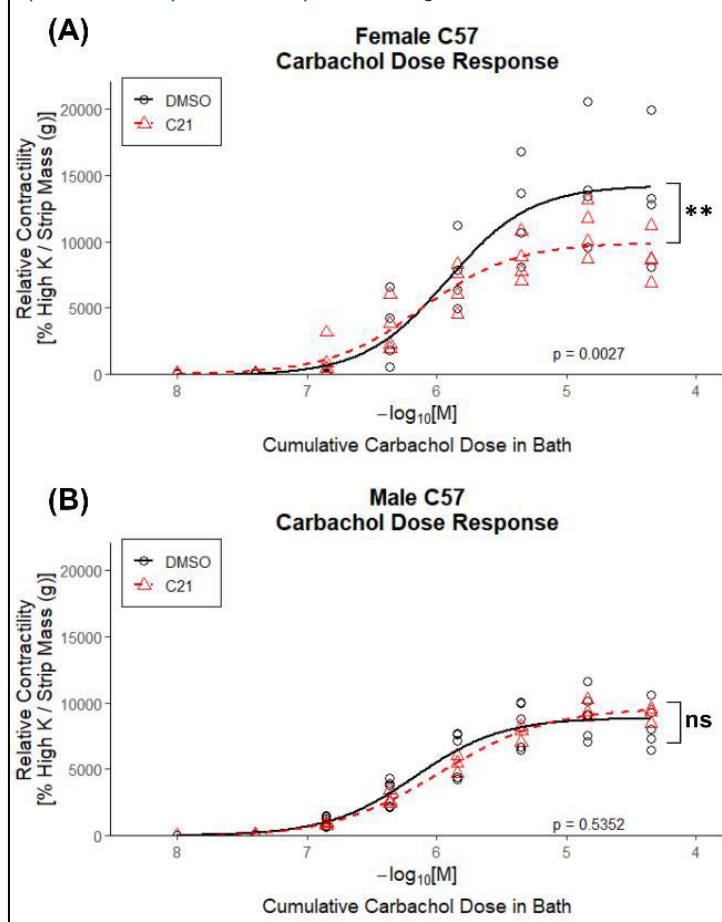
INTRODUCTION AND OBJECTIVE: One of the renin-angiotensin system receptors, the angiotensin II type 2 receptor (AT2R), is postulated to have vasodilatory and anti-inflammatory roles in various organ systems. Clinical trials have emerged for an AT2R agonist, Compound 21 (C21), to harness these protective properties.

However, AT2R's role in the bladder remains unclear. We sought to explore any potential effects of AT2R agonism with C21 on bladder contractions *ex vivo* in male and female mice. We hypothesized that C21 would reduce amplitudes of Ang II- and carbachol-induced contractions.

METHODS: Each mouse bladder was dissected into 2 strips and mounted in an organ bath for isometric tension recording. In the presence or absence of 10 μ M C21, angiotensin II (Ang II) or carbachol was administered. 70 mM KCl was administered pre- and post-testing to identify tissue desensitization. Male and female C57BL/6 mice were used experimentally, and AT2R gene (Agtr2)-null mutant mice were used as negative controls.

RESULTS: Our data have shown that in female bladder tissue, the presence of C21 significantly reduces the amplitude of Ang II- ($n=8$, $p=0.0039$) and carbachol- ($n=4$, $p=0.0027$, **Figure A**) induced contractions, while in male bladder tissue, C21 has no significant effect on Ang II- ($n=8$, $p=0.3849$) nor carbachol- ($n=3$, $p=0.5352$, **Figure B**) induced contractile amplitudes. In Agtr2-null female mice, C21 had no significant effect on carbachol-induced contractions ($n=4$, $p=0.2527$), suggesting C21 effects are due to the presence of AT2R.

FIGURE. Bladder strips treated with C21 (red dashed line) exhibited significantly reduced carbachol-induced contractions in females (A) but not in males (B) compared with strips treated with the vehicle DMSO (black solid line), based on 4-parameter logistic model fits.



CONCLUSIONS: Our results suggest a sex difference in the action of AT2R in murine angiotensin- and muscarinic- receptor driven bladder contractions, with capability to decrease amplitudes in female mice. We aim to continue these experiments in bladder tissue subjected to experimental autoimmune cystitis, which will evaluate AT2R as a potential pharmacological target for pervasive bladder diseases such as interstitial cystitis/bladder pain syndrome (IC/BPS).

HEALTH RELEVANCE: Investigating sex differences in Ang II signaling in the bladder through mouse models may further our

understanding of clinically observed sex differences in bladder diseases. Future research in this area may help to inform treatments for IC/BPS.

IN AGED RATS, PELVIC RADIATION DID NOT WORSEN VAGINAL COLLAGEN DEPOSITION, ELASTIN CONTENT, OR EPITHELIAL THICKNESS (poster #6)

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INTRODUCTION AND OBJECTIVE: In the US, over 19 million women are pelvic cancer survivors. Pelvic cancers are most commonly treated with radiotherapy (RT) and frequent side effects include vaginal stenosis, fibrosis, and dryness, which lead to sexual dysfunction and decreased quality of life. Additionally, most pelvic cancer survivors are post-menopausal. A previously established RT model in young rats showed that RT reduced vaginal blood flow, thinned vaginal epithelium, and increased vaginal contraction. However, it is unknown if the adverse effects of RT are greater in aged rats. This study will assess if RT differentially affects the vaginal epithelium and extracellular matrix in young and old rats.

METHODS: Young (12 weeks) and old (15 months) female Sprague-Dawley rats received a single dose of pelvic RT (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 stain was used to evaluate collagen deposition. Masson's trichrome stain was used to evaluate epithelial thickness, count epithelial layers, and identify epithelial cells (basal, intermediate, apical). Modified Hart's stain assessed elastin area. ImageJ was used to quantify epithelial thickness, collagen deposition, and elastin area. For all parameters, three representative images (40x) were taken, analyzed, and averaged. A two-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests compared groups.

RESULTS: Independent of RT, total vaginal collagen was increased in old compared to young rats ($62 \pm 10.53\%$ vs $38 \pm 11.7\%$, $p<0.05$). RT did not change total vaginal collagen in young or old rats. Similarly, 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 versus Sham ($p<0.05$). In all other groups, vaginal epithelial layer number did not change. Finally, total elastin was unaffected by RT in young or old rats. We are continuing to measure elastin length and fragmentation.

CONCLUSIONS: As previously established, aging increased vaginal collagen deposition. However, radiation in aged rats did not further alter vaginal collagen content, the epithelium, or elastin area. 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 the quality of life in survivors, increased understanding of the treatment-induced mechanisms of pathological vaginal smooth muscle and epithelial remodeling is essential to mitigate these adverse effects.

FUNDING: ISSWSH, UWCCC Support Grant P30 CA014520

PREPUBERTAL LEUPROLIDE TREATMENT ATTENUATES ANDROGEN RECEPTOR EXPRESSION IN THE VENTRAL PROSTATE OF ADOLESCENT SPRAGUE-DAWLEY RATS (poster #7)

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INTRODUCTION AND OBJECTIVE: Extended-release formulations of gonadotropin-releasing hormone (GnRH) agonists, like leuprolide acetate (LA), are used to halt puberty in patients with gender dysphoria by suppressing estrogen and androgen synthesis. These GnRH agonists, colloquially known as puberty blockers, delay the development of secondary sex characteristics, which effectively provides the patient with more time to explore their gender identity. These same drugs have been extensively studied in the treatment of advanced prostatic cancer, where they reliably reduce both androgen levels and prostate size. While these effects suggest a similar influence on the developing prostate, the impact of GnRH agonists on healthy prostatic tissue in the context of gender-affirming care remains largely unexplored. Thus, our objective was to investigate the short-term effects of a single prepubertal LA depot injection on prostate health in rats. We hypothesized that the development of the ventral prostate would be impaired due to reduced androgen receptor (AR) expression and regulation.

METHODS: Sprague-Dawley rats were subcutaneously injected once with either the LA depot (0.6 mg, n=12) or vehicle control (n=12) on postnatal day (PND) 23. At PND 44, three weeks after drug administration, ventral prostate tissue was collected for biometric, histological, and molecular analysis.

RESULTS: At euthanasia, LA males showed a significant reduction in ventral prostate normalized mass ($p=0.004$), and trended towards decreased body mass ($p=0.074$). Although serum testosterone levels were unchanged, LA treatment significantly reduced AR protein expression within the ventral prostate epithelium. Preliminary evidence suggests that the cell proliferation marker, Ki67, was also reduced in the epithelium of LA-treated animals compared to controls.

CONCLUSIONS: Collectively, these findings demonstrate that a single LA prepubertal injection significantly impairs ventral prostate development by potentially suppressing cell proliferation and AR signaling during the treatment period. Additional histological and molecular analyses are underway to fully ascertain prostate health. Furthermore, the persistence of these effects following drug clearance remains to be determined.

HEALTH RELEVANCE: This study contributes foundational insights on the effects of prepubertal GnRH agonist therapy on prostate health, which helps to inform clinical decisions in gender-affirming care for transgender and nonbinary youth.

PRENATAL EXPOSURE TO ESTROGENIC ENDOCRINE DISRUPTORS LEADS TO TRANSGENERATIONAL ALTERATIONS IN ESTROGEN RECEPTOR EXPRESSION AND SIGNALING (poster #8)

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INTRODUCTION AND OBJECTIVE: Prior research has shown inappropriate prenatal estrogen exposure alters prostate development and increases susceptibility to disease later in life. Yet, the transgenerational effects of prenatal estrogen exposure on estrogen receptor (ER) signaling remain unclear. Given the association of ER α to BPH (benign prostatic hyperplasia) and the antiproliferative effects of ER β in the prostate, we hypothesize prenatal estrogen exposure increases ER α signaling and reduces ER β signaling transgenerationally.

METHODS: CD-1 mice, known to be less sensitive to estrogen exposure in adulthood, were implanted with 17 β -estradiol (E2), ethinyl estradiol (EE2), BPS, and corn oil (control) in utero to produce F1, F2, and F3 generations. At 2 months, a subset received testosterone (T) and E2 to induce BPH. We assessed steroid hormone metabolism via multiplex fluorescent immunohistochemistry

targeting ER α , ER β , CYP7B1, AKR1C1, E-cadherin, and COX-2 in the anterior prostate (AP) and examined ER β signaling through AKR1C1, an enzyme that produces ER β agonists, and CYP7B1, an enzyme that degrades ER β agonists.

RESULTS: AP ER α alpha signal intensity increased with prenatal BPS exposure and with and without T+E2 treatment in F3 vs F1. AP ER β signal intensity decreased with prenatal E2 exposure and T+E2 treatment in F2 vs F1 and F3 vs F1. This decrease was supported by a decrease in AKR1C1 and CYP7B1 intensity in F3 vs F1 mice prenatally exposed to E2.

CONCLUSIONS: Our findings suggest prenatal exposure of BPS increases ER α signaling in the F3 generation, an effect which may be independent of T+E2 treatment and may increase susceptibility to developing BPH. The prenatal exposure of E2 with T+E2 treatment appears to sensitize AP to decreased ER β signaling and to lower accumulations of ER β agonists in the F3 generation, alterations which may increase prostatic proliferation.

HEALTH RELEVANCE: Our results support the proposition that prenatal estrogen exposure may contribute to the higher incidence of BPH in African American (AA) men compared to European American men, a difference which appears to be independent of socioeconomic factors. Given evidence this population is exposed to higher levels of maternal estrogens *in utero*, elucidating the etiology of BPH may help improve prevention and treatment approaches for AA men.

DEVELOPMENT OF A 3D BLADDER ORGANOID MODEL FROM MOUSE UROEPITHELIAL CELLS (poster #9)

Silvia Grant, PhD, Christina Bergemann, PhD, Nazema Y Siddiqui, MD, MHSc

Duke University, Department of Obstetrics and Gynecology, Division of Urogynecology

INTRODUCTION AND OBJECTIVE: Recurrent urinary tract infections (rUTIs) are a major health concern in postmenopausal women, with recurrence rates near 50%. Repeated antibiotic exposure contributes to antimicrobial resistance, highlighting the need for alternative strategies. Estrogen hormone therapy and probiotics, particularly *Lactobacillus crispatus*, show promise in restoring a healthy urogenital microbiome. However, mechanistic understanding of host-microbiome interactions in the bladder is limited by the lack of physiologically relevant models. Given recent emphasis on non-animal model systems (NAMS), we anticipate future studies will use human tissue models. However, to translate findings from rodent studies, it would be helpful to co-develop rodent and human tissue models that allow for cross-comparison of findings. This study aims to develop a 3D bladder organoid model from mouse uroepithelial cells to enable controlled investigations of microbial colonization, epithelial responses, and hormonal modulation.

METHODS: Bladders will be harvested from germ-free female C57BL/6 mice, and luminal uroepithelial cells will be isolated using TrypLE-mediated dissociation (Fig. 1). Cells will be embedded in Cultrex Basement Membrane Extract and seeded as 3D domes in 24-well plates. Organoids will be cultured in defined medium containing FGF7, FGF10, A83-01, B27, and Y-27632, with regular passaging or shearing to maintain growth. Differentiation and identity will be validated via immunostaining for uroplakin and cytokeratin 7. These organoids will be used to study *Lactobacillus crispatus* colonization and the influence of steroid hormones (e.g., estrogen and progesterone) on host-microbe interactions.

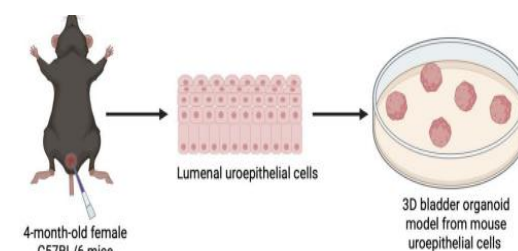


Figure 1. 3D Bladder Organoid creation. Simplified flow-chart of the design of 3D bladder organoids using luminal uroepithelial cells from four-month-old female germ-free C57BL/6 mice.

ANTICIPATED RESULTS: We expect to generate mature, stratified bladder organoids that recapitulate key features of the uroepithelium. These organoids will provide a reproducible, physiologically relevant platform for investigating microbial colonization dynamics and epithelial responses to hormonal modulation.

CONCLUSIONS: This model lays the groundwork for generating analogous human bladder organoids from patient-derived uroepithelial cells, enabling translational studies of host-microbe interactions.

HEALTH RELEVANCE: By modeling the postmenopausal urogenital niche, future research will inform development of microbiome-based interventions to prevent recurrent UTIs in women.

ESTROGEN-MEDIATED PROTECTION OF THE UROEPITHELIUM AGAINST UROPATHOGENIC *ESCHERICHIA COLI* (poster #10)

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) affect over 60% of women and cost an estimated \$2 billion annually in the United States. Understanding host-pathogen interactions driving infection is essential for developing preventive strategies. Vaginal estradiol (E2) is an effective therapy for recurrent UTIs, yet its mechanisms remain unclear. This study examined the effects of E2 and estrogen receptor (ER) subtypes—ER α , ER β , and GPR30 (GPER)—on Uropathogenic *Escherichia coli* (UPEC) adherence, invasion, and innate immune responses in urothelial cells.

METHODS: Human bladder carcinoma cells (HTB-9) were treated with 100 nM E2, or E2 following pretreatment with ER α antagonist Fulvestrant (100 nM), ER β antagonist PHTPP (100 nM), GPER antagonist G-15 (100 nM), or vehicle (VEH). UPEC strain UTI-89 was cultured for 16 h at 37 °C and added to HTB-9 cells at a multiplicity of infection (MOI) of 0.4. After treatment, adherence, invasion, qPCR, and cell viability assays were performed. Serial dilutions determined viable UTI-89 counts (CFU/mL). Three biological (12 technical) replicates were used. Data were analyzed by unpaired t-test or one-way ANOVA with Tukey's post-test ($p < 0.05$).

RESULTS: E2 significantly reduced UPEC adherence (E2 vs VEH $p < 0.001$) and invasion (E2 vs VEH $p < 0.001$) in HTB-9 cells. Fulvestrant attenuated E2's ability to inhibit bacterial growth both adherence (E2 vs E2 + Fulvestrant $p < 0.001$) or invasion (E2 vs E2 + Fulvestrant $p < 0.01$). PHTPP and GPER did not alter E2 bacterial growth inhibition. E2 treatment significantly upregulated the expression of *ers1* ($p < 0.0001$), *ers2* ($p < 0.001$), and *gper* ($p < 0.05$) compared to VEH, with *ers1* (ER α) showing the most pronounced (8-fold) increase in response to E2 treatment. The antagonists abrogated the E2 effect. Fulvestrant treatment downregulated *ers1* expression. E2 also upregulated mRNA expression of AMPs *s100a7* ($p < 0.01$), *slpi* ($p < 0.05$), *defb103b* ($p < 0.01$) and *defb4a* ($p < 0.05$) when compared to VEH. Fulvestrant significantly blocked the E2-induced upregulation of these AMPs.

CONCLUSIONS: Estradiol reduced UPEC adherence and invasion in bladder epithelial cells through an ER α -dependent mechanism and increased AMP expression. Fulvestrant blocked both antibacterial and AMP responses, confirming ER α as the primary mediator of E2's protective effects. These results identify ER α -dependent signaling as a key pathway by which E2 enhances

urothelial defense and suggest potential therapeutic targets for recurrent UTIs.

HEALTH RELEVANCE: ER α -dependent estrogen signaling reinforces uroepithelial defense, providing a biological basis for the clinical effectiveness of vaginal estrogen therapy in preventing recurrent urinary tract infections.

EFFECTS OF PROPHAGE INDUCTION ON BACTERIAL COMPETITION IN THE URINARY MICROBIOME (poster #11)

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INTRODUCTION AND OBJECTIVE: Lactobacilli are prevalent in the female urobiome and can inhibit the invasion of uropathogens. Due to increasing concerns about antibiotic resistance in urinary tract infection (UTI), alternative treatment options such as probiotic lactobacilli have been studied. However, it remains unclear how urinary lactobacilli interact with one another and how the introduction of new probiotic strains might affect these interactions. Prior work showed the inhibition of *Lactobacillus delbrueckii* by *Lactocaseibacillus rhamnosus* likely through a cell dependent mechanism. Genomic analyses have revealed the presence of a temperate prophage, JCL1032, in urinary *L. delbrueckii* strains, potentially influencing bacterial fitness and competitive interactions. This study aims to examine whether prophage induction in *L. delbrueckii* impacts bacterial competition in the urinary tract.

METHODS: Urinary and non-urinary *L. delbrueckii* strains will be exposed to UV light and mitomycin C to induce prophage activation. Co-culture experiments of *L. delbrueckii* strains with *L. rhamnosus* and its spent media will assess changes in bacterial growth using optical density measurements. Phage induction will be confirmed via plaque assays. Bioinformatics analyses of genome sequences of urinary *L. delbrueckii* and isolates from non-urine sources will be used to identify prophage presence and integration patterns.

RESULTS: We established near complete whole genome sequences of three urinary *L. delbrueckii* isolates. These isolates harbor plasmid carrying JCL1032 prophage and are inhibited by several isolates of *L. rhamnosus*. In our ongoing work testing for induction of this prophage, we hypothesize that prophage induction will reduce *L. delbrueckii* viability in co-culture with *L. rhamnosus* and that JCL1032 prophage will be more frequently present in urinary isolates of *L. delbrueckii* correlating with specific inhibition patterns.

CONCLUSIONS: This study will contribute to the understanding of prophage-directed bacterial competition and could lead to discovery of targeted probiotic approaches for promoting a healthy urinary microbiome while suppressing risk for UTI in susceptible populations.

HEALTH RELEVANCE: Due to increasing antibiotic resistance in clinical isolates of uropathogens, it is important to consider alternative preventive and therapeutic approaches using emerging knowledge of human urobiome composition and function. This work considers basic microbial interactions that will help determine which of the urobiome-based interventions is the most promising to develop.

USING GENERATIVE ARTIFICIAL INTELLIGENCE TO CHARACTERIZE PRACTICE PATTERNS: METHENAMINE USAGE AMONG KIDNEY TRANSPLANT RECIPIENTS (poster #12)

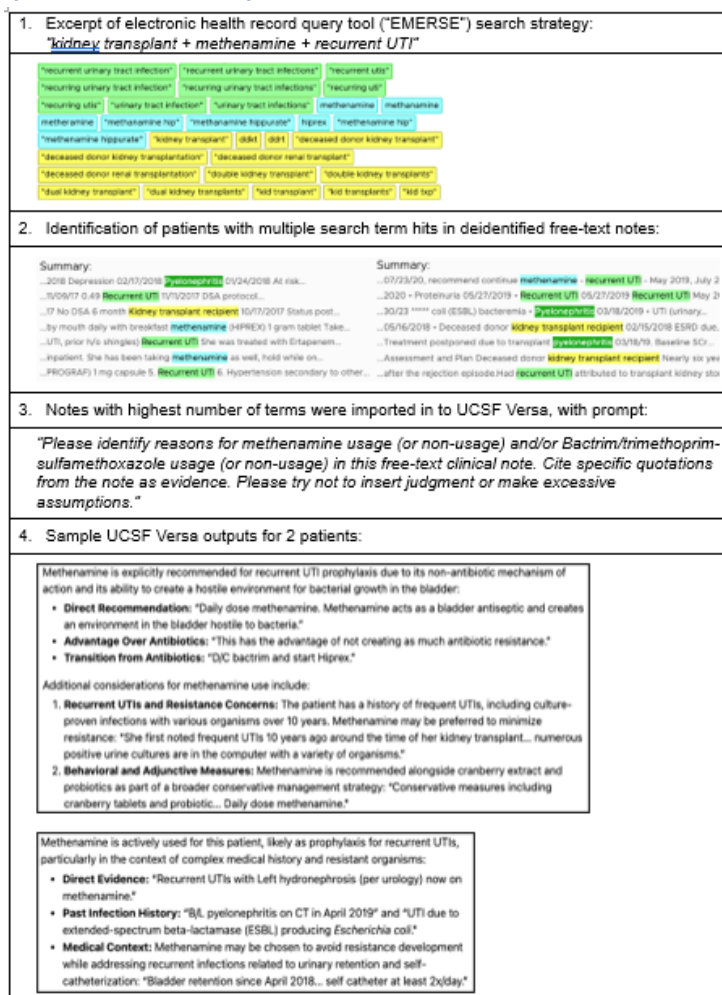
Alexandra Bicki, MD, MPH¹ Alison Huang, MD, MPhil^{2,3,4} Deborah Adey, MD,⁵ Joseph Liao, MD,⁶ Elaine Ku, MD, MAS^{1,4,5}

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INTRODUCTION AND OBJECTIVE: Daily oral methenamine, a urinary antiseptic, may reduce patients' potential for developing urinary tract infections (UTIs) with resistant organisms. Up to 80% of kidney transplant recipients experience UTIs. However, only two retrospective studies have evaluated methenamine's efficacy in this population. The objective of this study was to pilot the use of generative artificial intelligence (genAI) to identify drivers of practice patterns related to methenamine use for UTI prophylaxis after kidney transplant.

METHODS: Two UCSF systems were utilized: EMERSE, an electronic health record (EHR) query tool comprised of >100 million deidentified clinical notes that does not require IRB approval for use, and Versa, a genAI platform running GPT-4 certified to process sensitive/protected health information. We utilized EMERSE to identify kidney transplant recipients with EHR mentions of recurrent UTIs and methenamine. We then prompted Versa to determine providers' rationale for methenamine use (Figure). As the "gold standard", one author (AB) conducted independent thematic synthesis of the same clinical notes.

FIGURE. Process of characterizing methenamine practice patterns using a generative artificial intelligence framework



RESULTS: Within 8 minutes, we generated a cohort of 502 patients from 2012-2024 meeting the search criteria (80% female, 33% Hispanic/Latino, 75% age >50 years). The ability to sort notes with the most mentions of methenamine efficiently yielded patients who had actively taken the drug (vs. those for whom it was only briefly discussed). GenAI's indication for methenamine use agreed with the gold standard particularly for patients with prior extended-spectrum beta-lactamase infections and/or atypical genitourinary anatomy. Infectious Disease and Urology notes were the most comprehensive

in addressing methenamine rationale. Cranberry and Vitamin C were identified as potentially valuable adjunctive treatments.

CONCLUSIONS: An EHR query tool coupled with a secure genAI platform can quickly synthesize lengthy free-text clinical notes. Together, the systems facilitated cohort generation and accurately identified interdisciplinary care patterns and leading reasons for methenamine use.

HEALTH RELEVANCE: This framework enabled rapid understanding of practice patterns for an infrequently used medication in a high-risk population. This approach could be useful for identifying patients for simulated trials, for whom traditional randomized controlled trial recruitment is challenging.

NOTCH SIGNALING DRIVES RENAL UROTHELIUM DIFFERENTIATION AND BARRIER MATURATION IN URINARY TRACT OBSTRUCTION (UTO) (poster #13)

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INTRODUCTION AND OBJECTIVE: Childhood urinary tract obstruction (UTO) is a leading cause of chronic kidney disease in children and is met with limited treatment options. The renal urothelium mounts a protective adaptation during UTO, characterized by the expansion of uroplakin (UPK⁺) cells. The upstream pathways that govern renal urothelium differentiation and UPK expression remain poorly understood. Based on single-cell RNA sequencing predictions and known roles of NOTCH in bladder urothelial differentiation, we hypothesized that NOTCH promotes differentiation and UPK expression in the renal urothelium. Our goal is to understand the role of Notch signaling in renal urothelium.

METHODS: Kidney urothelium-derived organoids (KUDOs) were generated, and RNAseq was used to define the urothelial transcriptome in *Krt5^{CreERT2};Rbpj^{fl/fl}* (Notch^{i-LOF}), *Krt5^{CreERT2};R26^{N1-IC}* (Notch^{i-GOF}) and control (*Cre-*) conditions. Gene ontology (GO) enrichment analysis was used to define the functional consequences of Notch signaling manipulation. Upstream transcriptional regulators were predicted using ChEA3 and findings were validated using western blot (WB). UTO was modeled using unilateral ureteral obstruction (UUO) in Notch^{i-LOF}, Notch^{i-GOF} and control mice. Kidneys were collected at postoperative day (POD)7 and urothelium was evaluated using IF.

RESULTS: RNAseq identified 1,013 DEGs in Notch^{i-GOF} vs. Notch^{i-LOF} KUDOs. Upregulated genes included *Upk(1a,2,3b)*, *Krt20*, *Pparg* and *Grhl3*, while downregulated genes included *Jag2*, *Fgfr2*, *Wnt(4,10a,3a)*, *Krt(17,4)* and *Cdkn2c*. ChEA3 analysis of the 527 upregulated genes predicted master regulators of urothelial differentiation and barrier formation (PPARγ and GRHL3), whereas regulators of the 486 downregulated genes included E2F family members and mitotic drivers. Although PPARγ and GRHL3 were nearly absent in renal urothelium at baseline, both were induced during UTO, and were modulated by Notch activity.

CONCLUSIONS: Our findings demonstrate that Notch signaling is a key driver of renal urothelium differentiation, activating transcriptional programs that reinforce urothelial barrier integrity and promote UPK expression. Additional studies will define the specific Notch ligands and receptors mediating this differentiation program and assess whether Notch activation confers functional barrier reinforcement and renoprotection in UTO.

HEALTH RELEVANCE: Understanding the cellular and molecular basis of the kidney's response to UTO is critical for developing therapies that go beyond surgical decompression.

PURINERGIC RECEPTOR ACTIVATION CONSTRICTS URINARY BLADDER ARTERIOLES VIA P2X1 (poster #14)

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INTRODUCTION AND OBJECTIVE: Bladder dysfunction affects millions of people, but few effective treatments are available. Changes in bladder function correlate to changes in blood flow, which makes the vasculature a novel target for treating bladder dysfunction. Surprisingly, bladder blood flow is not regulated by myogenic tone like in other organs, suggesting other mechanisms regulate bladder blood flow. ATP is a potent vasoconstrictor released during bladder filling that could alter bladder vascular tone. Thus, we sought to determine which purinergic P2X receptors are present in urinary bladder arterioles and if they regulate purinergic vasoconstriction.

METHODS: Urinary bladder arterioles from 10 to 16-week-old male C57Bl/6 mice were isolated and used for gene expression assays or pressure myography. To measure mRNA expression, vessels were first enzymatically dissociated. Then, endothelial and vascular smooth muscle cells were isolated for measurement of *P2rx1-7*, *Acta2* (smooth muscle marker), and *Nos3* (endothelial marker) mRNA by single-cell qRT-PCR. For pressure myography experiments, isolated bladder arterioles were cannulated for measurement of diameter in response to the purinergic receptor agonist α,β Methylene-ATP (α,β Me-ATP; 100 nM). In some experiments, the endothelium was denuded via air bubble through the lumen. The presence or absence of endothelium was verified by dilation to the muscarinic receptor agonist carbachol (1 μ M). Constriction to α,β Me-ATP was measured before and after exposure to the nonselective purinergic receptor antagonist PPADS (100 μ M), or the selective P2X1 antagonist NF 279 (10 μ M).

RESULTS: Vascular smooth muscle cells expressed *P2rx1*, *P2rx4*, and *P2rx5* mRNA. In endothelial cells, *P2rx1*, *P2rx4*, and *P2rx7* were expressed. In pressure myography experiments, α,β Me-ATP produced a rapidly desensitizing constriction that was blocked by PPADS and NF 279. Interestingly, PPADS alone produced a constriction that was augmented in endothelium-denuded vessels. This was specific to bladder arterioles, as PPADS produced a significantly smaller constriction in mesenteric arterioles.

CONCLUSIONS: Based on these data, activating P2X1 receptors constricts urinary bladder arterioles and intrinsic P2X activity may help maintain urinary arteriolar dilation. Overall, these data introduce purinergic signaling as a modulator of bladder blood flow, and future experiments will further explore these mechanisms.

HEALTH RELEVANCE: This project aims to reframe bladder dysfunction as a vascular issue, and the findings of this project demonstrate that purinergic signaling can potentially be used to modulate blood flow in the bladder.

MULTI-OMIC INTEGRATION REVEALS MICROBIAL AND METABOLOMIC SIGNATURES OF LACTOBACILLUS-PREDOMINATED UROBIOMES (poster #15)

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⁵Division of Urogynecology, Oregon Health & Science University, Portland Oregon

INTRODUCTION AND OBJECTIVE: Postmenopausal women experience higher rates of lower urinary tract disorders, potentially linked urobiome dysbiosis. In premenopausal women, commensal *Lactobacillus* species typically predominate and promote urinary tract health. The molecular mechanisms underlying this protection, and the species-specific contributions of *Lactobacilli* remain unclear. We applied a multi-omic approach to identify signatures associated with

Lactobacillus-predominated (L-pred) versus *Lactobacillus*-minority (L-min) urobiomes and evaluated these differences.

METHODS: Catheterized urine samples from 166 women (Duke IRB Pro00083917; OHSU IRB 10729) underwent DNA isolation, 16S rRNA V4 sequencing (MiSeq), *Lactobacillus* species identification via LoopSeq synthetic long-read sequencing and untargeted metabolomics using ultra-high-performance liquid chromatography and orbitrap mass spectrometer. Urobiomes were classified as L-pred, (> 50% *Lactobacillus*) or L-min, (<1%). Multi-omic features were identified using sparse generalized canonical correlation analysis (sgCCA; MintTea v1.1.1) and validated with DEseq2 (v1.48.2). Species-level comparisons were conducted for *L. crispatus*, *L. iners*, and *L. gasseri*.

RESULTS: Species-level data were recovered for 138 participants. Of these, there were 43 L-pred and 42 L-min. Four associative modules differentiated L-pred from L-min, with differences in 7 bacteria and 3 metabolites. Mod 1 showed a 2.4 log-fold increase in carboxylic acid in L-min ($p < 0.001$), while Homovanillic acid increased 0.5 log-fold in L-pred ($p = 0.5$). Comparing *L. iners* vs. *L. crispatus* revealed one module with >1.5 log-fold increase in *Gardnerella* species in the *L. iners* group ($p < 0.001$).

CONCLUSIONS: MintTea's integrative multi-omic analysis identifies system-level omic features linked to urobiome composition and potential protective mechanisms. Consensus modules spanning the microbiome and metabolome suggest that *Lactobacillus*-predominant urobiomes confer molecular signatures of protection, with species-specific variations in mechanism.

HEALTH RELEVANCE: Defining the molecular pathways by which urobiome *Lactobacilli* promote urinary tract health is critical for developing strategies to prevent lower urinary tract disorders in women.

MAPPING BLADDER AND TIBIAL NERVE CONVERGENCE IN SPINAL CORD USING PSEUDORABIES VIRUS TRACING AND TIBIAL NERVE STIMULATION (poster #16)

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INTRODUCTION AND OBJECTIVE: Tibial nerve stimulation (TNS) improves symptoms of overactive bladder (OAB), but the underlying mechanism of therapeutic benefit remains unclear. In particular, where in the spinal cord tibial and bladder neurons converge, and the type of these neurons remains unclear. This gap limits rational optimization of stimulation parameters and the development of future neuromodulation strategies. Our aim is to trace where these pathways converge in the spinal cord and the cells responsible for this mechanism.

METHODS: In female rats, we injected pseudorabies viruses (PRV, Bartha Strain), a retrograde transsynaptic tracer with green (PRV-152, GFP) or red (PRV-614, RFP) fluorescent protein into peripheral targets (one in the tibial nerve, one in the detrusor). Dorsal root ganglia (DRG; L1-S1) and spinal cord (L1-S1) segments were collected and investigated using Immunohistochemistry (IHC). In another cohort, PRV-152 was injected into the detrusor and then the right tibial nerve was stimulated at a 1x motor threshold (1T) for 30 min at 20 Hz. Following stimulation, the L1-S1 spinal cord was collected and assessed for convergence of PRV with neuronal activity from stimulation (c-Fos) using IHC.

RESULTS: PRV-labeled neurons were consistently found in lumbosacral DRG. We saw dual PRV labeling of neurons in lumbosacral DRG (mostly at L6-S1), most likely indicating a tibial sensory to bladder motor pathway (i.e. transsynaptic labeling from bladder motor neurons, as opposed to a shared sensory neuron),

although this result varied across animals. Following TNS, we observed c-Fos and PRV- GFP co-localization in lumbosacral spinal cord and ipsilateral (to stimulation) c-Fos expression in the lumbosacral spinal cord, supporting functional engagement of circuits near the segments identified by PRV.

CONCLUSIONS: In summary, PRV tracing reliably marks bladder and tibial pathways in lumbosacral DRG and supports a focus on L6–S1 for spinal convergence. The ipsilateral c-Fos response to TNS further suggests segment-specific activation of these pathways. Next, we will use Herpes simplex virus (H-129), an anterograde transsynaptic tracer to trace afferent connections across spinal synapses. This will help clarify neural connections between the tibial nerve and bladder in the spinal cord. We also plan on using RNAscope to clarify the type of interneurons linking these two areas.

HEALTH RELEVANCE: By tracing where bladder and tibial pathways meet in the spinal cord, this work will support more targeted neuromodulation strategies for OAB and related bladder disorders.

STRENGTHENING COLLABORATION IN NON-MALIGNANT UROLOGY RESEARCH USING A NOVEL, CUSTOM-BUILT MOBILE PHONE APP (poster #17)

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INTRODUCTION AND OBJECTIVE: The CAIRIBU (Collaborating for the Advancement of Interdisciplinary Research in Benign Urology) Community connects and engages researchers across >200 institutions and organizations to foster collaboration and innovation. However, community engagement and dialogue among investigators from different disciplines and institutions is limited by fragmented tools, inconsistent participation, and cumbersome communication

mechanisms. We designed, developed, and evaluated a mobile app – CAIRIBU Connect – to strengthen networking, resource sharing, communication, collaboration, and research visibility within the CAIRIBU Community and with investigators from the broader urology research community.

METHODS: Using a user-centered design approach, the CAIRIBU app was built with React Native and Firebase to support both iOS and Android devices. Core features, based on initial feedback from CAIRIBU Community leaders, included a member directory, discussion forums, event listings, and a content hub for shared resources (FIGURE). Iterative feedback was collected through testing and weekly meetings of CAIRIBU Interactions Core staff and CAIRIBU Community investigators. User engagement metrics (e.g., post creation, event attendance, and discussion participation) and qualitative feedback mechanisms were set up to assess usability and impact.

RESULTS: “Phase Two” formal usability testing and data collection are ongoing. It is anticipated that the CAIRIBU app will demonstrate strong usability, accessibility, and engagement. We expect users will find the interface intuitive and that integrated features such as the discussion board, event listings, and member directory will promote active participation and information sharing. Based on initial feedback, we believe the app will reduce barriers to communication and increase collaboration opportunities between researchers who use it.

CONCLUSIONS: The CAIRIBU app provides a scalable and effective platform to enhance interdisciplinary collaboration and engagement. The integration of communication, event management, and discussion tools into a single app is anticipated to promote greater participation, a stronger sense of community, and more visibility for non-malignant urology research.

HEALTH RELEVANCE: By improving collaboration and information sharing, the CAIRIBU app may contribute to faster dissemination of scientific findings, improvements in patient care and public health, and greater awareness of non-malignant urology research.

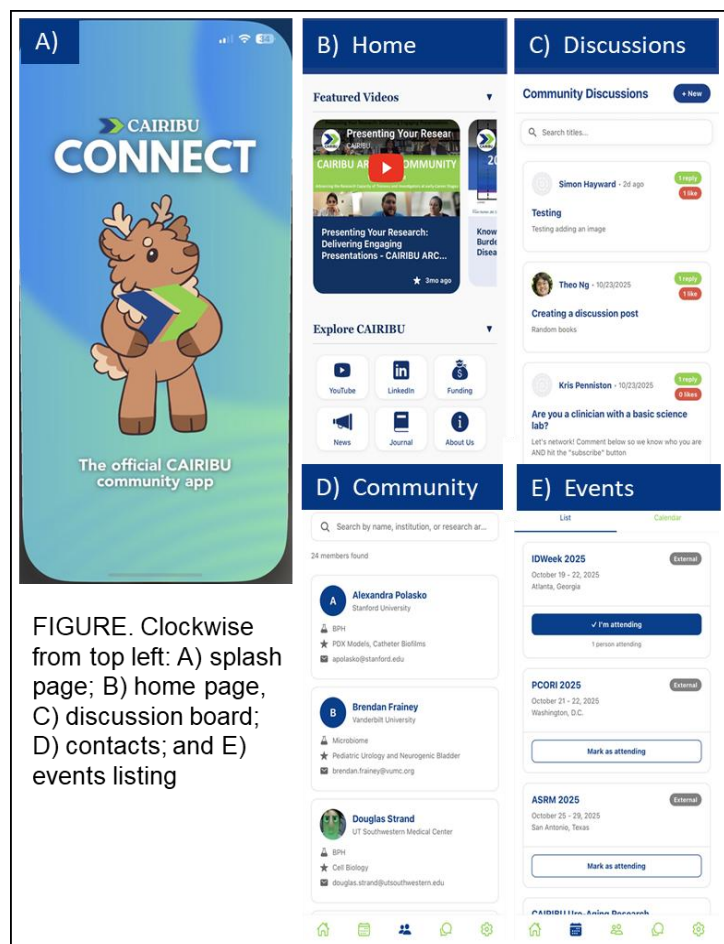


FIGURE. Clockwise from top left: A) splash page; B) home page; C) discussion board; D) contacts; and E) events listing

NON-INVASIVE ASSESSMENT OF BLADDER DIVERTICULUM BIOMECHANICS USING DYNAMIC MAGNETIC RESONANCE IMAGING (poster #18)

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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.

T CELL EXHAUSTION IN BLADDER TERTIARY LYMPHOID TISSUES ACROSS THE LIFESPAN (poster #19)

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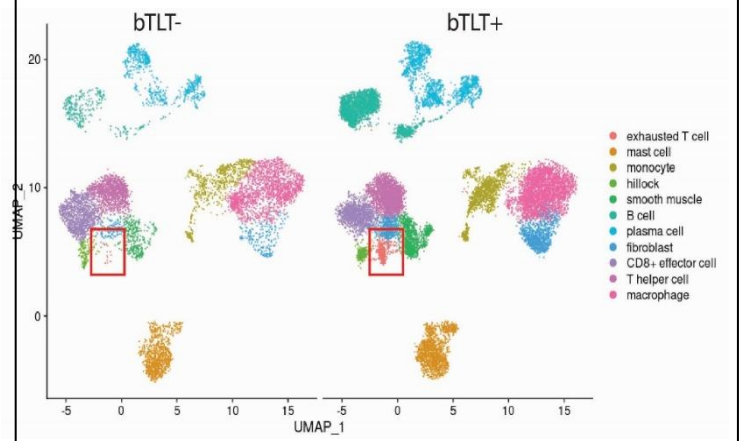
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INTRODUCTION AND OBJECTIVES: Declining estrogen during menopause is implicated in increased susceptibility to recurrent urinary tract infection (rUTI). Estrogen also modulates the immune system to elicit anti-inflammatory effects and is associated with improved immune function. Tertiary Lymphoid Tissues (TLTs) are ectopic lymphoid tissues containing T and B cells and drive antigen-specific immune responses at sites of chronic inflammation. TLTs in the bladder (termed bTLTs) are associated with worse rUTI outcomes. The prevalence and function of human bTLTs are largely unknown. Our goal is to determine the prevalence, cellular composition, and expression profile of bladders with and without TLTs across the lifespan and associated with rUTI.

METHODS: Formalin-fixed, paraffin-embedded (FFPE) bladder specimens from males (n = 70) and females (n = 87) aged 18-71 years were sourced from an active biorepository. Tissues from the neck, mid, and dome regions of each bladder were sectioned and stained with hematoxylin and eosin. bTLTs were quantified across bladder regions, sexes, and the lifespan. We selected FFPE specimens from 3 postmenopausal female cohorts (N=4/cohort) including 1) biopsies from women with rUTI with bTLTs, and age matched donor bladders 2) +bTLTs and 3) -bTLTs for single cell RNA sequencing (scRNA-seq) via 10x Genomics Chromium Flex. ScRNA-seq data were analyzed in Seurat.

RESULTS: bTLTs were observed in 43% of female and 8.6% of male specimens. There was no difference in bTLT incidence in pre- versus postmenopausal women. scRNA-seq revealed that bladders with bTLTs had 3 times as many T-cells than those without bTLTs. The most differential features between single cell profiles of bTLT+ versus bTLT- tissues were proliferative (Ki67+), exhausted T cells (PD-1 and CTLA-4+) in bTLT+ bladders (Figure). In bTLT+ bladders, 33% of T-cells displayed exhaustion markers in contrast to 4% in bladders without bTLTs.

FIGURE. bTLT(+) and bTLT(-) leukocytes separately clustered to specific cell types. The major delineating transcript is CTLA-4, which is associated with T cell exhaustion.



CONCLUSIONS: Exhausted T-cells are associated with impaired immune function, and their identification in bTLTs suggests a possible mechanism by which these lymphoid structures may contribute to worse rUTI outcomes in women.

HEALTH RELEVANCE: The finding that T-cell exhaustion may be associated with bTLTs and rUTI susceptibility in women may open the door to new immune-modulatory strategies for rUTI management. Drugs targeting T-cell exhaustion are widely used to improve bladder cancer outcomes and could become an important tool in managing rUTI.

BEYOND THE BLADDER: AN UNPREDICTABLE PHENOTYPE IN UROPLAKIN-2 CRE MICE (poster #20)

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INTRODUCTION AND OBJECTIVE: Urothelium plays an important role in sensing mechanical stretch and chemical stimuli. We aimed to show that the activation of the inhibitory Designed Receptor Exclusively Activated by Designed Drugs (DREADDs) in the urothelial cells would lead mice's bladder to be less sensitive to normal distention and store higher urine volumes.

METHODS: 10 weeks old mice UPK2Cre+hM4D(Gi) expressing the DREADD into the urothelial layer or UPK2Cre-hM4D(Gi) were used in our study. Mice were randomized to either receive saline or the DREADD agonist Clozapine-N-Oxide (5mg/kg i.p. CNO). Next, they were subjected to the UroVoid for 24h to evaluate the latency to the first void, inter-micturition interval, and total number of voids. We also performed cystometry, immunohistochemistry in the bladder, dorsal root ganglia (DRG), pelvic ganglia (PG), spinal cord (SC) and brainstem, whereas quantitative polymerase chain reaction (qPCR) for Cre was performed in the DRG, PG and urothelium. Data were analyzed using GraphPad Prism v.10 considering a 5% likelihood of type-I error. Results are presented as mean ± SD. All experiments met the guidelines for animal research.

RESULTS: As predicted, UPK2Cre+hM4D(Gi) mice showed a higher latency to the first void in response to CNO injection (p=0.004), a reduced inter-micturition interval (p=0.006), but no changes in the number of voids (p>0.05). Importantly, no differences were observed among UPK2Cre-hM4D(Gi) that received either saline or CNO. Surprisingly, we noticed that CNO injection in UPK2Cre+hM4D(Gi) mice triggered a phenotype characterized by an apparent disruption of respiratory patterns, tremors, and sustained immobility. We then wondered whether cystometry in anesthetized animals would account for differences between groups. Although we found no difference in the cystometric parameters during saline infusion, CNO

to Cre+ mice was lethal. This prompted us to investigate potential off-target expression of the GFP reporter and Cre. Besides the bladder, we noticed GFP+ cells into the DRG, PG and to a lesser extent to the SC and brainstem regions involved in respiratory and autonomic control. qPCR also confirmed Cre expression in DRG, PG and urothelium.

CONCLUSIONS: Collectively our results demonstrate that UPK2Cre+ mice have an unconventional physiological response when challenged with CNO, suggesting that there could be recombination of lox-sites followed by DREADD activation apart from urothelium. Future studies using this knock-in strain should take into consideration potential off-target expression to avoid result misinterpretation.

HEALTH RELEVANCE: Our study suggests that manipulating bladder physiology could be an efficient approach to treat conditions such as overactive bladder and incontinence.

ESTABLISHING *IN VIVO* AND *IN VITRO* PLATFORMS TO INVESTIGATE MICROBIAL CONTRIBUTIONS OF BENZO[A]PYRENE METABOLISM AND BLADDER DISEASES (poster #21)

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INTRODUCTION AND OBJECTIVE: Interactions between host, environmental chemicals, and microbes play a critical role in bladder health and disease, including bladder cancer (BC). Polycyclic aromatic hydrocarbons (PAHs) are byproducts of incomplete combustion and are prevalent in charred foods, automobile exhaust, and cigarette smoke, with diet and tobacco as primary exposure routes, and have been linked to BC. Benzo[a]pyrene, a prototypical PAH, can be metabolized into secondary metabolites that induce carcinogenic, mutagenic, and pro-inflammatory responses in host tissues. While human cells are known to metabolize BaP, it remains unclear whether bladder-associated microbes can metabolize BaP into reactive intermediates and contribute to carcinogenic or mutagenic effects in host tissues. Importantly, previous studies have shown compositional differences between the urinary microbiome of healthy individuals and those with BC, suggesting potential microbial contributions to disease pathogenesis.

METHODS: To address this gap, we developed a combined *in vivo* and *in vitro* platform to investigate microbial contributions to BaP metabolism and its impact on bladder health. *In vivo*, mice (N = 36) were gavaged daily with BaP for four weeks, and fecal and urine samples were collected weekly for targeted metabolomics and metagenomic sequencing. In a parallel *in vitro* study, bladder-associated microbial isolates (*Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*) were individually co-cultured in the presence of but sans contact with urothelial cells (HBLAK) in a trans-well system, enabling BaP diffusion between compartments. Cells were analyzed for cell viability and cytotoxicity to determine the impacts of microbial metabolism and BaP exposures on cell health. Spent media will be analyzed for BaP and its metabolites via targeted metabolomics.

RESULTS: In the *in vivo* mouse study, we anticipate distinct microbial community differences between treatment groups, characterized by an increased abundance of taxa involved in BaP and secondary metabolite metabolism. For the *in vitro* transwell experiments, we expect a reduction in cell viability and an increase in cytotoxicity for cells exposed to microbes and BaP.

CONCLUSIONS: This methodological framework enables controlled assessment of chemical-microbe-host interactions and PAH metabolism across biological systems, providing a foundation for

elucidating microbial contributions to bladder health and bladder cancer.

HEALTH RELEVANCE: Understanding how bladder-associated microbes metabolize carcinogenic compounds like benzo[a]pyrene may reveal mechanisms driving recurrence in bladder diseases and inform strategies to prevent disease reemergence.

FATTY ACID AUXOTROPHY AN ESSENTIAL METABOLIC FEATURE OF FEMALE UROGENITAL LACTOBACILLI (poster #22)

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INTRODUCTION AND OBJECTIVES: A healthy female urogenital microbiome (FUM) is dominated by species of the genus *Lactobacillus* (*Lb*), like *L. crispatus* and *L. gasseri*, that produce D-lactate, bacteriocins, hydrogen peroxide to competitively exclude pathogens. Disruption of FUM *Lb* may increase susceptibility to recurrent urinary tract infections. A major hurdle for probiotic approaches to restore a healthy FUM is achieving stable colonization of probiotic strains, which requires detailed knowledge of their metabolism. However, the metabolism of FUM *Lb* species remain understudied. We found that FUM *Lb* largely lack the canonical FAB operon for type II fatty acid (FA) biosynthesis. We therefore sought to determine if FUM *Lb* are FA auxotrophs, the extent of FA auxotrophy among the Lactobacillaceae family, and types of FAs utilized by FUM *Lb* including host FAs.

METHODS: We performed bacterial growth assays in modified DeMan Rogosa Sharpe media and chemically defined media (CDM) with different FA supplements with isolates of FUM *Lb* species and species of Lactobacillaceae associated with vertebrate hosts. We performed tracing assays with ¹³C glucose followed by bacterial lipid extraction and GC-MS of fatty acid methyl esters to test for de novo FA biosynthesis. Tracing assays were also performed by supplementing lipids extracted from human bladder epithelial cells (5637 and T24) grown on ¹³C glucose to different Lactobacillaceae species to evaluate utilization of bladder-derived lipids.

RESULTS: FUM *Lb* including species from genera *Lactobacillus* and *Limosilactobacillus* cannot perform de novo FA biosynthesis, whereas non-urogenital species can synthesize FAs and incorporate carbon from ¹³C glucose directly into their FAs. Both *L. crispatus* and *L. gasseri* utilize unsaturated FAs of different chain lengths and isomerizations. Although saturated FAs alone do not stimulate growth, *L. crispatus* and *L. gasseri* use saturated FAs when supplemented with 10% oleic acid. Importantly, tracing of ¹³C-labeled bladder epithelial cell lipids showed that *L. crispatus* and *L. gasseri* directly incorporate host FAs and their membrane FA composition is highly similar to that of host epithelial membranes.

CONCLUSIONS: FA auxotrophy exhibited by urogenital *Lactobacillus* and *Limosilactobacillus* species distinguish them from other Lactobacillaceae family members supporting the hypothesis of close co-evolution between FUM *Lb* and the female urogenital tract. Incorporation of host FAs also suggest that FA availability in the host environment shapes *Lb* membrane composition and symbiotic function.

HEALTH RELEVANCE: Our findings define an essential metabolic relationship between FUM *Lb* and their host that will prove important in designing effective prebiotic and probiotic therapies for female health.

CROSSTALK BETWEEN THREE TWO-COMPONENT SIGNALING SYSTEMS MEDIATES ANTIBIOTIC RESISTANCE IN *KLEBSIELLA PNEUMONIAE* (poster #23)

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INTRODUCTION AND OBJECTIVE: Two-component systems (TCS) are transcriptional regulatory systems employed by bacteria to adjust their physiology in response to fluctuating conditions. TCSs sense many signals, from changes in nutrient levels, temperature, growth state, or internal stressors. Importantly, this regulatory activity can also be co-opted to drive the development and spread of antibiotic resistance. Antimicrobial resistance is a severe public health burden. Especially concerning are MDR infections, which restrict treatment options and significantly increase mortality risk. MDR *Klebsiella pneumoniae* (*Kp*) is of especially high concern; it is a major cause of nosocomial respiratory and urinary tract infections (UTIs). Notably, *Kp* harbors three TCSs that have been shown to induce resistance to polymyxin antibiotics, one of the last-resort treatments for highly drug-resistant infections: PhoPQ, PmrAB, and CrrAB.

Genomic analyses of polymyxin resistant (PR) clinical isolates of MDR *Kp* have identified significant diversity in the mutational profiles that lead to resistance. In particular, a majority of isolates simultaneously harbor more than one activating mutation in *phoPQ*, *pmrAB*, or *crrAB*. Notably, minimum inhibitory concentration (MIC) testing of isogenic mutants harboring these TCS mutations show stepwise increases in PR. Further, it has been shown that two connector proteins, PmrD and CrrC, work to induce crosstalk between all three TCSs via unknown mechanisms. Together, this suggests that these regulatory systems function cooperatively to induce resistance.

METHODS: To delineate the mechanisms underlying crosstalk between each TCS, I performed RNA-sequencing (RNAseq). I utilized one clinically-identified mutation in CrrB, *crrB**L94M, known to cause high levels of PR in MDR *Kp*. In this background, I systematically perturbed each component of the regulatory network and assessed the consequences for signaling and resistance.

RESULTS: Here I present a global analysis of the PhoPQ-PmrABD-CrrABC regulatory network in MDR *Kp*.

CONCLUSIONS: This work represents a comprehensive analysis of the transcriptional profiles attributed to each component of the network, to disentangle how all three regulatory systems work together to induce antibiotic resistance.

HEALTH RELEVANCE: MDR Gram-negative bacteria, including *Kp*, are a major threat to human health worldwide. Understanding how regulatory signaling systems promote antibiotic resistance will provide practical benefits by uncovering novel ways to interfere with the growth of MDR *Kp* and related pathogens.

DEVELOPMENT OF A XENIUM SPATIAL TRANSCRIPTOMICS ANALYSIS PIPELINE TO INVESTIGATE BENIGN PROSTATIC HYPERPLASIA TISSUE MICROARRAYS (poster #24)

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INTRODUCTION AND OBJECTIVE: It is poorly understood whether the pathological heterogeneity of human Benign Prostatic Hyperplasia (BPH) is associated with clinical outcomes. The objective of this study is to develop an analysis pipeline to associate specific BPH cellular and molecular phenotypes with outcomes data

using the Xenium spatial transcriptomics 5K prime assay on a newly developed tissue microarray (TMA).

METHODS: Duplicate cores were punched from 101 Formalin Fixed, Paraffin Embedded (FFPE) blocks representing 16 normal prostate specimens and 85 BPH specimens to create a TMA. Cores included urethral, transition zone, and peripheral zone from young healthy prostate as well as cores taken from distinct stromal and epithelial phenotypes within BPH specimens from patients across distinct medical and surgical treatments. Transcriptomic and spatial imaging data were generated with a pre-designed panel of 5,000 genes and an additional custom 100-gene panel. An analysis pipeline was developed with the Seurat package in R and completes a few main steps, including separating cores into individual objects and combining core-level and clinical data into the Xenium framework. Seurat-compatible data processing allows for use of more familiar single-cell analysis methods including quality control, batch correction, cell type annotation, and differential gene expression (DEG).

RESULTS: One core with low quality was removed. Sample level batch correction was not necessary. Automated transfer of cell type labels from single-cell RNA-sequencing data was unsuccessful due to poor correlation ($R^2=0.54$) between data types. Accordingly, manual separation of cell types through lineage-based unsupervised clustering was used and produced the expected proportion of cell types (28.80% epithelia, 19.28% leukocyte, 47.44% stroma, 4.48% endothelia).

CONCLUSIONS: This dataset and analysis pipeline will allow us to investigate fundamental questions about the molecular and cellular composition of BPH phenotypes and whether specific phenotypes are associated with key clinical features such as response to medical therapy.

HEALTH RELEVANCE: The response of BPH patients to medical therapy is mixed. The production of cell type-specific molecular data at scale may provide evidence that specific clinical or pathological phenotypes are predictive of therapeutic failure, resulting in personalized approaches to clinical practice.

COMPARATIVE ANALYSIS OF HUMAN PROSTATE SINGLE-CELL TRANSCRIPTOMES FROM FRESH AND FFPE TISSUE (poster #25)

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INTRODUCTION AND OBJECTIVE: Single-cell RNA sequencing (scRNA-seq) has revolutionized our understanding of human prostate cellular heterogeneity. However, the logistics of fresh human tissue collection and profiling can be challenging. The recently developed 10x Genomics Single Cell RNA Flex platform enables single nuclear transcriptomic profiling from formalin-fixed paraffin-embedded (FFPE) tissue. We aimed to systematically compare cellular composition and gene expression profiles obtained from fresh human prostate tissue versus matched FFPE blocks using this new chemistry.

METHODS: Fresh prostate was digested into a single cell suspension for conventional 10x Genomics Chromium 3' scRNA-seq per previous protocols. FFPE sections of prostate were checked by DV200 analysis for RNA quality and then digested into a single nuclear suspension using the 10x Genomics Single Cell RNA Flex workflow. Data were processed using Cell Ranger and Seurat for quality control, normalization, and clustering. Cell-type annotation was performed using canonical prostate epithelial, stromal, and immune markers. Comparative analyses included cell-type proportions, gene detection rates, and transcriptional correlation across matched cell populations.

RESULTS: Fresh and FFPE-derived datasets revealed similar proportions of epithelial, stromal, and immune cell clusters. Gene expression correlation between matched cell types showed strong correlation indicating robust transcriptomic concordance. While FFPE-derived libraries showed lower UMI and gene counts per cell, major lineage-defining markers were robustly detected. Batch-correction and integration analyses demonstrated that FFPE data could be co-analyzed with fresh datasets without loss of biological resolution.

CONCLUSIONS: Our results validate the feasibility of applying 10x Genomics Flex chemistry to archived prostate FFPE tissues and demonstrate that key cellular and molecular features of the prostate are preserved. This enables retrospective single-cell studies on large clinical cohorts, bridging molecular insights with patient outcomes.

HEALTH RELEVANCE: Leveraging FFPE tissues for single-cell transcriptomics expands access to biobanked human prostate specimens, accelerating translational discoveries in benign and malignant prostate disease.

SELECTIVE ELIMINATION OF SENESCENT CELLS IMPROVES LOWER URINARY TRACT SYMPTOMS (poster #26)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is the non-malignant growth of the prostate gland, which can lead to the development of lower urinary tract symptoms (LUTS). Autopsies show that 90% of men over 80 are affected by histological BPH, making it one of the most prevalent age-related conditions in the United States. BPH progression is known to involve androgen signaling and smooth muscle dysfunction. However, recent studies suggest that fibrosis and age-mediated cellular senescence in the lower urinary tract may also play a role. Although unable to divide, senescent cells release pro-inflammatory and pro-fibrotic factors into neighboring tissues that cause dysfunction. We hypothesized that eliminating senescent cells would reduce fibrosis and improve lower urinary tract function.

METHODS: Twenty-four-month-old p16-3MR reporter mice were treated with ganciclovir (GCV) or vehicle for four weeks to ablate senescent cells. Lower urinary tract function was assessed weekly using void spot assays, and senescent cell presence was quantified via in vivo bioluminescence imaging. At necropsy, prostate lobes and bladder tissues were collected for histological analysis. Tissue fibrosis and apoptosis were evaluated using picrosirius red (PSR) staining under polarized light and TUNEL assays, respectively.

RESULTS: GCV-treated mice exhibited a significant reduction in total void spot count compared to vehicle-treated mice ($p < 0.01$), indicating improved urinary function. Quantification of senescent cell clearance showed a non-significant trend toward decreased bioluminescence in GCV-treated mice ($p = 0.2154$). PSR assessment showed a trend toward decreased thick, mature collagen fibers in GCV-treated bladder tissue ($p = 0.0868$) but not prostates. No differences in apoptosis were observed between treatment groups.

CONCLUSIONS: Senescent cell clearance improved urinary function in aged mice, perhaps through mechanisms beyond local prostate and bladder changes. Upstream tissues such as the brain, spinal cord, and peripheral nerves play critical roles in urinary function and should be further explored. Furthermore, the prostatic urethra, distal to the prostate and bladder, is a conserved region between humans and mice and is implicated in clinical BPH. Previous studies show this region is susceptible to fibrotic remodeling. Future studies will examine the prostatic urethra and upstream tissues. Our findings support targeting senescence in the aging prostate, either preventatively or in combination with standard therapies, to improve medical management of BPH/LUTS.

HEALTH RELEVANCE: Targeting senescent cells may provide a novel therapeutic approach to improve lower urinary tract symptoms and quality of life in aging men with BPH.

NEUROKININ A INCREASES BLADDER MECHANICAL COMPLIANCE AND AUGMENTS TRANSIENT PRESSURE EVENTS (poster #27)

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INTRODUCTION AND OBJECTIVES: The mast cell activator compound 48/80 increases bladder mechanical compliance and transient pressure event (TPE) rate of rise, causing sensory hyperactivity and overactive bladder independent of mast cells. However, as a non-endogenous molecule with complex pharmacology, it lacks relevance for physiological mechanisms. Thus, we aimed to identify an endogenous pathway that recapitulates the biomechanical and sensory effects of compound 48/80. Compound 48/80's effects were reversed by antagonists that also target tachykinin receptors. Tachykinins, such as substance P and neurokinin A (NKA), are widely distributed in the genitourinary tract and stimulate muscle contraction and sensory processes via the neurokinin receptors NK1, NK2 and NK3. We hypothesized that endogenous tachykinin signaling replicates compound 48/80's effects.

METHODS: Bladders from C57BL/6 mice (8-12 weeks old) were dissected and mounted in the Pentaplanar Reflected Image Macroscopy (PRIM) system, which allows for concurrent measurement of infused volume, intravesical pressure, and bladder geometry during ex vivo bladder filling. These data were then used to calculate bladder wall stiffness, mechanical compliance, and leading slope of TPEs as the bladder fills. We first tested the NK1 agonist substance P (10 μ M) in the presence and absence of the NK1 antagonist aprepitant (1 μ M), NK2 antagonist saredutant (5 nM) and NK3 antagonist osanetant (200 nM). Next, we tested the NK2 agonist neurokinin A (5 nM) in the absence or presence of NK2 antagonist saredutant. Lastly, we tested if saredutant could prevent the effects of compound 48/80. Statistical analysis was performed using paired t-tests and one-way ANOVA.

RESULTS: Substance P did not alter bladder compliance but did increase TPEs via NK2 receptor dependent pathways (unpaired t test, $p < 0.05$). NKA fully recapitulated compound 48/80's effects by increasing compliance and enhancing TPE rate of rise in NK2 dependent manner. Surprisingly, the NK2 antagonist saredutant also abolished compound 48/80-induced responses (one-way ANOVA, $p < 0.05$) implicating the NKA-NK2 axis as an endogenous modulator of bladder biomechanics and sensory outflow.

CONCLUSIONS: Our data uncovered that neurokinin A, acting via NK2 receptors, is capable of regulating bladder wall mechanical compliance and TPEs that drive sensory outflow during filling. Future experiments will determine the mechanism by which NKA alters bladder compliance and TPEs.

HEALTH RELEVANCE: This advances our understanding of tachykinin signaling and their role in LUTS pathophysiology and suggests NK2 as a potential therapeutic target.

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SURVIVAL OF THE SWEETEST: WHICH UROGENITAL BACTERIA CAN SURVIVE ONLY ON UROMODULIN? (poster #28)

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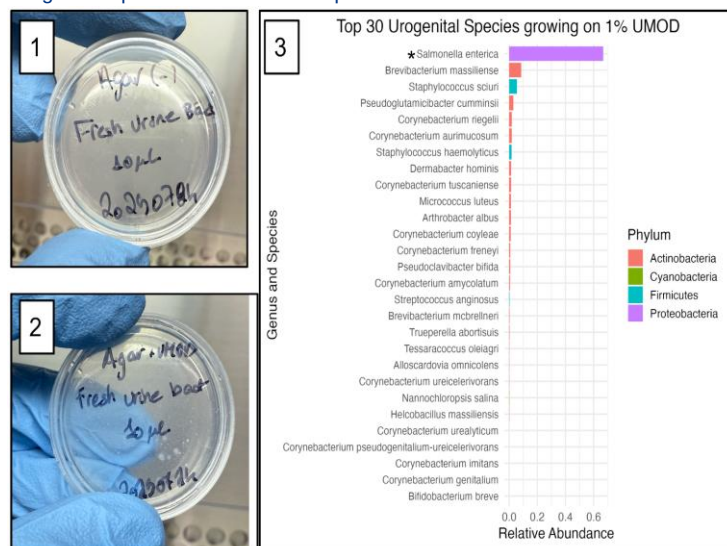
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INTRODUCTION AND OBJECTIVE: Uromodulin (UMOD) is the most abundant glycoprotein in human urine, acting as a ligand for

uropathogenic *Escherichia coli* via its *N*-glycans. Given that bladder microbes, including *Bifidobacterium longum*, have the genetic capacity to digest *N*-glycans, we aimed to test which urogenital bacteria from voided urine can survive on UMOD as the primary carbon source.

METHODS: Voided urine was collected from a female donor (Lewis & Clark College IRB 2025-06), and urogenital bacteria were pelleted at 3,000 g for 15 minutes at 4°C, then resuspended in 500 µL of sterile phosphate buffer saline. 10 µL of bacterial pellets were plated on sterile 1.5% agar only or supplemented with 2% of 0.22 µm-filtered ammonium bicarbonate purified UMOD (n=3). Plates were incubated at 37°C in 10% CO₂ for 7 days. Colonies were picked, pooled, and preserved in DNA/RNA Shield (Zymo), then sent to ZymoBIOMICS Targeted Sequencing Service for sequencing with 16S rRNA universal primers, and bioinformatics analysis.

FIGURE. Identification of female urogenital bacteria surviving on UMOD
1: Urinary pellets; **2:** UMOD + Urinary pellets; **3:** Relative abundance of urogenital species. * indicates the presence in the +/- controls.



RESULTS: No colonies were observed on agar only, but 3–5 colonies were observed on each UMOD-agar plate. Except for *Nannochloropsis salina* (microalgae), all identified species were previously observed in the urogenital microbiome (Saenz et al. 2024). The most abundant species was *Salmonella enterica* (66.82%), followed by *Brevibacterium massiliense* (8.54%) and *Staphylococcus sciuri* (5.54%); however, the presence of *S. enterica* was also noted in both positive and negative controls. Notably, *Bifidobacterium breve* and *Streptococcus anginosus* bacteria – associated with host *N*-glycans digestion – were also observed.

CONCLUSIONS: This preliminary study is the first to demonstrate that a subset of urogenital bacteria from voided urinary pellets can utilize UMOD as a sole carbon source. Our work in progress involves diversifying the urinary samples (e.g., genders) and growth conditions (e.g., strictly anaerobic).

HEALTH RELEVANCE: These findings advance our understanding of the urogenital microbial metabolism *vis-à-vis* host urinary glycoproteins.

CHARACTERIZING RADIATION-INDUCED MICROVASCULAR DAMAGE IN VITRO TOWARD A TISSUE ENGINEERED BLADDER MODEL OF RADIATION CYSTITIS (poster #29)

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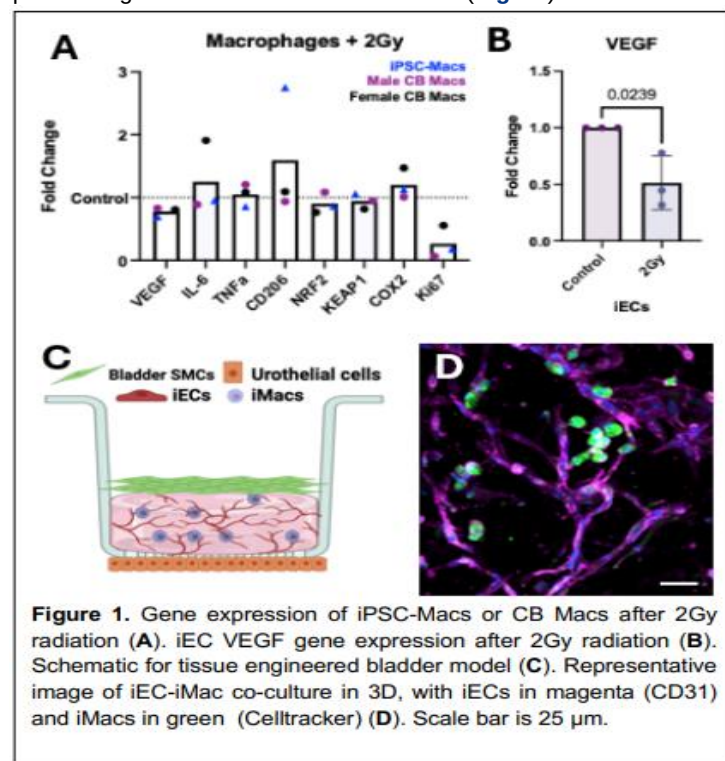
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INTRODUCTION AND OBJECTIVE: Radiation cystitis (RC) is a side effect of pelvic radiation where patients have painful, frequent, and urgent urination. It is thought that radiation-induced damage to the

bladder urothelium leads to inflammation, fibrosis, and microvascular damage. However, the lack of in vitro models makes studying mechanisms of RC challenging, requiring expensive and long-term in vivo work. To address this gap, we are developing a tissue engineered (TE) model of the bladder to find and test new therapeutic targets. We first sought to characterize the effects of a clinically relevant dose of radiation on induced pluripotent stem cell (iPSC) derived macrophages (iMacs) and endothelial cells (iECs). Then, we sought to establish a 3D iEC-iMac co-culture as the first building block of our TE bladder model.

METHODS: Cord blood (CB) derived macrophages, iMacs, and iECs were exposed to 2Gy X-ray radiation using a CIX3 cabinet irradiator. Cells were then cultured for 24h. RNA was isolated and converted to cDNA prior to polymerase chain reaction (PCR) with gene specific primers and SYBR green. For 3D studies, iECs were encapsulated in collagen-hyaluronic acid gels for 3 days prior to the addition of iMacs in suspension above the gel for 24h.

RESULTS: After exposure to 2Gy radiation, both iMacs and CB Macs show downregulation of VEGF and Ki67 (Fig 1A). Notably, VEGF gene expression is significantly downregulated in iECs after 2Gy radiation (Fig 1B). iEC morphology was not visually altered by 2Gy radiation (not shown). Toward our TE bladder model (Fig 1C), successful 3D iEC-iMac co-culture has been achieved, with iMacs penetrating established vascular networks (Fig 1D).



CONCLUSIONS: Our data suggests that radiation decreases VEGF gene expression by both iECs and iMacs within 24 hours. This could be a mechanism to drive microvascular damage and prevent regeneration in radiation cystitis. As VEGF is highly expressed by the urothelium, examining VEGF gene expression in urothelial cells after radiation is of high priority. Additionally, examining this mechanism in our complete TE bladder model will provide more insight into bladder cell crosstalk post-radiation.

HEALTH RELEVANCE: Radiation cystitis significantly impacts long-term quality of life for patients who undergo pelvic radiation for cancer treatment, even years after remission. By developing an in vitro model of the bladder wall for mechanistic testing, we hope to uncover new therapeutic targets to prevent or reduce the symptoms of radiation cystitis.

ACCELERATING DELPHI CONSENSUS: COMPARING AI AND HUMAN PERFORMANCE IN QUALITATIVE CODING FOR HYPOSPADIAS COUNSELING (poster #30)

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INTRODUCTION: Accurate and efficient qualitative coding is essential for generating statements in modified Delphi research. Artificial intelligence (AI) language models offer rapid data processing and thematic synthesis, while human coders excel in preserving nuanced meaning. Understanding the strengths and limitations of each approach can optimize consensus-building, particularly for complex clinical counseling topics such as hypospadias complications.

METHODS: We conducted a comparative analysis of coding performance between a novice human coder and ChatGPT (OpenAI) using 115–116 qualitative comments on hypospadias counseling themes. Each coded raw comments to generate candidate Delphi statements addressing surgical risks, follow-up, sexual health, and communication strategies. Coding outputs were evaluated for completeness (coverage of key themes), accuracy (fidelity to source comments), and synthesis quality (actionability and clarity of statements), with expert review of interpretive errors.

RESULTS: AI completed thematic coding and statement synthesis in 1 minute 29 seconds, compared to approximately 3 hours for the human coder. AI outputs capture all major thematic domains-mechanism, risk factors, management, follow-up, and communication- demonstrating high completeness and fidelity. Occasional interpretive additions were noted, especially in rare complication contexts. Suggested evaluation metrics include Number of major/minor themes accurately coded, proportion of raw codes captured without omission, expert-rated statement clarity and synthesis, and frequency of interpretive errors or subjective addition.

CONCLUSIONS: A human-in-the-loop model, combining rapid AI coding with targeted human oversight, optimizes qualitative synthesis for modified Delphi studies. This approach accelerates statement generation, enhances thematic standardization, and minimizes coder bias- supporting more efficient and reliable consensus development.

HEALTH RELEVANCE: Efficiently generating high-quality Delphi statements enables timely development of clinical guidelines and communication strategies. For hypospadias counseling, integrating AI could help standardize discussions around surgical risks, psychosocial considerations, and follow-up, ultimately improving shared decision-making and patient-centered care.

DEFINING WHAT TO SAY AND WHEN: A MODIFIED DELPHI CONSENSUS ON HYPOSPADIAS COUNSELING (poster #94)

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INTRODUCTION AND OBJECTIVES: Hypospadias affects approximately 1 in 200 live male births and carries some of the highest complication rates in pediatric urology - exceeding 50% in severe cases. Families frequently report long-term concerns about

urinary and penile function, unmet expectations, psychosocial challenges, and decisional conflict. Despite existing decision aids and patient-reported outcome measures, counseling remains highly variable across clinicians and developmental stages. There is a critical need for consensus-based, age-specific counseling guidelines to support informed decision-making and improve care continuity.

METHODS: Using a modified Delphi approach, participants will complete up to three survey rounds evaluating statements on content, timing, and delivery of hypospadias counseling. Respondents will rate agreement, importance, and provide feedback. Participants include pediatric and adult urologists (North America and Europe), pediatricians, and experts in medical law and bioethics. Round 1 statements are developed from literature review and expert input. Statements achieving ≥75% agreement will be considered consensus; those not meeting the threshold will be revised and re-rated in subsequent rounds.

RESULTS: Consensus analysis will identify key priorities for counseling across developmental stages. Example Round 1 statements address core themes such as surgical risks, functional outcomes, follow-up expectations, psychosocial considerations, and communication strategies (Table). Revised statements will inform a standardized counseling guide.

Table 1. Examples of Round 1 statements presented to participants for rating. Statements address key domains in hypospadias counseling, including content, timing, and delivery, to guide consensus development through subsequent Delphi rounds.

Condition	Summary of Themes	Candidate Delphi Statement
Urethrocutaneous Fistula	Mechanism, timing, risk factors, management, communication style	Families should be informed about timing, risk factors, and management of fistula, including likelihood of outpatient repair.
Meatal Stenosis	Etiology (scar tissue), consequences, need for revision, communication nuances.	Counseling should address cause, functional impact, and potential need for revision surgery.
Urethral Diverticulum	Mechanism (outpouching), risk factors (multi-stage), consequences (dribbling, Urinary tract infection)	Families should be informed about diverticulum risk, particularly in complex repairs, and its potential urinary consequences.
Urethral Stricture	Mechanism (scarring), consequences (obstruction), risk factors (prior repair).	Surgeons should explain risk and potential functional impact of urethral stricture following repair.
Urinary Problems	Manifestations, follow-up needs.	Families should receive individualized counseling about urinary stream changes and follow-up plans
Need for Future Surgery	Indications, probability, follow-up importance.	Counseling should include severity-specific risks of further surgery and the need for long-term follow-up.
Impact on Sexual Health	Fertility, sexual function, psychosocial implications, communication style.	Counseling should address fertility and sexual function clearly, differentiating mild from severe hypospadias outcomes.

CONCLUSIONS: Consensus-based, age-specific counseling guidelines have the potential to standardize clinical communication, align expectations, and provide families with clear, developmentally appropriate information. This may enhance patient self-perception, reduce decisional regret, and improve long-term functional and psychosocial outcomes.

HEALTH RELEVANCE: Hypospadias is common and associated with significant surgical and psychosocial challenges. Standardized counseling can address existing variability, ensuring families receive consistent information that supports informed decisions and improves long-term care quality. This variability in counseling may influence patients' awareness of potential intermediate and longterm complications.

CHARACTERIZATION OF NORMAL BLADDER T1 AND T2 RELAXATION TIMES AT 1.5T AND 3T USING 3D MAGNETIC RESONANCE FINGERPRINTING (poster #31)

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INTRODUCTION AND OBJECTIVES: Magnetic Resonance Fingerprinting (MRF) is an emerging imaging framework that enables simultaneous quantitative mapping of multiple tissue properties. While MRF has been developed and applied in several organ systems, its use in the bladder has been limited. We developed a high-resolution 3D MRF approach for whole-bladder mapping, and applied it to characterize normal bladder wall tissue properties values in asymptomatic adults on both 1.5T and 3T MRI scanners.

METHODS: MRF datasets were acquired at 1x1x3 mm³ resolution during an 11.8 min scan in sagittal orientation. Subjects voided immediately before each MRI. Global T₁ and T₂ values were measured over the entire bladder wall. Regional T₁ and T₂ values were measured within regions of interest (ROIs) placed in the trigone, base, dome, anterior, and posterior walls at the midline. Two additional ROIs were drawn in slices 20 mm to the right and left of the trigone. Bladder wall thickness (BWT) was measured both globally and regionally using synthetic T2-weighted images derived from the MRF maps. The mean and standard deviation of T₁, T₂, and BWT were calculated across subjects, and associations with sex and age were investigated.

RESULTS: Data from 29 participants' MRF scans were analyzed (15 female; ages 18-78 years). Of these, 17 underwent back-to-back MRI exams at 1.5T and 3T using the same MRF sequence. Global bladder T₁ values were 984±53 ms at 1.5T and 1486±118 ms at 3T, and T₂ values were 51.4±5.6 ms at 1.5T and 43.5±4.0 ms at 3T. Consistent with expected MRI physics, T₁ values were significantly higher at 3T versus 1.5T, while T₂ values were significantly lower at 3T versus 1.5T. Global BWT was 4.5±1.6 mm at 1.5T and 4.3±1.6 mm at 3T, with no significant differences between field strengths. There were no statistical differences in T₁, T₂, or BWT values based on sex, although a trend was observed with higher BWT in men (Panel 1). Global T₁ and BWT showed no correlation with age, while a weak positive correlation with age was identified for T₂ values at both field strengths (Panel 2).

CONCLUSIONS: MRF provides characteristic and reproducible data between 1.5T and 3T field strengths.

HEALTH RELEVANCE: This study demonstrates the feasibility of high-resolution 3D bladder MRF at both 1.5T and 3T, provides normative T₁ and T₂ relaxation measurements, and offers preliminary insights into age- and sex-related differences in healthy adults. This work establishes a foundation for future studies to apply MRF to assess bladder wall remodeling in patient populations.

Financial Funding: NIDDK K-12 Uro EPI (PI SARMA), NHLBI HL163030

COMPARING SEQUENCING APPROACHES AND TEMPORAL DYNAMICS TO ELUCIDATE THE CLINICAL UTILITY OF THE PEDIATRIC UROBIOME (poster #32)

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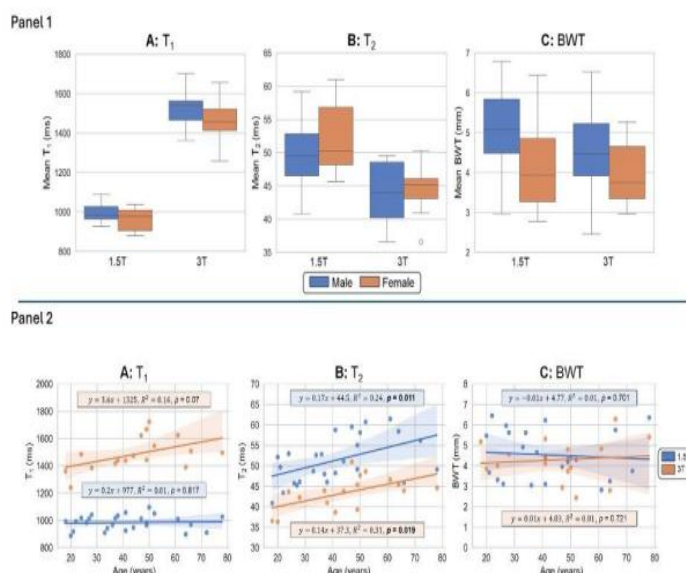
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BACKGROUND: Urinary tract infections (UTIs) are the most common outpatient infection in the United States and represent one of the most serious bacterial infections encountered by pediatricians. Up to 50% of children that have experienced recurrent UTIs (rUTIs) have chronic health consequences. Despite this burden, the pediatric urobiome remains understudied, particularly in relation to its temporal stability, compositional differences between children with and without rUTIs and representative sampling practices.

METHODS: Two studies were conducted to investigate pediatric urobiome stability and methodological impacts on microbial detection (Duke IRB Pro00110530 and Pro00100031). In Study 1, six children provided voided urine samples on days 1–5, 14, 21, 28, and 60. The samples underwent 0.22 µm filtration to collect biomass and DNA was isolated and prepared for 16S rRNA V4 sequencing (MiSeq). In Study 2, 20 voided urine samples were collected from children - 10 with no UTI history and 10 with a history of rUTI. DNA was extracted following 0.22 µm filtration and sequenced using two methods: 16S rRNA V4 sequencing (MiSeq) and synthetic long-read sequencing (LoopSeq).

RESULTS: Among the participants in Study 1, relative abundance profiles remained stable over time, with the predominant taxa within each individual persisting across multiple time points. Alpha diversity showed limited fluctuation, for example Shannon diversity varied less than 0.5 between the first and final collections, including in one participant who experienced an E. coli perturbation early in sampling. The predominant phyla were Firmicutes and Bacteroidota, with persistent genera including Prevotella, Actinotignum, Varibaculum, and Porphyromonas. In Study 2, LoopSeq provided greater species-level resolution and identified decreased Corynebacterium and increased Finegoldia in the rUTI cohort. Actinobacteriota were more abundant in MiSeq data, while Proteobacteria appeared only in LoopSeq data. Across methods 86 genera were detected with both, 13 were unique to LoopSeq and 78 were unique to MiSeq; 34 of the MiSeq-unique genera represented potential environmental contaminants, reflecting the higher specificity and reduced contaminant amplification with LoopSeq.

CONCLUSIONS: The pediatric urobiome appears stable over two months within individuals, suggesting that cross sectional or limited sampling frequency is sufficient for urobiome studies. Sequencing methodology substantially influences microbial detection, with long-read approaches offering superior taxonomic resolution and lower amounts of environmental genera. These findings support the use of long-read sequencing for distinguishing disease-state phenotypes or monitoring responses to therapeutic interventions.



Panel 1: Sex differences in bladder MRF measurements. Global measurements of (A) T₁, (B) T₂, and (C) BWT at 1.5T and 3T are presented for male and female subjects.

Panel 2: Association between bladder MRF relaxation times and BWT with age. Measurements of global (A) T₁, (B) T₂, and (C) BWT using 1.5T MRF and 3T MRF are displayed as a function of subject age in years. The best-fit regression line is reported, along with the coefficient of determination (R² value) and p-value. The shaded areas indicate the 95% confidence interval for the regression.

ROLE OF MEMBRANE TRANSPORTERS IN TRANSEPITHELIAL OXALATE SECRETION USING A SIMPLE, GENETICALLY-MODIFIABLE RENAL EPITHELIUM (poster #33)

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INTRODUCTION, OBJECTIVE: 70-80% of kidney stones are calcium oxalate (CaOx). Yet, our understanding of CaOx stone formation is incomplete limiting treatment options (e.g., lithotripsy, percutaneous nephrolithotomy). Using mammalian kidney models, three enzymes were identified to cause hyperoxaluria, and urinary supersaturation is implicated as a CaOx stone driver. However, complexities of mammalian transepithelial oxalate (ox²⁻) movements (gut, kidney) hinder determining cause-effect relationships.

METHODS: Therefore, we use a functionally similar, simple genetic model (*Drosophila* Malpighian tubules, MT) to measure renal CaOx crystallization (NaOx feeding), membrane transport (intracellular pH) and ox²⁻ secretion. Candidate transporter-channel genes are tested for transepithelial oxalate secretion with genespecific knockdown or knockout using CRIMIC (CRISPR-Mediated Integration Cassette) alleles in MTs due to comparable function to mammalian nephrons.

RESULTS: MTs comprise two epithelial cell types, Stellate and Principal cells, with each transporting different solutes from the hemolymph to MT lumen. Our lab identified several membrane transporters involved: Prestin (Slc26a6, apical), Neat (Slc26a1-like, basal) and Ndae1 (Slc4a8-like, basal). Neat (2HCO₃/ox²⁻-exchange) and NDAE1 (Na⁺/2HCO₃-/Cl⁻ exchange) functionally combine to takeup hemolymph ox²⁻. Prestin then secretes ox²⁻ via Cl⁻/ox²⁻-exchange to create luminal MT CaOx crystals. Prestin or Neat knockdowns in principal cells decrease CaOx crystals. NDAE1 knockdown in principal cells decreases CaOx crystals and HCO₃-transport (A), however, stellate cell knockdown of NDAE1 increases CaOx crystals like AQP (Drip, Prip)-knockdowns and enhances HCO₃-transport (B).

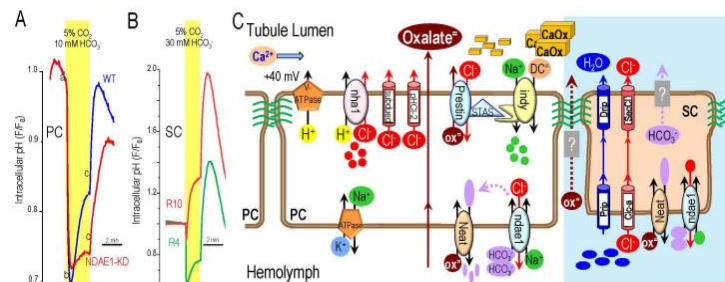


Figure 3: Malpighian principal and stellate cells both contribute to bicarbonate and oxalate transport. (A) Principal cell HCO₃- transport (recovery) is due to NDAE1 activity. (B) Some stellate cells have robust HCO₃- influx. (C) Epithelial transporters moving oxalate and HCO₃-.

CONCLUSIONS, FUTURE: Neat and NDAE1 work together to takeup ox²⁻ then Prestin secretes ox²⁻ into the MT lumen (C). Using CRIMIC alleles, we will measure CaOx crystals, ox²⁻ secretion and pH regulation (pH sensors).

HEALTH RELEVANCE: These experiments will replace knocked-out *Drosophila* genes by a human homolog cDNA to measure functional recovery. Humanizing this simple epithelial will allow functional impact testing of variants of unknown significance (VUS) in human genes.

DEVELOPMENT OF A LOW-COST, RELIABLE CATHETER FOR URETHRAL PRESSURE PROFILE MEASUREMENTS IN ANESTHETIZED RATS (poster #34)

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INTRODUCTION AND OBJECTIVE: Preclinical urethral pressure profile (UPP) techniques are often limited by cost, consistency, and reproducibility. We developed a low-cost, side-hole infusion catheter system equipped with an automatic puller and characterized its function in anesthetized female rats.

METHODS: Custom made 3Fr catheters with a hole 0.3 mm in diameter, 5 mm from the tip were fabricated from Braintree Scientific (\$40). Since the sensing port is located on the side rather than the tip, it captures pressure exerted by the urethral wall while avoiding interference from bladder pressure. During withdrawal the side hole aligns with different segments of the urethra enabling spatial mapping of urethral pressure. The catheter is connected to an infusion pump and pressure transducer. To characterize the side-hole catheter, we examined the impact of varying the saline infusion rate (n=3), as well as the number of pulls (n=4; ~20 pulls/rat, post-hoc analysis of impact of varying pull count). Motor pudendal nerve stimulation (PNS; n=8) and euthanasia (n=13) served as positive and negative controls.

Additionally, the impact of adding an additional 0.3 g/kg of urethane was assessed (n=17). UPPs were analyzed for maximum urethral pressure (MUP) and area under the curve (AUC).

RESULTS: MUP rose rapidly at low infusion rates, as the saline formed a stable water column for sensing, and plateaued beyond 10 mL/hr. We thus used a slightly higher value, 12 mL/hr, in subsequent testing. MUP measurements converged to within 10% of the final value (average across all trials/pulls) within 2-4 trials. As expected, PNS increased both metrics to ~130% of baseline; post-euthanasia pressures were significantly reduced, confirming physiological origin. Providing additional urethane significantly reduced MUP and AUC (p<0.01). We also used a solid-state catheter (Millar, SPR-407, 2Fr) to measure UPPs. Catheter measurements showed low MUP and AUC, likely due to the recessed sensor design suited for fluid-filled chambers (e.g., bladder) rather than muscle pressure (e.g., urethra). After the catheter broke, we chose not to pursue further use of this type of catheter given the high cost (~\$4000) and sub-optimal design.

CONCLUSIONS: This side-hole catheter system generates reproducible, physiologically responsive UPPs while reducing cost compared to solid-state devices. Findings highlight the impact of anesthesia depth on UPP and provide a practical tool for rigorous preclinical studies. Future work will test awake and disease models to expand translational relevance.

HEALTH RELEVANCE: Reliable and accessible UPP methods will enable broader investigation of urethral dysfunction, a major contributor to urinary incontinence, and support the development of improved therapies.

EVALUATING THE ROLE OF PRO-INFLAMMATORY AGENTS IN MALE URETHRAL SEROTONIN SECRETION (poster #35)

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INTRODUCTION AND OBJECTIVE: Lower urinary tract dysfunction (LUTD) impacts up to 90% of men over the age of 75. Alpha adrenergic blockers are largely effective at relaxing urinary tract smooth muscle to relieve LUTD, but some men have persistent symptoms despite using them. Through a locally mediated mechanism, serotonin causes contractions in an *E. coli* infected female urethra and escalates LUTD in male mice. This study sought to identify a link between bacterial and viral derived inflammatory agents, as well as intrinsic inflammatory cytokines, and serotonin secretion within the male urethra.

METHODS: Prostatic urethras were isolated from 8–9-week-old male C57BL/6J mice. Urethras were then placed in 400 µL of Krebs (130 mM NaCl, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.18 mM MgSO₄, 14.9 mM NaHCO₃, 5.6 mM glucose, and 1.56 mM CaCl₂ in water) and incubated at 37°C for 30 minutes in a humidified chamber containing 5% CO₂. This was followed by a 35-minute incubation in 400 µL Krebs and 10 µM fluoxetine (FLX). Tissues were then incubated for

an additional hour in Krebs alone (control), or Krebs containing 10 μ M FLX and IL1B (12 pg/mL), IL6 (50 pg/mL), IL10 (.5 pg/mL), TNF α (50 pg/mL), lipopolysaccharide (LPS, 0.5 mg/mL, mimics gram negative bacteria), lipoteichoic acid (LTA .1 μ g/mL, mimics gram positive bacteria), or polyinosinic-polycytidylic acid (poly(I:C, 10 μ g/mL, mimics viral RNA). The supernatant was then collected and frozen for testing in a serotonin ELISA. We will use immunofluorescent staining to confirm the presence of cytokine receptors on neuroendocrine cells.

RESULTS: Preliminary results indicate that the passive secretion of serotonin in Krebs+FLX alone falls beneath the assay limit of detection (0.95 nM). LPS induces serotonin secretion ($p < 0.0001$, $n = 3$). IL6 and CXCL10 resulted in serotonin secretion below the assay limit of detection. We expect LTA and poly(I:C) to stimulate similar responses as LPS, and for IL1B and TNF α to stimulate little to no serotonin secretion.

CONCLUSIONS: This assay provides a novel method of observing the role of serotonin and neuroendocrine cells in the prostatic urethra. We expect extrinsic inflammatory agents, such as LPS, LTA, and poly(I:C), to cause serotonin release from neuroendocrine cells, indicating that viral or bacterial infection in the prostatic urethra could increase local serotonin output and serotonergic contractions. We will use this model to inform further investigations into neuroendocrine cell mechanics.

HEALTH RELEVANCE: LUTD treatments fail to help a subpopulation of men, indicating a need to identify novel targetable pathways. Understanding what triggers the release of serotonin in the prostatic urethra is the first step in uncovering the role of the serotonin pathway in treatment-resistant LUTD.

VALIDATING MOUSE MODELS OF BENIGN PROSTATIC HYPERPLASIA THROUGH URINARY FUNCTION ASSESSMENT (poster #36)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a non-malignant prostate enlargement affecting most aging men and causing lower urinary tract symptoms (LUTS). Despite its high prevalence and impact on quality of life, current treatments remain limited. Advancing BPH research requires reliable experimental tools, yet mouse models remain insufficiently characterized and compared. To address this gap, we compared urinary function in 3 mouse models as part of the Endeavor Health System P20 Forward project.

METHODS: For the steroid hormone imbalance model (T+E2), C57BL/6 mice received subcutaneous pellets containing 25 mg testosterone and 2.5 mg estradiol for 4 weeks. For the high-fat diet (HFD) model, C57BL/6 mice were fed a 60% fat diet and controls received regular 13.4% fat chow for 16 weeks. The 3rd model was the use of non-obese diabetic mice (NOD/ShiLtJ) and control ICR/HaJ mice and mice were aged to 26 weeks. At study completion, prostates and bladder were harvested, weighed and normalized to body weight. Urinary function was assessed by void spot assays (VSA) and Uroflow (UF, Med Associates Urovoid System) biweekly.

RESULTS: In the T+E2 model, mice showed urinary dysfunction, with voiding frequency ~2-fold higher and mean voided volume nearly doubled relative to controls, plus a ~4-fold rise in voids by week 4 on UF. Prostate lobe weights increased (most notably ventral and lateral lobes, 4- and 3-fold), and a ~2-fold increase in bladder weight. In contrast, HFD mice showed a ~1.5-fold lower voiding frequency and voided volume by week 16, with reduced dorsal and anterior lobe

weights (~1.2-fold) and ~1.4-fold decrease in bladder weight. Preliminary NOD data showed ~3-fold higher voiding frequency over control but ~2-fold reduced voided mass.

CONCLUSIONS: These findings demonstrate that the T+E2 and HFD models both show lower urinary tract dysfunction (LUTD), but differ in presentation, reflecting different phenotypes. The T+E2 model mirrors obstructive voiding and bladder enlargement, whereas the HFD model shows reduced voiding activity and prostate lobe weight, latter potentially indicating a high degree of inflammation. Together, these results suggest that the use of multiple models may better capture the heterogeneity of BPH-related urinary dysfunction and enhance the relevance of preclinical studies.

HEALTH RELEVANCE: Characterization of animal models of BPH are key to understanding their similarities and differences to human disease and preclinical testing of future therapies.

GRP78-MEDIATED ENDOPLASMIC RETICULUM STRESS CORRELATES WITH FIBROSIS IN BENIGN PROSTATIC HYPERPLASIA (poster #95)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a nonmalignant enlargement of the prostate gland characterized by epithelial and stromal proliferation affecting up to 80% of men in their 80s. BPH is classified as a multifactorial disease, involving epithelial and stromal growth, inflammation, fibrosis, and epithelial barrier dysfunction. Emerging evidence suggests that disturbances in protein homeostasis and the subsequent activation of endoplasmic reticulum (ER) stress pathways may contribute to BPH pathology. Glucose-regulated protein 78 (GRP78), a key regulator of ER stress, is upregulated in BPH, and has been implicated in proliferation, apoptosis, and epithelial-mesenchymal transition (EMT). However, published data are limited to in vitro studies or secondary findings related to treatments, with little focus on ER stress contributing to BPH development. In this study, we aimed to identify the BPH pathological subtype associated with increased ER stress.

METHODS: Prostate tissues ($n = 25$) were obtained from BPH patients who underwent Holmium Laser Enucleation of the Prostate (HoLEP) surgery. To assess fibrosis, collagen accumulation was quantified using Picrosirius Red staining and fluorescence microscopy. Prostate size information was determined from patient charts (MRI and/or ultrasound). GRP78 expression was assessed by immunohistochemistry, and all imaging was analyzed using InForm software (Akoya Biosciences). Correlations were evaluated using Spearman's rank correlation.

RESULTS: GRP78 expression was positively correlated with collagen accumulation ($r = 0.4269$, $p = 0.0333$) and negatively correlated with prostate size ($r = -0.4700$, $p = 0.0205$). Moreover, collagen accumulation showed a negative correlation with prostate size ($r = -0.6162$, $p = 0.0013$).

CONCLUSIONS: Our results indicate that ER stress, measured by GRP78, is associated with fibrosis and smaller prostate size in BPH. This small fibrotic subtype of BPH may therefore have a distinct etiology from the classical adenomatous BPH, initiated by epithelial ER stress that ultimately promotes epithelial-stromal crosstalk and increased stromal extracellular matrix deposition. Our work also identifies GRP78 and ER stress as novel therapeutic targets in BPH.

HEALTH RELEVANCE: Understanding how ER stress contributes to BPH may reveal new therapeutic strategies to prevent fibrosis and

block disease progression, potentially improving urinary symptoms in men with BPH.

THE RELEVANCE OF AGE IN MIDURETHRAL SLING SURGERY FOR OLDER FEMALES: A SYSTEMATIC REVIEW (poster #37)

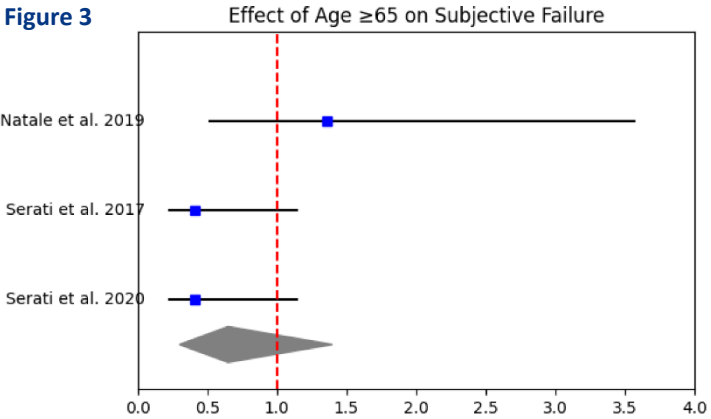
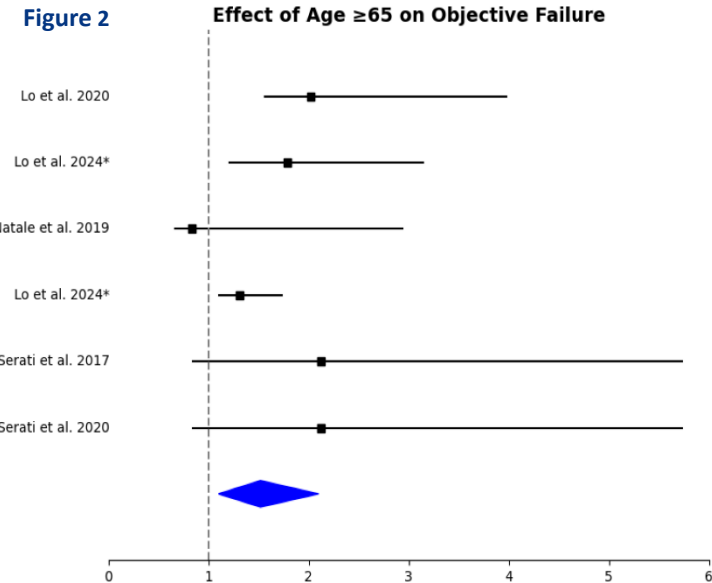
Candace Parker-Autry, MD, Joseph Huffman, BS, Katherine Woodburn, MD, Amr El Haraki, MD, Danielle Lauture, BS

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INTRODUCTION AND OBJECTIVE: Urinary incontinence (UI) is increasingly common and disproportionately affects women. Treatment of stress urinary incontinence (SUI) with midurethral sling (MUS) has been shown to be less effective in older women. This review aims to assess the effect of age on MUS treatment outcomes in older women and characterize the literature surrounding this topic.

METHODS: A scoping review search strategy targeting studies of procedure-based treatments for UI was developed. This search was focused to studies of populations aged 65+ and on studies published since 2014. Studies were excluded if they focused on conservative treatments, neurogenic UI/OAB, included concomitant prolapse surgery, or lacked an age-based analysis. Scoping and systematic reviews were excluded. For this systematic review, only studies on MUS were included.

RESULTS: 101 studies were included in the scoping review of procedure-based treatments of UI. 53/101 (52.5%) studies were focused on MUS. 38/53 (71.7%) included studies were level 3 studies, 14/53 (26.4%) studies were level 2, and 1 study was level 1. Treatment outcome measures were diverse with the cough stress test and UDI-6 being most common (Figure 1). Forest plots depict the effect of age ≥65 or ≥66 years on treatment failure (Figures 2 and 3). We anticipate performing more data analyses on other included studies with different age cutoffs.



CONCLUSIONS: The efficacy of MUS in treating SUI in older women is highly varied in the literature. Many different treatment outcomes are used to measure MUS efficacy. Age-based analyses of treatment outcomes are lacking in level 1 studies, as the majority of our sample is comprised of level 2 and 3 studies.

HEALTH RELEVANCE: Age is a factor worth considering when prescribing MUS to treat SUI in older women. More level 1 and 2 studies with age-based analyses of treatment outcomes are necessary to determine the effect of age on MUS outcomes in older women.

LONGITUDINAL STUDY OF URINARY MICROBIOME AND BIOMARKERS AMONG POSTMENOPAUSAL WOMEN WITH AND WITHOUT OVERACTIVE BLADDER (poster #96)

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¹University of Michigan ²Central Michigan University

INTRODUCTION: Overactive Bladder (OAB) is a condition characterized by lower urinary tract symptoms and disproportionately impacts postmenopausal women, yet its etiology is relatively unknown. We aimed to longitudinally characterize the urinary biomarkers in postmenopausal women with OAB compared to healthy controls to potentially uncover the biological signatures of OAB.

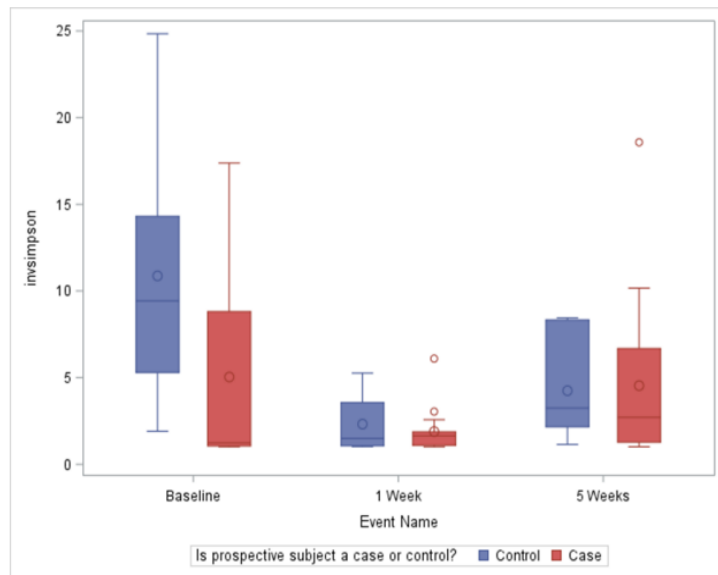
METHODS: A prospective longitudinal study of urine samples of postmenopausal women at least 55 years old with a clinical diagnosis of OAB based on LURN-SI-10 (n=25) and controls (n=11) was conducted over 5 weeks, at 3 different timepoints. Sterile, intermittent catheterization was performed at each visit to collect 120 mL of urine. Urinalysis, urine culture, urinary biomarker and microbiome analysis, and V4 region of the 16S rRNA amplicon sequencing were performed on all samples.

RESULTS: LURN-SI-29 score was significantly higher in cases compared to controls at all timepoints (p<0.001). Biomarker analysis showed no significant difference in Choline/Creatinine (Ch/Cr) or Acetylcholine/Creatinine (Ach/Cr) between cases and controls at any time. There was no significant difference in any of these ratios within the cases or controls over time. At baseline, microbiome analysis found a lower alpha diversity in cases compared to controls (4.5 vs 8.6, p=0.038), however, there was no significant difference at week 1 or week 5 (Figure 1, next page). Alpha diversity decreased significantly over time among the controls (p=0.0095), yet there was no significant difference over time within the cases (p=0.70). There were no correlations between microbiome and biomarkers at any point.

CONCLUSIONS: Postmenopausal women with OAB had less microbial richness (Alpha diversity) in catheterized urine compared to controls, though this difference did not hold longitudinally due to lower (Alpha diversity) in controls after initial catheterization. We found no

difference in Choline or Acetylcholine levels in postmenopausal women with and without OAB symptoms.

Figure 1. Alpha diversity in cases vs. controls at each timepoint.



HEALTH RELEVANCE: On catheterized urine specimens, postmenopausal women with OAB had less urinary microbial diversity compared to controls. After initial catheterization, the microbial richness of controls decreased, which may have implications for the role of sterile intermittent catheterization on changes of the urinary microbiome.

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SLEEP FRAGMENTATION INDUCES URINARY DYSFUNCTION AND PROSTATIC INFLAMMATION IN MICE (poster #38)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a non-malignant growth of the prostate gland characterized by the proliferation of epithelial and stromal cells within the transition zone accompanied by chronic inflammation and fibrosis. BPH patients present with various symptoms, collectively known as lower urinary tract symptoms (LUTS). The most bothersome symptom is nocturia, or getting up at night to urinate, which may result in sleep fragmentation (SF). SF has been found to worsen inflammation after stroke and in cardiovascular disease. Therefore, we hypothesize that SF will similarly contribute to inflammation and urinary dysfunction in BPH.

METHODS: C57BL/6J mice were housed in SF chambers for twelve weeks where an automated bar moved across the bottom of the chamber every 2 minutes during the light period (6am-6pm) to interrupt their sleep. Control mice were housed in normal cages. Every second week, the mice underwent a void spot assay (VSA), where they were individually housed for 4 hours in a cage fitted with filter paper. Filter papers were analyzed with Void Whizzard. After the 12-week experiment, mice were euthanized, and the bladder and prostate lobes were weighed, fixed, and embedded in paraffin. Inflammation was determined by immunohistochemistry using anti-CD45 antibody and fibrosis was assessed with Picrosirius Red (PSR) staining. CD45 images were analyzed by counting positively stained

cells, and PSR was quantified using fluorescence intensity, and all were normalized to tissue area. Groups were compared using Mann-Whitney test.

RESULTS: After 12 weeks, bladder weights were significantly increased in the SF group (15% increase, $p=0.012$) compared to controls. VSA showed that there was a significant decrease in both voiding volume (1.8-fold decrease, $p=0.022$) and void count (2-fold decrease, $p=0.022$) in the SF group compared to controls at 12 weeks. CD45+ count in the dorsal lobe was significantly increased ($p=0.004$) in the SF group. PSR staining is currently being imaged and analyzed.

CONCLUSIONS: The increase in bladder weights with the decrease in voiding volume points towards hypertrophy of the bladder caused by bladder outlet obstruction. SF alone also increased immune cell counts, suggesting that SF may exacerbate BPH/LUTS. Further work will provide more detailed insight into the changing immune landscape in this model and identify how BPH is worsened by sleep fragmentation.

HEALTH RELEVANCE: Understanding how SF worsens BPH/LUTS will draw greater attention to treating nocturia and improving sleep hygiene.

EFFECTS OF DEVELOPMENTAL EXPOSURE TO 3'-DICHLOROBIPHENYL (PCB11) ON MRNA EXPRESSION AND VOIDING PHYSIOLOGY IN C57BL6/J MICE (poster #39)

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INTRODUCTION AND OBJECTIVE: Polychlorinated biphenyls (PCBs) are pollutants whose resistance to degradation makes legacy sources an issue. PCB 11 is inadvertently produced as a byproduct of industrial processes, to which human exposure is of emerging concern. PCB exposure has been linked to adverse health effects, though impact on lower urinary tract function is understudied. Previously, we demonstrated that *in utero* and lactational exposure to an environmentally relevant PCB mixture, including PCB 11, altered RNA expression of receptors required for proper bladder function and increased the number of small volume void spots in offspring mice of both sexes. Whether these effects are due to a single congener, or the mixture is unknown. Here, we test the effects of developmental exposure to PCB 11 on bladder function in wild type C57BL6/J mice.

METHODS: Wild type C57BL6/J female mice were dosed with 0, 0.1, 1 or 6 mg/kg/d PCB 11, with tissues from resulting offspring collected postnatally (P35). qPCR of bladder tissue was conducted to analyze expression of big potassium (BK) channels, muscarinic and purinergic receptors, which are important for bladder contractility. RNA relative abundance was analyzed using one way ANOVA with Dunnett's multiple comparisons test. Voiding physiology of offspring was examined at P42 using void spot assay. VoidWhizzard was used to examine the number and size of voids. Group differences in spot size were determined using repeated measures two-way ANOVA with Tukey's multiple comparisons test.

RESULTS: BK channel component *Kcnma1* RNA is upregulated relative to vehicle in 1.0 and 6.0 mg/kg/d dose groups ($n=5$), with *Kcnmb2* RNA upregulated in the 1.0 mg/kg dose group ($n=5$) for male, but not female, bladder. This suggests the effects of PCB 11 on RNA expression in bladder are dose and sex dependent. Current voiding data suggests PCB 11 exposure increases the number of small spots from offspring of both sexes at the 0.1 mg/kg/d dose group compared to control ($n=2-8$ males, $n=2-5$ females).

CONCLUSIONS: Despite changes in RNA expression, altered voiding physiology is not observed in 1.0 or 6.0 mg/kg/d male bladders at P42, while an increase in small voids is observed in 0.1

mg/kg/d of both sexes. This may suggest differences in clearance of PCB 11 between lower and higher dosed offspring, where the latter may clear the contaminant faster, preventing PCB 11 induced changes to voiding.

HEALTH RELEVANCE: Exposure to environmental LUTS contributors occurs daily; however, the mechanisms by which they contribute to LUTS are poorly understood. This work, aiming to elucidate PCB 11's impact on the lower urinary tract, may encourage more targeted therapeutic approaches to improve LUTS through strategies to reduce exposure, and prevent or treat symptoms in a subset of patients.

SEX-SPECIFIC PROTEOSTASIS AND UROTHELIAL RESPONSES TO SENOLYTIC THERAPY IN THE AGED BLADDER (poster #40)

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INTRODUCTION AND OBJECTIVE Lower urinary tract dysfunction increases with age and disproportionately affects women, yet the molecular mechanisms underlying this sex bias remain poorly defined. The aging bladder plays a central role in this, and our previous work identified increased cellular senescence, oxidative stress, and activation of the unfolded protein response (UPR) as key features. We investigated the therapeutic potential of a senolytic combination, dasatinib and quercetin (D&Q), in aged (25-month-old) male and female Diversity Outbred (DO) mice, to assess sex differences and whether D&Q could improve bladder health by modulating ER stress.

METHODS Whole bladder samples of DO mice treated with a combination of 5 mg/kg/day of Dasatinib and 50mg/kg/day of Quercetin or vehicle control from 20-24 months of age were provided by the Jackson Laboratory. Western blotting, immunofluorescence, and H&E staining were used to assess molecular and anatomical changes in aged male and female bladders.

RESULTS We identified marked baseline sex differences in UPR and ER-associated degradation (ERAD) proteins: aged females exhibited higher PERK-pathway components, while males showed enhanced ERAD markers and greater autophagic flux. Aged female bladders also had increased urothelial thickness but lower uroplakin IIIA (UPKIIIa) expression than males, indicating incomplete differentiation of umbrella cells. D&Q treatment induced sex-specific alterations—upregulating eIF2 α and ERLEC1 in females, ubiquitin in males, and reducing CHOP in males. UPKIIIa expression improved in aged females treated with D&Q treatment.

CONCLUSIONS Our findings reveal sex-specific differences in proteostasis regulators that may underlie vulnerabilities in bladder aging. Elevated PERK pathway activation in females suggests greater ER-stress sensitivity, whereas higher ERAD activity in males may confer adaptive resilience. D&Q treatment selectively altered proteostasis markers in a sex-specific manner, notably an increase in expression of ERLEC1, a protein involved in maintenance of membrane integrity, in aged females. D&Q treatment also improved UPKIIIa expression in aged females, indicating a potential benefit of treatment for urothelial barrier integrity. In contrast, male bladders showed limited response to treatment. These results indicate that senolytic therapy may differentially enhance aspects of bladder function in females, possibly through effects on proteostasis or epithelial differentiation, and emphasize the importance of incorporating sex as a biological variable in therapeutic design.

HEALTH RELEVANCE Our research provides an important foundation for future investigations into sex-specific mechanisms of bladder aging and impact of seno-therapeutics.

INVESTIGATING THE RELATIONSHIP BETWEEN P2X3 AND CGRP EXPRESSION AND REFERRED PAIN IN INTERSTITIAL CYSTITIS (poster #41)

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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 UCPS, 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.

POTENTIAL ROLE FOR RETINOIC ACID SIGNALING IN RENAL UROTHELIAL REMODELING DURING CONGENITAL AND ACQUIRED URINARY TRACT OBSTRUCTION (poster #42)

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INTRODUCTION AND OBJECTIVE: Urinary tract obstruction (UTO) is the leading cause of chronic and endstage renal disease (CKD/ESRD) in children. There are no therapeutics that prevent the

progression of CKD, but renoprotective adaptations may explain positive outcomes. Increased Uroplakin (UPK) expression and urothelial plaque formation protect the kidneys from obstructive injury, but the mechanisms underlying urothelial remodeling are incompletely understood. We hypothesize that the urothelial transcriptome would unveil pathways responsible for remodeling.

METHODS: Megabladder (Mgb-/-) mice were used to model congenital UTO. Incorporation of a Upk2Cre;R26zsGreen enabled FACS enrichment of renal urothelium cells in Mgb-/-;Upk2Cre;R26zsGreen mice with hydronephrosis and Upk2Cre;R26zsGreen controls. RNASeq and bioinformatics examination were performed to determine the impact of congenital UTO on urothelial gene expression and predict urothelial transcriptional regulators. Unilateral ureteral obstruction (UO) served as an acquired model of UTO, and kidneys were collected on postoperative days (POD) 2 and 7. We developed a novel renal urothelial organoid model to manipulate the retinoic acid signaling pathway in vitro. Urothelial lineage markers and retinoid signaling molecules were evaluated by immunofluorescence microscopy.

RESULTS: We identified 88 differentially expressed genes in UPK+ cells from Mgb-/-;Upk2Cre;R26zsGreen vs. Upk2Cre;R26zsGreen kidneys. Pathway analysis indicated activation of retinol and retinoid metabolic processes (Cyp11b1, Adh7, and Rbp4). IF validation confirmed that CYP11B1 and ADH7, and other retinoic acid molecules, RBP1 and ALDH1A1, are expressed in the Mgb-/- and UO renal urothelium. We localized ADH7, CYP11B1, and RBP1 to superficial and intermediate urothelial cells, and ALDH1A1 to intermediate and basal cells. In our novel renal urothelial organoid model, the inhibition of retinoic acid signaling via BMS-493 increased UPK expression ($p = 0.0094$) and organoid area ($p = 0.0069$) compared to carrier-treated controls. In vivo dosing of BMS-493 during UO leads to an increase in FABP4 expression, a marker of urothelial differentiation and indicator of superficial cells.

CONCLUSIONS: Congenital and acquired UTO trigger activation of retinoic acid signaling in the renal urothelium. Pharmacologic inhibition of retinoic acid in vivo and in our novel renal urothelial organoids represents a renoprotective mechanism during UTO that likely regulates UPK expression and will require additional preclinical investigation.

HEALTH RELEVANCE: These findings advance our knowledge of renoprotective adaptations to UTO within the renal urothelium and incite curiosity in how retinoic acid regulates UPK expression.

PREOPERATIVE PELVIC FLOOR PHYSICAL THERAPY MAY DECREASE TRANSIENT STRESS URINARY INCONTINENCE AFTER HOLMIUM LASER ENUCLEATION OF THE PROSTATE: INTERIM ANALYSIS OF MULTI-INSTITUTIONAL RANDOMIZED CLINICAL TRIAL (poster #43)

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INTRODUCTION AND OBJECTIVE: Holmium laser enucleation of the prostate (HoLEP) is a highly effective and durable treatment for benign prostatic hyperplasia (BPH). Postoperatively, 20% - 40% of patients may experience transient stress urinary incontinence (tSUI), impacting quality of life. We hypothesized that initiating pelvic floor physical therapy (PFPT) preoperatively could reduce postoperative tSUI rates and shorten the time to regain urinary continence.

METHODS: In this multi-institutional randomized controlled trial, patients scheduled for HoLEP were recruited and randomized in a 1:1 ratio to either the standard of care (SOC), initiating PFPT post-HoLEP, or to an intervention arm (IARM), starting PFPT 4 weeks prior to HoLEP with 3 daily sets of 10 Kegel exercises. Participants were educated on the correct technique for exercises, received weekly postoperative phone calls to ensure adherence, with additional

preoperative reminders for the IARM. A similar surgical technique with early apical release was used in all cases. Follow-up evaluations included pad usage, post-void residual volume (PVR), and American Urological Association Symptom Score (AUASS). Continence was defined as the use of no pads.

RESULTS: Seventy-two patients were included in the interim analysis, with 38 randomized to IARM and 34 to SOC. Median prostate size and operative time were comparable between groups (Table 1, $p > 0.05$ for both). However, patients in the IARM group had significantly higher rates of previous BPH interventions and a greater average weight of resected adenoma compared to the SOC group (22% vs. 2.9%; 69 [31–97] vs. 47 [31–64], respectively; $p < 0.05$ for both).

Table1. Pre-, Peri- and Post-operative Patient Characteristics and Outcomes.

Variables	Intervention Arm, n=38	Standard of Care Arm, n=34	p-value
Age, years, median (IQR)	71 (64 – 75)	69 (63 – 76)	0.7
BMI, kg/m ² , median (IQR)	28 (25 – 32)	28 (25 – 33)	>0.9
Prostate size, cm ³ , median (IQR)	113 (79 – 154)	95 (63 – 125)	0.13
Preop CIC, n (%)	4 (11%)	4 (12%)	>0.9
Preop use of alpha-blockers, n (%)	35 (92%)	30 (91%)	>0.9
Preop use of 5ARIs, n (%)	15 (39%)	14 (44%)	0.7
Preop PVR, mL, median (IQR)	90 (24 – 167)	43 (11 – 200)	0.4
History of previous BPH interventions, n (%)	8 (22%)	1 (2.9%)	0.03
Preop AUASS, median (IQR)	16 (13 – 20)	14 (13 – 23)	0.8
Total procedure time, minutes, median (IQR)	119 (91 – 156)	119 (91 – 145)	0.5
EBL, mL, median (IQR)	50 (25 – 50)	50 (20 – 50)	0.6
Pathological weight of resected adenoma, gr, median (IQR)	69 (31 – 97)	47 (31 – 64)	0.04
Postop PVR, mL, median (IQR)	10 (1 – 21)	12 (0 – 44)	0.6
Postop immediate continence, n (%)	14 (37%)	6 (18%)	0.3
Postop continence at 1-week, n (%)	27 (71%)	19 (56%)	0.2
Any incontinence at 1-month, n (%)	5 (13%)	8 (24%)	0.2
Transient stress urinary incontinence, n (%)	4 (11)	8 (24%)	0.2
Postop AUASS, median (IQR)	5 (3 – 7)	5.5 (3 – 9)	0.8

Abbreviations: IQR – Interquartile Range, BMI – Body Mass Index, CIC – Clean Intermittent Catheterization, 5ARI – 5- α Reductase Inhibitor, PVR – Postvoid Residual Volume, BPH – Benign Prostatic Hyperplasia, AUASS – American Urological Association Symptom Score, EBL – Estimated Blood Loss.

CONCLUSIONS: One-month preoperative PFPT may help reduce post-HoLEP incontinence. Further analysis of the final cohort is needed to assess its role more accurately.

HEALTH RELEVANCE: TSUI is a potential adverse effect of the HoLEP procedure that negatively impacts patients' quality of life. Patient-initiated preoperative PFPT is a low-risk, cost-effective practice that may facilitate earlier return to continence and improve patient satisfaction.

CYSTITIS MAY INDUCE UNIQUE MOLECULAR CHANGES IN L3 DORSAL ROOT GANGLIA (poster #44)

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INTRODUCTION AND OBJECTIVE: Interstitial cystitis is bladder disease characterized by increased urinary urgency, frequency, and pelvic pain, and many patients report somatic pain extending beyond the pelvic region. Animal models of cystitis replicate this secondary or referred pain, demonstrating hind paw skin hypersensitivity. Most cystitis studies in mice focus on bladder-innervating L5-L6 dorsal root ganglia (DRG) but rarely examine paw-innervating L3 DRG. Along with L5-L6 DRG, previous work in the lab found that cystitis caused hyperexcitability of L3 DRG neurons. Notably, L3 DRG distinctly had the largest neurophysiologic changes. However, it is unclear if cystitis has other, unique effects on pain-related molecular changes. We hypothesized that cystitis increases expression of pain-related markers P2X3 and CGRP within L3 DRG, including in paw afferents.

METHODS: Adult female C57BL/6 mice received hind paw Fast Blue tracer injections before being randomized to saline (control) or acrolein (cystitis) bladder instillations. Cystitis was induced via intravesical acrolein instillation. Von Frey testing was used to assess paw hypersensitivity in vivo, prior to euthanasia for tissue collection. L3-L6 DRG were collected post-fixation and immunostained for pain-related protein markers P2X3 (a purinoreceptor) and CGRP (a peptidergic marker).

RESULTS: CGRP expression in L3 DRG was significantly increased in cystitis mice compared to controls ($p = 0.0171$), while P2X3 expression showed no change ($p = 0.2402$). Within paw-labeled neurons, P2X3 and CGRP expression were not significantly increased after cystitis. Colocalization of Fast Blue paw tracer with P2X3 and CGRP in L3 did not change with cystitis ($p = 0.2485$, $p = 0.2660$). However, cystitis increased CGRP expression in neurofilament heavy (NFH) neurons, with NFH and CGRP colocalization significantly greater in cystitis DRG compared to controls ($p = 0.0290$, Mann-Whitney test). NFH and P2X3 colocalization trended towards an increase but did not reach significance ($p = 0.0599$).

CONCLUSIONS: L3 DRG represents a potential site of cross-talk between bladder and paw neurons, undergoing molecular changes following cystitis. Increased CGRP expression and enhanced NFH-CGRP colocalization suggest altered signaling in large-diameter neurons that may contribute to secondary pain. Changes in paw-innervating neurons did not reach significance, but these trends highlight molecular pathways that warrant further investigation. Ongoing analysis will explore changes in protein colocalization in L3 DRG and correlations to paw sensitivity.

HEALTH RELEVANCE: Understanding the molecular changes in L3 DRG may help improve pain management strategies and enhance quality of life for individuals with interstitial cystitis.

DEVELOPMENT OF HUMANIZED MOUSE MODELS FOR STUDIES OF THE UROGENITAL MICROBIOME (poster #45)

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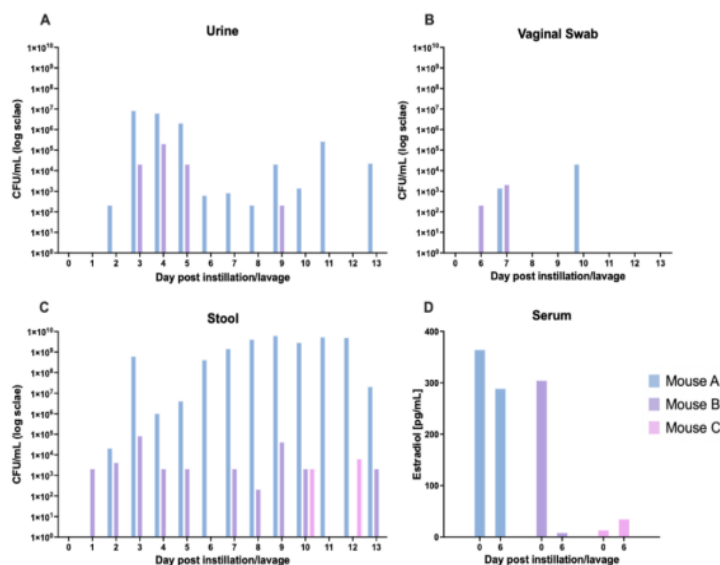
INTRODUCTION AND OBJECTIVE: Postmenopausal women disproportionately experience recurrent urinary tract infections (rUTI), and estrogen hormone therapy (EHT) has been linked to protection through enrichment of *Lactobacillus crispatus*. However, mechanistic studies are limited by the lack of animal models that replicate the human urogenital microbiome. Our objective was to develop a germ-free, humanized mouse model for future studies.

METHODS: Four female C57BL/6 germ-free mice underwent bilateral ovariectomy (OVX); three survived to the pellet insertion stage and were implanted with either a 21-day β -estradiol (EHT+) or placebo pellet. 11 days post-OVX, mice were inoculated via vaginal lavage and bladder instillation with several urinary *L. crispatus* strains derived from human females. Longitudinal urine, fecal, and vaginal samples were collected for 13 days and analyzed for colony-forming units (CFU) via serial dilutions on MRS agar plates. Blood samples were collected via transcardiac aspiration to evaluate serum estradiol levels.

RESULTS: *L. crispatus* colonization was variable through the observation period and by niche (Figure). Colonization was most consistent in fecal and urinary niches, with substantial variability in vaginal swab samples. Despite standardized EHT exposure, serum estradiol levels varied significantly, and estradiol levels were directly correlated with urinary and fecal colonization.

CONCLUSIONS: Findings suggest that host factors and estradiol status influence colonization efficiency and persistence. Study

limitations included the small cohort size and *Staphylococcus hominis* contamination during the observation period, but provided proof-of-concept that *L. crispatus* can colonize the urogenital and gastrointestinal tracts of OVX gnotobiotic mice. Ongoing studies with larger cohorts and 60-day long-release EHT pellets will further investigate these interactions.



Colonization and estradiol levels. Bacteria were quantified using the serial dilution method to estimate colony forming units per milliliter (CFU/mL) and are displayed per mouse and per day post-inoculation. A) *L. crispatus* in urine; B) *L. crispatus* from vaginal swabs; C) *L. crispatus* levels from stool; D) Estradiol levels (in picograms/mL) from Mouse & Rat ALPCO ELISA

HEALTH RELEVANCE: This humanized mouse model is promising in its use as a model system to investigate rUTI prevention strategies for women.

LESSONS IN CONSTRUCTING A MULTIMODAL CELL ATLAS OF HUMAN LOWER URINARY TRACT (poster #46)

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INTRODUCTION AND OBJECTIVE: The production of single cell atlases has spurred a deeper understanding of the cellular pathogenesis of human disease. Comprehensive multimodal atlases spanning multiple organs across sex and age can reveal biology that would otherwise be missed by bulk approaches. Here, we discuss the generation of a multimodal reference cell atlas of normal human lower urinary tract, including obstacles and solutions. By sharing this experience, we hope to aid other groups in the production of similar high-quality reference and/or query datasets.

METHODS: We collected the lower urinary tract from healthy human male and female organ donors, dissected them into separate organs (bladder, prostate, ureter, urethra), and embedded the tissue in OCT. Nuclei were isolated using the S2 Genomics Singulator 200 for paired single nucleus RNA+ATAC sequencing with the 10x Genomics Multiome kit. We tested several in vitro and in silico protocols to determine which produced the highest quality data. Data quality was assessed separately for RNA and ATAC libraries and consisted of several metrics for each.

RESULTS: We generated 130 libraries (65 RNA+ATAC pairs) from 35 donors. Manual nuclei isolation resulted in high ambient RNA and low yields requiring an automated approach with the S2 Genomics Singulator. Even with this automated isolation, high ambient RNA contamination was observed in a tissue-dependent manner, especially in bladder. Bladder nuclei suspensions were loaded with debris and displayed especially poor ATAC quality control metrics. Magnetic sorting with anti-nucleus beads removed debris but did not yield sufficient nuclei for sequencing. Flow cytometry of 7-AAD-stained nuclei prior to permeabilization markedly improved ambient RNA levels, tissue debris, and ATAC quality. We found that Cell

Ranger-ARC frequently yielded nuclei counts that strongly disagreed with our loading. An alternative method, EmptyDropsMultiome, produced counts more aligned with our expectations, rescuing 7 samples that would have been omitted. We have now produced 37 high quality library pairs to construct a multimodal normal human reference atlas of the lower urinary tract.

CONCLUSIONS: The production of linked RNA+ATAC single cell atlases is a complex process, and protocol optimization for sample preparation and data processing is still ongoing. Although different tissues require boutique solutions, we hope that our experience can inform others who plan to produce similar resources.

HEALTH RELEVANCE: High quality reference data are needed to understand disease but creating these references can be very challenging and expensive. Here we describe what we've learned while creating these types of reference to help other groups save time and money.

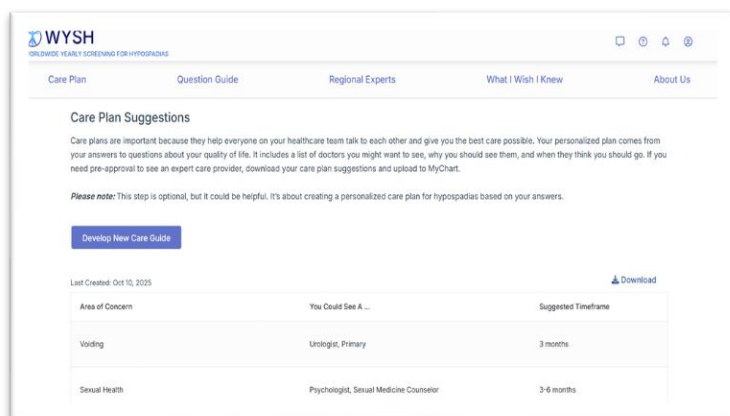
UTILIZING SYSTEM USABILITY SCREENING TO COUNTER BARRIERS TO LONG-TERM CARE FOR PATIENTS WITH HYPOSPADIAS (poster #47)

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University of Wisconsin-Madison

INTRODUCTION AND OBJECTIVE: Hypospadias is currently cited to affect 1/125 live male births in the U.S. Surgical repair (across techniques and severity) is generally performed when the patient is between 6-18 months old with a goal of increased psychosexual development and lower complication rates. However, less than 1/3 of surgeons who perform Hypospadias Repair report following up with their patients into adulthood, indicating an inability to observe accurate surgical results. This is exacerbated by the lack of a singular, universal measure to evaluate functional and psychosexual characteristics. Our study objective is to create an application that has high usability across varying degrees of health literacy and e-health literacy to increase quality of long-term care for patients who have undergone Hypospadias Repair Surgery.

METHODS: Using a Modified Delphi Method, we developed a questionnaire of sixty-five statements to be rated on a five-point Likert Scale (Never - Always). This questionnaire built the backbone of our application – Worldwide Yearly Screening for Hypospadias (WYSH). Recruiting patients from clinical settings and previous studies, we are interviewing patients over a secure video conference. This consists of accessibility testing of facets of the application, timing task completion, system usability testing post-interview, and a three-week and three-month follow-up.



RESULTS: Preliminary feedback of the application has shown reliability of the questionnaire, high usability scoring, suggestions on refinement, and overall positive responses. Current system usability scales show a high perceived usability with participants of medium and high e-health literacy scoring (using eHeals assessment);

however, we will continue analysis as we predict this will possibly impact sustainability.

CONCLUSIONS: Creating a patient-reported outcomes measure through rigorous, iterative rounds of a Modified Delphi and mixed-method interviews, including qualitative insights and quantitative scoring, increases quality of care for patients with Hypospadias and Hypospadias Repair. Compounded with tools to aid in timeline for seeking care, finding experts, and answers to common patient questions - we are striving for the goal of high healthcare feasibility through the WYSH application.

HEALTH RELEVANCE: Increasing access to personal health history, disease information, recommendations of care, and relevant providers in the area reduces a large burden on clinicians and patients, alike.

CO-CULTURING OF URINARY LACTOBACILLI AND UROPATHOGENIC E. COLI SUGGEST CELL AND METABOLITE INTERACTIONS (poster #48)

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) are among the most common bacterial infections, predominantly caused by uropathogenic *Escherichia coli* (UPEC). The estimated total U.S. cost to treat UTIs over the course of 20 years is \$25.5 billion, highlighting the substantial economic and health burden associated with these infections. The rising antibiotic resistance in UPEC encourages researchers to search for alternative strategies to prevent and manage UTIs. Recent advances in the urinary microbiome research revealed lactobacilli, a lactic acid-producing bacteria, are abundant in the female urinary tract and might be engaging in defending against uropathogens. This study aims to identify the method by which lactobacilli inhibits UPEC growth.

METHODS: Urinary isolates of lactobacilli were co-cultured with UPEC in different media formulations to assess inhibition patterns. Growth inhibition was measured on MRS, LB and other agars using well-diffusion assay and colony counting. To test effects of media components on inhibition, specific compounds were added or eliminated from the media. Light microscopy was used to characterize morphology of bacteria in co-cultures of lactobacilli and UPEC.

RESULTS: Multiple urinary lactobacilli isolates inhibit UPEC growth in MRS broth but not in the LB broth. Small molecules were identified in the MRS media as possible co-inhibitory factors, such as acetate. The results suggest that UPEC growth is hindered by small molecules present in broth, such as acetate, in addition to the catabolites produced by lactobacilli. Further characterization revealed that *L. gasseri* forms stable co-aggregates with UPEC cells, suggesting potential physical interaction that contributes to inhibition. Upon further testing it was observed that the two species have more complex interactions when found in different media with UPEC supporting growth of lactobacilli.

CONCLUSIONS: These findings suggest that heterofermentative lactobacilli might inhibit UPEC growth through catabolite repression, in addition to other ways, through direct aggregation and acid-mediated effects. Ongoing work aims to reveal how these interactions influence the balance between these two bacteria within the urinary tract.

HEALTH RELEVANCE: Understanding how commensal and pathogenic bacterial species interaction in the urinary tract can inform the development of probiotic or microbiome-based therapies to reduce UTI recurrence and antibiotic dependence.

PCR-BASED TESTING FOR COMPLEX URINARY SYMPTOMS (poster #49)

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INTRODUCTION AND OBJECTIVE: Given concerns about lack of precision in standard urine cultures (SUC), we aimed to critically assess initial experiences with one PCR-based diagnostic test in a clinical population.

METHODS: This is a retrospective case series of females at a single institution where PCR-based testing with a pooled antibiotic sensitivity test (P-AST; Guidance UTI® by Pathnostics) was implemented for complex symptom presentations. Descriptive statistics, proportions receiving antibiotics, and concordance with SUC were evaluated based on electronic medical records (EMR).

RESULTS: From 8/2023 – 11/2024, a total of 2,023 females with history of recurrent urinary tract infection (rUTI) and 95 with history of interstitial cystitis (IC) were seen. Of 309 Guidance UTI® tests sent in this population for diagnostic evaluation of urinary symptoms, EMR diagnoses included rUTI in 247 (79.9%), IC in 67 (21.7%), and overactive bladder in 126 (40.8%). Test results are summarized in **Fig. 1** with a median of one (range 1-7) organisms identified on positive tests. Antibiotics were prescribed or altered based on 56.3% of all PCR-based results. New IC diagnoses were assigned to 6 patients (2%) patients after testing. SUC was obtained within 30 days of Guidance UTI® in 71.2% of cases; of these, 20.3% were Mixed

Figure 1.

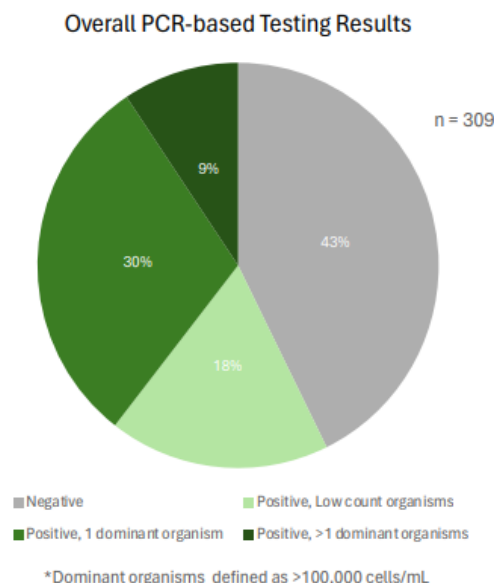
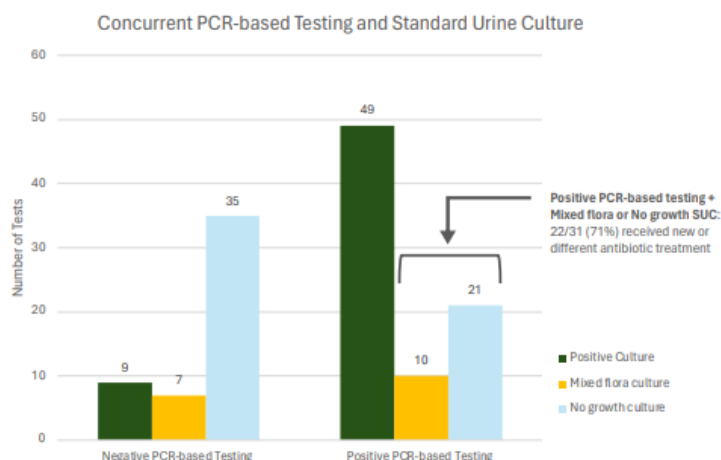


Figure 2.



flora and 40.1% resulted in no growth. In 131 instances SUC and Guidance UTI® were sent on the same day, allowing for direct comparison. Characteristics of concurrent tests are demonstrated in **Fig. 2**.

CONCLUSION: In a specialty population with complex symptoms, PCR-based testing was often negative, even when sent for suspicion for UTI. In some patients with persistent symptoms preceded by negative or mixed flora SUC results, PCR-based testing led to new antibiotic prescriptions.

HEALTH RELEVANCE: In women with complex symptom presentations, PCR-based testing may provide additional information to guide clinical care.

URINARY INCONTINENCE IN ALZHEIMER'S DEMENTIA MAY BE ATTRIBUTED TO AMYLOID- β DEPOSITION IN THE LUMBAR AND SACRAL SPINAL CORD (poster #52)

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INTRODUCTION AND OBJECTIVE: Urinary incontinence (UI) is a prevalent non-cognitive complication of Alzheimer's disease (AD) and is associated with reduced quality of life. Medical management of UI in AD poses a challenge: acetylcholinesterase inhibitors used to slow cognitive decline can worsen lower urinary tract dysfunction (LUTD), while anticholinergics used for UI can impair cognition and counteract AD therapies. UI in AD patients is often attributed to functional incontinence; however, evidence suggests that amyloid- β (A β) plaques within central micturition centers may contribute, leaving the relationship between AD pathology and LUTD unclear. In this study, we aimed to characterize LUTD in a transgenic AD mouse model and determine whether A β deposition within the lumbar and sacral spinal cord and peripheral nerves provides a mechanistic basis for UI in the setting of AD.

METHODS: To investigate the contribution of A β pathology to LUTD, aged (24 months) male and female APP/PS1 transgenic mice, a model of A β pathology, and non-transgenic littermate controls, were evaluated. Voiding behavior was assessed by void spot assay (VSA) and UroFlowmetry. Urodynamics were performed in urethane-anesthetized animals via cystometrograms (CMG) to measure bladder mechanics. Following functional studies, spinal cords, sacral nerve bundles, and bladder tissues were harvested and analyzed using immunohistochemistry and histological methods to assess A β deposition and its relationship to gliosis, nerve pathology, and fibrosis.

RESULTS: Transgenic APP/PS1 mice exhibited more severe LUTD compared to controls. Male mice demonstrated a significantly increased void spot count on VSA ($p < 0.05$) and a significant decreased stream rating on UroFlowmetry ($p < 0.01$), consistent with droplet-like voiding. Female mice showed a trend in increased void spot count ($p = 0.073$). Tissue analyses revealed A β deposition within the lumbar and sacral spinal cord, including the central canal, perineural spaces, perineural lymphatic vessels. A β deposits were localized to areas of gliosis, implicating neuroinflammation in dysfunction of pathways regulating micturition. CMG showed reduced bladder compliance ($p < 0.05$), while all other parameters were unchanged, suggesting that altered afferent signaling or detrusor dysfunction may contribute to the observed functional abnormalities.

CONCLUSIONS: LUTD in AD may be attributed to A β -mediated pathology in the lumbar and sacral spinal cord, and the peripheral nerves involved in micturition rather than being explained solely by functional UI.

HEALTH RELEVANCE: Identification of A β pathology within spinal pathways reframes UI in Alzheimer's disease as pathology-driven rather than purely functional, highlighting a novel therapeutic target.

OLEIC ACID SUPPLEMENTATION IN MICE WITH LOWER URINARY TRACT DYSFUNCTION (poster #53)

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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 dysfunction 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 two models of LUTD. We hypothesize that OA supplementation will alter extracellular collagen and proteins in the fatty acid oxidation pathway.

METHODS: C57BL/6J aged mice (24-25 month old) were treated with 40 mg/kg/day OA (voluntary feeding) over eight weeks (6x weekly). Young (8 weeks old) C57BL/6J mice were implanted with compressed testosterone and 17 β -estradiol (TE2) subcutaneously and also treated with OA over eight weeks. Void spot assays were performed weekly, then mice were euthanized, and tissues were harvested. Prostate and bladder tissues were stained using picosirius 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) and HADHB (Abcam, 1:1500). Using Inform, we quantified CPT1A and HADHB percent positivity.

RESULTS: CPT1A transporters are significantly increased in aged mouse anterior prostate (AP) compared to young AP (p=0.0001) but are not significantly altered in aged mice treated with OA. HADHB is not significantly altered with age or OA supplementation. Neither CPT1A nor HADHB are significantly altered with TE2 treatment or OA supplementation. There was no significant difference in fibrillar collagen in OA treated bladder or prostate lobes when compared to untreated tissues for either LUTD model.

CONCLUSIONS: This study shows the effects of OA in aged mice and TE2 treated mice. OA does not alter collagen content in the prostate or bladder of aged or TE2 treated mice. Surprisingly, CPT1A expression increased with age, which may explain why fatty acid supplementation caused no further increase in expression. Given that OA doesn't alter protein expression in TE2 mice, this suggests that oleic acid supplementation is altering urinary function through a different mechanism. More experiments examining fatty acid oxidation and other mechanisms are required to understand OA in the prostate.

HEALTH RELEVANCE: This research uses two preclinical models of LUTD to evaluate a treatment option targeting mitochondrial dysfunction. Evaluating new treatments is of critical importance given BPH/LUTS patients often fail existing therapies.

TRANSIENT RECEPTOR POTENTIAL MELASTATIN TYPE 8 AND VANILLOID TYPE 1 CHANNELS ARE INVOLVED IN MOUSE URINARY BLADDER MICTURITION (poster #54)

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INTRODUCTION AND OBJECTIVE: Transient receptor potential ion channels such as melastatin type 8 (TRPM8) and vanilloid type 1 (TRPV1) are temperature and chemical sensors involved in

nociception. TRPM8 is sensitive to cool temperatures (8°-28°C) and chemically induced by menthol/mint. TRPV1 is sensitive to high temperatures (>42°C) and is also activated capsaicin. They are distributed throughout the urinary bladder in afferent nerves and smooth muscle cells; however, their exact role in micturition remains unknown. Sensitized TRPV1 channels increase afferent nerve signaling and impact bladder sensation, but it is unclear if TRPM8 and TRPV1 channels interact. We hypothesize TRPV1 and TRPM8 channel activation increases mechanical compliance in the urinary bladder.

METHODS: Bladders from male C57BL/6 mice (ages 10-14 weeks) were cannulated on the Pentaplanar Reflected Image Macroscopy (PRIM) System to simultaneously record intravesical pressure, geometry, and infused volume during ex vivo bladder filling. From these recordings, mechanical compliance is calculated as the Cauchy stress and stretch. To determine the implications of activating one or both channels on bladder compliance, experiments were performed in the presence of the TRPV1 agonist capsaicin (200 nM), the TRPM8 agonist menthol (600 μ M), or menthol and capsaicin together.

RESULTS: Capsaicin alone had no effect on bladder pressure, volume, or biomechanics. After menthol exposure, a larger volume was required to reach the same pressure. These bladders also stretched more for the same stress, indicating increased compliance. Surprisingly, the increase in compliance caused by menthol was reversed when capsaicin was added after menthol.

CONCLUSIONS: These findings suggest that TRPV1 and TRPM8 function in opposition to one another in terms of regulating bladder compliance during filling.

HEALTH RELEVANCE: Additional studies will determine the location of TRPV1 and TRPM8 in the bladder wall and possible mechanisms by which these channels can reversibly alter bladder compliance.

HOW VARIABLE IS THE URINARY MICROBIOTA OF HEALTHY DOGS OVER TIME? (poster #55)

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INTRODUCTION AND OBJECTIVE: Healthy urine was long thought to be sterile; however, advances in sequencing and expanded culturing techniques have challenged this concept. A few recent cross-sectional studies have characterized the urine microbiome (or urobiome) in healthy and diseased dogs. However, the stability of canine urine microbiota over time is unknown. Urine is a highly variable matrix that can change in pH, concentration, and composition within hours based on a dog's health, diet, and hydration status. Understanding if urine microbial profiles fluctuate within this changing environment will help drive more informed clinical decisions. Based on longitudinal culture results, we hypothesized that urinary microbiota would be relatively stable over time within individual dogs but would differ between dogs.

METHODS: To test this, mid-stream free catch urine was collected from 14 healthy dogs (7 female, 7 male) over 12 time points that ranged from hours, to days, to months apart. We also collected genital (vulvar or preputial) and perineal swabs and fecal samples from the same dogs at the same time points. DNA was extracted using Qiagen DNA Isolation Kits. The V4 region of the 16S rRNA gene was amplified and sequenced, and the sequence data was processed and analyzed using DADA2 and QIIME 2™. We used Jaccard, Bray Curtis, and UniFrac distances to assess microbial community similarity and stability within and between dogs.

RESULTS: While there was no significant difference in urine microbial communities over time (PERMANOVA, $p = 1.0$), we did observe significant variation in the urobiome by sex and dog (both PERMANOVA, $p = 0.001$).

CONCLUSIONS: These findings help define normal variation in urinary microbiota, as well as how the urinary microbiota compare to fecal, perineal and genital microbiota.

HEALTH RELEVANCE: Defining normal taxa and normal variation in the urobiota of healthy dogs can ultimately help us better define abnormal changes in disease states such as urinary tract infection and bladder cancer.

EXPLORING DEMOGRAPHIC AND CLINICAL PREDICTORS OF TREATMENT PREFERENCES FOR FEMALE SEXUAL DYSFUNCTION (poster #56)

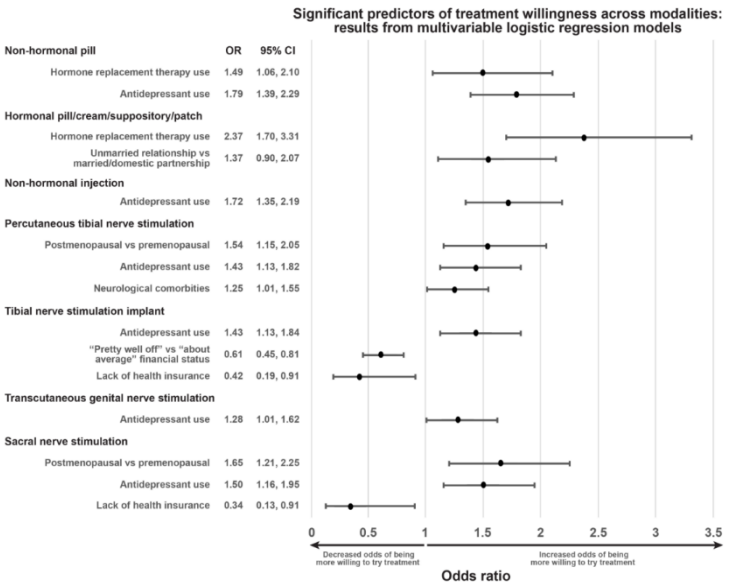
Mackenzie B. Moore^{1,2,3}, Caitlin Seibel⁴, Joanna Marantidis⁴, Hania Timek^{1,2}, Kelsey Francis^{2,5}, Samantha Godinez⁶, Giulia M. Ippolito⁴, Priyanka Gupta⁴, Tim M. Bruns^{1,2}

¹Department of Biomedical Engineering, University of Michigan, ²BioInterfaces Institute, University of Michigan, ³Medical College of Wisconsin, Milwaukee, WI, ⁴Department of Urology, University of Michigan, ⁵Undergraduate Program in Neuroscience, University of Michigan, Ann Arbor, MI, ⁶Wayne State University School of Medicine, Detroit, MI

INTRODUCTION AND OBJECTIVE: Treatment options for female sexual dysfunction (FSD) remain limited, and patient perspectives on these therapies are not well characterized. This study explores predictors of willingness to pursue different treatment modalities for FSD.

METHODS: We administered a 28-question online survey to adult female participants to collect demographic data, treatment preferences, and FSD status, with the 6-item Female Sexual Function Index (FSFI-6) used as a validated measure of sexual dysfunction. Participants rated their willingness to try seven treatments (hormonal and non-hormonal medications and neuromodulation options) on a 5-point scale ranging from 'not willing' (1) to 'very willing' (5). Ordinal logistic regression was used to assess the association between patient factors and willingness to try each treatment.

RESULTS: Of 934 participants (mean age 44 ± 15), 413 (44%) were sexually active (SA) with FSD, 280 (30%) were SA without FSD, 123 (13%) were SA without intercourse in the past 4 weeks, and 118 (13%) were not SA. Most participants were premenopausal (45%) or postmenopausal (36%). Non-hormonal pill had the highest percentage (69%) of participants rate their willingness 4 or 5 ('very willing'), followed by transcutaneous genital nerve stimulation (43%) and hormonal pill, cream, suppository or patch (37%). Hormonal options were rated an average score of neutral (2.7-3) across all



groups. Antidepressant use was associated with greater willingness to try almost all treatment types (Figure). Lack of health insurance was associated with reduced willingness to try tibial (OR 0.42, 95% CI 0.19–0.91) or sacral nerve stimulation implants (OR 0.34, 95% CI 0.13–0.91).

CONCLUSIONS: Preferences for treatments of FSD varied by demographic and clinical factors. Non-hormonal pills were the most preferred option.

HEALTH RELEVANCE: By highlighting factors that shape treatment preferences, these results provide guidance for developing and delivering therapies that are both acceptable and accessible to patients.

MURINE MODELS OF BENIGN PROSTATIC HYPERPLASIA: CURRENT RELEVANCE AND NEW PERSPECTIVES (poster #57)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a common, aging-related condition often associated with lower urinary tract symptoms (LUTS). Murine models have been developed that recapitulate key aspects of BPH pathophysiology in a tractable way, facilitating mechanistic understanding and therapeutic response. The objective of this review is to summarize murine models developed or applied in the past decade, highlight novel insights, and propose how such models may be paired with state-of-the-art and advancing technologies to accelerate future discovery.

METHODS: PubMed® was searched using "mouse prostate" and "BPH" as well as other keywords related to specific models such as "aging", "transgenic", and "PDX" for articles published prior to May 2025. We prioritized studies published within the past 10 years that used a model resulting in a novel finding about disease pathology and/or treatment.

RESULTS: Recent murine studies have yielded new mechanistic insights into BPH and lower urinary tract dysfunction (LUTD). Aging and hormone models have been utilized to reveal a role for reduced mitochondrial function in LUTD and test the impact of potential interventions such as exercise and oleic acid supplementation. Inflammatory models, such as *E.coli* instillation and diabetic models unveil IL-1, osteopontin, and CXCR4 signaling as key drivers of prostatic fibrosis. Transgenic models disrupting epithelial barrier function demonstrated that barrier loss could induce prostatic inflammation, fibrosis, and secondary LUTD; and that inhibition of mitochondrial ROS generation could reduce prostate weight and voiding frequency in mice with activated prolactin signaling. Finally, patient-derived xenografts preserved native stromal-epithelial crosstalk and hormone responsiveness, enabling predictive modeling of patient-specific therapeutic responses.

CONCLUSIONS: Mouse models of BPH remain keystone tools for bridging mechanistic studies with translational discovery, with recent work demonstrating the contribution of mitochondrial dysfunction, epithelial barrier dysfunction, and intraprostatic prolactin signaling to BPH and LUTD. Further refinement and engineering of new murine models, thoughtful selection of new means of longitudinal monitoring of prostate (dys)function and leveraging the latest omics technology to bridge current models to human relevance will accelerate our understanding of this dynamic and complex disease.

HEALTH RELEVANCE: In the past decade, mouse models have been used successfully to identify molecular drivers of human BPH, increasing our understanding of BPH pathogenesis and accelerating

progress toward identifying novel pharmacotherapies targeting heretofore unexplored signaling pathways and molecular targets that could be employed to improve BPH/LUTS patient outcomes and quality of life.

SEROTONIN-INDUCED CONTRACTIONS OF THE MALE MOUSE URETHRA ARE ALPHA BLOCKER INSENSITIVE (poster #58)

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INTRODUCTION AND OBJECTIVE: Lower urinary tract dysfunction (LUTD) affects over 40% of aging men and is associated with Benign Prostatic Hyperplasia (BPH) and prostate inflammation. BPH is treated with alpha blockers which relax prostatic and urethral smooth muscle but are not effective for everyone and do not address prostate inflammation. It was recently shown that the female mouse urethra secretes serotonin (5-HT) to drive contraction. This study hypothesizes that 5-HT contracts the male mouse urethra by a mechanism that is unresponsive to alpha blockers, and that the inflammatory cytokine, tumor necrosis factor-alpha (TNF α), which has been linked to prostate inflammation, enhances the male urethra's contractile response to 5-HT.

METHODS: Prostatic urethras were collected from 8–9-week-old male C57BL/6J mice, prostate lobes were removed, and trimmed urethras mounted on wire myograph pins to assess ex vivo tissue contractility. Krebs-Henseleit buffer was maintained at 37°C and perfused with carbogen. For alpha blocker studies, tissues were pre-incubated 20 min with alfuzosin (10 nM) or tamsulosin (1 nM) before phenylephrine (PE) (1 nM – 100 μ M) or 5-HT (100 nM – 3 mM) exposure. For inflammatory studies, tissues were pre-incubated 1 h with TNF α (50 pg/mL) before PE and 5HT contraction curves.

RESULTS: PE and 5-HT drive dose-dependent contractions of the prostatic urethra (**Fig. 1A-B**). Tamsulosin reduces the magnitude of PE- but not 5-HT contractions. Alfuzosin reduces the magnitude of PE contractions but only attenuated 5-HT contractions at the highest concentrations tested (**Fig. 1B**). Although 5-HT contractions did not match the magnitude of PE contractions, inflammation can modulate smooth muscle response. Pre-incubation with TNF α did not significantly alter PE contractions but increased the magnitude of 5-HT induced contractions (**Fig. 1C-D**).

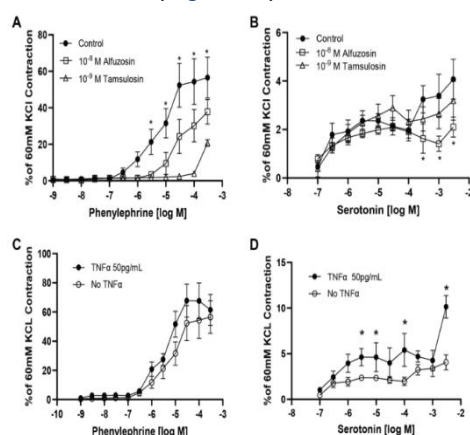


Figure 1. Dose–response curves for PE and 5-HT with and without alpha-blockers or TNF α pre-incubation. * indicate $p < 0.05$.

CONCLUSIONS: Alpha blockers reduced PE- but not 5-HT contractions (except at the highest concentrations with alfuzosin) in the male mouse prostatic urethra. In TNF α incubated tissue, 5-HT but not PE contractions are augmented, signaling that serotonergic pathways are specifically susceptible to inflammation.

HEALTH RELEVANCE: 5-HT mediated contractions represent an alternative pathway for urethral contraction that is amplified by inflammation. Targeting this pathway could be a step towards

effective care in patients with LUTD who are unresponsive to current medications.

URINARY BIOCHEMICAL ECOLOGY REVEALS MICROBIOME-METABOLITE INTERACTIONS AND METABOLIC MARKERS OF RECURRENT URINARY TRACT INFECTION (poster #59)

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INTRODUCTION: Recurrent urinary tract infections (rUTIs) remain a major clinical challenge, and their rising prevalence underscores the need to understand host–microbiome interactions that drive susceptibility. The ways in which the urinary microbiota engages with the local biochemical environment of the urogenital tract are not yet fully defined. Here, we integrate paired urinary metagenomic and quantitative metabolomic data to construct a microbe–metabolite association network of the female urinary microbiome and identify metabolic signatures of rUTI.

METHODS: We collected voided urine from a controlled cross-sectional cohort of postmenopausal women (n=75) divided into 3 groups based on rUTI history and current UTI status. We previously performed whole genome metagenomic sequencing (WGMS) to yield taxonomic and functional profiles. In matched samples, we performed targeted LC-MS/MS to profile urinary metabolites using validated MRM transitions for the quantification of >600 analytes.

RESULTS: By integrating metagenomic and metabolomic data, we identified distinct metabolites, such as methionine sulfoxide and trimethylamine oxide, that associate with variation in urinary microbial diversity. Coupled analyses of microbial and biochemical beta diversity reveal metabolite-linked ecological shifts that may shape rUTI susceptibility. We further define specific lipid species as biomarkers of active rUTI and recurrence risk. Notably, urinary deoxycholic acid (DCA), a microbially modified secondary bile acid, emerges from our analysis as a potential prognostic indicator of recurrence ($HR = 4$), suggesting that systemic metabolic processes influence urinary tract susceptibility and opening new opportunities for personalized management.

CONCLUSIONS: This integrative analysis reveals previously unrecognized links between the urinary microbiome and host biochemical environment in postmenopausal women. By connecting metagenomic profiles to quantitative metabolomic signatures, we demonstrate that specific microbial–metabolite networks underlie ecological states associated with protection or susceptibility to rUTI.

HEALTH RELEVANCE: The associations identified here are now providing critical insight as biomarkers of urobiome ecology, which we are now translating for more personalized approaches in the management of rUTI.

BUILDING COLLABORATIVE RESEARCH AGENDAS IN NON-MALIGNANT UROLOGY: DESIGN AND PROCESS OF A RESEARCH STRATEGY SPRINT (poster #60)

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INTRODUCTION AND OBJECTIVE: The Collaborating for the Advancement of Interdisciplinary Research in Benign Urology (CAIRIBU) Interactions Core piloted a 1.5-day virtual “research strategy sprint” with goals of exchanging knowledge, co-creating research priorities in benign urology, fostering interdisciplinary collaboration, and generating future meritorious grant applications.

The event included intensive working group meetings focused on 3 themes: urobiome, uro-aging, and urinary voiding/dysfunction.

METHODS: Leaders for each working group were identified from within the CAIRIBU Community and collaborated in the months prior to the event to set the sprint agenda and identify processes for knowledge gap analysis (FIGURE). The event was structured, time-limited, and highly interactive and included both didactic and experiential components. Working group discussions were facilitated; detailed notes and transcripts were generated. Invited speakers for each group set the stage for and stimulated discussion. The groups came together at the end of each day to share results and identify areas for future collaboration. An evaluation plan with both short- and long-term components was devised.

FRIDAY, JULY 18



SATURDAY, JULY 19



RESULTS: Groups identified knowledge gaps and research barriers, prioritized research ideas, developed specific aims, and assigned collaborative leads. Pre- and post-event surveys confirmed the value of collective problem-solving and enthusiasm for the process. Participants stated high interest in continued post-event working group interactions and identified specific supportive needs to be provided by CAIRIBU Interactions Core staff; these included coordinating and ensuring continuity between follow-up meetings, providing platforms and mechanisms for continued collaboration, and identifying appropriate funding mechanisms and timelines for emerging grant applications. Follow-up meetings are underway.

CONCLUSIONS: Our results suggest that similar processes around focused topics may assist other research communities, particularly in the current competitive and uncertain funding landscape, to engage in strategic, collaborative visioning toward long-term research agendas and grant applications.

HEALTH RELEVANCE: The sprint was effective in this multi-institutional community of investigators in building shared knowledge and momentum toward strategic research agendas. Longer-term outcomes will assess whether goals are achieved for new research funding that advances patient care.

LOW-RANK ADAPTATION–SPECIALIZED MULTIMODAL MODEL FOR STRUCTURED CT REPORTING OF NEPHROLITHIASIS (poster #61)

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INTRODUCTION AND OBJECTIVE: The rise of nephrolithiasis in the United States increases the demand for CT scans. Currently, CT reporting varies widely in style, specificity, and quality between radiologists, delaying downstream clinical decisions. We asked whether a multimodal large language model (LLM), MedGemma-4 Billion Parameters (4B), specialized with Low-Rank Adaptation (LoRA), can produce accurate and structured CT report elements for stones: presence, laterality, hydronephrosis, and size (coronal Y-dimension).

METHODS: We fine-tuned MedGemma-4B with small LoRA adapters where the base model was frozen. Starting with 240 non-contrast CT cases (n), 3 datasets were made: 174 train / 32 validation / 34 test. Every case fed the model a single standardized coronal slice (centered on the stone if known, otherwise mid-coronal) plus a fixed prompt. The model generated a four-line output: Stone detected, Stone Location (side), Size Y (mm), and Hydronephrosis. After generation, a text parser compared model predictions against ground truth from radiology reports. Unknown/Not reported items are excluded from the denominator for that metric. Primary endpoints were accuracy for stone presence, hydronephrosis, laterality, and size error (root mean squared error (RMSE), mean absolute error (MAE), and bias). Baseline model was compared using the same inputs.

RESULTS: On the test set (n=34), LoRA delivered better stone detection than the frozen baseline (F1: 0.735 vs 0.717; Accuracy: 0.618 vs 0.559) with near-perfect Recall (0.947). Hydronephrosis classification improved to perfect accuracy (n=14). Laterality remained moderate (LoRA 0.7 on n=30 scorable cases vs baseline 0.824 on n=34). Stone size (y) error remained comparable.

Endpoint	Metric	Baseline	LoRA	Denominator
Stone detection	Accuracy	0.559	0.618	34
	Precision	0.559	0.600	34
	Recall	1.000	0.947	34
	F1	0.717	0.735	34
Hydronephrosis	Accuracy	0.714	1.000	14
Laterality	Accuracy	0.824	0.700	34 (base) / 30 (LoRA)
Size (Y)	RMSE (mm)	13.458	13.702	19
	MAE (mm)	8.053	8.263	19
	Bias (mm)	-3.737	-4.368	19

CONCLUSIONS: The LoRA-specialized MedGemma-4B model outperformed the baseline for stone detection and hydronephrosis accuracy using one standardized coronal slice. The extension to anatomical location predictions would require larger and thoroughly annotated CT cohorts to measure location reliably. Additionally, results are limited by small per-endpoint denominators but show clear gains with a lightweight update.

HEALTH RELEVANCE: More consistent CT outputs for nephrolithiasis scans may shorten time toward treatment with minimal added burden on radiologists. With less variability in report style, this system could improve data quality for population studies.

SINGLE-CELL ANTIMICROBIAL SUSCEPTIBILITY TESTING VIA DROP-BASED MICROFLUIDICS FOR RESISTANT URINARY TRACT INFECTIONS (poster #62)

Maureen A. Pelissero,¹ Carter W. Hoffman,^{1,2} Connie B. Chang¹

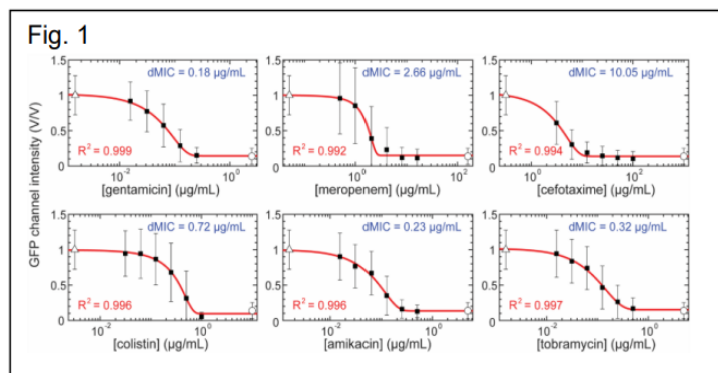
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INTRODUCTION AND OBJECTIVE: Antimicrobial resistance (AMR) is a critical global health threat, reducing treatment efficacy and

increasing mortality. Urinary tract infections (UTIs) are the most common AMR-related bacterial infections in the U.S., with 27% recurring within 6 months. The gold standard for antimicrobial susceptibility testing (AST), minimum inhibitory concentration (MIC), has key limitations. It requires dense bacterial cultures, masking resistant subgroups that may survive treatment and cause reinfection. It also relies on extensive bacterial growth, delaying results by 24-120 hours. These constraints hinder timely, effective intervention. We aim to develop a rapid, single-cell AST platform for UTIs that detects heteroresistance, tests multiple antibiotic conditions in parallel, and allows downstream analysis.

METHODS: We use drop-based microfluidics (DBM) to isolate single bacterial cells in picoliter drops containing media, antibiotics, and fluorescent barcodes. A custom fluorescence detection stand measures growth of Green fluorescent protein (GFP)-expressing *E. coli* HB101 across 36 antibiotic conditions in parallel to determine MIC. Drop sorting is performed via dielectrophoresis. Statistical analyses include confidence intervals, Pearson coefficients, and t-tests. We are integrating fiber optics into the platform to enable label-free detection via scattered light, simplifying optics, and enhancing portability.

RESULTS: We demonstrated multiplexed DBM AST on single GFP *E. coli* cells within 5 hours, revealing single-cell growth distributions that expose resistant subgroups and MIC (Figure 1). Drop sorting of fluorescent dye achieved 99% sensitivity and 99.9% specificity; sorting single GFP *E. coli* cells reached 96% sensitivity and 90% specificity. The optofluidic platform is expected to distinguish resistant and susceptible bacteria based on scattered light intensity, with results anticipated in under 2 hours.



CONCLUSIONS: DBM improves AST by enabling single-cell analysis, revealing heteroresistance missed by conventional methods, and reducing time to results. Drop sorting enables collection of resistant cells for further analysis.

HEALTH RELEVANCE: This technology supports rapid, precise treatment decisions for UTIs, advancing efforts to combat AMR.

ESTROGEN-DEPENDENT MECHANISMS ELEVATE UROTHELIAL C-FIBER DENSITY AND DRIVE TO OVERACTIVE BLADDER PATHOGENESIS IN FEMALE TYPE 1 DIABETIC AKITA MICE (poster #63)

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INTRODUCTION AND OBJECTIVE: Diabetes is associated with up to a 77% increase in the prevalence of overactive bladder (OAB) symptoms, making OAB the most common presentation in patients with diabetes. A hallmark of OAB is hyperexcitability of afferent bladder nerves, attributed to an extensive network of c-fiber nerves in the urothelium. Estrogen-dependent mechanisms partially regulate the growth of c-fiber nerves. In diabetic animal models, estrogen-dependent signaling pathways have been implicated in autonomic dysregulation, cardiac dysfunction, and benign prostatic hyperplasia. Yet, the influence of diabetes on estrogen-dependent mechanisms

regulating micturition remains unclear. We hypothesized that estrogen-dependent mechanisms drive urothelial c-fiber growth and diabetes-induced OAB. To test this hypothesis, we assessed in vivo bladder function and quantified urothelial c-fiber populations in gonadally intact and ovariectomized female type 1 diabetic Akita mice.

METHODS: Female type 1 diabetic Akita mice (C57BL/6J background) and non-diabetic C57BL/6J controls were either ovariectomized at 8 weeks of age or left gonadally intact. At 15 weeks of age, in vivo bladder function was assessed using awake-restrained cystometry to measure void volume and frequency (n=9-11 per group). Additionally, in all groups (n=6 per group), immunofluorescence labeling with calcitonin gene-related peptide was used to identify c-fiber nerves on transverse bladder sections. Labeled nerves were quantified and normalized to total urothelial area. Statistical significance (p<0.05) was calculated using a one-way analysis of variance with Tukey post hoc tests to compare all groups.

RESULTS: Diabetic mice exhibited significantly reduced void volume and increased voiding frequency compared to non-diabetic controls, consistent with signs of OAB. Diabetic mice also showed increased urothelial c-fiber density. In contrast, ovariectomized diabetic females failed to develop any discernible signs of OAB, displaying voiding parameters comparable to non-diabetic mice. Consistent with the absence of an OAB phenotype, ovariectomized diabetic mice displayed significantly fewer urothelial c-fibers compared to gonadally intact diabetic mice, with levels similar to non-diabetic controls. Ovariectomy had no significant effects on voiding or c-fiber density of non-diabetic controls.

CONCLUSIONS: Diabetes upregulates estrogen-dependent mechanisms that promote urothelial c-fiber growth and OAB development.

HEALTH RELEVANCE: Further elucidation of the precise mechanisms by which estrogens influence bladder innervation may inform novel therapeutic strategies to prevent or treat diabetes-associated OAB.

INTRACELLULAR ATP PRODUCTION AS A DRIVER OF CATHETER BIOFILM FORMATION IN CHRONICALLY CATHETERIZED PATIENTS (poster #64)

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INTRODUCTION AND OBJECTIVE: Catheter-associated urinary tract infections (CAUTIs) and biofilm-mediated catheter obstructions represent major complications of long-term catheterization, affecting nearly 50% of patients. Organisms from urine culture may not predict their potential to form biofilms on catheters. Our objective was to identify and characterize drivers of urinary catheter biofilm formation.

METHODS: Urethral Foley catheters (n=38; latex or silicone) were collected from chronically catheterized male patients at the Palo Alto VA Medical Center. Biofilms were removed by vortex-sonication into 1X PBS, cultured under aerobic and anaerobic conditions, and assayed for biofilm production (crystal violet), viability, turbidity, intracellular ATP, and protein content. Shotgun proteomics and 16S/metagenomics were used to identify enriched pathways, proteins, and microbial communities across high, moderate, and low biofilm-forming catheters. Protein-protein interaction networks were generated using STRING analysis.

RESULTS: Intracellular ATP correlated most significantly (R² = 0.76, p< 0.001) with catheter biofilm production (~14-fold higher in high vs. low biofilm formers), while CFU, total protein, or turbidity did not. Proteomic profiling revealed that in the high biofilm group, proteins

related to ATP metabolism (ATPA), ribosomal proteins (RPSL, RPSN), and chaperones (GROEL, DNAK) were increased in multiple uropathogens, while proteins supporting the Fo subunit (ATPF) were decreased. Network and GO enrichment analyses showed increased presence of energy investment (ATP generation, translation, nucleotide recycling) and reduced motility/transport pathways (PILS, OMPX). Human proteomic analyses showed suppression of host defense proteins in high biofilm catheters, suggesting reduced local immune activity. When *Enterococcus faecalis* ATCC 29212 was cultured under acidic conditions, intracellular ATP and biofilm significantly increased compared to normal pH, suggesting a charge-regulated mechanism of adhesion-induced ATP gain in biofilm formation.

CONCLUSIONS: Intracellular ATP accumulation in uropathogens is associated with high biofilm-forming catheters and is linked to metabolic shifts that promote resilience and matrix production. These findings support a model in which microbial attachment to catheter surfaces modulates bacterial proton motive force, elevating intracellular ATP and driving biofilm. Ongoing work is characterizing the genomic profiles of individual uropathogens to identify organism-specific mechanisms underlying these community-level proteomic signatures.

HEALTH RELEVANCE: Uropathogen intracellular ATP may be a potential therapeutic target in catheter biofilm prevention and provides a framework for studying interventions in long-term catheterized patients.

INTRINSIC G-PROTEIN COUPLED RECEPTOR ACTIVITY REGULATES MOUSE URINARY BLADDER PHASIC SMOOTH MUSCLE CONTRACTIONS, POTENTIALLY BY MODULATING MEMBRANE POTENTIAL (poster #65)

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INTRODUCTION AND OBJECTIVE: Detrusor smooth muscle (DSM) of the bladder exhibits action potentials leading to Ca^{2+} activity that propagates in waves to elicit phasic non-voiding contractions. Phasic contractions produce fluctuations in bladder pressure, called transient pressure events (TPE), and are associated with bursts of afferent nerve activity to the central nervous system that signal filling status. G protein-coupled receptors (GPCR) regulate bladder contraction and relaxation during micturition. This study investigates the role of GqPCR on TPE in isolated mouse urinary bladder. We hypothesized that GqPCR activity modulates DSM phasic contractions and inhibition reduces Ca^{2+} activity and TPE frequency and amplitude.

METHODS: Urinary bladders were isolated from C57Bl6/J mice. Bladders were attached to a syringe pump and inline pressure transducer to monitor intraluminal pressure, and partially filled to induce TPE. Recordings were analyzed with Spike2 software (Cambridge Electronic Design). Membrane potential (VM) was recorded in bladder sheets by impaling individual DSM cells with a glass microelectrode (Fig. 1).

RESULTS: Inhibition of GqPCR signaling with YM-254890 (YM, 500nM) decreased mean TPE frequency (88%, $p=0.001$), amplitude (95%, $p=0.035$), and intravesical pressure (86%, $p=0.023$) ($n=8$ bladders). Application of the Protein Kinase C (PKC) activator, Phorbol 12, 13-dibutyrate (PDBu, 100nM), rescued intravesical pressure ($p=0.227$) and produced a modest return in TPE amplitude ($p=0.132$) but not frequency ($p=0.010$) compared to baseline, suggesting both PKC and IP3 mediated Ca^{2+} release is involved. Measuring DSM VM with microelectrodes, YM appears to elicit hyperpolarization (Fig. 1) in 4 of 6 cells while PDBu depolarized 4 of

5 cells. Further analysis is required to determine if DSM action potential frequency or kinetics is altered.

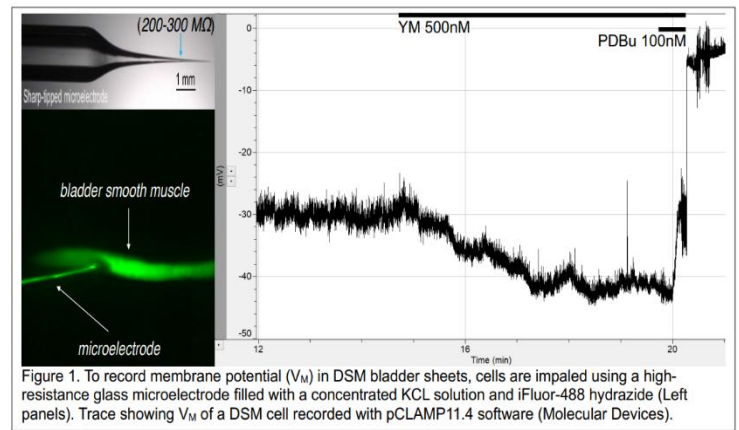


Figure 1. To record membrane potential (V_m) in DSM bladder sheets, cells are impaled using a high-resistance glass microelectrode filled with a concentrated KCL solution and iFluor-488 hydrazide (Left panels). Trace showing V_m of a DSM cell recorded with pCLAMP11.4 software (Molecular Devices).

CONCLUSIONS: These results suggest intrinsic GqPCR activity mediates DSM contractility, potentially through regulation of VM and/or action potentials. Potential GqPCR targets that could influence DSM excitability include K^+ , Ca^{2+} , and Ca^{2+} -activated chloride channels. More experiments are required to elucidate a mechanism.

HEALTH RELEVANCE: These results highlight the importance of GqPCR in the generation of phasic DSM electromechanical activity and sensory feedback to the CNS, addressing a knowledge gap in the field. This work can be applied to disease models of overactive and underactive bladder to help identify novel therapeutic targets.

LINEAGE TRACING REVEALS UNEXPECTED EXPRESSION OF OSR2-CRE IN THE EFFERENT DUCT EPITHELIUM (poster #66)

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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.

NERVE-EVOKED AND SPONTANEOUS BLADDER SMOOTH MUSCLE CONTRACTILITY IMPACTS OF POLYCHLORINATED BIPHENYLS (PCBS) EXPOSURE (poster #67)

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INTRODUCTION AND OBJECTIVE: BK channels (large-conductance Ca^{2+} -activated K^{+} channel) are outwardly rectifying K^{+} channels providing negative feedback for bladder smooth muscle contractions through membrane potential maintenance and shaping current rectification. BK channel opening is triggered by Ca^{2+} sparks through ryanodine receptors (RyRs, classical target of polychlorinated biphenyls (PCBs)) during bladder relaxation, and Ca^{2+} waves through cholinergic signaling during nerve-evoked contractions. Exposure to the environmental toxicant PCBs causes sex- and dose-dependent lower urinary tract dysfunction (LUTD) in mice. We hypothesize PCBs alter bladder contractile activity in part through increasing BK channel opening activity.

METHODS: Female nulliparous mice were dosed with a human-relevant PCB mixture at 0, 0.1, 1, or 6 mg/kg/d 2 week prior to mating until the offspring were weaned (P21). Offspring (~P42) were euthanized, and bladders were immediately collected. qPCR was performed to quantify cholinergic receptor, BK channel, and RyR RNA abundances. *Ex vivo* tissue baths were performed to measure bladder contraction using the following stimuli: nerve-evoked bladder contraction (electrical field stimulation (EFS, 1-50 Hz)), cholinergic receptor-mediated contraction (carbachol), EFS with BK channel opener (NS11021), and spontaneous contractions with RyR closer (ryanodine) and/or BK closer (paxilline). Analysis utilized one- or two-way ANOVA followed by multiple comparisons tests to analyze PCB exposure group effects compared to the sex-matched vehicle controls.

RESULTS: PCB exposed mice had sex- and dose-dependent impacts on cholinergic receptor expression and *ex vivo* bladder contractions. In female mice, PCBs diminished nerve-evoked bladder contractile strength, increased cholinergic receptor RNA abundance and sensitivity to direct cholinergic stimulation, made pharmacological BK opening more efficacious at diminishing the contractile strength of nerve-evoked contractions, and increased the RyR contributions to spontaneous bladder contractions leading to changes in BK-RyR coupling.

CONCLUSIONS: Human-relevant PCBs impact murine urinary voiding physiology in part through augmented myogenic and nerve-evoked contraction. PCBs may impact detrusor contractility through opening RyRs and increasing BK channel opening probability, leading to impacts on bladder contractile sensitivity and a urinary voiding phenotype reminiscent of overactive bladder symptoms in humans.

HEALTH RELEVANCE: Overactive bladder and augmented detrusor activity are clinically relevant adverse health outcomes yet

impacts of environmental chemicals on the molecular underpinnings of LUTD etiology is understudied.

CHRONIC LOSARTAN DELIVERY ATTENUATES ABDOMINAL HYPERSENSITIVITY AND BLADDER PRESSURES IN AN AUTOIMMUNE CYSTITIS ANIMAL MODEL (poster #68)

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INTRODUCTION AND OBJECTIVE: 3-7% of women in the United States suffer from Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS), a chronic pain condition. IC/BPS etiology is unclear, challenging to diagnose, and current treatments have side effects that outweigh the benefits; thus, there is an urgent need for more effective therapies. Although IC/BPS pathophysiology is unknown, there are various parallels with inflammatory diseases in other organs. It is well established that angiotensin signaling plays a role in tissue inflammation and fibrosis through the angiotensin type 1 receptor (AT1R) in the lungs, kidneys, heart, and liver. However, knowledge regarding AT1R and bladder function is limited. Therefore, we wanted to further investigate whether AT1R plays a role in bladder pathophysiology in an experimental autoimmune cystitis (EAC) animal model.

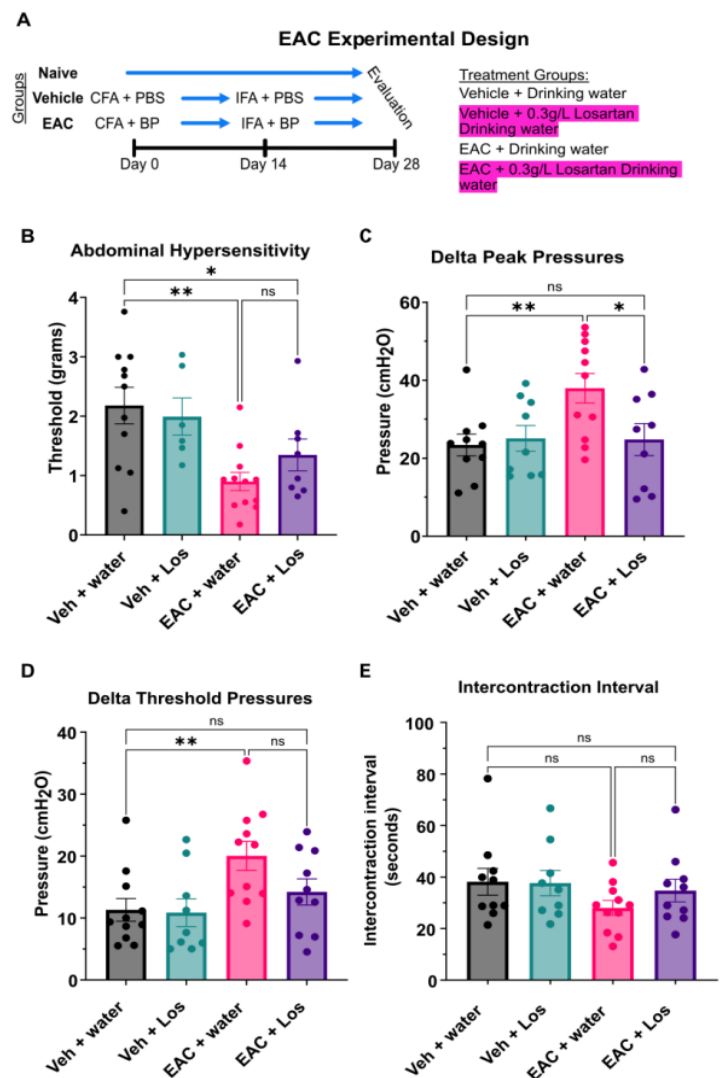


Figure 1. Chronic losartan delivery in an experimental autoimmune mouse model. EAC experimental design (A) in C57BL/6J female mice. Von frey mechanical hypersensitivity after the induction of EAC, with and without 0.3g/L losartan drinking water (B). Cystometry delta peak pressure (C), delta threshold pressure (D), and intercontraction interval (E).

METHODS: In naïve C57BL/6J bladder strips, we recorded ex vivo bladder contractile force in response to the angiotensin-II peptide and AT1R antagonism by the losartan metabolite, EXP3174. For in vivo experiments, C57BL/6J female mice were given bladder protein immunizations on experimental days 0 and 14 to induce an autoimmune response (A). 0.3g/L losartan, an FDA-approved AT1R antagonist, was administered in drinking water starting on experimental day 1 and continuing for 4 weeks. After the development of cystitis, we utilized UroVoid to assess voiding behavior and mechanical von Frey to assess abdominal hypersensitivity. After behavior data collection, we performed terminal cystometry in the same animals to evaluate urodynamics.

RESULTS: The mice with autoimmune cystitis have significantly increased abdominal sensitivity that is attenuated with the oral losartan treatment compared to controls (B). The EAC mice also have significant increases in the delta peak and threshold pressures that are attenuated with the losartan treatment (C, D). We found no statistical differences in intercontraction intervals between the four groups (E).

CONCLUSIONS: These findings suggest that AT1R contributes to heightened abdominal sensitivity and bladder pressure changes in an EAC mouse model that can be mitigated with chronic losartan delivery. More studies are needed to further evaluate the mechanism of AT1R in bladder pain and dysfunction.

HEALTH RELEVANCE: FDA-approved drugs that target angiotensin signaling could be of potential interest for chronic bladder inflammatory diseases.

PIEZO1 PROMOTES A PPAR γ -DIRECTED PROGRAM OF RENAL UROTHELIUM DIFFERENTIATION (poster #69)

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INTRODUCTION AND OBJECTIVE: Urinary tract obstruction (UTO) is a leading cause of pediatric chronic and end-stage kidney disease. Understanding the kidney's response to UTO is critical for developing therapies that go beyond mechanical decompression. Renal urothelium remodeling during UTO involves expansion of UPK⁺ cells, which are known to form urothelial plaques and tight junctions to enhance impermeability and tissue compliance. Renal urothelium expresses PIEZO1, yet despite increased mechanical stress being a hallmark of UTO, the role of mechanoreceptors in this context remains unexplored. We hypothesize that PIEZO1 activation promotes renal urothelium remodeling during UTO. Our goal is to determine how PIEZO1 regulates renal urothelium remodeling.

METHODS: Hoxb7Cre;Piezo1fl/fl (Piezo1cKO) mice were used to conditionally delete PIEZO1 in renal urothelium, and Piezo1fl/fl mice served as controls. Unilateral ureteral obstruction (UUO) was used to model UTO, and kidneys were collected at postoperative day (POD)7. To explore PIEZO1-regulated pathways, we performed scRNA-seq on Yoda1- and vehicle-treated kidney urothelium-derived organoids (KUDOs). PROGENy was used to infer active signaling pathways at single-cell resolution. RNAscope, immunofluorescence and Western blot were used to evaluate renal urothelium and KUDOs.

RESULTS: Piezo1cKO kidneys showed reduced UPK, attenuated renal parenchyma and increased suburothelial collagen deposition at UUO-POD7 compared to Piezo1fl/fl kidneys. Compared to carrier-treated KUDOs, Yoda1 treatment increased size, differentiation and activated pro-proliferative and survival pathways (MAPK, EGFR, PI3K). The Krt5⁺ cluster upregulated proliferation and cell cycle progression markers, while the Upk⁺ cluster upregulated barrier and urothelial differentiation genes including Pparg, a master regulator of

urothelial differentiation. Increased UPK, and PPAR γ were confirmed by WB.

CONCLUSIONS: This study demonstrates the critical roles of PIEZO1 in UTO-induced renal urothelium remodeling and suggests that PIEZO1 activation promotes renal urothelium growth, differentiation, and PPAR γ -associated barrier maturation programs.

HEALTH RELEVANCE: Understanding how the kidney's urothelium senses and adapts to UTO may reveal new therapeutic targets to preserve kidney function beyond surgical repair.

ASCENDING UTIS: HOW UROPATHOGENIC *E. COLI* USES CHEMOTAXIS TO COLONIZE THE BLADDER (poster #70)

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) are responsible for at least 7 million doctor visits and 100,000 hospitalizations per year. Currently, treatment is limited to antibiotics. Improving knowledge of how bacteria reach the bladder could lead to the development of new pharmacological targets and treatments for UTIs. We hypothesize uropathogenic *E. coli* (UPEC) use swarming, a coordinated and flagella-dependent surface movement that depends on host cues (chemotaxis or chemorepulsion) to ascend the urethra and colonize the bladder. We optimized a mouse model of ascending UTI in which UPEC are introduced into the distal urethra and ascends to colonize the bladder within four hours. This study tested the hypothesis that two genes required for swarming, aer (encoding a chemoreceptor) and tls (encoding a chemotaxis control protein), are required for bladder colonization by uropathogenic *E. coli* UTI89 in adult female C57BL/6J mice.

METHODS: Adult (6-8-week-old) female C57BL/6J mice are anesthetized before using a catheter to instill 5 μ L (OD 0.80, contains 5 x 10⁶ to 4.1 x 10⁷ CFUs) of *E. coli* UTI89/pComGFP, *E. coli* UTI89 Δ tls/pComGFP (a truncated mutant of the trg chemoreceptor involved in flagella rotation), or *E. coli* UTI89 Δ aer/pComGFP (an aer deletion mutant deficient in oxygen sensing), or saline into the distal portion of the urethra. All bacterial strains are transformed to confer kanamycin resistance. Mice are euthanized 4 hours after infection, a silk ligature is used to physically separate the bladder and urethra, each is removed, homogenized, serially diluted in saline, and plated on agar plates containing Kanamycin (50 μ L/mL). Bacteria are grown for 24 hours and bacterial colony forming units (CFUs) are counted.

EXPECTED RESULTS AND CONCLUSIONS: We hypothesize the hyper motile UTI89 Δ tls strain will more readily colonize the bladder, and the UTI89 Δ aer will less readily colonize the bladder, than the wild type (*E. coli* UTI89) strain.

HEALTH RELEVANCE: These studies will clarify how bacteria interact with the host to move from the urethra into the bladder. Understanding these mechanisms will improve insight into the bacterial factors that promote movement (swarming) and may identify new targets for preventing or treating urinary tract infections.

DEFINING THE METABOLIC PATHWAY FOR ALLULOSE UTILIZATION BY URINARY ENTEROCOCCUS FAECALIS (poster #71)

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INTRODUCTION AND OBJECTIVES *Enterococcus faecalis* is a Gram-positive bacterium prevalent in the gastrointestinal and female

urinary tracts. It is both a commensal and opportunistic pathogen causing endocarditis and urinary tract infection (UTI). *E. faecalis* colonizes and persists in diverse environments due to its metabolic flexibility and antimicrobial resistance. Allulose (D-psicose) is a rare sugar used as a low-calorie sweetener for individuals with diabetes. Allulose is not metabolized by humans and is excreted in urine, potentially creating a unique carbon source in the urinary tract. We identified an *E. faecalis* urine isolate (C130) that uses allulose as a sole carbon source. Allulose utilization has not been reported in *Enterococcus* spp. and the metabolic pathway is not defined. Our objective is to define the genetic determinants underlying this novel metabolic phenotype.

METHODS Biolog microarrays were used to screen carbon utilization across *E. faecalis* isolates. For kinetic growth assays, strains were grown in chemically defined media (CDM) with 0.5% allulose or glucose and optical density monitored for 40 hours at 35°C, 5% O₂, 10% CO₂ on a Cerillo Alto. Targeted deletions were generated by markerless gene exchange, and quantitative RT-PCR was used to measure gene expression. Adaptive mutations were identified by Illumina whole genome sequencing and Breseq analysis.

RESULTS *E. faecalis* C130 grew in CDM with allulose, but growth curves revealed a long lag phase during the first exposure to allulose. This lag phase was not observed for C130 previously exposed to allulose, even after multiple passages without allulose, suggesting a genetic adaptation. Genome analysis revealed a shared mutation in a plasmid-borne *deoR/glpR* gene, encoding a PTS system repressor, among allulose-adapted strains. Mutations were in the C-terminal substrate-sensing domain and were predicted to be loss of function. Indeed, deletion of this gene resulted in rapid growth in allulose without previous exposure. qPCR confirmed increased expression of PTS transporter genes during growth in allulose.

CONCLUSIONS We demonstrate for the first time that not only can *E. faecalis* use allulose as a sole carbon source but that this ability is gained through acquired mutations in the PTS regulatory protein *DeoR/GlpR*. This newly identified metabolic capacity likely reflects an adaptive mechanism to exploit rare sugars available in the urinary niche, enhancing colonization fitness and persistence.

HEALTH RELEVANCE The emergence of allulose-utilizing *E. faecalis* strains may have important clinical implications. As allulose consumption increases among diabetic individuals, this adaptation could provide an advantage for opportunistic *E. faecalis* strains in the urinary tract, promoting UTI risk.

FUNCTIONAL MRI ANALYSIS OF OVERACTIVE BLADDER: BRAIN AND BRAINSTEM MECHANISMS AND THE ROLE OF TIBIAL NERVE STIMULATION (poster #72)

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INTRODUCTION AND OBJECTIVE: Overactive bladder (OAB) affects nearly 1 in 3 women over the age of 60, leading to reduced quality of life, increased healthcare costs, and greater incidence of depression and sleep disturbances. Current neuromodulation therapies, such as sacral nerve stimulation (SNS) and tibial nerve stimulation (TNS), have shown mixed efficacy, are largely unoptimized, and have unclear mechanisms of action. The objective of this "in progress" study is to understand better how TNS changes brain/brainstem function.

METHODS: Experiments are conducted on female CD rats utilizing 9.4T functional MRI. Animals are anesthetized with urethane and have a catheter placed in the dome of the bladder. Cystometric data are collected, with a targeted time of 3-4 minutes per fill. We have begun to construct an analysis pipeline utilizing General Linear Models and correlation analysis. Once we have confirmed our ability to record high-quality signals in our areas of interest (periaqueductal

gray or PAG and Pontine Micturition Center or PMC), and understand how many trials are necessary to get a good signal-to-noise ratio, we will measure brain/brainstem activity before, during, and after TNS. Additionally, to better understand brain function, we are interested in examining the change in PAG activity, over time, as the bladder fills, whereas traditionally studies have focused solely on differences in PAG activity between full and empty bladder states.

RESULTS: Custom software has been written to collect and plot bladder pressure data from a MRI safe amplifier (SAII, output via serial port). Data from 6 rats, without stimulation, has been collected. Each functional imaging session lasted approximately 90 minutes and consisted of approximately 18 fills. Analysis of the collected signals is in progress. We hypothesize that TNS inhibits bladder afferents, meaning that a higher bladder volume is necessary for the PAG to trigger micturition. Alternatively TNS may inhibit PMC activation, in which case we would observe no change in the PAG/bladder volume relationship, and higher PAG activity would be necessary to trigger micturition.

CONCLUSIONS: By studying changes in brain/brainstem activation with TNS we expect to understand better the mechanisms of action of TNS. Such insights may guide future studies to develop optimized neuromodulation strategies and may ultimately help with matching the appropriate therapy (SNS, TNS, etc.) to individuals.

HEALTH RELEVANCE: This study provides insights into the neural mechanisms of bladder control, specifically the role of tibial nerve stimulation, which is critical for improving neuromodulation strategies for treating OAB.

EFFECT OF ESTROGEN-SIGNALING VIA NEURONS OF BARRINGTON'S NUCLEUS ON LOWER URINARY TRACT FUNCTION (poster #73)

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INTRODUCTION AND OBJECTIVE: Barrington's nucleus (Bar), a brainstem nucleus with estrogen receptor-expressing neurons, drives micturition by innervating motor neurons that promote synergistic contraction of the detrusor muscle and relaxation of the external urethral sphincter (EUS). Bar is integral to the central switch between urine storage and voiding phases. Loss of ovarian function and lower estradiol levels after menopause are associated with increased prevalence of urinary incontinence, yet the mechanism of action of estrogen as a modulator of lower urinary tract (LUT) function remains underexplored. This study characterizes the functional importance of estrogen receptor alpha (Esr1)-expressing neurons of Bar and will elucidate the modulatory role of estrogen signaling via brainstem neurons in LUT function. The objective of this research is to gain a better understanding of the mechanisms by which changes in central hormone signaling influence female urogynecologic health.

METHODS: To ablate Esr1-expressing neurons of Bar, diphtheria toxin A (DTA) fragment carried by an adeno-associated viral vector (AAV DIO-DTA-mCherry) was injected bilaterally into Bar of Esr1-IRES-Cre/+; L10-GFP young adult female mice (Bar^{Esr1}-DTA). Lower urinary tract function including void frequency, volume, and location was recorded using micturition video thermography (MVT). To selectively target estrogen signaling via Esr1, the receptors were knocked out from Esr1+ neurons by bilateral Cre injection into Bar of female Esr1 lox/lox mice. This allowed for the selective excision of exon3 from the Esr1 gene and resulting loss of Esr1 protein expression (Esr1-KO). Mice underwent MVT to characterize LUT function. Brain tissue was collected and precise and bilateral injection of the AAVs into Bar was confirmed by fluorescence microscopy.

RESULTS: Following DTA ablation, Bar^{Esr1}-DTA mice exhibited a strong increase in the number of small, abnormal voids characterized as leaks compared to pre-DTA behavior. However, Bar Esr1-KO mice

did not have a significant change in the number of normal voids or leaks compared to their baseline. We are increasing cohort sizes to better understand the changes in micturition behavior following targeted knockout of *Esr1* and sequential absence of estrogen signaling via Bar neurons.

CONCLUSIONS: DTA ablation of Bar^{Esr1} neurons produces a urinary retention phenotype similar to the phenotype observed when the total glutamatergic Bar neuron population is ablated, suggesting that the *Esr1* receptor is expressed by Bar neurons with functional importance to lower urinary tract behavior. However, the normal voiding behavior upon targeted excision of the *Esr1* receptor from Bar neurons suggests that the voiding phenotype observed following DTA ablation of the Bar^{Esr1} neurons may not be independently driven by signaling via the *Esr1* receptor.

HEALTH RELEVANCE: This research underscores that the lower urinary tract is susceptible to hormonal signaling pathways at the level of brainstem neurons. More research is needed to understand the function of *Esr1* signaling in Bar neurons and to elucidate whether hormonal modulation contributes to changes in LUT symptomatology observed at major endocrinologic timepoints such as menopause.

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THE INTERPLAY OF MEAL-TIMING AND URINARY OXALATE EXCRETION (poster #74)

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INTRODUCTION AND OBJECTIVE: Kidney Stone Disease (KSD) is a common urological disease impacting quality of life and placing a financial burden on healthcare systems. Most kidney stones are composed of calcium oxalate and increased urinary oxalate (UOx) excretion is a major risk factor for such stones. A major source of UOx excretion is endogenous hepatic oxalate synthesis. The liver functions in a strong circadian rhythm with much of its transcriptome regulated by the molecular clock, being influenced by external cues—including meal-timing. Clock disruptions are associated with several metabolic disorders. Despite the liver functioning in a strong circadian rhythm, the relationship between circadian rhythm and hepatic oxalate synthesis and UOx remains unclear.

METHODS: 10-week-old C57BL/6 wild-type male and female mice (n=24, 12 male, 12 female) underwent 3 weeks of time restricted feeding (TRF), with 12 mice (6 male, 6 female) receiving food only during their active period (active TRF, aTRF), and the other 12 receiving food only during their inactive period (inactive TRF, iTRF). Mice were singly housed in metabolic cages, given 2 days of acclimation followed by 3 days of 12-hour urine collections. UOx was analyzed by ion chromatography mass spectrometry and creatinine by a colorimetric assay.

RESULTS: Male and female aTRF and iTRF mice body weight remained stable while in metabolic cages, with no difference in food intake. Urine volume and water intake displayed a diurnal pattern, with higher urine volume and water intake seen when they had access to food in male and female iTRF and aTRF groups. There were no differences in urine creatinine. UOx was higher in male iTRF mice compared to male aTRF mice (pTRF = 0.0002, $\bar{x}$ iTRF = 87.3 μ g/12 Hr, $\bar{x}$ aTRF = 41.1 μ g/12 Hr), whereas in females, TRF was not a significant factor (pTRF=0.3409).

CONCLUSIONS: UOx synthesis and excretion appear to increase in male mice that have had their molecular clock aberrated by mistiming of food, whereas female mice show a resistance to such disruptions.

HEALTH RELEVANCE: With KSD placing immense burdens on healthcare systems, novel treatments are critical to mitigate costs and improve quality of life. We seek to gain better understanding of the role the molecular circadian clock plays in regulating endogenous oxalate synthesis and UOx excretion.

INVESTIGATING CHANGES IN GANGLION MACROPHAGES IN AN INDUCED CYSTITIS MOUSE MODEL (poster #75)

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INTRODUCTION AND OBJECTIVE: Urologic chronic pelvic pain syndrome (UCPPS) is characterized by pain in the pelvic region accompanied by bladder pain and urinary dysfunction without an identifiable cause. While it is known that bladder macrophages are important in bladder inflammation, or cystitis, resident ganglion macrophages within dorsal root ganglia (DRG) are rarely studied and their role in urologic pain is unknown. Our objective is to investigate whether cystitis changes ganglion macrophages. We hypothesized ganglion macrophages will be larger and more numerous after cystitis.

METHODS: We induced cystitis by instilling acrolein into the bladder via a transurethral catheter. We perfused the mice at 8 days, a time point that has not yet been examined since voiding dysfunction has resolved, but we find other cystitis phenotypes persist. We dissected T13- S1 DRGs from fixed spinal columns, sectioned and immunostained for IBA1 (macrophages) and DAPI (nuclei). Z-stacks were taken with a 3D Thunder microscope (Leica) and compressed in extended depth of field images. Analysis performed using NIS Elements. We looked at macrophage morphology, distribution, and IBA signal intensity. Statistical analyses included Mann-Whitney and Kolmogorov-Smirnov test (GraphPad Prism 9.4.1) and linear mixed effects model and Tukey-Kramer Multiple Comparisons test (SAS PROC MIXED version 9.4).

RESULTS: After cystitis, ganglion macrophages were smaller (area, p=0.0070). Regardless of treatment, L3 macrophages were larger than L5 macrophages (area, p=0.0092). In L5 and S1 (bladder innervating), macrophages that overlapped/touched neurons, termed peri-neuronal macrophages, overlapped with smaller neurons (area size) after cystitis (p<0.0001). This was not observed in L3 and L4 (non-bladder innervating) macrophages. L3 and L5 macrophages have higher signal intensity, suggesting they become more activated after cystitis (p<0.0001). Analysis of T13-L2 DRGs is ongoing.

CONCLUSIONS: Cystitis significantly decreased ganglion macrophages size. Peri-neuronal macrophages were more likely to be overlapping smaller neurons. IBA1 intensity analysis suggested that ganglion macrophage populations vary in response across DRGs. These results suggest ganglion macrophages do not return to normal by 8 days after induced cystitis.

HEALTH RELEVANCE: Long lasting changes in ganglion macrophages offering a possible novel mechanism (and therapeutic target) for persistent bladder dysfunction and pain.

CLINICAL CORRELATES AND PROGNOSTIC VALUE OF AGATSTON-BASED NEPHROCALCINOSIS QUANTIFICATION IN PRIMARY HYPEROXALURIA TYPE 1 (poster #76)

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INTRODUCTION AND OBJECTIVE: In primary hyperoxaluria type 1 (PH1), nephrocalcinosis is an independent risk factor for end-stage kidney disease (ESKD); however, a standardized quantitative imaging metric for assessing nephrocalcinosis has been lacking. In this study, we adapted a CT-derived Agatston framework to quantify renal nephrocalcinosis and evaluated its clinical correlates and prognostic value for ESKD among patients with PH1 participating in the Rare Kidney Stone Consortium (RKSC) PH Registry.

METHODS: Non-contrast renal CT scans were processed to generate Agatston scores (AS); reader adjudication was used to exclude renal stones. When available, ultrasound (US) cortical and medullary grades were recorded. The agreement between US and CT assessments was evaluated using Spearman correlation. Associations between longitudinal clinical variables and AS were estimated using linear mixed models. The prognostic value of AS and other covariates for ESKD was assessed with time-dependent Cox models.

RESULTS: A total of 78 patients with 244 CT follow-up scans and a median follow-up duration of 71.6 months (25th, 75th percentile: 3.3–165.9) were included in this study. AS was found to be a feasible and reproducible method for quantifying nephrocalcinosis. Total bilateral US grades showed a moderate correlation with AS (Spearman $r = 0.63$, $P=0.002$). AS was significantly associated with G170R mutation status, plasma oxalate levels, and age. In multivariable time-dependent Cox analyses, follow-up eGFR, plasma oxalate, and AS were independently linked to the risk of ESKD. A simplified ESKD risk prediction nomogram incorporating these three predictors demonstrated excellent discrimination (C-index 0.862; AUC ranged from 0.772 to 0.962).

CONCLUSIONS: Agatston-based quantification provides an objective, reproducible, and scalable method for assessing nephrocalcinosis that is independently associated with the risk of ESKD in PH1.

HEALTH RELEVANCE: These findings support the use of Agatston scoring in imaging follow-up, risk stratification, and the implementation of earlier, more individualized clinical management strategies.

DATA LINKAGE FROM THE PKIDS STUDY AND PCORNET COMMON DATA MODEL: A MODEL FOR EXPANDING DATA INFRASTRUCTURE IN CLINICAL RESEARCH (poster #50)

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INTRODUCTION AND OBJECTIVE: Collecting and analyzing multiple data sources can expand the impact of clinical trials and produce evidence to improve real world care. We sought to integrate data from the Pediatric Kidney Stone (PKIDS) clinical trial with patient-level linkage to the PCORnet common data model and 24-hour urinary chemistry data.

METHODS: The PKIDS Study was a prospective non-randomized clinical trial across 31 hospitals in the United States and Canada that enrolled 1290 patients aged 8-21 undergoing ureteroscopy, shockwave lithotripsy, and percutaneous nephrolithotomy from March 2020 to July 2023. Data collected included patient, stone, institution, and surgeon characteristics as well as stone clearance and patient-reported outcomes within 4 months of treatment. PCORnet is a research infrastructure of 8 clinical data research networks leveraging a common data model (CDM) using electronic health data. We linked patient-level data of participants in the PKIDS study with patient-level data from the PCORnet CDM, including diagnosis codes, health care visits, surgical interventions, lab orders and results, and medication prescriptions, as well as 24-hr urine chemistry data (Litholink, LabCorp).

RESULTS: Of the enrolled patients, 1222 were eligible for review as 30 and 32 withdrew from the study and did not receive surgery, respectively. For 14 sites, PKIDS-PCORnet data linkage was feasible for a total of 794 PKIDS participants. Cohort descriptions of the

linked, unlinked, and total PKIDS participants are shown in the [Table](#). Regarding the 24-hr urine chemistries, 541 (44.3%) PKIDS patients had at least one valid study available. The percentage of data missingness across urinary oxalate, creatinine, citrate, calcium, volume and supersaturations of calcium oxalate and phosphate ranged from 0% to 3.5%.

Table: Description of both linked and unlinked cohorts of PKIDS participants. IQR – interquartile range; PCNL – percutaneous nephrolithotomy; SWL – shockwave lithotripsy; URS – ureteroscopy

Characteristics		PKIDS Non PCORnet N=421	PKIDS PCORnet N=794	Total N=1222
Median age, (IQR) - year		15.4 (12.4, 17.2)	15.6 (12.5, 17.3)	15.6 (12.5, 17.3)
Age ranges, No. (%)	8-11 years	65 (15.7%)	114 (14.6%)	180 (15%)
	12-15 years	120 (29.0%)	229 (29.2%)	350 (29.1%)
	16-18 years	166 (40.1%)	318 (40.7%)	487 (40.5%)
	19-21 years	63 (15.2%)	120 (15.3%)	185 (15.4%)
Female biologic sex, No. (%)	Female	254 (60.3%)	470 (59.2%)	727 (59.4%)
Race, No. (%)	White	304 (72.2%)	622 (78.3%)	932 (76.3%)
	Black	12 (2.9%)	31 (3.9%)	43 (3.5%)
	Asian	6 (1.4%)	10 (1.3%)	16 (1.3%)
	More than 1 reported	27 (6.4%)	30 (3.8%)	57 (4.7%)
	Other	23 (5.5%)	21 (2.6%)	44 (3.6%)
	Missing/unknown	47 (11.2%)	79 (9.9%)	127 (10.3%)
Ethnicity, No. (%)	Hispanic	50 (11.9%)	98 (12.3%)	148 (12.1%)
	Non-Hispanic	285 (67.7%)	580 (73.1%)	871 (71.3%)
	Missing/unknown	50 (11.9%)	73 (9.1%)	124 (10.1%)
	Other	36 (8.6%)	43 (5.4%)	79 (6.5%)
Comorbid conditions, No. (%)	Neurogenic bladder	30 (7.1%)	73 (9.2%)	104 (8.5%)
	Ventilator-dependent	4 (1.0%)	13 (1.6%)	17 (1.4%)
	Neuromuscular disorder	55 (13.1%)	122 (15.3%)	179 (14.6%)
	Hematologic disorder	17 (4.0%)	42 (5.3%)	59 (4.8%)
	Oxygen support	7 (1.7%)	27 (3.4%)	35 (2.9%)
	Cardiac risk factors	15 (3.6%)	26 (3.3%)	41 (3.3%)
	Structural central nervous system abnormality	12 (2.9%)	23 (2.9%)	36 (2.9%)
	Developmental delay	45 (10.7%)	124 (15.6%)	170 (13.9%)
	Epilepsy	22 (5.2%)	67 (8.4%)	90 (7.4%)
Surgical Intervention, No. (%)	PCNL	29 (6.9%)	57 (7.1%)	87 (7.1%)
	SWL	85 (20.2%)	102 (12.8%)	187 (15.3%)
	URS	307 (72.9%)	636 (80.1%)	949 (77.7%)

CONCLUSION: Through PKIDS-PCORnet data linkage, 65% of participants had clinical data augmented beyond the study timeframe including laboratory values and health care utilization. Additionally, nearly half of patients had 24-hour urine studies available for augmented data. These data linkages will be foundational for secondary analyses including prediction models for progression of residual fragments following surgical intervention.

HEALTH RELEVANCE: Clinical trial data is typically limited to single use. Linkage of clinical trial data with data derived from clinical care

creates new opportunities for assessment of longer-term outcomes and ancillary real-world studies.

POST-OPERATIVE TRAJECTORIES OF PAIN INTERFERENCE AND INTENSITY AFTER URETEROSCOPY FOR NEPHROLITHIASIS IN YOUTH: A PKIDS STUDY (poster #77)

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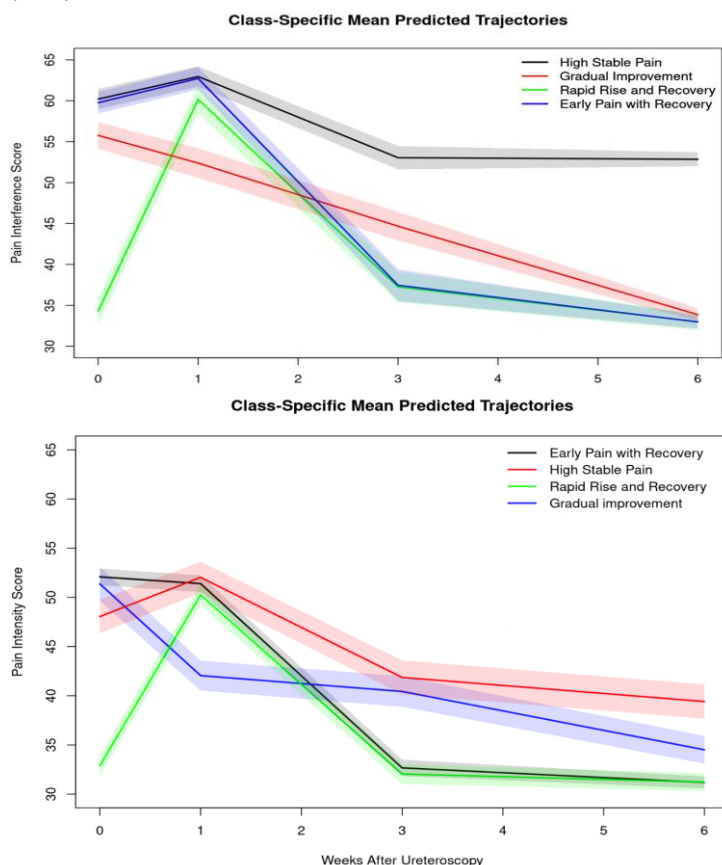
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INTRODUCTION AND OBJECTIVE: Youth with nephrolithiasis frequently undergo ureteroscopy to treat their stones, yet post-operative recovery courses are variable. We assessed the trajectories of pain interference and pain intensity following ureteroscopy and examined clinical predictors of trajectory group membership.

METHODS: Participants aged 8-21 years from 31 centers in North America who underwent ureteroscopy between 2020 and 2023 as part of a prospective observational study were included. Outcomes of post-operative pain interference and pain intensity were evaluated at baseline and at weeks 1, 3, and 6 using Patient-Reported Outcomes Measurement Information System questionnaires. Group-based trajectory models were used to identify distinct recovery patterns. Classification trees identified predictors of trajectory group membership.

RESULTS: Among 823 participants undergoing ureteroscopy (median age 15.6 years; 65% female), 667 had stents which were

Figure: Top: Recovery Trajectories of Pain Interference Following Ureteroscopy. A four-class model (class descriptor with corresponding class prevalence) was used to map pain interference change in patients: High Stable Pain (24.8%); Gradual Improvement (26.8%); Rapid Rise and Recovery (23.6%); Early Pain with Recovery (24.8%). **Bottom:** Recovery Trajectories of Pain Intensity. A four-class model (class descriptor with corresponding class prevalence) was used to map pain intensity change in patients: Early Pain with Recovery (33.8%); High Stable Pain (31.1%); Rapid Rise and Recovery (12.5%); Gradual Improvement (22.6%).



removed a median of 5 days post-surgery. Four distinct trajectory groups were identified for each outcome (see **Figure**). The groups included: High Stable Pain, Gradual Improvement, Rapid Rise and Recovery, and Early Pain with Recovery. For pain interference, the four groups were equally divided among the cohort. For pain intensity, nearly a third of the cohort were in each of the High Stable Pain and Early Pain with Recovery groups. For both pain interference and intensity, significant clinical predictors of group membership were age, baseline pain interference/intensity scores, baseline anxiety, and largest stone size.

CONCLUSIONS: We identified distinct recovery trajectories for pain interference and intensity after ureteroscopy with variable prevalences in our cohort. We also found significant clinical factors associated with group membership for pain interference and intensity. These findings highlight the heterogeneity in recovery and underscore the need for personalized pain management strategies.

HEALTH RELEVANCE: Ureteroscopy for treatment of kidney stones is often associated with high pain interference and intensity after surgery, usually due to an indwelling ureteral stent. In this study, we assessed different post-operative recovery trajectories for pain interference and intensity and their associated clinical factors, which moves towards personalizing outcomes for youth with kidney stones.

SPATIAL TRANSCRIPTOMIC MAPPING OF THE HUMAN BLADDER USING XENIUM TECHNOLOGY (poster #78)

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INTRODUCTION AND OBJECTIVE: Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic pain condition that severely affects quality of life, yet treatment remains challenging. Molecular diagnostic approaches have advanced mechanism-based therapies for other chronic pain disorders, but remain limited in IC/BPS. An avenue to improve targeted therapies is to better understand local cell-to-cell communication that occurs in the bladder, in particular, the communication between non-neuronal cells and neurons. To address this gap, we performed high-plex spatial transcriptomics using the Xenium Prime 5K Human Pan Tissue and Pathways Panel (10X Genomics, Pleasanton, CA), expanded with 100 custom genes relevant to bladder biology. Our objective was to comprehensively profile healthy human bladder tissues from male and female donors.

METHODS: Paraffin-embedded bladder tissue sections were mounted on separate Xenium slides at 5 µm thickness. Xenium Cell Segmentation was used to assign transcripts to individual cells. Immunofluorescence (IF) staining for PGP9.5 (ab302578, Abcam, Waltham, MA) was performed to investigate cell-to-cell interactions near nerve endings. The IF signal was imaged using a slide scanner (Evident Scientific, Waltham, MA) and images were converted to OME-TIFF using QuPath. Images were spatially aligned with Xenium transcript data and visualized using Xenium Explorer.

RESULTS: We identified diverse cell populations across the bladder tissue layers, including urothelium, lamina propria, and detrusor smooth muscle. Using clustering analysis, we identified 16 different cell types including smooth muscle cells, fibroblasts, mast cells, etc. Custom gene groups included markers for fibroblast function, purinergic receptors, TRPV channels, cholinergic signaling, and immune modulation. These targets provide new insights into cell-to-cell communication and neurogenic regulation within bladder physiology.

CONCLUSIONS: This study used the Xenium 5k panel expanded with 100 custom genes to generate a detailed spatial transcriptomic profile of healthy human bladder. Future studies will be conducted using this technology to expand our understanding of bladder pathophysiology using mouse models.

HEALTH RELEVANCE: This study offers valuable spatial insights into the human bladder and provides a translational foundation for basic science research.

NEURO-UROLOGICAL MECHANISMS UNDERLYING LOWER URINARY TRACT DYSFUNCTION IN AUTISM SPECTRUM (poster #79)

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INTRODUCTION AND OBJECTIVE: Lower Urinary Tract Symptoms (LUTS) are reported as a comorbidity in 77% of individuals with Autism Spectrum Disorder (ASD), yet treatment strategies remain limited. A lack of societal and behavioral awareness, along with alterations in neuronal connectivity within the central and/or peripheral nervous systems that regulate voiding, likely contribute to ASD-associated LUTS. However, the exact mechanisms remain unresolved. To begin addressing this gap, we investigate the severity of ASD and LUTS phenotypes in a mouse model of ASD. We then assess the potential of zinc supplementation as a therapeutic strategy for reducing ASD-associated LUTS.

METHODS: The Shank3B (B6.129-Shank3tm2Gfng/J) (Shank3B⁻) mouse model of ASD was used to assess ASD-related behaviors via the three-chamber social approach, repetitive grooming test, and olfactory habituation test. Voiding function was evaluated using the Void Spot Assay (VSA). Behavioral endpoints were correlated to determine whether LUTS severity aligns with ASD severity, mirroring patterns observed in human ASD. A separate cohort was generated from dams fed either low-zinc (30 ppm) or normal-zinc (60 ppm) chow, with offspring maintained on the same diet until six weeks of age. Voiding in six-week-old offspring was assessed using VSA, followed by anesthetized cystometry with tandem pelvic nerve recordings. Dorsal Root Ganglia (DRG) from these mice were cultured to assess morphology and electrical activity.

RESULTS: ASD behavioral assays were validated: wild-type (WT) mice exhibited the strongest social behaviors, heterozygous Shank3B⁻ mice showed intermediate behaviors, and homozygous Shank3B⁻ mice displayed the least social interaction, repetitive grooming, and odor discrimination. Sex differences were noted—male heterozygous Shank3B⁻ mice spent more time grooming than WT males, a pattern not observed in females. VSA results indicated that both heterozygous and homozygous Shank3B⁻ male and female mice produced fewer small urine spots compared to WT controls.

CONCLUSIONS: *Shank3* mutations result in altered social behavior, consistent with expectations. Heterozygous mice exhibit an intermediate phenotype that may be modulated by gene-environment interactions, such as zinc supplementation. Shank3B⁻ mice of both sexes produce fewer small urine spots than WT counterparts, suggesting altered voiding behavior.

HEALTH RELEVANCE: Understanding the pathophysiology of ASD and its comorbidities, such as LUTS, along with modifiable risk factors like micronutrient status, may inform improved treatment strategies.

PATERNAL TRANSGENERATIONAL EFFECTS OF PRENATAL ESTROGEN EXPOSURE INCREASES SUSCEPTIBILITY TO URINARY FREQUENCY IN MOUSE MODELS OF BENIGN PROSTATIC HYPERPLASIA (poster #80)

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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) as well as the 2+/3+ (p<0.01, 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, p<0.01) from F2 to F3 (Fig. 1).

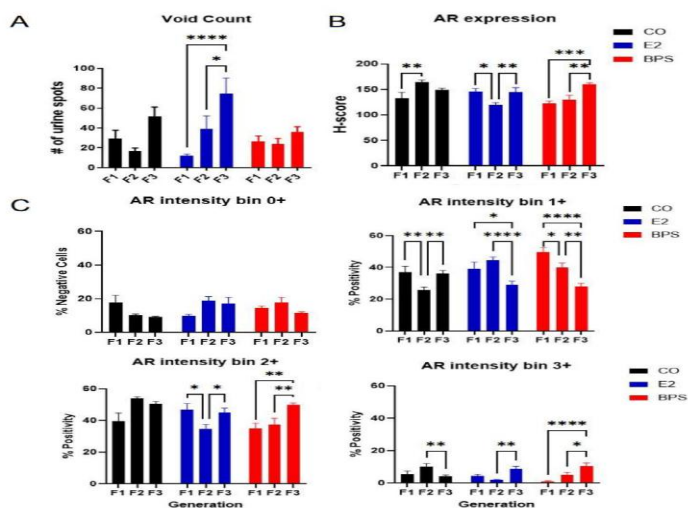


Figure 1. Paternal transgenerational impact of BPS and E2 on urinary frequency and AR expression in three generations of CD-1 mice. Urinary frequency was measured via void spot assays (VSAs) on the fourth week of T+E2 treatment (A). AR expression in the anterior prostate measured by H-score (B). AR expression 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.

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. Future studies will investigate the molecular mechanism(s) of AR regulation and their downstream targets/consequences.

HEALTH RELEVANCE: This work identifies putative epigenetic changes involved in the development of BPH/LUTS. Furthermore, it provides evidence that the manifestation of BPH is influenced by environmental factors that adversely affect healthcare and well-being.

PCB EXPOSURE AFFECTS URETHRA CONTRACTILITY IN A DEVELOPMENTAL EXPOSURE MODEL (poster #81)

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INTRODUCTION AND OBJECTIVE: Polychlorinated biphenyls (PCBs) are contaminants which permeate our environment and bodies and are known to have various adverse health effects. PCB-induced changes to the lower urinary tract are of interest due to evidence that PCB exposure during uterine development and throughout lactation induces lower urinary tract dysfunction (LUTD) in mouse models. While the bladder mechanics of LUTD are commonly examined, the role of urethral function in these phenotypes is underexplored. Urethral muscle contractile properties contribute to continence and have been shown to play a role in urinary tract infection risk. Here we sought to test whether in utero and lactational exposure to PCBs alters the contractile function or morphology of the urethra.

METHODS: Urethral smooth muscle contractility was assessed *ex vivo* using wire myography. Test animals were 6-week-old male and female offspring were exposed to 0, 0.1, 1 or 6 mg/kg/day to an environmentally relevant PCB mixture in utero and during lactation. Ring sections of the proximal urethra were prepared in an *ex vivo* bath assay and contractile strength tested in the presence of a muscle interstitial cell agonist (serotonin) and a smooth muscle alpha-adrenergic agonist (phenylephrine) and normalized to KCl responses. Responses were analyzed with one- or two-way ANOVA followed by multiple comparisons tests for PCB exposure group effects compared to the sex-matched vehicle controls. Proximal urethras were also collected for structure analysis using immunohistochemistry and RNA quantification using qPCR.

RESULTS: Male mice exposed to 0.1 mg/kg/day PCB showed significantly lower contractile strength in response to interstitial cell contractile agonist serotonin compared to vehicle control ($n=5$, $p<0.0002$), and females are trending similarly ($n=5$, $p<0.4$). PCB exposure did not impact urethral contractile strength when directly stimulating alpha-adrenergic receptors with phenylephrine. We also determined that there is no PCB effect on the width of the female urethra muscle, stroma, lumen, epithelium, and overall structure width ($n=4$, $p>0.5$), suggesting that the contractile differences are not due to structural changes in urethra tissue composition in females. Male urethra structure analysis is ongoing, as well as qPCR analysis of serotonin pathway related receptors in both sexes.

CONCLUSIONS: These results suggest that voiding dysfunction induced by developmental PCB exposure may arise from not only previously observed changes in bladder contractility but may also extend to changes in urethra contractility.

HEALTH RELEVANCE: LUTD afflicts a large proportion of the population, and the urethra is not well understood. Altered urethral activity may explain some symptoms of lower urinary tract dysfunction in humans, which may be an effect of environmental chemical exposure.

CALCIUM IMAGING REVEALS CYSTITIS-INDUCED INCREASES IN NEUROEXCITABILITY OF INTACT LUMBAR SENSORY GANGLIA (poster #82)

Emily L. Tran¹, Sara A. Stuedemann¹, Qianqian Zhao², and LaTasha K. Crawford¹

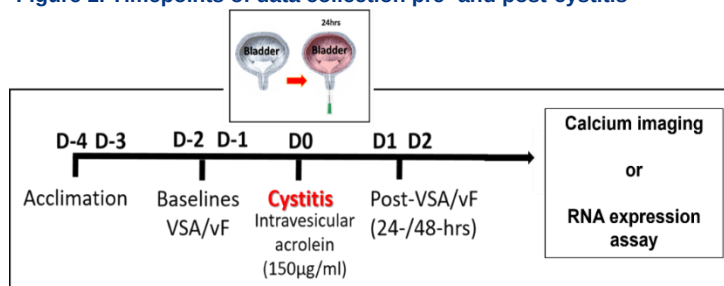
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INTRODUCTION AND OBJECTIVE: It is unclear why urologic pain conditions like interstitial bladder inflammation, termed cystitis, are accompanied by somatic pain, including sensory neuropathy in lower limb skin. Notably, similar referred pain in hind paw skin is found in animal models of cystitis. Sensory ganglia are sites of communication between sensory neurons and potentially visceros-somatic cross-talk. Using *ex vivo* calcium imaging of intact sensory ganglia, we tested the hypothesis that cystitis increases neuroexcitability, including neurons that do not innervate the bladder.

METHODS: T13-L6 DRG of C57BL/6J mice were examined after 0.1% Cholera Toxin-B with Alexa Fluor™ 488 Conjugate injections in

bladders and 0.5% Fast Blue injections in hind paws. Cystitis induction and experimental design is detailed in **Figure 1**. *Ex vivo* calcium imaging was conducted on whole L3-L6 sensory ganglia responding to 30mM KCl. T-stacks (videos) were acquired on an upright microscope (Leica Microsystems). Fluorescence intensity was quantified using NIS Elements-AR Software with NIS.ai module (Nikon). Statistical analysis of response amplitudes were analyzed with linear Mixed-effect model using SAS PROC MIXED. A separate set of experiments utilized nCounter technology (Bruker) to explore patterns of neuroinflammatory gene expression.

Figure 1. Timepoints of data collection pre- and post-cystitis



RESULTS: Dual retrograde tracer studies in mice confirmed bladder and hind paw neurons can be co-housed in L3, L5-L6 sensory ganglia, providing ample opportunity for cross-talk. In separate experiments, hind paw hypersensitivity was demonstrated in mice after cystitis. Calcium imaging revealed cystitis induced neuronal hyperexcitability in bladder-innervating L5-L6 ganglia. Notably, cystitis caused even greater hyperexcitability in paw-innervating L3 ganglia. A screen of RNA expression confirmed that, in addition to L5 and L6 ganglia, L3 are also altered after cystitis.

CONCLUSIONS: Cystitis increases neuron excitability of bladder- and paw-innervating DRG most when both populations are present. Patterns of neuroinflammatory gene expression provided guidance for future studies on the molecular underpinnings of cystitis-induced neuronal hyperexcitability. In particular, L3 ganglia demonstrate distinct changes after cystitis.

HEALTH RELEVANCE: Hyperexcitability of sensory neurons is known to drive pain sensed in innervated tissues. Our findings may represent a novel mechanism of referred pain after cystitis that lies outside of the central nervous system.

INVESTIGATING THE LINK BETWEEN ENVIRONMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBS) AND DEVELOPMENT OF ACQUIRED URINARY INCONTINENCE IN DOGS (poster #83)

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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. USMI risk factors include genetic predisposition, loss of hormones after removal of ovaries, increasing age, tail docking, adiposity, and environmental exposure. Polychlorinated biphenyls (PCBs) are environmental pollutants with neurotoxic and endocrine-disrupting effects. Previous data reported UI-like signs in adult mice exposed to PCBs during development. We hypothesized that dogs with USMI would have a higher serum concentration of PCBs than dogs without urinary incontinence.

METHODS: We used biobanked serum samples from the Golden Retriever Lifetime Study, a longitudinal health study conducted by the

Morris Animal Foundation. The cases were female dogs with a diagnosis of USMI (n=8). Serum samples were from the year of diagnosis. Cases were matched with controls of the same breed, age, sex, sterilization status, geographic location, and sample collection year (n=8). All 209 PCB congeners were measured by gas chromatography-tandem mass spectrometry (GC-MS/MS). Because several PCB concentrations were lower than the method of detection limit, data were analyzed using a two-sample t-test comparing the sum of detected PCB congeners in each group (cases vs controls). We analyzed total PCBs, and subsets according to toxic activity (estrogenic/anti-estrogenic, ryanodine receptor, dioxin-like/non-dioxin-like). We also analyzed the MARBLES mixture reported to produce an incontinent phenotype in female mice.

RESULTS: Detection frequencies (DF) and PCB concentrations are found in **Table 1**. There were no statistically significant differences between case and control groups (**Table 2**).

Table 1. PCB concentrations (ng/g) and detection frequencies (DF) between cases and controls

PCBs	Cases (n=8)		Controls (n=8)	
	Mean ± SEM	DF	Mean ± SEM	DF
Total	0.040 ± 0.019	75%	0.063 ± 0.021	100%
Estrogenic	0.006 ± 0.003	37.5%	0.024 ± 0.011	75%
Anti-estrogenic	0.011 ± 0.005	62.5%	0.015 ± 0.004	100%
Dioxin like	0.001 ± 0.001	12.5%	0.002 ± 0.002	25%
Non-dioxin like	0.039 ± 0.019	75%	0.062 ± 0.020	100%
Ryanodine Receptor	0.016 ± 0.007	37.5%	0.036 ± 0.013	75%
MARBLES mixture	0.012 ± 0.005	75%	0.030 ± 0.011	100%

Mean ± SEM (Standard Error of the Mean) were calculated based on data above method of detection limit. DF is the number of dogs with at least one PCB detected from the category divided by all dogs in the group (# dogs with PCBs detected/8).

Table 2. Results of T-test for sum of PCB congeners detected between cases and controls

PCBs	Cases (n=8)	Controls (n=8)	P-value
Total	4.38 ± 1.69	5.50 ± 1.27	0.60
Estrogenic	0.88 ± 0.48	1.63 ± 0.56	0.33
Anti-estrogenic	1.13 ± 0.48	1.75 ± 0.31	0.29
Dioxin like	0.25 ± 0.25	0.13 ± 0.13	0.66
Non-dioxin like	4.13 ± 1.61	5.38 ± 1.21	0.54
Ryanodine Receptor	1.75 ± 0.67	2.88 ± 0.64	0.25
MARBLES mixture	1.25 ± 0.45	2.38 ± 0.53	0.13

Data is presented as mean ± SEM (Standard Error of the Mean).

CONCLUSIONS: Our study did not find evidence that serum PCB concentrations relate to acquired UI in dogs. Small sample size, sample type, age, diet, and location of animals may have contributed to this finding. More research is needed.

HEALTH RELEVANCE: PCB exposure may impact a variety of health conditions in dogs and humans. Because dogs share environments with their human owners, they are a valuable model for environmental exposure research.

AGE-STRATIFIED CLINICAL PRESENTATIONS OF CHILDREN AND ADOLESCENTS EVALUATED FOR POSSIBLE URINARY TRACT INFECTION (poster #84)

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INTRODUCTION AND OBJECTIVE: Initial pediatric UTI diagnoses are less accurate for older children, even though they report urinary tract symptoms more reliably. Our objective was to assess the extent of urine testing performed based on localizing urinary tract symptoms across age groups.

METHODS: This is a cross-sectional study of visits to 3 emergency departments and 1 urgent care clinic from January 1, 2022-December 31, 2023. Using electronic health records, we selected index visits by patients 2 months-17 years old who had a urinalysis performed and were discharged home. We excluded patients with prior urologic surgery, immunodeficiency and other comorbidities, and patients receiving antibiotics before testing. We abstracted symptoms/signs using definitions based on Unified Medical Language System concepts. Age categories were pre-defined based on developmental stages. We assessed associations between age and any reported urinary tract symptoms (vs. none) using the Cochran-Armitage test for trend, then chi-squared tests for pairwise comparisons between adjacent age groups.

RESULTS: Of 1150 visits, there were 878 (76%) by >2 year-old children and 748 (65%) by female children. Age-stratified symptoms/signs are shown in **Table 1**. There was a trend in the proportion with urinary tract symptoms by age group (Cochran-Armitage test, p<0.001). Compared to 2 month-<2 year old children, more 2-<6 year old children had urinary tract symptoms (8.5% vs. 48%, p<0.001). There was no further increase in the proportion with urinary tract symptoms between 2-<6 year old and 6-<12 year old groups (48% vs. 48%, p=0.88) or between 6-<12 year old and 12-17 year old groups (48% vs. 50%, p=0.60). Abdominal pain was the predominant symptom in the two oldest age groups.

Table 1. Symptoms and signs at emergency or urgent care visits with urine testing performed, by pediatric age group

Documented symptom or sign n (%) of visits	Age group			
	2 months-<2 years n=272	2-<6 years n=278	6-<12 years n=286	12-17 years n=314
Any urinary tract symptom ^a	23 (8.5%)	134 (48%)	136 (48%)	156 (50%)
Specific urinary tract symptoms				
Dysuria	9 (3.3%)	106 (38%)	89 (31%)	65 (21%)
Incontinence	0	11 (4.0%)	9 (3.2%)	1 (0.3%)
Suprapubic pain/tenderness	1 (0.4%)	15 (5.4%)	41 (14%)	78 (25%)
Urgency	0	11 (4.0%)	8 (2.8%)	19 (6.1%)
Frequency	3 (1.1%)	32 (12%)	21 (7.3%)	31 (9.9%)
Hesitancy	2 (0.7%)	5 (1.8%)	7 (2.5%)	14 (5.5%)
Foul smelling urine	9 (0.4%)	13 (4.7%)	7 (2.5%)	2 (0.6%)
Hematuria	1 (0.4%)	4 (1.4%)	13 (4.6%)	13 (4.1%)
Flank pain/tenderness	0	0	4 (1.4%)	42 (13%)
Other symptoms/signs				
Fever ^b	254 (93%)	155 (56%)	107 (37%)	43 (14%)
Back pain ^c	1 (1%)	8 (2.9%)	19 (6.6%)	27 (8.6%)
Abdominal pain/tenderness ^d	13 (4.8%)	119 (43%)	181 (63%)	183 (58%)
Vomiting	94 (35%)	106 (38%)	97 (34%)	88 (28%)

^aAny urinary tract symptom: one or more of the specific urinary tract symptoms listed below was documented.
^bFever: either documented symptom in visit history or measured at visit (temperature ≥ 100.4°F).
^cBack pain: if described as generalized or affecting location other than flank (flank pain/tenderness was considered a urinary tract symptom).
^dAbdominal pain: if described as generalized or affecting other location other than suprapubic area (suprapubic pain/tenderness was considered a urinary tract symptom).

CONCLUSIONS: Urine testing was more often directed based on localizing symptoms in children outside of infancy. However, we did not find a progressive increase in evaluation based on localizing symptoms across older age groups. Even when urine tests are performed for other indications, these testing patterns may lead to more incidental positive screening results given lower prevalence of UTI in older children.

HEALTH RELEVANCE: Decisions about UTI evaluation impact care and outcomes for many pediatric acute undifferentiated illnesses. Our findings highlight a need to better support these decisions for older children and adolescents, as they comprise the majority of those evaluated and many are evaluated for symptoms that overlap with other serious conditions.

KIDNEY STONE FATIGUE CHARACTERIZATION FOR OPTIMAL LASER LITHOTRIPSY SETTINGS (poster #85)

Grant West¹, Pei Zhong¹, Michael Lipkin², John Dolbow¹

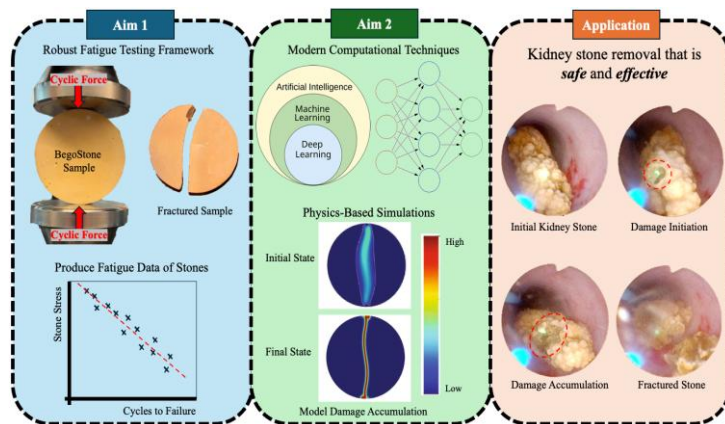
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INTRODUCTION AND OBJECTIVE: The cases of urinary stone disease (USD) are increasing due to different factors, including rising obesity rates and occupational stress. Laser lithotripsy, the most prevalent stone intervention, utilizes pulsating lasers to ablate kidney stones. Modern laser advancements offer a wide range of adjustable parameters—an opportunity to optimize treatments for each patient, but also a source of uncertainty that can lead to thermal injury and ureteral stricture. To truly optimize laser settings, a physics-based understanding of stone failure during laser exposure is required. However, without reliable experimental data to describe how stones

fracture and fatigue under cyclic loading, such modeling remains incomplete. The study's objective is to develop a robust method to characterize the fracture and cyclic fatigue strengths of kidney stones. Findings from this initial, ongoing work will inform later aims, developing analytic models that predict damage initiation and growth during procedures.

METHODS: Due to kidney stone scarcity, Begostone – a material with similar mechanical properties – will be used to develop the testing methods. Both materials are brittle and difficult to grip; therefore, standard uniaxial tension tests do not accurately capture their behavior. To address this, the study adapts the standardized Brazilian tensile test, which avoids the need for sample gripping. After converging on a robust method for measuring the fracture and fatigue strengths, real kidney stones will be mechanically characterized using the testing framework.

RESULTS: This work aims to provide a Begostone fracture strength dataset using various modes of force application. Later, the cyclic testing will provide an empirical relationship between cyclic force amplitudes and the number of cycles to failure in Begostone. Finally, fracture and fatigue strength datasets will be generated for various kidney stone types.



CONCLUSIONS: The study establishes robust methods for measuring tensile and fatigue properties in brittle materials. These datasets inform future analytic and computational models predicting damage nucleation and growth. Clinically, these models can be implemented as software guiding lithotripsy procedures.

HEALTH RELEVANCE: This project aims to provide a mechanistic basis for kidney stone fatigue failures induced via laser lithotripsy. In practice, this reduces thermal injuries and strictures while improving operating room efficiency.

INVESTIGATING THE USE OF OXYGEN CONCENTRATORS AS AN ALTERNATIVE OXYGEN SUPPLY IN MYOGRAPHY EXPERIMENTS (poster #86)

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INTRODUCTION & OBJECTIVE: Myography experiments assess the function and reactivity of tissues *ex vivo* by measuring isometric tension and isobaric diameter. Commercially available oxygen cylinders (tanks) contain a mixture of 95% O₂ and 5% CO₂ and remain the gold standard for tissue bath experiments. Tanks are often expensive to refill and require specific regulators. Oxygen concentrators (O₂C) filter oxygen from atmospheric air to produce 90-95% O₂. We hypothesize that O₂C may be a secondary, cost-effective alternative to aerate tissue baths in myography experiments.

METHODS: Aorta, mesenteric arteries, penis, and bladder were dissected from adult male Wistar rats (500-700 g; n=3). Aorta and mesenteric arteries were cut into ~2-3 mm rings, and penis and

bladder tissues were cut into strips and mounted (in Krebs) onto multi-myography systems. All tissues were run in duplicate from the same rat under both O₂ conditions, except for the penis. We assessed contraction to potassium chloride (KCl; 120mM), phenylephrine (PE; 10⁻⁹-3x10⁻⁵M) or acetylcholine (ACh; 10⁻⁹-3x10⁻⁵M, bladder). We precontracted vascular tissues with PE (10⁻⁶M) and added increasing concentrations (10⁻⁹-3x10⁻⁵M) of ACh or sodium nitroprusside (SNP), to measure endothelial-dependent and independent responses. Electric field stimulation (EFS; 1-64 Hz; n=2) assessed nerve-mediated contractions in the bladder and penis. Data were analyzed using Student's t-test and RM Two-Way ANOVA with multiple comparisons test.

RESULTS: There was no difference in maximal contraction to KCl in the aorta, mesentery, penis, and bladder under both O₂ conditions. With O₂C, aortic rings had a slight increase in contraction to PE (3x10⁻⁷ and 3x10⁻⁶M, p<0.05), whereas mesenteric arteries and penis responses to PE were unchanged. In the bladder, ACh-induced contractions were greater in baths aerated by the tank vs O₂C at the highest concentrations tested (p<0.05). In the penis and bladder, EFS-induced contraction was similar at all frequencies except at 64 Hz, which was higher in the penis with O₂C (p<0.05). Finally, relaxation to both ACh and SNP was unchanged in the aorta, mesentery and penis under both O₂ conditions.

CONCLUSIONS: We investigated the use of an O₂C in myography and suggest that an O₂C may serve as a suitable alternative to tank-derived oxygen. These results will be confirmed in a larger cohort of animals and the impact on pH or reactive oxygen species generation will be determined.

HEALTH RELEVANCE: To identify cellular and molecular mechanisms of disease, myography assesses vascular and muscular reactivity to pharmacological agents. The accuracy of a myography experiment is highly dependent on a constant flow of oxygen to mimic *in vivo* conditions.

BURST-PATTERNED SACRAL NERVE STIMULATION FOR BOWEL FUNCTION IN THE SPINAL CORD INJURED RAT (poster #87)

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INTRODUCTION: Spinal cord injury has body-wide consequences, and 80% of individuals living with SCI exhibit fecal incontinence or chronic constipation due to neurogenic bowel dysfunction. Although sacral nerve stimulation (SNS) applied with standard clinical parameters (continual stimulation at low frequency and amplitude) can treat fecal incontinence, it is less successful in chronic constipation. In preclinical studies examining non-neurogenic chronic constipation, burst-patterned SNS decreased colon transit times through generation of coordinated contractions, and this approach may also have utility in cases of SCI-induced constipation.

METHODS: Male and female Sprague Dawley rats (n=6, n=5) underwent moderate thoracic spinal cord contusions. At four-weeks post-injury, SNS was performed along with anorectal manometry to determine the impact of SNS pattern on rectal tone and function. Three SNS patterns were tested during 5-minute periods; 14 Hz continuous stimulation, 18 Hz stimulation for 30 seconds with 80 second inter-burst intervals, and 20 Hz stimulation for 40 seconds with 20 second inter-burst intervals. Each pattern was tested both with and without rectal distention and three within-subject replications were performed. Pressure recordings were taken at 4, 5, and 6 cm from the anal verge in each animal and were analyzed for the number of unique contractions, the number of propagating contractions, duration of contractions, area under the curve (AUC), and the maximum contractile amplitude.

RESULTS: A Friedman rank-sum test revealed that SNS had a significant effect on the number of unique contractions (p=0.029), and periods without stimulation exhibited a greater number of

contractions (9.83 ± 6.69) compared to periods with 18 Hz burst stimulation (6.44 ± 4.52). AUC and maximum contractile amplitude decreased with SNS. During periods with rectal distention, SNS did not have a statistically significant effect on outcomes, however, there was a notable decrease in the number of unique contractions during 18 Hz burst stimulation (12.50 ± 6.84 to 9.77 ± 5.70), but an increase in propagating contractions (0.22 ± 0.29 to 1 ± 2.48).

CONCLUSIONS: SNS applied in a burst-pattern may not help with chronic constipation in cases of SCI, however, it may provide alternate stimulation parameters that can be used to help with fecal incontinence.

HEALTH RELEVANCE: Spinal cord injury and other neurodegenerative diseases that impact bowel function are quite heterogeneous. Establishing alternative stimulation paradigms for SNS may increase the efficacy of this intervention and thus improve bowel function and overall quality of life.

GLYCOPROTEOME ANALYSIS OF BENIGN PROSTATIC HYPERPLASIA (poster #88)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a common age-related condition and often leads to lower urinary tract symptoms and reduced quality of life in men. Although extensively studied, its molecular mechanisms remain unclear. Protein glycosylation regulates extracellular matrix (ECM) organization and signaling; however, global glycoproteomic alterations in BPH are largely unexplored. This study aimed to perform glycoproteome analysis of human BPH tissue to elucidate molecular pathways and glycoprotein alterations using quantitative mass spectrometry.

METHODS: Prostate tissues from healthy donors and patients with BPH were homogenized. Proteins were digested into peptides, and DiLeu-labeled glycopeptides were enriched using hydroxyethyl-HILIC SPE tips. Samples were analyzed using an Orbitrap Fusion Lumos mass spectrometer coupled to a Dionex Ultimate 3000 UPLC system. Raw data files were processed and searched using Proteome Discoverer.

RESULTS: A total of 1180 N-glycopeptides from 227 N-glycoproteins were identified in human prostate tissues. Hierarchical clustering revealed distinct glycoform expression patterns between BPH and control samples. Functional network analysis linked the altered glycoproteins to stromal expansion and fibrosis, consistent with BPH pathology. Notably, a glycoform of protein O60568 was significantly downregulated, indicating altered glycosylation regulation. The glycan-protein network showed balanced distributions of sialylation, fucosylation, and high-mannose structures, whereas complex/hybrid species were less frequent, highlighting broad glycosylation remodeling in BPH.

CONCLUSIONS: These results reveal widespread alterations in protein N-glycosylation in BPH. The findings underscore glycosylation remodeling as a key molecular feature of BPH pathogenesis and highlight specific glycoforms as potential biomarkers for further investigation.

HEALTH RELEVANCE: This study holds promise for identifying novel biomarkers and therapeutic targets for BPH.

DELAYED OR FORGONE CARE FOR URINARY TRACT INFECTION SYMPTOMS AMONG EMERGING ADULT WOMEN (poster #51)

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INTRODUCTION AND OBJECTIVE: Despite the high prevalence of urinary tract infections (UTIs) among young women, limited research has explored care-seeking behavior, including whether and why some individuals delay or do not seek care for UTI symptoms. This study examined delayed or forgone care for UTI symptoms among emerging adults aged 18–29, analyzing differences by sociodemographic characteristics and symptoms.

METHODS: We analyzed survey data collected May 2024–March 2025 in a supplementary study to a cluster randomized trial in California and Texas. The analytic sample included 381 women aged 19–29 who reported experiencing typical symptoms of uncomplicated UTI within the past year (urgency, frequency, dysuria, or hematuria). The outcome variable, based on self-reported care-seeking for UTI symptoms, included three categories: (1) delayed or forgone care due to perceived barriers, (2) delayed or forgone care due to perceived lack of need, and (3) received care without delay. We used mixed-effects multinomial logistic regression for clustered data to examine predictors of delayed or forgone care.

RESULTS: The average participant age was 22.6 years, 56% identified as Hispanic, 21% were uninsured, and 33% lacked a usual source of care. Overall, 39% reported delaying or forgoing medical care for UTI symptoms due to access barriers, and 41% did so due to lack of perceived need for care. In adjusted analyses, younger age (RRR: 0.77, 95% CI: 0.65–0.91) and lack of a usual source of care (RRR: 1.99, 95% CI: 1.04–3.82) were associated with higher relative risk of delayed or forgone care due to access barriers. Younger age also was associated with higher relative risk of delayed or forgone care due to lack of need (RRR: 0.82, 95% CI: 0.69–0.97). Reporting hematuria (RRR: 0.33, 95% CI: 0.14–0.82) was linked to lower relative risk of delayed or forgone care due to access barriers, and hematuria (RRR: 0.26, 95% CI: 0.09–0.71) and dysuria (RRR: 0.26, 95% CI: 0.14–0.47) were linked to lower risk of delayed or forgone care due to lack of need.

CONCLUSIONS: Delayed or forgone care for UTI symptoms was common among emerging adult women, driven by both access barriers and lack of perceived need. Younger age and absence of a usual source of care were key predictors of delayed or forgone care due to access barriers, while experiencing hematuria or dysuria, symptoms that may be more distressing, was associated with seeking care promptly. Efforts to improve UTI care access should address structural barriers and knowledge of when care is warranted.

HEALTH RELEVANCE: Both access and symptom perception influence delaying or forgoing UTI care. Interventions addressing barriers and promoting awareness of when medical evaluation is needed could reduce care delays and prevent UTI complications.

TELEHEALTH VS. IN-PERSON VISITS: DIAGNOSTIC PRACTICES FOR URINARY TRACT INFECTIONS IN EMERGING ADULT WOMEN (poster #89)

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) and sexually transmitted infections (STIs) are common among emerging adult women (ages 18–29) and often present with overlapping symptoms, making accurate diagnosis challenging. With the expansion of telehealth, diagnostic practices for urinary symptoms may differ from those used in in-person care. This study compared diagnostic practices for UTI among emerging adult women receiving telehealth vs. in-person care.

METHODS: We analyzed survey data collected between May 2024 and March 2025 as part of a supplementary study to a cluster randomized trial in community colleges in California and Texas. The analytic sample included 193 women aged 19–29 who had been diagnosed with a UTI in the past year. Binary outcomes captured whether a urine test or STI test was ordered and whether a healthcare professional asked about sexual history. We used mixed-effects logistic regression for clustered data to examine differences in diagnostic practices by care modality (telehealth vs. in-person), as well as by sociodemographic characteristics (age, race and ethnicity, insurance status, usual source of care), sexual risk factors (new sexual partner, multiple partners), presence of abnormal vaginal discharge, and history of UTI and STI.

RESULTS: Participants' mean age was 22.8 years; 57% identified as Hispanic, 73% reported a history of UTI, and 24% a history of STI. Overall, 18% received UTI care via telehealth. At diagnosis, 84% reported urine testing, 55% STI testing, and 68% sexual health history-taking. Among all covariates examined, only the care modality was significantly associated with whether urine testing was ordered (predicted probability: 28.6% telehealth vs. 96.2% in-person, $p < 0.001$). In multivariate regression, telehealth visits were associated with significantly lower predicted probabilities of sexual health history-taking (42.8% vs. 73.5%, $p < 0.001$) and STI testing (29.1% vs. 61.3%, $p < 0.001$). Younger age was also predictive of sexual health history-taking ($p < 0.05$) and STI testing ($p < 0.05$).

CONCLUSIONS: Diagnostic practices for UTI differ by care modality. Lower rates of sexual health history-taking and STI testing during telehealth visits suggest a potential risk of missed STI diagnoses among young women receiving UTI care virtually.

HEALTH RELEVANCE: Young women receiving telehealth UTI care are less likely to have sexual health history assessment or STI screening. These gaps warrant further attention, as accurate diagnosis of UTIs and STIs is critical for reducing individual harms and addressing major public health challenges, including the STI epidemic disproportionately impacting emerging adults and rising antimicrobial resistance.

RARE VARIANT ASSOCIATION STUDY IDENTIFIES SLC34A3 AS THE TOP HIT GENE FOR KIDNEY STONES (poster #90)

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INTRODUCTION AND OBJECTIVE: Kidney stone disease (USD) is a common, debilitating, and recurrent disorder affecting one in every eleven individuals in the U.S. population. Although more than 40 monogenic causes have been identified, much of the heritable risk remains unexplained.

METHODS: To further understand genetic contribution, we performed a rare-variant association study (RVAS) using sequencing data from 5,998 USD cases and 37,745 controls of European ancestry in the Mayo Clinic Biobank (MCBB). Variants were aggregated into masks based on predicted pathogenicity and allele frequency, and gene-based tests were conducted with REGENIE, adjusting for age, sex, and ancestry. The exome-wide significance threshold was set at $P < 2.6 \times 10^{-6}$.

RESULTS: Among 18,894 genes tested, SLC34A3, which encodes the type IIc sodium phosphate cotransporter (NaPi-IIc), was the top

hit with $-\log_{10}P = 6.213$ ($P \approx 6.1 \times 10^{-7}$); ACAT (Aggregated Cauchy Association Test) $-\log_{10}P = 6.655$ ($P \approx 2.2 \times 10^{-7}$), exceeding the exome-wide threshold. The signal was driven primarily by rare loss of function and pathogenic missense variants, with strongest evidence under loss of function enriched masks (Mask 1). Sex stratified analyses demonstrated that the association was most prominent in males ($-\log_{10}P = 5.018$) while more modest in females ($-\log_{10}P = 1.903$).

CONCLUSIONS: This study identifies SLC34A3 as the strongest gene level association in our RVAS study. Our findings extend the role of SLC34A3 beyond recessive hereditary hypophosphatemic rickets with hypercalciuria (HHRH), highlighting its broader contribution to stone formation in adults and emphasizing phosphate metabolism in kidney stone pathogenesis. These results support SLC34A3 testing as a potential tool for precision diagnosis and targeted management of recurrent stone formers.

HEALTH RELEVANCE: Identifying the genetic risks for the development of kidney stones will help anticipate and mitigate the health, economic, and social burdens associated with the condition.

IDENTIFICATION OF G QUADRUPLEX AS A BINDING SITE OF DEAD-BOX HELICASE 3 ON ANDROGEN RECEPTOR MRNA (poster #91)

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INTRODUCTION AND OBJECTIVE: The androgen receptor (AR) is a key transcription factor that regulates prostate development, homeostasis, and disease progression. Aberrant AR expression and signaling are hallmarks of prostatic disorders, including benign prostatic hyperplasia (BPH)/lower urinary tract dysfunctions (LUTS) and prostate cancer. Previous studies have shown that prostatic AR declines with age, prostate cancer progression, and 5-alpha-reductase inhibitor resistance in BPH. It has been reported that an RNA-binding protein, DEAD-box helicase 3 (DDX3), translationally regulates AR protein expression, which might be a mechanism leading to AR protein reduction during aging. Others have demonstrated that DDX3 interacts with transcripts via RNA G quadruplex (rG4) structure, providing a potential mechanism for DDX3:AR mRNA binding. We hypothesize that DDX3 may recognize rG4 sequences within AR mRNA. In this study, we investigate whether rG4s within AR mRNA are present and test their ability to interact with DDX3 directly.

METHODS: The rG4-forming sequences were predicted using a web server, and the top three hits were synthesized *in vitro*. Their ability to form rG4 was examined through the thioflavin T fluorescence assay. The DDX3 protein was purified from HEK 293T cells using plasmid transfection and HA-tag purification. The binding patterns between DDX3 and putative rG4-forming sequences were determined by electrophoretic mobility shift assay (EMSA).

RESULTS: Seven rG4-forming sequences were predicted within AR mRNA. The three synthesized RNA oligos adopt rG4 structures *in vitro*. EMSA suggested direct binding between the synthesized rG4-forming sequences and purified DDX3 protein *in vitro*.

CONCLUSION: AR mRNA harbors rG4-forming sequences that are capable of direct binding to DDX3 *in vitro*.

HEALTH RELEVANCE: Our discovery of a DDX3:AR mRNA molecular mechanism of decreasing AR protein expression provides a means to alter AR function in the prostate. Pharmacological

inhibition of DDX3 is possible and may offer a therapeutic method to reverse the decreases in AR protein observed during aging/disease process, potentially helping to alleviate LUTS and/or treat prostate cancer.

THE PHOSPHODIESTERASE-5 INHIBITOR TADALAFIL BLOCKS SEROTONIN-MEDIATED CONTRACTIONS IN MALE MICE URETHRA (poster #92)

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INTRODUCTION AND OBJECTIVE: Lower Urinary Tract Symptoms (LUTS) commonly occur in aging men, reducing quality of life. LUTS is often treated with alpha-adrenergic blockers to reduce prostatic and urethral tone, but this approach is not always effective. Phosphodiesterase-5 inhibitors are sometimes used with alpha blockers to treat LUTS, but which men are more likely to benefit from this combination therapy is unclear. In female mice urethra, Serotonin (5-HT) causes smooth muscle contractions by a mechanism that does not rely on neuronal activity. Our preliminary data support the notion that 5-HT also contracts the male urethra by a mechanism that is resistant to alpha blockers. The purpose of this study is to test the hypothesis that tadalafil blocks 5-HT-mediated contractions of the male mice urethra.

METHODS: Prostatic urethras will be dissected from eight-week-old C57BL/6J male mice (group sizes of 5 are anticipated). Tissues will be mounted onto wire myograph pins and maintained in individual chambers with 37°C Krebs buffer solution (NaCl 130 mM, KCl 4.7 mM, KH₂PO₄ 1.18 mM, MgSO₄ 1.18 mM, NaHCO₃ 14.9 mM, Glucose 5.6 mM, CaCl₂ 1.56 mM, pH 7.4), aerated with 95% O₂ and 5% CO₂. Chambers will be washed with Krebs buffer every 15 minutes and between treatments. 60 mM KCl in Krebs will be administered to chambers to verify vitality and obtain maximum contraction response for data normalization. Urethras will be exposed to graded concentrations of alpha-adrenoreceptor agonist phenylephrine (0.1-300 µM) and relaxed with tadalafil (1µM and 10µM). Next, urethras will be exposed to graded concentrations of phenylephrine (0.1-300 µM) or 5-HT (100 nM-3 mM). Finally, urethras will be incubated with 10 µM tadalafil for 20 minutes and graded concentrations of phenylephrine (0.1-300 µM) or 5-HT (100 nM-3 mM) will be added to the tadalafil-containing Krebs buffer. Data will be analyzed using GraphPad Prism.

RESULTS: We expect that phenylephrine and 5-HT will cause contractions in male mice urethra and that tadalafil will block 5-HT induced contractions. We also expect that tadalafil will relax phenylephrine-induced contractions, resulting in a rightward shift in the concentration-response curve compared to phenylephrine alone.

CONCLUSIONS: If pre-incubation of tadalafil can block 5-HT-induced contractions in male mice urethra, this may indicate that tadalafil may be more beneficial than alpha blockers under conditions when serotonin is released from the urethra, such as urethral inflammation.

HEALTH RELEVANCE: Tadalafil may be useful in precision therapy of male LUTS, specifically in the subset of men with prostate inflammation who are not effectively treated by alpha blockers.
