



CAIRIBU - Collaborating for the Advancement of
Interdisciplinary Research in Benign Urology

www.cairibu.urology.wisc.edu

2024 Annual CAIRIBU Meeting

December 4-6
Milwaukee, WI

Abstract Booklet

CAIRIBU U24 Interactions Core PI and Director:

Kristina L. Penniston, PhD
penn@urology.wisc.edu

NIH/NIDDK Leadership:

Christopher Mullins, PhD
Project Scientist
mullinsc@extra.niddk.nih.gov

Deepak Nihalani, PhD
Program Official
deepak.nihalani@nih.gov



STATEMENT OF CONFIDENTIALITY

The annual CAIRIBU meeting is specifically designed to showcase the work of trainees and early-stage investigators. **As such, much of the work herein contains unpublished data and/or work in progress.** Please regard the research reflected in this abstract booklet and otherwise presented at this meeting as privileged. Your confidence is much appreciated.



(G1-12) ELECTRICAL STIMULATION TREATMENT FOR SENSORY DYSFUNCTION IN WOMEN WITH UNDERACTIVE BLADDER

Em Abbott,¹ Cindy Amundsen,² Eric Gonzalez¹, Warren M Grill,¹

¹Duke University, Biomedical Engineering, Durham, NC; ²Duke Health, Urogynecology, Durham, NC

INTRODUCTION: Underactive bladder (UAB) affects nearly 40% of the aging population. It involves detrusor underactivity and/or reduced sensory nerve activity leading to lower urinary tract symptoms (LUTS) including pressure, urinary frequency, poor urinary stream, and incomplete emptying. Behavioral management often fails to alleviate LUTS and no medications have proven to be effective. Thus, patients must rely on clean intermittent catheterization (CIC), a task which is not always achievable and impacts quality of life. We hypothesized that sensory dysfunction contributes to UAB and explored whether targeted electrical stimulation of the bladder or urethra could improve bladder emptying and LUTS.

METHODS: Non-neurogenic women with UAB diagnosed by urodynamics were recruited (IRB# Pro00106457, ClinicalTrials.gov: NCT04516434) and screened for bothersome urinary symptoms and poor voiding efficiency. At the study visit and one week following the stimulation session participants completed the Comprehensive Assessment of Self-Reported Urinary Symptoms (CASUS). We normalized subscales of urinary frequency, bladder sensation, voiding effort, urinary flow, and quality of life (range 0-100; higher scores indicated worse symptoms). Current perception thresholds (CPT) were assessed with an electrode catheter in the bladder or urethra with sinusoidal stimuli delivered at 5, 250, and 2,000Hz. Based on CPT values and clinical presentation, participants received either continuous intravesical or intraurethral stimulation. Urodynamics (UDS) assessed bladder function after stimulation compared to prior clinical UDS results.

RESULTS: Twenty women symptomatic with UAB for 18±22 months participated: 8 received intraurethral stimulation (67yo ± 14) and 12 received intravesical stimulation (69yo ± 11). The sample population was 85% White, 15% Black or African American, 55% self-catheterized, and 65% had recurrent urinary tract infections (UTIs). Seventy percent of the participants reached the maximum CPT output at all frequencies (9.99mA). No significant differences in CPT amplitude were observed between bladder vs urethra at 5Hz (8.25±3.56 vs 7.95±3.86, p=0.56), 250Hz (9.61±1.69 vs 9.14±2.41, p=0.47), or 2000Hz (9.77±0.95 vs 9.99±0.00, p=0.78). However, CPT values were lower at slower frequency of 5Hz compared to higher frequencies (p < 0.05, pairwise ANOVA type III corrected for multiple comparisons). CASUS scores showed no significant change between the baseline vs after stimulation for bladder (55±13 vs 52±10) or urethra (48±16 vs 44±12).

CONCLUSIONS: Electrical stimulation of the bladder or urethra showed variable responses in our population of women poor sensation. This technology may be more effective in individuals with less severe sensory loss.

FUNDING: K01 DK120632; NIH KURC K12DK100024

(G2-1) MITOCHONDRIAL DYSFUNCTION AND THE AGING PROSTATE: CHARACTERIZING MITOCHONDRIAL HETEROPLASMY

Alexis E. Adrian¹, Gregory J. Tranah², Scott R. Bauer³, Douglas W. Strand⁴, William A. Ricke¹

¹University of Wisconsin—Madison, School of Medicine and Public Health, Madison, WI ²California Pacific Medical Center Research Institute, San Francisco, CA; ³Department of Medicine, Urology, Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California; ⁴University of Texas Southwestern Medical Center, Dallas, TX; USA

INTRODUCTION: Lower urinary tract symptoms due to benign prostatic hyperplasia (LUTS/BPH) are a pervasive condition. Nearly 90% of men aged 50-80 experience some degree of urinary symptoms which reduce quality of life. Although age is the number one risk factor, the contribution of aging processes in the development of LUTS/BPH remains unknown. Mitochondrial dysfunction has been implicated in other diseases of aging, and recently, in LUTS/BPH. Our recent studies suggest that changes in the oxidative phosphorylation (OXPHOS) pathway may contribute to disease. Mitochondrial DNA (mtDNA) encodes for 13 proteins in the OXPHOS pathway. mtDNA is prone to damage and heteroplasmy, or the presence of

two or more distinct genomes in a given tissue. Mitochondrial heteroplasmy is associated with functional OXPHOS defects and aging. The objective of this study is to compare mitochondrial heteroplasmy trends in donor and BPH prostate tissue to better understand how changes in mitochondria contribute to LUTS/BPH.

METHODS: Frozen prostate samples were acquired from young organ donors (n=11) and BPH surgeries (n=8). Genomic DNA was extracted and mtDNA was enriched through whole mtDNA hybridization capture-based target enrichment. Whole mitochondrial genome sequencing was performed on the Illumina PE150 platform by CD Genomics. Resulting sequences were mapped to a mitochondrial reference genome and frequency calls for each base pair were determined. Sites with heteroplasmy frequencies occurring between 5- 95% were identified for each sample. The loci and corresponding coding region were identified and compared.

RESULTS: BPH prostate samples showed a trending increase in the number of heteroplasmic sites per sample. In the 11 control samples, 46 total sites were identified. In the 8 BPH tissues, 83 mutations were identified. When sorted by coding region 30% of mutations in BPH tissues were for genes encoding for complex I proteins, while only 15% were attributed to complex I in donor samples.

CONCLUSION: Despite these preliminary studies being statistically underpowered, they reveal interesting trends. First, a higher level of mitochondrial heteroplasmy in BPH samples compared to controls. A larger proportion of heteroplasmy sites encode for complex I proteins in BPH tissue than in donor samples. This suggests that complex I mutations could contribute to mitochondrial dysfunction in LUTS/BPH, but further analysis is needed.

RELEVANCE: Mitochondrial heteroplasmy is a potentially novel disease driver that requires further investigation. If validated, it could serve as a future disease stratifying biomarker. Additionally, these findings also support complex I as a potential disease contributor and may suggest a role for oxidative stress in disease pathology.

(G2-24) MODULATION OF BLADDER CONTRACTION AND EXTERNAL URETHRAL SPHINCTER MUSCLE ACTIVITY BY LOCAL INJECTION OF ADENO-ASSOCIATED VIRUS

^{1,2}Firoj Alom, ¹Kyle J Ratcliff, ^{1,2,3,4}Aaron D Mickle

¹Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, ²Department of Physiology, Medical College of Wisconsin, Milwaukee WI, ³Department of Neurosurgery, Medical College of Wisconsin, Milwaukee WI, ⁴Department of Urology, Medical College of Wisconsin, Milwaukee, WI.

INTRODUCTION AND OBJECTIVES: Spinal cord injury (SCI) patients frequently suffer from dyssynergia between the bladder and the external urethral sphincter (EUS) muscle function. This dyssynergia occurs due to the SCI affecting micturition circuits that control the coordination of the bladder and EUS. Electrical neuromodulation of the lower urinary tract remains an attractive therapeutic target to stimulate the impaired neuronal circuit, but it has yet to be fully deployed clinically. Neuromodulation using optogenetic is more selective than electrical stimulation and allows for longer stimulation without undesirable side effects. The present study was conducted to investigate optogenetic-based neuronal coordination of the micturition circuits that control the bladder and EUS to initiate successful voids in SCI patients.

METHODS: We injected AAV9-CAG-chrmine-Oscarlet and AAVretro-CAG-chrmine-Oscarlet directly into the bladder and EUS of naive rats. After 5-7 weeks, we performed cystometry to evaluate the functional modulation of bladder (contraction) and EUS (EUS EMG bursting) by application of red light which activates chrmine. At the end of cystometry, we perfused the rats and collected tissues for histological evaluation of chrmine transduction into the peripheral neurons.

RESULTS: Single fill cystometry showed that the bladder took less time to initiate void when stimulated with red light. Illumination of EUS with red light produces EUS EMG bursting events. Illumination of EUS with green light produces no EUS EMG bursting. This result confirms that EUS EMG bursting events produced by red light is not an artifact and is producing due to activation of chrmine (Figure 1). We confirmed the retrograde expression of chrmine-Oscarlet by histology.

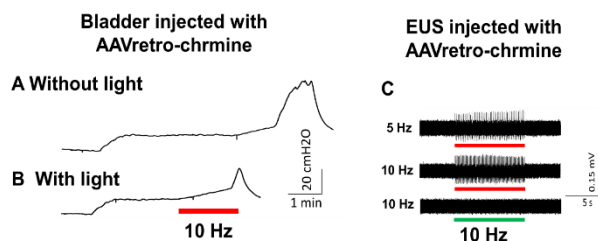


Figure 1. AAVretro-chrmine can modulate bladder and EUS function in rats. Single fill cystometry showed that activation of chrmine with red light initiates void earlier (B) compared to no light application (A). Illumination of EUS with red light produced EUS EMG bursting, whereas green light showed no bursting events, indicating chrmine-specific action for the EUS bursting event (C).

CONCLUSIONS: Our preliminary data suggests that local injection of AAV9-CAG-chrmine or AAVretro-CAG-chrmine could be effective in transducing neurons and modulating the bladder contraction and EUS EMG bursting events. Future studies need to explore different stimulation parameters and neuromodulation strategies that will induce detrusor contraction and, at the same time, initiate EUS EMG bursting for a successful void in the SCI model of rats.

HEALTH RELEVANCE: We aim to improve optogenetic-based neuromodulation techniques targeting lower urinary tract function.

(G1-26) SPATIALLY RESOLVED BLADDER TRANSCRIPTOMICS DIFFERENTIALLY EXPRESSED GENES IN MEGABLADDER (MGB) MICE

Nathalia G. Amado¹, Nixon Raj¹, Israel Cotzomi-Ortega¹, Yuriko Sanchez-Zamora¹, Kelly Grounds¹, Rollin Li¹, Xin Wang¹, Matthew Cannon², Juan de Dios Ruiz Rosado¹, Kirk McHugh³, Ashley Jackson¹, Emily Theisen⁴, Benjamin Stanton⁴, and Linda A. Baker¹

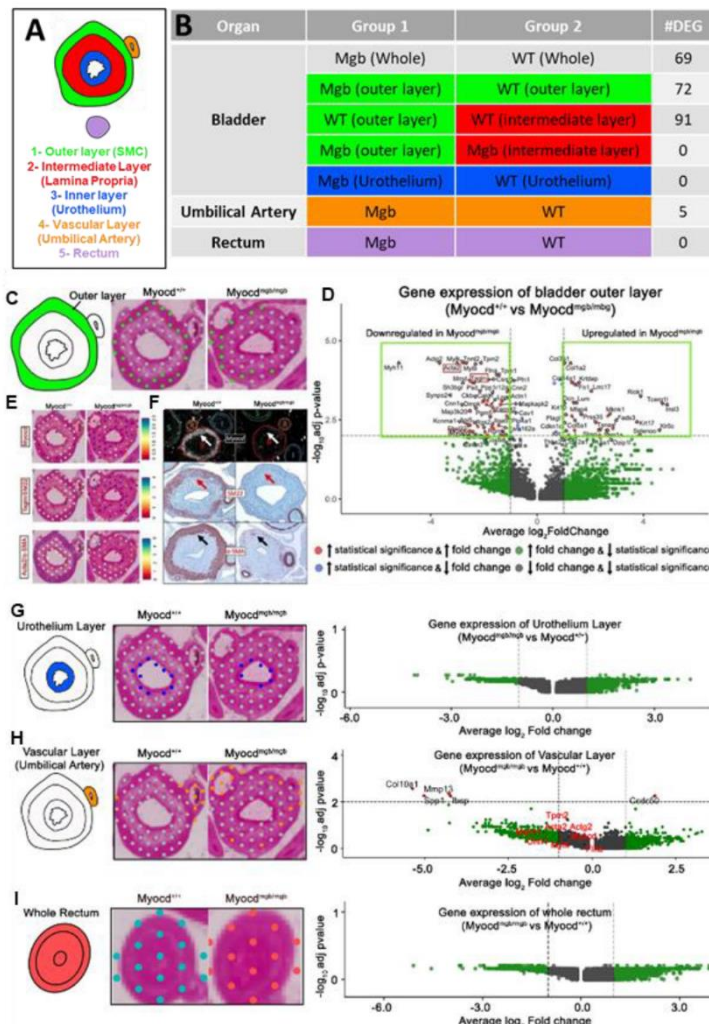
¹The Kidney and Urinary Tract Center, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH. ²Steve and Cindy Rasmussen Institute for Genomic Medicine at Nationwide Children's Hospital, Columbus, OH. ³Department of Anatomy, Ohio State University, Columbus, OH. ⁴Center for Childhood Cancer at the Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH; USA.

INTRODUCTION AND OBJECTIVE: The myocardin (MYOCD) gene, crucial for detrusor smooth muscle cell (SMC) differentiation, causes a Prune Belly Syndrome-like disorder when mutated. Within the nucleus, MYOCD protein co-activates SMC contractile genes, transforming embryonic mesenchyme into SMCs. Myocd knockout mice die in embryonic stages from vascular malformations, but Mgb mice, with a disrupted intergenic bladder-specific Myocd regulatory region, exhibit normal visceral and vascular SMCs except in the detrusor. This study uses spatial transcriptomics (ST) to investigate gene expression differences in SMC differentiation between wild type (WT) and Mgb E15.5 embryos during detrusorgensis.

METHODS: WT and Mgb E15.5 embryos were formalin-fixed, paraffin-embedded, and cross-sectioned. Slices were placed on 10X Genomics Visium mRNA ST slides. After library preparation, tissue was Illumina NovaSeq6000 sequenced. ST data were processed and statistically analyzed with SPATA2, Space Ranger-2.1.1, and Seurat-5.0.3. Gene Ontology analysis identified functional top up- and down-regulated gene transcripts, with validation by RNAScope (RS) or immunofluorescence (IF).

RESULTS: Transcript clustering of the umbilical arteries and whole bladders identified four spatial regions in WT and Mgb embryos: 1) outer (SMC), 2) intermediate, 3) inner (urothelial), and 4) vascular layers. Differential gene expression (DEG) analysis between the outer layers of WT and Mgb bladders revealed significant downregulation of SMC genes and upregulation of collagen and fibroblast-related genes in Mgb bladders. These findings were confirmed by RS and IF. Significant DEGs were observed between the WT intermediate and outer layers, but no DEG were detected between these layers in Mgb, indicating disrupted outer layer detrusorgensis. No significant gene expression changes were found in the urothelial layers between Mgb and WT. Additionally, the vascular layer and rectal visceral SMCs showed unaltered gene expression patterns in Mgb mice.

Fig.1: ST Comparisons of E15.5 organs invested with SMCs (A) Cartoon representation of the 4 clusters derived from the bladder (1-Outer layer (SMC), 2- Intermediate layer (Lamina Propria), 3- Inner layer (Urothelium), 4- Vascular layer (umbilical artery)) and one cluster derived from rectum. (B) Table with the list of ST Comparisons performed. (C) Cartoon representation showing the outer layer and the same cluster spots spatially highlighted on E15.5 bladder sections of WT and Mgb. (D) Volcano plot of differential gene expression analysis of bladder outer layer of Mgb vs. WT. The x-axis shows the average log2 fold change in each gene's expression and the y-axis gives the log transformed FDR-adjusted p-values, indicating strength of statistical significance of the change. Red dots indicate genes with high fold change and high statistical significance, with upregulated genes in Mgb within the upper right green box and downregulated genes in Mgb within the upper left green box. Blue, green, and gray dots indicate genes that did not pass the filtration criteria of both average log2 Fold Change > 1 and FDR-adjusted p-value < 1e-2. (E) The spatial location of expression levels of 3 classic smooth muscle marker genes (Myocd, Tagln and Acta2) in WT vs. Mgb bladders. The expression levels are indicated by the color of the spots. (F) Secondary validation of 3 dysregulated smooth muscle marker genes. Myocd mRNA downregulation is validated by RNAScope in Mgb bladders. Immunofluorescence of Tagln/SM22 and Acta2/αSMA validates their downregulation in Mgb bladders. (G) Urothelial, (H) Vascular and (I) Rectum layer. Cartoon representation showing the urothelium, vascular and rectum layer. The urothelial, vascular and rectum cluster spots spatially highlighted on sections from WT and Mgb mice. Volcano plot showing no differential gene expression between Mgb and WT in Urothelium, Vascular and Rectum layers.



CONCLUSIONS & HEALTH RELEVANCE: ST comparing WT to Myocd-deficient Mgb mice has confirmed 1) bladder-specific SMC gene dysregulation, since gut and vascular SMC is not altered, and 2) Myocd-deficiency induced upregulation of collagen and fibroblast-related genes and downregulation of SMC genes in Mgb outer layers indicating absent SMC differentiation. These findings provide an unbiased look at Myocd's role in detrusorgensis and open new avenues for targeted therapeutic interventions.



(G2-27) FLNA^{C2152R/Y} MUTANT MICE RECAPITULATE THE PRUNE BELLY SYNDROME (PBS) BLADDER PHENOTYPE AND SHOW BLADDER CIRCADIAN CLOCK DYSREGULATION

Nathalia G. Amado¹, Justin Wobser¹, Nixon Raj¹, Thomas J. Egeland², Sujay S. Ithychanda³, Luis Sifuentes Dominguez⁴, Nida S. Iqbal², Alexandria N. Fusco², Oumoukhairi Camara¹, Monica Ridlon⁵, Ashley Jackson¹, Kimberly P. Keil-Steitz⁵, Jun Qin³, Roy Zent⁶, and Linda A. Baker^{1,2}.

¹The Kidney and Urinary Tract Center, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, USA.

²Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX. ³Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio. ⁴Department of Medicine, Gastroenterology Division, University of Texas Southwestern Medical Center, Dallas, TX. ⁵Department of Comparative Bioscience, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI. ⁶Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee; USA.

INTRODUCTION AND OBJECTIVE: We identified 4 humans with PBS having X-linked, pathogenic FLNA missense mutations, including the severe FLNA p.C2160R gain-of-function mutation disrupting regulatory protein binding sites. The FLNA protein is highly expressed in detrusor smooth muscle cells (SMC) and crucial for SMC regulation. To elucidate mechanistic causality, analogous Flna^{C2152R/Y} (F-2152) mice were generated using Crispr-Cas9, allowing assessment of this mutation's impact on bladder form and function.

METHODS: 2-month-old adult male offspring (WT and F-2152) were evaluated for bladder anatomy, gene expression, and function. 24-hour metabolic cage measured voided urine volume and frequency (UroVoid). *Ex vivo* organ bath assays assessed bladder contractility using electrical field stimulation (EFS), cholinergic and purinergic agonists. Bladder bulk RNA-seq compared WT and F-2152 male mice transcriptomes. β -III-tubulin and VACHT expression were measured via western blot (WB). Mann-Whitney tested group differences with $p < 0.05$ considered significant.

RESULTS: Compared to WT, F-2152 males had significant bladder differences, with 28% increased weight and 40% increased detrusor thickness. On bladder natural filling, F-2152 males had 172% larger mean voided volumes per void, and 57% fewer voids per 24hrs. Bladder contractility assays demonstrated normally functioning cholinergic and purinergic pathways suggesting normal muscle function, but F-2152 bladders showed a 24% decrease in EFS response at 50Hz, indicating diminished bladder neural input. Consistently, F-2152 bladders showed significantly reduced neuronal markers by WB. Bulk RNA-seq revealed upregulation of circadian rhythm genes in F-2152 mouse bladders, which are known to affect daytime vs nighttime voiding frequency. When comparing daytime vs

nighttime voiding, WT mice had 63% nocturnal voids. F-2152 mice lost these voiding differences between day and night, averaging 45% nocturnal voids and suggesting this FLNA mutation impacts bladder circadian rhythm.

CONCLUSIONS & HEALTH RELEVANCE: Adult male mutant mice carrying the analogous human PBS missense mutation F-2152 exhibited PBS-like thick-walled bladder with functionally large capacity and infrequent voiding. These bladders show alteration of circadian rhythm gene expression with altered day/night voiding patterns. Collectively, these results highlight the complex interplay between molecular, structural, and functional factors contributing to PBS bladder dysfunction in F-2152 mice.

(G2-25) EVALUATING THE EFFECTS OF ANGIOTENSIN II TYPE 2 RECEPTOR AGONISM ON BLADDER CONTRACTILITY

Hannah A Anderson^{1,2}, Firoj Alam³, Aaron D Mickle¹⁻⁴

¹Joint Department of Biomedical Engineering, Medical College of Wisconsin and Marquette University, Milwaukee, WI, ²Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, ³Department of Neurosurgery, Medical College of Wisconsin, Milwaukee, WI, ⁴Department of Urology, Medical College of Wisconsin, Milwaukee, WI

INTRODUCTION AND OBJECTIVE: Angiotensin II (Ang II), the main effector molecule of the renin-angiotensin system, acts on two of its receptors, Ang II type 1 receptor (AT1R) and Ang II type 2 receptor (AT2R). AT1R and AT2R activity have been shown to cause effects on major organ systems, which has resulted in the widespread usage of AT1R-targeting drugs and the emergence of clinical trials for C21, an AT2R agonist. The roles of AT1R and AT2R should be considered in bladder physiology and pathophysiology, as bladder diseases pose a substantial health burden with limited treatment options. While it is understood that AT2R generally has a protective role in other organ systems, limited studies consider the role of AT2R in the bladder. However, previous studies have suggested that bladder tissue is sensitive to changes in AT2R activity. We aimed to characterize the role of AT2R agonism in bladder contractions through *ex vivo* investigations in the bladders of naive female mice. We hypothesized that AT2R agonism would reduce the amplitude of Ang II-induced bladder contractions, following patterns in supporting literature.

METHODS: Two longitudinal bladder strips were prepared from each mouse and mounted in a 10 mL organ bath for isometric tension recording. Following equilibration, reproducible control contractions were obtained by administration of 70 mM KCl. Ang II-induced contractions were induced with or without the presence of 10 μ M C21. Finally, a contraction was induced with 70 mM KCl to account for any potential tissue desensitization. Following the experiment, we quantified the changes in amplitude of each contractile response.

RESULTS: Our preliminary data has supported our hypothesis, showing that the presence of C21 significantly reduces the amplitude of Ang II-induced bladder contractions ($n=3$, $p=0.0392$ in paired t-test).

CONCLUSIONS: Our results begin to define a role of AT2R in bladder contractions, potentially with a role in mediating bladder contractions. However, further studies are needed to determine its role in bladder physiology.

HEALTH RELEVANCE: Through this objective, we address the fundamental gap in knowledge regarding the role of AT2R in bladder physiology, thereby contributing to the ongoing investigation of the role of renin-angiotensin signaling in the bladder. The long-term goal of progress in this area of research is to inform improved therapeutic options for patients with bladder diseases.

(G1-22) GENOME-WIDE ASSOCIATION ANALYSIS OF ACQUIRED URINARY INCONTINENCE IN DOGS IDENTIFIES ASSOCIATION WITH AN UNCHARACTERIZED LONG NONCODING RNA

Lauren A. Baker

Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI, USA

INTRODUCTION AND OBJECTIVE: Urethral sphincter mechanism incompetence (USMI) is the most common acquired urinary incontinence (UI)

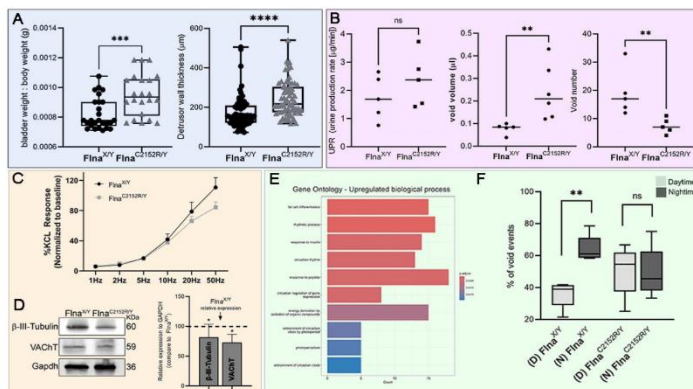


Figure-1: Alterations in Bladder Function and Gene Expression in Flna^{C2152R/Y} (F-2152) Male Mice. (A) 2MO bladder-relative weight quantification: Each dot represents one mouse/bladder. 2MO detrusor muscle thickness quantification: Each dot represents one measurement, with each bladder measured six times in random locations. (B) Urine production rate, void volume, and number of voids measured by UroVoid over 24 hours: Each dot represents one mouse ($n=10$). (C) Percent maximal contractile response normalized to baseline for applied electrical field stimulation at 1, 2, 5, 10, 20, and 50 Hz ($n=5$). (D) Western Blot for β -III-Tubulin and VACHT with densitometry graph. GAPDH was used as a control ($n=3$). (E) Gene ontology analysis of upregulated biological processes from whole bladder bulk RNA-seq. (F) Percentage of voids during the daytime vs. nighttime measured by 24-hour UroVoid assay ($n=10$) (D=daytime and N=nighttime). Mann-Whitney was used to calculate significance where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

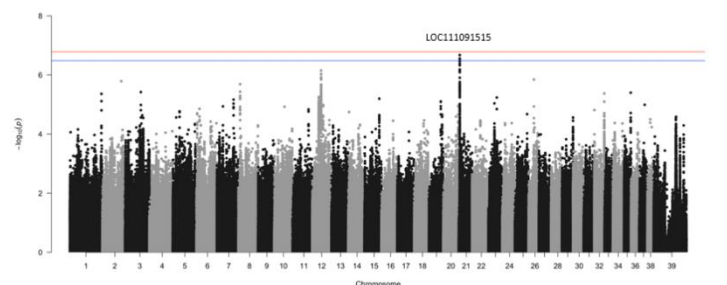


in female pet dogs, with clinical signs similar to stress or mixed incontinence in women. While loss of reproductive hormones after sterilization surgery (spay) is a major contributing factor, breed-specific predisposition indicates an important genetic contribution to USMI. Defining the genetic contribution to UI in dogs may reveal underlying mechanisms that make tissues more susceptible to hormone-driven changes that lead to UI. The purpose of the present investigation was to identify genetic associations with USMI in a mixed population of aging female dogs.

METHODS: This research utilized publicly available data collected by the Dog Aging Project (DAP): a large open-access longitudinal study of aging in dogs. Cases were spayed female dogs with owner-reported UI. Owner comments were evaluated to include only dogs with a USMI diagnosis vs. other causes of UI (e.g. ectopic ureter). Controls were spayed female dogs >40lbs, ≥10 years of age, with no history of urinary disease. Single nucleotide polymorphism (SNP) data underwent quality control filtering in PLINK v. 2.0. A linear mixed model genome-wide association study (GWAS) was performed using R package 'gaston' v. 1.6. Genome-wide significance was determined using a Bonferroni correction for the calculated number of haplotype blocks in the population using PLINK: 0.05/303,237=1.6E-07. Regions in linkage (linkage disequilibrium (LD) $r^2 > 0.5$) with top SNPs were defined using results-based LD clumping in PLINK.

RESULTS: The final dataset contained 6,986,675 SNPs with minor allele frequency >0.1 and genotyping rate >96% from 57 case and 157 control dogs. A SNP at chr21:4112444 closely approached the threshold for genome-wide significance ($P=2.12E-07$). Several SNPs in near-perfect LD with the index SNP ($r^2=0.99$) were within the boundaries of a long noncoding RNA (lncRNA), LOC111091515 (Figure 1).

Figure 1. A Manhattan plot of $-\log_{10}$ p-values from genome-wide association analysis of dogs with and without acquired urinary incontinence



CONCLUSIONS: Though it did not pass the significance threshold, the chr21 locus overlapping LOC111091515 was identified in a previous analysis of DAP data (32 cases, 99 controls) and became more significant with the addition of dogs from this latest data release. This supports a true association with USMI in dogs. Because the functional role of LOC111091515 is unknown, its relevance is unclear. More research is needed to validate this association and further characterize the function of LOC111091515.

HEALTH RELEVANCE: We identified a genetic association with UI in pet dogs that may be relevant to human health.

(G1-10) COLLABORATING FOR THE ADVANCEMENT OF INTERDISCIPLINARY RESEARCH IN BENIGN UROLOGY COLLABORATIVE SUMMIT: AN EARLY-STAGE INVESTIGATOR- CENTERED APPROACH FOR CATALYZING CONNECTIONS TO ADVANCE UROLOGY RESEARCH

Anna E Barrett, Mariana M Coughlin, MS, Kristina L Penniston, PhD

Department of Urology, University of Wisconsin School of Medicine and Public Health, Madison, WI

INTRODUCTION AND OBJECTIVE: The CAIRIBU U24 Interactions Core (CAIRIBU = Collaborating for the Advancement of Interdisciplinary Research in Benign Urology) was formed in 2020 with objectives to raise the visibility of urology research and to facilitate transdisciplinary research collaborations. The CAIRIBU Interactions Core developed and evaluated a 1½ day "Collaborative Summit" to determine if new connections could be fostered, specifically among early-stage investigators (ESIs).

METHODS: Although anyone within the urology community was invited to attend the event, it was designed primarily for a group of CAIRIBU-affiliated ESIs ("key participants"). The ESIs provided a short description of their research and listened to three "State of the Science" talks that identified what is known about specific research areas: lower urinary tract dysfunction, infection/urobiome, and neuro-urological and pain processes in the urinary tract. Within breakout sessions for each topic, attendees participated in a "Speed of Light Thinking" activity that involved several stages of generating research ideas, both privately and as a group. These ideas were then shared broadly.

RESULTS: Participants (n=113) from 53 unique institutions attended virtually or in-person. Most (53%) were ESIs (n=34) (including key participants, n=17) and graduate or undergraduate students (n=26). Responses to a post-event survey demonstrated that 100% strongly agreed or agreed they gained new knowledge; 95% strongly agreed or agreed they expanded their research networks. A 3-month follow-up survey of key participants (response rate 76%) demonstrated a total of 48 connections formed with other key participants (3.7 connections/key participant). Additionally, 75% cited sustained collaborations (e.g., discussions, feedback on research ideas, sharing resources/techniques, or working on manuscripts/grants) with other Summit attendees.

CONCLUSIONS: We piloted a new ESI-centered initiative to critically examine and discuss research needs within areas relevant to the broader CAIRIBU Community. Discussions were robust and critical knowledge gaps that can be addressed in future work were identified. Further follow-up surveys will assess the outcomes of Summit-generated collaborations. The CAIRIBU Collaborative Summit was an effective mechanism for facilitating knowledge exchange and spurring ESI-centered collaborations.

HEALTH RELEVANCE: By employing innovative approaches to address knowledge gaps, interdisciplinary research may accelerate the development of new diagnostics and treatments for urologic conditions and improve patient care. ESI-centered initiatives to foster and sustain interdisciplinary collaborative research can hasten and potentiate these efforts.

(G2-4) INVESTIGATING LONG CHAIN UNSATURATED FATTY ACIDS AS MITOCHONDRIAL MODULATORS IN THE PROSTATE

Natalie J Boucher, Alexis E Adrian, William A Ricke

Department of Urology, George M. O'Brien Center of Research Excellence, University of Wisconsin-Madison, Madison, Wisconsin, USA.

INTRODUCTION AND OBJECTIVE: Benign prostate hyperplasia (BPH), and the associated lower urinary tract symptoms (LUTS), is a disease of aging which affects 90% of men by their eighties. A hallmark of aging, mitochondrial dysfunction, may contribute to disease progression. Specifically, mitochondrial dysfunction is associated with fibrosis in other diseases of aging and needs further investigation in LUTS/BPH. We have previously shown that oleic acid (OA) ameliorates mitochondrial dysfunction and mitigates the pro-fibrotic environment. This study investigates the potential of other long chain unsaturated fatty acids in reducing mitochondrial dysfunction in prostate stromal cells.

METHODS: Human prostate stromal (BHPs1) cells were treated with vehicle, a complex I inhibitor (25nM rotenone), and rotenone in combination with the fatty acid of interest. A dose-response curve for palmitoleic acid (PA), linoleic acid (LA), and α-linolenic acid (ALA) was generated by measuring the oxygen consumption rate (OCR) on Resipher in rotenone treated cells. The most effective doses of PA, LA, and ALA were further tested on complex I-inhibited BHPs1 cells. Immunocytochemistry was used to measure collagen deposition (COL1A1). Mitochondrial function was measured with Resipher and MitoTracker.

RESULTS: Complex I inhibition in BHPs1 cells resulted in a decreased OCR compared to the vehicle control. Treatment with 60nM PA, 75nM LA, and 25nM ALA were found to be most effective in rescuing the respiration rate, with the established 100 nM oleic acid serving as a positive control. ICC for extracellular COL1A1 suggests LA, ALA and PA may be effective in reducing extracellular collagen. MitoTracker results are expected to show an increase in mitochondrial function when treated with ALA, PA, and LA.

CONCLUSIONS: These preliminary results show promising trends, but further experiments must be performed to determine the biological and



statistical significance of these findings. The enhanced respiration in BHPrS1 cells treated with PA, LA, and ALA indicates a potential protective role of these fatty acids against rotenone induced mitochondrial dysfunction. This is further supported by the rescue of mitochondrial function. Additionally, the potential of long chain fatty acids as anti-fibrotic intervention requires further investigation.

HEALTH RELEVANCE: Fatty acid derivatives are an exciting area of anti-fibrotic research. This study suggests fatty acids other than oleic acid may be effective. This research could lead to novel dietary or therapeutic strategies for managing BPH and the associated lower urinary tract symptoms, ultimately enhancing the quality of life for aging men.

(G2-5) ZC3H4, A TRANSCRIPTION TERMINATION FACTOR, IS A NOVEL REGULATOR OF PROSTATE STROMAL CELL METABOLIC HOMEOSTASIS AND STROMAL TISSUE REMODELING

Mia Cararrini¹, Teresa T. Liu², Kegan O. Skaltsky^{1,2}, Nathalie El-Khoury¹, Alexander Chang³, Vishal Soman³, Ailing Liu⁴, Nnamdi Iherijika¹, Uma Chandran³, William A. Ricke², Donald B. DeFranco^{1,5}, and Laura E. Pasca^{1,6*}

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, ²Department of Urology, University of Wisconsin, Madison, WI, ³Department of Biomedical Informatics, University of Pittsburgh, Pittsburgh, PA, ⁴Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, ⁵Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA, ⁶UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA; USA

INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is an aging-related disease characterized by benign expansion of the prostate that can lead to the development of lower urinary tract symptoms (LUTS). Although the mechanisms by which aging-related changes contribute to BPH/LUTS are not fully understood, we previously showed that one hallmark of aging, mitochondrial dysfunction, could contribute to prostate fibrosis [1]. Recently, spurious extragenic transcription has been observed in aged and senescent cells [2]. We seek to determine whether ZC3H4, a novel regulator of extragenic transcription, could alter prostate stromal cell senescence and homeostasis, contributing to BPH/LUTS.

METHODS: Benign prostate stromal cells (BHPrS1) were subjected to glucose deprivation to increase their reliance on oxidative phosphorylation and mitochondrial Complex I (CI) inhibition via rotenone. Cell growth and intracellular ATP levels were measured as well as senescence using flow cytometry and Sudan Black-B staining. Cell adhesion, attachment and fibronectin expression were also assessed. Assays were repeated in BHPrS1 cells with siRNA knockdown of ZC3H4, an anoikis resistance marker and regulator of genome-wide extragenic transcriptional termination. Immunofluorescent staining of ZC3H4 was performed on a prostate tissue microarray [3]. Comparisons between groups were calculated using one- or two-way ANOVA as appropriate.

RESULTS: Glycolytic restriction sensitized BHPrS1 cells to rotenone-induced mitochondrial stress, resulting in reduced proliferation, increased senescence, loss of cellular adhesion and fibronectin induction, potentially impacting cell-cell and cell-substratum interactions. ZC3H4 was significantly downregulated in BPH tissue. ZC3H4 knockdown mimicked the impact of CI inhibition and glycolytic restriction on cell adhesion, senescence and proliferation.

CONCLUSIONS: ZC3H4, a protein that may be involved in aging-dependent increase in spurious extragenic transcription, may impact metabolic homeostasis in prostate stromal cells contributing to stromal cell remodeling that drives BPH development.

HEALTH RELEVANCE: Aging-related mitochondrial stress may contribute to BPH development and progression. Reducing mitochondrial stress and/or promoting ZC3H4 function could reduce BPH-related prostate stromal cell senescence, extracellular matrix remodeling and fibrosis.

(G2-10) T CELL EXHAUSTION IN FEMALE BLADDER TERTIARY LYMPHOID TISSUES ACROSS THE LIFESPAN

Samuel A. Cornelius,¹ Jashkaran Gadhi,² Douglas W. Strand,^{1,2} Nicole J. De Nisco^{1,2}

¹University of Texas at Dallas, Department of Biological Sciences, Richardson, TX; ²University of Texas Southwestern Medical center, Department of Urology, Dallas, TX

INTRODUCTION AND OBJECTIVE: Decreased estrogen production at menopause has been implicated for increased susceptibility to recurrent urinary tract infection (rUTI). Bladder Tertiary Lymphoid Tissues (bTLTs) are germinal centers with large populations of T and B cells and are associated with worse rUTI outcomes. Our goal is to characterize the prevalence, cellular composition, and expression profile of bTLTs in women across the lifespan and in association with rUTI.

METHODS: Over 100 formalin-fixed, paraffin-embedded (FFPE) bladder specimens from females ranging from 18 to 71 years old were sourced from an active biorepository. Tissues were sectioned and subjected to hematoxylin and eosin staining. bTLTs were identified histologically, and their number and size were quantified across the lifespan. A total of six FFPE bladder tissue blocks (3 bTLT+ and 3 bTLT-, age matched) from postmenopausal women (ages 55-71) were profiled with the 10x Genomics Chromium Flex single cell RNA sequencing to create a cellular atlas of bladder specimens with and without bTLTs.

RESULTS: In bTLT+ samples, leukocytes accounted for 68.5% of total cells profiled. In contrast, leukocytes accounted for only 31.5% of cells from bTLT- samples. Similarly, samples with bTLTs contained approximately 3X more T-cells than samples without bTLTs. Strikingly, one of the largest differential features between the single cell profiles of bTLT+ versus bTLT-tissues was the presence of proliferative, exhausted T cells in bTLT+ bladders (**Figure 1**). Exhausted T cells were characterized by upregulation of PD-1 and CTLA-4 as well as the proliferation marker Ki67. In bTLT+ bladders, 33% of T-cells displayed markers of exhaustion, in contrast to just 4% of T-cells in bladders without bTLTs.

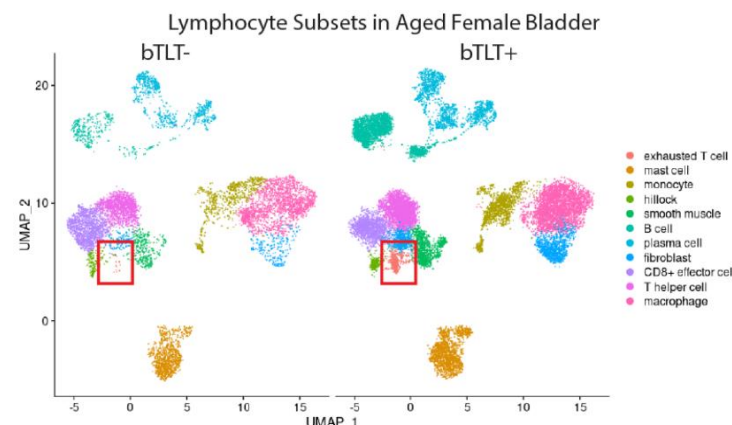


Figure 1: bTLT(+) and bTLT(-) leukocytes separately clustered to specific cell types. The major delineating transcript is CTLA-4, which is associated with T cell exhaustion.

CONCLUSIONS: Exhausted T-cells are associated with states of chronic infection and inflammation in other organ systems. The identification of exhausted T cells in bTLTs may explain why, despite containing proliferative T-cell populations, bTLTs are associated with worse rUTI outcomes in postmenopausal 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.



(G2-16) FATTY ACID AUXOTROPHY AS A DEFINING METABOLIC ADAPTATION OF UROGENITAL LACTOBACILLI

Hrishikesh Dalvi¹, Michael L. Neugent PhD¹, Ceejay Saenz¹, Jessica Komarovskiy¹, Katherine Lim¹, Eve Spiro, Jessica Gauch¹, Nicole J. De Nisco PhD^{1,2}

¹The University of Texas at Dallas, Department of Biological Sciences, Richardson, TX; ²The University of Texas Southwestern Medical Center, Department of Urology, Dallas, TX

Introduction and Objectives: The female urogenital microbiome (FUM) is predominated by specific species of the genus *Lactobacillus* (Lb) that are adapted to mammalian hosts. *Lactobacillus crispatus* and *Lactobacillus gasseri*, for example, produce D-lactate, hydrogen peroxide, and bacteriocins that chemically modify the environment to inhibit pathogen colonization. FUM dysbiosis is associated with recurrent urinary tract infection, urinary incontinence, and overactive bladder. Despite their importance to female urogenital health, the metabolic requirements of FUM *Lactobacillus* species remain largely undefined. Through gene synteny analysis we identified the absence of the *Fab* operon—responsible for type II fatty acid (FA) biosynthesis—in key FUM lactobacilli. These data combined with preliminary growth experiments led us to hypothesize that FUM *Lactobacillus* species are FA auxotrophs. The objective of this work was to rigorously test the hypothesis that FUM *Lactobacillus* species depend on external, host-derived FAs and cannot perform de novo FA biosynthesis.

Methods: We used growth assays in chemically defined media (CDM) to explore the metabolic requirements of FUM *Lactobacillus* species versus related genera in the family Lactobacillaceae, including *Lactocaseibacillus*, *Ligilactobacillus*, *Limosilactobacillus* and *Lactiplantibacillus*. Metabolic tracing experiments using universally labelled ¹³C glucose were conducted to measure de novo FA biosynthesis. Experiments evaluating *Lactobacillus* utilization and incorporation of host lipids were performed using lipids extracted from human 5637 bladder epithelial cells via Bligh-Dyer extraction.

Results: We found that FUM *Lactobacillus* species, including *L. crispatus* and *L. gasseri*, are FA auxotrophs and cannot perform de novo FA biosynthesis while species belonging non-urogenital genera were not. We further found that FUM *Lactobacillus* species can utilize either cis or trans unsaturated FAs and directly incorporate them into their membranes. Furthermore, FUM *Lactobacillus* species utilize and directly incorporate diverse host-derived FAs into their membranes.

Conclusions: Our results strongly support the hypothesis that FA auxotrophy defines FUM *Lactobacillus* species from other related genera and may represent a metabolic adaptation to the female urogenital tract. Our findings that FUM *Lactobacillus* species directly incorporate host-derived FAs also suggest that their membrane composition and function are shaped by the host environment.

Health Relevance: By expanding our understanding of the metabolic relationship between FUM lactobacilli and the human host, these findings will further the rational design of effective prebiotic and probiotic therapies to improve female urogenital health.

(G2-11) ELUCIDATING THE FUNCTION OF THE CELL-MEDIATED ADAPTIVE IMMUNE RESPONSE IN POSTMENOPAUSAL WOMEN WITH RECURRENT URINARY TRACT INFECTION

Jashkaran G. Gadhvi^{1,2}, Fatima Khan², Samuel A. Cornelius², Ujjaini Basu², Tahmineh Ebrahimzadeh², Douglas W. Strand², Phillippe E. Zimmern¹, Nicole J. De Nisco^{1,2}

¹Department of Urology, UT Southwestern Medical Center, Dallas, TX

²Department of Biological Sciences, University of Texas at Dallas, Richardson, TX

INTRODUCTION AND OBJECTIVE: There is an urgent need for new therapies for urinary tract infection (UTI) to address the growing threat of multidrug-resistant bacteria. Advanced age is a significant risk factor for recurrent UTI (rUTI), particularly among postmenopausal women. The mechanisms involved in the initiation of adaptive immunity during rUTI, and indeed the full nature of the adaptive immune response within the human

bladder, remain unclear. The objective of this study is to fill this critical gap in knowledge by defining the cellular and humoral adaptive immune response to rUTI in postmenopausal women by leveraging direct access to patient samples.

METHODS: After IRB approval and informed consent, bladder biopsies were taken under anesthesia. Biopsies were taken from uninflamed regions (C1) and inflamed regions (I1). Immunohistochemical analysis was conducted on control bladder tissues as well as bladder biopsies from rUTI patients. Immunofluorescence was used to detect CD4+ and CD8+ T-cells, CD20+ B cells and CD68+ Macrophages within the bladder. Urine samples were collected from these biopsied women as well as from postmenopausal women in 3 additional groups (Never UTI, rUTI Remission, rUTI Relapse) and analyzed for cytokine and immunoglobulin levels via the flow cytometry-based Biolegend LEGENDplex assay.

RESULTS: Among the most common immunological structures in the bladder tissues of rUTI patients were lymphoid follicles, also called bladder tertiary lymphoid tissues (bTLTs) containing both B and T-cells, including plasma B-cells. We observed that CD4+ T-helper cells significantly outnumbered CD8+ cytotoxic T-cells in both lymphoid follicles and the bladder epithelium. We detected high titers of immunoglobulins IgG1, IgG2, IgG3 and IgA in women with active UTI (rUTI Relapse) suggesting an active humoral response. However, our examination of TH cytokines demonstrated no apparent bias in TH1 or TH2 responses in women with active UTI with both IL-2 and IL-6 cytokines being elevated in the rUTI Relapse group.

CONCLUSIONS: Our data supports the hypothesis that there is an active adaptive immune response that can be detected in the bladder and the urine of postmenopausal women with rUTI. However, this response which includes high levels of urinary immunoglobulins and the formation of bTLTs, is unable to efficiently resolve rUTI and impart sterilizing immunity.

HEALTH RELEVANCE: These findings suggest a robust, yet somehow unproductive adaptive immune response occurs in postmenopausal women with rUTI and highlights the need for development of immunomodulatory therapies to improve bladder adaptive immune responses and rUTI in this vulnerable population.

(G2-15) HEMATURIA SUPPRESSES UROTHELIAL CELL DEATH AND INFLAMMATION

Aryan Ghotra, Uddhav Neupane, Katherine Xu, Rachel Sturley, Tian Shen, Jonathan Barasch

Columbia O'Brien Center

INTRODUCTION AND OBJECTIVE: Hematuria, a common symptom of urinary tract infections (UTIs), results from inflammation of the urinary tract and entrance of red blood cells (RBCs) into the urine. Hematuria is considered dangerous for its potential to induce bacteria growth and reaction oxygen species (ROS) production in host epithelial cells (urothelial cells). In this project, we investigate the effects of hematuria on the urothelium in the context of a UTI.

METHODS: C57BL6 mice were anesthetized and inoculated with 10E7 CFUs of uropathogenic *E. coli* (UPEC) into bladders using a soft tip catheter. Hematuria was simulated by phenyl-hydrazine (PHZ)-induced hemolysis into the serum followed by renal overflow into the urine.

RESULTS: We confirm that UTI treatment causes superficial cell shedding. However, PHZ treatment in addition to the UTI unexpectedly ablated urothelial exfoliation despite increased intracellular bacterial colony formation. Furthermore, immunoblots of urothelial lysates indicated that hematuria suppresses pyroptosis markers including caspase1 and 11 cleavage and Nlrp3 translation. Hematuria also attenuated inflammation as demonstrated by reduced cytokine expression. To find a possible mechanism of this hematuria rescue effect, we considered the heme metabolic gene – Hmox1. Hmox1 was highly expressed in the urothelium and markedly increased in UTI settings. However, strangely, hematuria suppressed Hmox1 transcription and translation suggesting a type of inflammatory role for Hmox1. Furthermore, Hmox1 -/- mice recapitulated the hematuria rescue. Looking more upstream, Hrg1 -/-, a heme endolysosomal transporter, also showed demonstrable hematuria rescue suggesting some type of extracellular signaling heme. Given reduced inflammation, we considered Tlr4 – a key pathogen recognition



receptor (PRR) known to bind to both LPS and heme. Amazingly, C3H/HeJ mice with a loss of function Tlr4 mutation were not rescued by PHZ treatment. Thus, hematuria not only interferes with Tlr4 signaling, but also requires the protein to produce its cell survival phenotype.

CONCLUSIONS: Hematuria, originally thought to be a frightening symptom, turns out to be anti-inflammatory and pro-survival to urothelial cells. In fact, we discovered that this epithelium contains a heme machine eating urinary heme collected from the apical surface, metabolizing it, and subsequently producing CO. However, the mechanism of this hematuria rescue is not dependent on the heme machine, rather on an extracellular PRR— Tlr4.

HEALTH RELEVANCE: Hematuria was found to be both anti-inflammatory and anti-cell death in the urothelium. A novel pathway was identified by which urothelial cells shed in response to pathogen invasion in the bladder that is dependent on Tlr4 and heme.

(G2-31) SUBSTANCE P MODULATES BLADDER TRANSIENT PRESSURE EVENTS VIA NEUROKININ-2 AND NEUROKININ-3 RECEPTORS WITHOUT ALTERING THE MECHANICAL COMPLIANCE

Ashika Goel,^{1*} Sara Roccabianca,² Nathan R. Tykocki¹

¹ Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI; ² Department of Mechanical Engineering and Materials Science, Washington University in St. Louis, MO, USA

INTRODUCTION AND OBJECTIVE: Bladder filling is accompanied by non-voiding contractions, which drive transient pressure events (TPEs) that stimulate sensory nerve activity. Substance P is a neuropeptide derived from sensory nerves that triggers muscle contraction and bladder inflammation. Its preferred receptor is the neurokinin-1 (NK1) receptor, but it can also activate NK2, NK3, and Mrgprb2 receptors. The exact mechanism by which substance P mediates bladder function remains unclear. We tested the hypothesis that substance P alters TPEs and mechanical compliance via activating neurokinin-1 receptors.

METHODS: Bladders from C57BL/6 mice (8 – 12 weeks old) were dissected and mounted in the novel Pentaplanar Reflected Image Macroscopy (PRIM) system, which allows for concurrent measurement of infused volume, intravesical pressure, bladder stiffness, and bladder compliance during ex vivo bladder filling. These data we then used to calculate the leading slope and peak amplitude of TPEs as the bladder fills. We also calculated wall stress and stretch during filling. Measurements were recorded in the presence of substance P (10 μ M) alone, or in the presence of Substance P after incubation with the NK1 receptor antagonist aprepitant (1 μ M), the NK2 receptor antagonist saredutant (5 nM), or the NK3 receptor antagonist osanetant (200 nM). Statistical analysis was performed using paired t tests and two-way repeated measures ANOVA.

RESULTS: Substance P alone markedly increased the peak amplitude and leading slope of TPEs without affecting mechanical compliance (paired t test, $p < 0.05$). Antagonism of NK2 and NK3 receptors, rather than the preferred NK1 receptor, led to a significant decrease in TPE leading slope (two-way RM ANOVA, $p < 0.05$ vs substance P). Mechanical compliance remained unaltered by neurokinin receptor antagonism.

CONCLUSION: These results suggest that changes in TPEs caused by substance P are collectively driven by NK2 and NK3 receptors and are independent of NK1 receptors. Future experiments will determine the mechanism that leads to the release of substance P in bladder dysfunction and the role of Mrgprb2 receptors in regulating bladder dysfunction.

HEALTH RELEVANCE: These findings provide new insights into the complex role of substance P and neurokinin receptors in bladder function and may help develop new treatment options.

Funded by NIH R01-DK135696, R01-DK119615 and P01-HL152951.

(G1-7) APPLICATION OF A LOW-COST, RELIABLE CATHETER FOR URETHRAL PRESSURE PROFILE IN ANESTHETIZED RATS

Wei He, James Hokanson

Medical College of Wisconsin, Milwaukee, WI

Introduction: The Urethral Pressure Profile (UPP) is a valuable tool for diagnosing and assessing urethral dysfunction but requires costly specialized equipment, and is rarely studied in animal models. This study aims to develop an inexpensive, reliable method for recording UPP in rats.

Methods: Female CD rats ($n=7$) were anesthetized with urethane (1.2 g/kg). UPP recordings were compared between a custom side-hole catheter (3 Fr, Braintree Scientific), a standard end-hole catheter (PE-50) and an expensive solid-state pressure catheter (2 Fr, Millar). A custom puller was created to withdraw the catheter at a fixed rate. To assess the impact of infusion rate 15 different rates (0.1–38 ml/hr) were tested in 3 rats. Each rate was tested 3x in pseudo-random order. For each infusion rate the maximum urethral pressure (MUP) was calculated. To evaluate the consistency of the side-hole catheter compared to the solid-state catheter, back-to-back recordings were performed on 4 rats, with approximately 20 pulls from each. Additionally every hour the end-hole catheter was also tested in one trial. The same puller speed and infusion rate was maintained across all trials. We then asked how many trials were needed before the average MUP was within 10% of the average across all trials. For all possible group sizes random sampling of the trials was performed, the resulting MUPs averaged, forming a distribution of MUPs for each group size. For each distribution the number of samples that were within 10% of the final average MUP (across all trials) was computed.

Results: The relationship between infusion rates and MUP for the side-hole catheter showed a rapid increase from 0.1 to 10 ml/hr, followed by a slower increase from 10 to 38 ml/hr. The side-hole catheter recorded a significantly higher MUP (mean: 38.4 cmH₂O) compared to the end-hole catheter (18.4 cmH₂O, $P = 0.019$) and the solid-state catheter (26.6 cmH₂O, $P = 0.043$). The side-hole catheter had 90% of its trial groupings within 10% of the final MUP in 4 trials or less, whereas the solid-state catheter required between 6 and 10 (depending on the experiment).

Conclusions: Our side-hole catheter setup with custom puller is able to record urethral pressure profiles that generally look like the results obtained with a solid-state transducer, albeit with slightly higher average pressures, but with higher consistency. Future work remains to demonstrate its utility in evaluating changes in urethral function.

(G2-13) ANALYSIS OF COMPETITION AMONGST LACTOBACILLI ISOLATED FROM THE URINARY TRACT OF POST-MENOPAUSAL WOMEN

Kaylie Hintze¹, Layla Jeries², Tatyana A. Sysoeva¹

¹Department of Biological Sciences, The University of Alabama Huntsville, Huntsville, AL, USA

INTRODUCTION AND OBJECTIVE: The rise of antibiotic resistant urinary tract infections (UTIs) has become a rising concern particularly in post-menopausal women as recurrent UTI is one of the most frequently diagnosed infections. There is a need to find alternative treatment methods that do not rely on antibiotics. One of these investigated methods, is the use of lactic acid producing bacteria such as lactobacilli. Lactobacilli belong to several genera of urinary bacteria that are common in pre-menopausal women and are shown inhibit urinary pathogens *in vitro*. However, it is not known if lactobacilli interact competitively with one another and what could be the potential impact of introducing nonnative lactobacilli in the urinary microbiome's species biodiversity.

METHODS: Lactobacilli strains isolated from post-menopausal women were used in well-inhibition assays to assess possible competitive behavior between lactobacilli species. Both whole bacteria cell culture and spent medium filtrate were tested for inhibition. Zones of inhibition were measured using plate images and GIMP software and significance was calculated using



Student T-tests. To conduct genetic comparison between species and strains, total genomic DNA was isolated and sequenced for consecutive hybrid assembly of the genomes.

RESULTS: Competitive behavior was observed between several lactobacilli pairs. Particularly, *L. rhamnosus* and *L. animalis* strains inhibited the growth of *L. delbrueckii*. Additionally, *L. animalis* was significantly inhibited by *L. delbrueckii* and *L. gasseri* strains. This inhibition only appeared in the whole cell tests and was absent in spent medium filtrate, suggesting that the observed interactions rely on the presence of bacterial cells. Genetic analysis shows differences including production of diverse bacteriocins by each species. Additionally, there appears to be a prophage in the three tested *L. delbrueckii* strains that is not found in any of the other lactobacillus species in our collection of urinary isolates.

CONCLUSIONS: Interspecies interactions were observed likely due to the genetic differences between species. However, it can be concluded that whatever mechanism that is used in inhibition requires cell activation rather than a passive mechanism due to the lack of inhibition with cell-free filtrate. Future research will determine what bacteriocins or another antimicrobial property is responsible for these apparent inhibitory interactions amongst tested species.

HEALTH RELEVANCE: The discovery and characterization of interbacterial competition between lactobacilli is important for our understanding of the urinary microbiome ecology and designing efficient probiotics or other biologicals for UTI prevention and treatment.

(G1-20) DOES THE NUMBER OF URINE VOIDS AND/OR URINE SPECIFIC GRAVITY PREDICT 24 HOUR URINE VOLUME?

Anabelle Hoffman¹ and Kristina L. Penniston²

¹University of Wisconsin-Madison Summer Program for Undergraduate Urology Research; ²University of Wisconsin School of Medicine and Public Health, Department of Urology; Madison, WI

INTRODUCTION AND OBJECTIVE: Higher urine volume (U_{vol}) decreases urine supersaturation and thus prevents kidney stone formation. Patients are typically advised to aim for daily U_{vol} of ≥ 2 liters (L). But without directly measuring 24 hour U_{vol} , they cannot know on a day-to-day basis if they reach the goal. Patients' self-efficacy in stone prevention could be enhanced if they could predict their daily U_{vol} . We assessed if number of daily urine voids correlated with total U_{vol} .

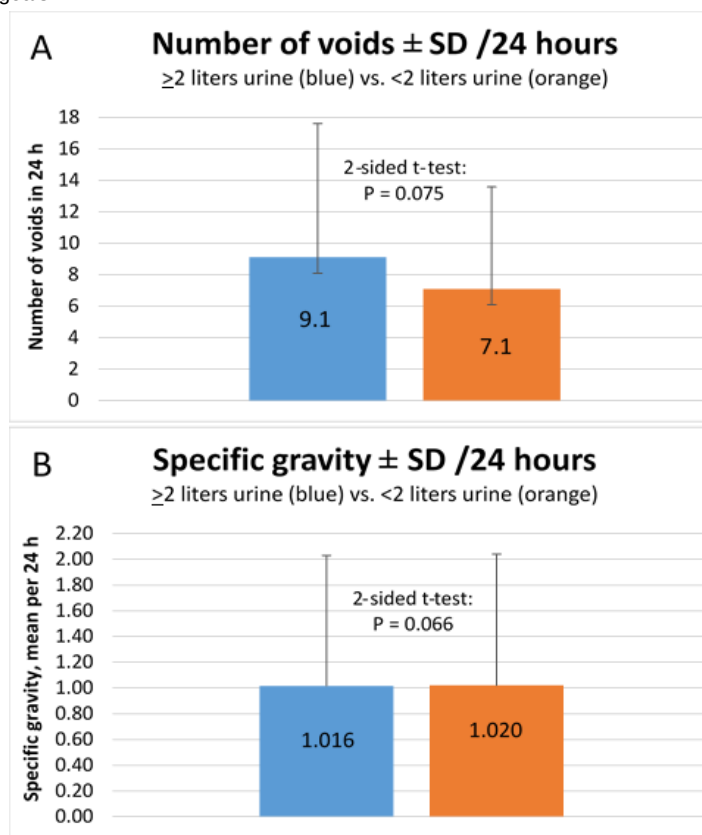
METHODS: A convenience sample of healthy participants were provided a urine cup, urine log, and dipsticks to measure specific gravity (SG). For each day, participants recorded U_{vol} each time they voided and entered the data on the log. They also recorded urine SG during voids at standardized times – within 1 hour after midday meal, within 1 hour after evening meal, before bed, and upon waking. Number of voids and U_{vol} were calculated; SG was averaged across all 4 measures.

RESULTS: Participants ($n=17$; 65% female; age 22-50 years) recorded 22 days of observations and were divided into 2 categories: those who voided ≥ 2 L (mean U_{vol}/day 2.75 ± 0.61 L) and those who voided < 2 L (mean U_{vol}/day 1.54 ± 0.26 L). The number of voids was not different (9.1 ± 2.6 for ≥ 2 L and 7.1 ± 2.4 for < 2 L; **FIGURE, panel A**). While there was a moderately strong correlation between the number of voids and U_{vol} among those with < 2 L of urine ($R=.52$), there was no such correlation for those with ≥ 2 L ($R=0.11$). For urine SG, there was a trend for difference between groups (1.016 for ≥ 2 L and 1.020 for < 2 L; $P=.066$; **FIGURE, panel B**). Mean urine SG and total daily U_{vol} was strongly correlated for those with ≥ 2 L urine ($R=.77$). For those with < 2 L urine, however, there only a weak correlation ($R=.37$).

CONCLUSIONS: We found that ≤ 7 urine voids/day predicts failure to reach at least 2 L U_{vol} . This knowledge could be incorporated into clinical counseling for patients needing to increase U_{vol} for stone prevention. Urine SG, on the other hand, at least from dipstick measures and/or from spot urine voids, may not be a useful biofeedback mechanism for predicting sufficiently high U_{vol} . Studies in a larger group of participants are needed to confirm these results.

PUBLIC HEALTH RELEVANCE: Approximately 1 in 11 people are affected by kidney stones, and there is no cure. Higher U_{vol} is the #1 way to prevent

stones. While patients cannot easily measure their own daily U_{vol} to ensure ≥ 2 L, they may be able to track the number of urine voids within a day, resulting in a potentially useful mechanism for gauging progress toward U_{vol} goals.



(G2-7) EPITHELIUM-DERIVED FACTORS MEDIATING LUMINAL MACROPHAGE TRANSLOCATION: INVESTIGATING THE ROLE OF CXCL17 IN BENIGN PROSTATIC HYPERPLASIA

Thaissa L. Horne,^{1,2,*} M.S., Samantha J. McGuire,^{1,2,*} M.S., Dita Julianingsih,^{1,2} M.S., Mariah A. Jensen-Wachspress,^{1,2} B.S., Samara Silver,^{1,2} B.S., Julius Nyalwidhe,^{1,2} Ph.D., Petra Popovics,^{1,2} Ph.D.

¹Department of Biomedical and Translational Sciences, Macon and Joan Brock Virginia Health Sciences at Old Dominion University; ²Leroy T. Canoles Jr. Cancer Research Center, Macon and Joan Brock Virginia Health Sciences at Old Dominion University *contributed equally

INTRODUCTION AND OBJECTIVE: Benign Prostatic Hyperplasia (BPH) is a non-malignant enlargement of the prostate gland affecting aging men and associated with Lower Urinary Tract Symptoms (LUTS). While the exact etiology is unclear, it has been shown that chronic inflammation and steroid hormone imbalance triggers prostatic pathological changes and urinary dysfunction in mice. We previously demonstrated that hormone imbalance produced in mice expands prostatic macrophages and drives translocation into the prostate lumen, where they differentiate into lipid-enriched foam cells. We also identified that foam cells and epithelial lipid accumulation are linked to LUTS/BPH, though the exact mechanisms and their impact on urinary function remain unclear. We hypothesize that steroid hormone imbalance stimulates the expression of cytokines in luminal epithelial cells which drives macrophage translocation and urinary dysfunction.

METHODS: Our mouse model for BPH is generated by steroid hormone imbalance through subcutaneous implantation of pellets containing 25 mg testosterone and 2.5 mg estradiol (T+E2) and collected ventral prostates two weeks later. Cells were dissociated and loaded on Chromium Next GEM (7000 cells/sample). Samples were sequenced on a NextSeq2000 and confirmed with *in situ* hybridization (ISH). *Cxcl17*^{-/-} mice were obtained from the Knock-out Mouse Project at University of California, Davis (Genentech ID



UNQ473) and were bred to obtain *Cxcl17*^{+/+} (WT) and *Cxcl17*^{-/-} (*Cxcl17*-KO) for pellet implantation. Urinary function is currently being assessed bi-weekly using a 6-unit Urovoid system. Prostate tissues will be collected at 2- and 12-weeks post-implantation for histological analysis.

RESULTS: sc-RNA-seq identified a unique increase in *Cxcl17* chemokine in epithelial cells which was confirmed by ISH. Uroflow analysis of wild-type and *Cxcl17*-KO mice showed no differences in basal urinary function, including total voiding number, average volume or flow rate before implantation. We are currently tracking post-surgery changes in urinary function and will analyze pathological differences, including foam cell formation, at week 12.

CONCLUSIONS: Our data suggest that *Cxcl17* is upregulated in response to steroid hormone imbalance, suggesting that it is the primary driver of macrophage luminal translocation in the prostate, a process necessary for foam cell formation. The loss of *Cxcl17* is also expected to affect urinary function. Completion of this study will confirm the *Cxcl17*-dependence of this process but will also inform about the role of foam cell formation in urinary dysfunction.

HEALTH RELEVANCE: The findings will identify molecular targets for potential therapeutic interventions, advancing our understanding of BPH pathophysiology and LUTS.

(G1-4) UROGENITAL ORGAN CHIPS: PRECLINICAL HUMAN IN VITRO MODELS FOR ADVANCING UROLOGICAL RESEARCH

Zohreh Izadifar^{1,2}

¹Urology Department, Boston Children's Hospital, Department of Surgery, Harvard Medical School, Boston, MA; ²Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA

INTRODUCTION AND OBJECTIVE: The incomplete understanding of many urogenital conditions (e.g., UTIs, IC/PBS, BV) has led to ineffective treatments focused on symptom management. The interplay of host mucosa, resident microbiome, and chemical (e.g., sex hormones) and biomechanical factors (e.g., micturition) makes studying urogenital organs physiology and pathophysiology challenging. Traditional animal models and cell cultures do not fully replicate the human urogenital mucosa microenvironment. Organ-on-chip (Organ Chip) technology offers the ability to recreate human tissue and organ structures and functions in vitro, with unprecedented physiological responses closer to human biology than conventional models. Our objective is to leverage Organ Chip technology to develop clinically relevant in vitro models of the female urinary and genital tract organs for both basic and translational urogenital research.

METHODS: We developed microfluidic-based human cervix and vagina models (Cervix Chip and Vagina Chip), consisting of two parallel microchannels separated by a microporous membrane. Primary human cervical or vaginal epithelial and stromal cells were cultured on opposite sides of the membrane under physiological flow conditions for 12 days, forming a 3D epithelium-stromal interface. These chip models were evaluated for their responses to steroid hormones, environmental cues, and interactions with healthy (*Lactobacillus crispatus*) or pathogenic (*Gardnerella vaginalis*) bacterial communities using a multidimensional molecular, biochemical, and image-based analysis approach.

RESULTS: Both the Vagina and Cervix Chips formed functional mucosal barriers with cellular and molecular responses to hormones, microenvironmental factors, and bacterial stimuli, closely mimicking in vivo behavior. The Cervix Chip produced abundant, hormone-responsive mucus with compositional and physical properties comparable to clinical samples. Co-culture with bacterial communities recreated host-microbiome interactions, revealing significant differences in tissue immune responses, barrier integrity, and mucus composition, reflecting healthy and dysbiotic conditions seen clinically. We are extending this approach to develop Organ Chip models of the bladder and urethra.

CONCLUSION: Organ Chip technology can create preclinical models of the human vagina and cervix, enabling the study of host-microbiome interactions under healthy and diseased conditions. These models offer a robust platform for developing and testing new therapeutic interventions to improve women's urogenital health.

HEALTH RELEVANCE: Urogenital conditions affect 20-50% of women and can lead to serious health outcomes. Human urogenital Organ Chips provide innovative tools to investigate the mechanisms of these conditions and support the development of more effective treatments for millions of women worldwide.

(G2-3) THE EFFECT OF SLEEP FRAGMENTATION ON PROSTATE PATHOLOGY

Mariah Jensen-Wachspress, BS^{1,2,3}, Dita Julianingsih, MS^{1,2}, Samara Silver, BS^{1,2}, Kasun Gunawardena, BS^{1,2}, Ilya Sobol, MD^{4,5}, Larry Sanford, PhD^{1,3}, Petra Popovics, PhD^{1,2,3}

¹Department of Biomedical and Translational Sciences, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA

²Leroy T. Canoles Jr. Cancer Research Center, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA

³Center for Integrative Neuroscience and Inflammatory Diseases, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA;

⁴Department of Urology, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA; ⁵Urology of Virginia, Suffolk, VA

INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is the enlargement of the epithelium and stroma in the transitional zone of the prostate gland. It is common in men over 50, with prevalence exceeding 90% by age 70. Prostate enlargement can cause urethral obstruction, leading to lower urinary tract symptoms, the most troublesome of which is nocturia, resulting in sleep fragmentation (SF). Inflammation is often present in BPH, though its pathogenesis remains uncertain and is likely multifactorial, involving factors such as infections, diet, and hormonal changes. Since SF is known to cause inflammation in cardiovascular and neuroinflammatory diseases, it is plausible that it also contributes to inflammation in BPH. Thus, our study aimed to determine whether SF leads to prostatic inflammation and to examine the correlation between immune cell infiltration and nocturia in BPH patients.

METHODS: Three-month-old C57BL/6J mice were housed in a SF chamber, where an automated moving rod disrupted their sleep every two minutes from 6 a.m. to 6 p.m. for three months. Following euthanasia, the four prostate lobes (ventral, anterior, lateral, and dorsal) were harvested. Prostate tissue from BPH patients undergoing Holmium Laser Enucleation of the Prostate was also collected, and the patients completed the International Prostate Symptom Score questionnaire to assess frequency of nocturia events. Inflammation levels were evaluated using immunohistochemistry for CD45, with images captured on a Mantra Quantitative Pathology Workstation. CD45+ cells were manually counted in mice and quantified using Inform software in human samples. Differences between SF and control mice were analyzed using the Mann-Whitney t-test, and a linear regression analysis was performed to assess the correlation between nocturia and CD45+ cells in BPH patients.

RESULTS: There were significant increases in CD45+ cells in the ventral (2.6-fold, p=0.0020), anterior (2.5-fold, p=0.0127), lateral (1.7-fold, p=0.0127), and dorsal (1.8-fold, p=0.0047) prostate lobes of SF mice (n=5) compared to controls (n=10). In BPH patients, CD45+ cell counts exhibited a trend towards a positive correlation with nocturia events (r=0.2569, p=0.2306, n=6).

CONCLUSION: The findings in this study using a mouse SF model suggest that sleep fragmentation promotes pro-inflammatory changes in the prostate. Although our pilot human study suggested a potential link between prostatic inflammation and nocturia, further research with larger sample sizes is needed and currently underway to confirm these findings and to understand the underlying immunologic pathology of BPH.

HEALTH RELEVANCE: This study will show novel insights into the connection between sleep quality and development or progression of BPH.



(G2-18) MAST CELL- SPECIFIC G- PROTEIN COUPLED RECEPTOR MRGPRB2 REGULATE BLADDER IMMUNITY DURING URINARY TRACT INFECTIONS

Waris Muhammad Khuwaja¹, Nicole De Nisco¹, Dustin P. Green², Xintong Dong¹

¹Department of Biological Sciences, University of Texas at Dallas, Richardson, TX, USA ²Department of Neuroscience, University of Texas Medical Branch, Galveston, TX, USA

INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) are among the most common bacterial infections and disproportionately impact women. My thesis project focuses on revealing the bladder immune mechanisms against UPEC infections, with the hope of identifying novel drug targets to treat UTIs without the use of antibiotics. We are targeting a G protein-coupled receptor called Mrgprb2 on mast cells to identify its involvement in UTI. Mast cells are tissue-resident granulocytes primarily associated with allergy and type 2 immunity but were also shown to be involved in anti- bacterial immunity in the skin. Our goal is to reveal the functions of Mrgprb2 and its human homolog MRGPRX2 in bladder immunity and pain.

METHODS: We infected WT (C57BL/6), and *Mrgprb2*-KO mice with 10⁶ CFUs of UPEC strain UTI89 via transurethral catheterization. After 24 hours of infection, we collected the bladders and performed CFU enumeration, qPCR, and RNA-sequencing to identify genes and pathways regulated by this mast cell-specific GPCR.

RESULTS: I discovered that G protein-coupled receptor, Mrgprb2, suppresses bladder immunity. Mutant mice lacking *Mrgprb2* are better at clearing UPEC infections. qPCR analysis revealed that in the absence of Mrgprb2, the bladder expressed higher Th17 cytokine *Il17a* and pro-inflammatory chemokine *Cxcl2* which may contribute to enhanced clearance of bacterial infections. We have submitted samples for RNA-sequencing to identify all differentially expressed genes and pathways between WT and *Mrgprb2* mutant bladders.

CONCLUSIONS: The results have highlighted the role of mast cells during UTI. Mrgprb2 inhibits type 17 immunity during UTI. In the absence of Mrgprb2, IL-17 expression is higher and UPEC is more efficiently cleared. Our results have for the first time shown the involvement of Mrgprb2 in bladder immunity.

HEALTH RELEVANCE: Mrgprb2 and its human homologue MRGPRX2 are being investigated as drug targets for pseudoallergic reactions and skin diseases. Antagonists for these receptors might be relevant for UTI treatment as well by enhancing bladder immunity and improving UTI patient outcomes.

(G1-1) EXOSOMES FOR ATROPHY: ACELLULAR REGENERATIVE BIOLOGIC FOR ESTROGEN-DEPRIVATION VAGINAL ATROPHY IN A PORCINE MODEL

Audrey Aitelli BS¹, Cassandra Kisby MD MS^{1,2}

¹Duke School of Medicine, ²Duke Health, Department of Ob/Gyn, Division of Urogynecology

INTRODUCTION AND OBJECTIVE: Female vaginal dryness causes feelings of discomfort, dyspareunia, and difficulty with sexual intercourse; it results from atrophy of vaginal tissues either from post-menopausal state or estrogen deprivation from procedures or pharmacologic treatments such as aromatase inhibitors for breast cancer. Vaginal estrogen cream is the gold standard for treatment; however, select patients cannot use vaginal estrogen due to risk factors such as a history of estrogen receptor positive breast cancer. Our objective is to evaluate the efficacy of a regenerative biologic, exosomes, in a porcine model for possible translation as a clinical treatment for vaginal atrophy.

METHODS: We completed a bilateral oophorectomy in 8 York-shire-crossed pigs to eliminate ovarian estrogen production over the 12-week study period. Animals received a baseline injection of exosomes suspended in hyaluronic acid (HA) or hyaluronic acid-alone. Exosomes were injected in a grid pattern into the fibromuscular tissue of the vagina. Tissues were harvested at 12 weeks and gross photography was taken. Tissues were stained for H&E, Masson's Trichrome, and PAS per standard protocols. Analysis is underway and we plan to evaluate the following outcomes: comparison of gross images

for rugae, count and depth of vaginal papillae, neovascularization per high power field, glycogen content on PAS, epithelial thickness on histology, and collagen content and organization.

RESULTS: Forthcoming

CONCLUSIONS: This preliminary study is an exploratory use of exosomes for vaginal tissue regeneration and possible use as a treatment for vaginal atrophy due to estrogen deprivation. Prior study of the use of exosomes on the vagina has shown increased neovascularization and epithelial thickness. We will report our findings at the meeting.

HEALTH RELEVANCE: Non-estrogen treatments are needed for vaginal atrophy given current therapies involve minimally effective vaginal moisturizers and vaginal lasers that have significant out of pocket cost to the patient. An injectable regenerative biologic would give patients options for increased lubrication, comfort, and sexual satisfaction, without the increased risks associated with estrogen exposure in vulnerable populations.

(G1-5) ACCESSIBLE WORKFLOWS FOR ANALYSIS AND VISUALIZATION OF SINGLE CELL SEQUENCING DATA

John T Labin, Douglas W Strand

Department of Urology, UT Southwestern Medical Center, Dallas, TX

INTRODUCTION AND OBJECTIVE: The Strand lab uses single cell RNA sequencing (scRNAseq) to produce cellular reference atlases of normal and diseased human lower urinary tract. A common use case is integration of experimental data for annotation or comparison to our references. Successful integration requires both the experimental and reference data to have been processed in a similar manner. To aid users in preparing their query data for integration with our reference atlases, as well as analysis and visualization of their own scRNAseq data, we have designed a "boilerplate" analysis pipeline.

METHODS: This pipeline has been developed as four nextflow workflows: (1) *cellranger-count* (alignment and counting of 10x Genomics scRNAseq data), (2) *cellbender* (ambient RNA correction), (3) *scRNA-QC* (doublet detection, filtering, normalization, and clustering), and (4) *CZ CELLxGENE* (interactive visualization). These workflows are modular, such that users can chain them together into a pipeline or use them individually. Each workflow exposes a subset of parameters with sensible defaults provided. The workflows are version-controlled and containerized to improve reproducibility. The workflows were tested using both human and mouse publicly available data from 10x Genomics and a variety of parameter combinations.

RESULTS: Each workflow is designed to add functionality to the underlying software to improve the user experience. The *cellranger-count* workflow allows for submission of multiple samples at once. The *cellbender* workflow includes an extra step to produce data that is compatible with the Seurat toolkit for downstream analysis. The *scRNA-QC* workflow utilizes adaptive thresholding to improve filtering, and optionally performs batch correction. The *CZ CELLxGENE* workflow includes the Visualization in Plugin (VIP) to provide users with additional options for plotting and analysis. When chained together into a single pipeline, users can begin with FASTQ files and end with an interactive visualization of their data processed in a manner similar to Strand lab methods.

CONCLUSIONS: These workflows will help to improve the accessibility of Strand lab reference atlases, as well as general scRNAseq analysis and visualization. This collection of workflow repositories can be found at the Strand Lab Astrocyte workflow GitLab page (<https://git.biohpc.swmed.edu/astrocyte/workflows/strand-lab/>). Containers used in the workflows are publicly available at each GitLab project's container registry page.

HEALTH RELEVANCE: These workflows adhere to NIH FAIR principles (Findable, Accessible, Interoperable, Reusable) and promote democratization of our reference data. This will allow more groups to glean deeper insights into their own scRNAseq data.



(G2-2) AGE-MEDIATED CELLULAR SENEESCENCE MAY DRIVE TISSUE DYSFUNCTION IN THE AGING PROSTATE MANIFESTING AS BENIGN PROSTATIC HYPERPLASIA AND LOWER URINARY TRACT DYSFUNCTION

Ajinkya R. Limkar^{1,2}, Alexis E. Adrian¹, Natalie J. Boucher³, William A. Ricke^{3,4}

¹Molecular and Cellular Pharmacology Graduate Program, University of Wisconsin-Madison; ²Medical Scientist Training Program, University of Wisconsin School of Medicine and Public Health; ³Department of Urology, University of Wisconsin-Madison; ⁴George M. O'Brien Research Center of Excellence, University of Wisconsin School of Medicine and Public Health

INTRODUCTION AND OBJECTIVE: Benign Prostatic Hyperplasia (BPH) and Lower Urinary Tract Dysfunction (LUTD) are diseases of aging. As with other diseases of aging, cellular senescence has been shown to play a key role in their development. Senescent cells, characterized by expression of p16^{INK4A} and p21^{WAF1/CIP1}, accumulate within the aging prostate and contribute to tissue dysfunction through the secretion of the Senescence-Associated Secretory Phenotype (SASP). The SASP has been implicated in cellular proliferation, smooth muscle dysfunction, and extracellular matrix remodeling, all of which are central to the pathophysiology of BPH/LUTD. We aim to explore the relationship between cellular senescence and LUTD in both *in vitro* and *in vivo* models of senescence.

METHODS: Human prostate stromal cells (BHPs1) were induced into senescence using doxorubicin or rotenone. Senescence was confirmed by senescence-associated-beta-galactosidase (SA-β-gal) and p21 expression. Mitochondrial dysfunction, a hallmark of senescent cells, was characterized by MitoTracker staining and analysis of cellular respiration by Resipher. Young (2-month) and aged (24-month) p16-3MR reporter mice were used to assess the relationship between senescence and LUTD. *In vivo* luminescence imaging tracked the accumulation of senescent cells in the mouse urogenital tract. LUTD was evaluated using the void spot assay and its correlation with luminescence was analyzed. Additionally, young p16-3MR mice implanted with testosterone and estradiol (T+E2) were utilized to assess the progression of senescence and its relationship to LUTD.

RESULTS: In BHPs1 cells, both doxorubicin and rotenone induced cellular senescence, as evidenced by morphological changes, positive SA-β-gal, and p21 expression. Senescent cells exhibited mitochondrial dysfunction by increased MitoTracker signal and reduced respiration. Compared to young, aged p16-3MR mice exhibited LUTD (p=0.006) and greater lower abdominal luminescence (p=0.031), with a positive correlation between luminescence intensity and LUTD severity. Similarly, in the T+E2 aging mouse model, lower abdominal luminescence increased as mice developed LUTD.

CONCLUSIONS: Preliminary data from the p16-3MR mouse model supports a link between BPH/LUTD and cellular senescence. Additionally, *in vitro* doxorubicin and rotenone models of induced senescence may be used to investigate the phenotypic, metabolic, and secretory profiles of senescent prostate cells and how these alterations contribute to tissue dysfunction in the aging prostate.

HEALTH RELEVANCE: Targeting senescence in the aging-prostate, either as a preventative or in combination with gold-standard treatments, holds potential to improve management of patients with BPH/LUTD.

(G1-29) TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 CHANNEL MRNA IS EXPRESSED THROUGHOUT THE MOUSE URINARY BLADDER

Marlene E. Masino,^{1,2} Emma Flood,² Bhanuteja Madhu,² Osvaldo J. Vega Rodriguez,² Nathan R. Tykocki^{1,2}

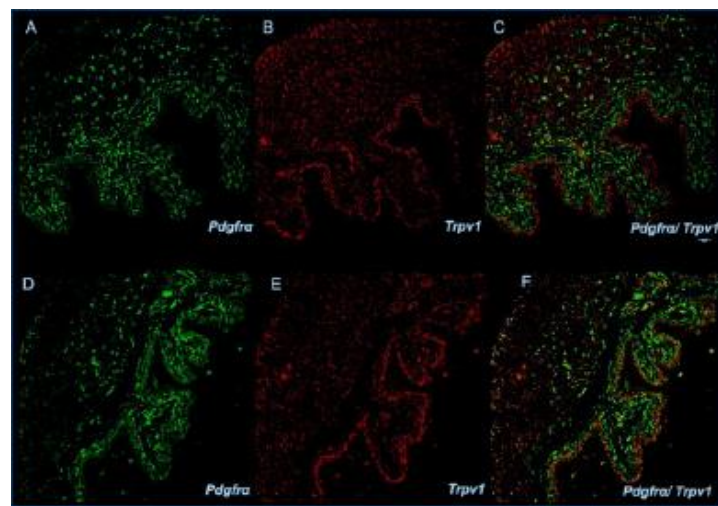
¹Neuroscience Program, Michigan State University, East Lansing, MI USA; ²Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI USA

INTRODUCTION AND OBJECTIVE: Transient receptor potential vanilloid type 1 (TRPV1) channels are implicated in nociception and may become sensitized during inflammation, resulting in increased release of pro-inflammatory neuropeptides. In the urinary bladder, TRPV1 channels are distributed throughout afferent nerves and vascular smooth muscle

cells, but their expression in other cell types within the bladder is controversial. As such, there may be an interdependent mechanism driving interstitial cell and sensory afferent nerve sensitization. While genetically-encoded indicator mice provide evidence for TRPV1-expressing interstitial fibroblasts in the bladder wall, it is unknown if these cells continue to express TRPV1 channels after development. Additionally, sex differences in bladder TRPV1 channel expression have not been thoroughly explored. We hypothesize bladder interstitial fibroblasts express *Trpv1* mRNA after development in male and female mice.

METHODS: Bladders from male and female C57Bl/6 mice (ages 10 – 14 weeks) were isolated, fixed, paraffin embedded and sectioned onto glass slides. Thereafter, RNAscope was used to visualize urinary bladder mRNA expression patterns for *Trpv1* and the fibroblast/interstitial cell marker *Pdgfra*.

RESULTS: While female mice appeared to have more *Pdgfra*-expressing cells in the bladder wall, *Trpv1* and *Pdgfra* were co-expressed throughout the bladder of both male and female mice (fig. 1). Surprisingly, in both sexes *Trpv1* mRNA was expressed throughout the bladder wall and epithelium in cells where *Pdgfra* mRNA was absent. No statistically significant differences in fluorescence were observed between the sexes.



CONCLUSION: Together, these data suggest *Trpv1* mRNA is expressed in multiple bladder cell types, including interstitial fibroblasts, past development.

HEALTH RELEVANCE: These *Trpv1* mRNA positive cells could contribute to the feed-forward sensitization of afferent nerves in the urinary bladder. These data also reveal potential sex differences in bladder fibroblast populations that may account for differences in bladder sensation.

Funded by NIH R01-DK135696, R01-DK119615, and P01-HL152951.

(G2-9) UNTARGETED SPATIAL MAPPING OF PROTEIN BIOMARKERS IN A MODEL OF BENIGN PROSTATIC HYPERPLASIA

Hannah N. Miles^{1,2,3}, Lauren Fields⁴, William A. Ricke^{1,2,3,5}, Lingjun Li^{1,4}

¹School of Pharmacy, ²Department of Urology, ³George M. O'Brien Urology Research Center, ⁴Department of Chemistry, ⁵School of Medicine and Public Health, University of Wisconsin-Madison

Introduction and Objectives: Global proteomics via mass spectrometry (MS) has established itself as a mainstay in biomarker research, allowing investigators to discover novel disease biomarkers and quantify known biomarkers of interest in any given sample type. One main limitation of global proteomics is the loss of spatial information, as global analyses are performed using LC-MS/MS. Mass spectrometry imaging (MSI) has grown in recent years and allows for spatial mapping of multiple analytes, ranging from peptides, metabolites and lipids to post-translational modifications. However, MS/MS confirmation can only be done in a targeted fashion similar to antibody-based imaging approaches. Here, we focused on developing an



automated, open-source platform to integrate MSI and LC-MS/MS data for untargeted profiling of disease markers contributing to BPH.

Methods: A hormone-induced mouse model of aging and lower urinary tract dysfunction (LUTD) was used as previously described. At experimental endpoint, mice were euthanized and lower urinary tracts were collected. Bladder tissues collected from mice were formalin fixed and paraffin embedded (FFPE) prior to sectioning onto ITO-coated slides. Tissues were dewaxed and underwent antigen retrieval followed by tryptic digestion and matrix application for matrix-assisted laser desorption ionization (MALDI)-MS analysis using a Bruker RapifleX at a spatial resolution of 25 μ m. A serial tissue section was prepared similarly until matrix application, at which point digestion was quenched and peptides were extracted, desalted, and resuspended for LC-MS/MS confirmation using a Thermo Q-Exactive HF mass spectrometer.

Results: Using MSight, we were able to identify up to 2500 proteins per tissue section, depending on tissue type and sample amount injected for LC-MS/MS. One particular protein of interest in our studies is Col1a1, as increases in collagen deposition can contribute to fibrosis in BPH progression. Here, we were able to accurately map 31 unique peptides for Col1a1 on bladder tissue sections in our hormone model. Continued results should demonstrate significant differences in biomarker concentration when comparing untreated mouse tissue sections to our hormone-induced model.

Conclusions: MSight allows for high throughput, untargeted profiling of proteins on FFPE tissue samples of interest, increasing the utility of MSI in translational studies. Current efforts are focusing on integration of additional omics datasets into the platform and application to clinical patient samples.

Health Relevance: This open-platform allows for high throughput, untargeted profiling of proteins on FFPE tissue samples of interest, increasing the utility of MSI in clinical biomarker discovery.

(G1-14) NO STRINGS ATTACHED: EVALUATION OF THE PATIENT EXPERIENCE OF STENTING WITH AN EXTRACTION STRING

Golena Fernandez Moncaleano¹, Jennifer M. Thelus¹, Stephanie Daignault-Newton¹, Jerison Ross¹, David Wenzler², Jeremy Konheim³, Casey A. Dauw¹, Khurshid R. Ghani¹, for the Michigan Urological Surgery Improvement Collaborative, Ann Arbor, MI

¹University of Michigan, ²Michigan State University College of Human Medicine and Comprehensive-Urology, ³IHA-Urology

INTRODUCTION AND OBJECTIVE: Ureteral stents with extraction strings are used for convenient removal and short dwell time. However, some surgeons believe it may be more painful for patients and increase complications. To date, no studies have examined the experience of patients undergoing ureteroscopy with strings vs. no string vs. stent omission via validated patient-reported outcomes (PROs). We assessed this in a multicenter cohort with the goal to inform patient care.

METHODS: Using the Michigan Urological Surgical Improvement Collaborative (MUSIC) clinical registry, we identified ureteroscopy and stone intervention procedures with PROs across centers in Michigan from 2021-2023. Patients completed online questionnaires of PROMIS[®] pain intensity and pain interference at 3 timepoints: preoperatively, 7-10 days, and 4-6 weeks after surgery. Three groups were assessed: stent with string, stent without string, and stent omission. All stented patients had stents removed before day 7-10 responses. Our primary outcome was difference in symptoms at 7-10 days. Using multivariable linear regression, we compared PROs after adjusting for pre-surgery score, age, gender, pre-stenting, stone size, stone location, and chronic pain. We also compared 30-day post-operative emergency department (ED) visits.

RESULTS: We identified 536 ureteroscopies with PRO data from 9 centers: stent with strings N=200, stent without string N=128, stent omission N=208. Mean age was 60; 49% male. Mean stone size was 7mm (IQR: 5-10). At day 7-10, patients with a string reported lower pain intensity in the preceding 7 days, compared to patients without a string (Fig 1A; 54.2 vs 56.5, p=0.04). Patients undergoing stent omission reported the lowest pain intensity. Differences in pain interference were not statistically significant between patients with string vs. no string at 7-10 days (Fig 1B; 59.7 vs 61.4, p=0.18). Stent omission patients had the lowest ED visit rate (4.8%); there were no

differences in ED visits between patients with string (9%) vs. no string (7.8%) (p=0.2).

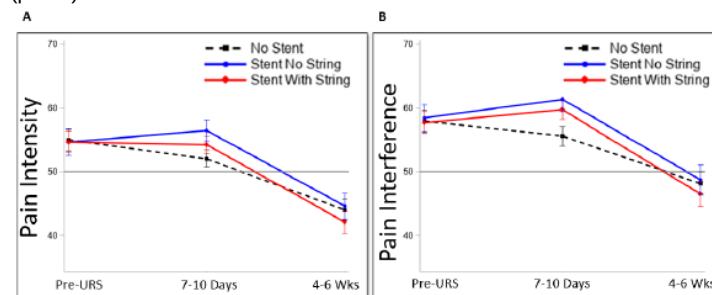


Figure 1. PROMIS[®] pain intensity (A) and pain interference (B) from patients treated with ureteroscopy for stones in the Michigan Urological Surgery Improvement Collaborative. PROs were assessed in three cohorts: stent with string (red, n=200), stent with no string (blue, n=128), and stent omission (dashed black line, n=208); at three time points. For the pain scores, clinically important differences range from 2 to 3 points.

CONCLUSIONS: We found no clinically significant differences in pain intensity or interference in patients with a string vs. no string, and no difference in ED visits.

HEALTH RELEVANCE: This information can guide patients as they make a decision on whether to consider an extraction string strategy.

Sources of Funding: Blue Cross and Blue Shield of Michigan

(G1-15) WHO'S AT RISK? NEW POSTOPERATIVE HYDRONEPHROSIS IN URETEROSCOPY PATIENTS WITHOUT PRIOR HYDRONEPHROSIS

Golena Fernandez Moncaleano¹, Russell E.N. Becker¹, Sabir Meah¹, Stephanie Daignault-Newton¹, Jennifer Thelus¹, Jerison Ross¹, Richard Sarle², David Wenzler³, Brian Seifman⁴, Casey A. Dauw¹, Khurshid R. Ghani¹ for the Michigan Urological Surgery Improvement Collaborative.

¹University of Michigan, ²Sparrow Medical Group, ³Michigan State University College of Human Medicine and Comprehensive-Urology, ⁴Michigan Institute of Urology

INTRODUCTION AND OBJECTIVE: In the United States low imaging rates after ureteroscopy (URS) are well documented. Previous studies indicate that new postoperative hydronephrosis can occur in up to 2.9% of cases and can be associated with renal compromise. Predicting patients at greatest risk for new postoperative hydronephrosis could inform imaging guidelines. We used data from a clinical registry to better understand new onset postoperative hydronephrosis and associated risk factors.

METHODS: Using the Reducing Operative Complications from Kidney Stones (ROCKS), a statewide clinical registry of diverse practices from the Michigan Urological Surgery Improvement Collaborative (MUSIC), we retrospectively identified all URS for stone treatment between Q3 2020 and Q2 2023. Since our primary goal was to identify new postoperative hydronephrosis, we included patients who had preoperative imaging such as computed tomography (CT) or ultrasound (US) and did not have preoperative hydronephrosis. New postoperative hydronephrosis was defined as hydronephrosis seen more than 4 weeks postoperatively. We then compared demographic and clinical variables from patients with new postoperative hydronephrosis with patients who did not develop hydronephrosis using Pearson's Chi-squared, Wilcoxon rank sum and Fisher's exact testing. Small sample size precluded logistic regression analysis.

RESULTS: Preoperative CT or US from 31 diverse practices was available for 4742 patients prior to URS of which 1627 also had postoperative imaging within 60 days of surgery. New postoperative hydronephrosis was seen in 87 (5.3%) patients more than 4 weeks following surgery. Among them, 40 patients (2.45%) had transient hydronephrosis, 6 (0.36%) had strictures, 5 (0.3%) had residual stones, 2 (0.12%) had hematomas and 11 (2.02%) had other causes. Factors significantly associated with new postoperative hydronephrosis after 4 weeks included increasing stone size, use of a ureteral access sheath, ureteral stent placement after surgery, increasing stent dwell time and presence of residual stones (Table).

CONCLUSIONS: New onset of new postoperative hydronephrosis in patients with no preoperative hydronephrosis occurs 1 in 20 postoperatively imaged



patients. Nearly half percent of these are transient. Very few strictures were noted in our series.

Characteristic	Post-Operative Hydronephrosis		p-value ²
	No Hydronephrosis, N = 1,423 ¹	Hydronephrosis Beyond 4 Weeks, N = 87 ¹	
Gender			0.7
Male	664 (47%)	39 (45%)	
Female	759 (53%)	48 (55%)	
Age	62 (50, 71)	63 (54, 74)	0.076
BMI	30 (26, 35)	29 (26, 34)	0.3
Charlson Score	0.00 (0.00, 1.00)	0.00 (0.00, 1.00)	0.4
Charlson Score Category			0.7
0	917 (64%)	60 (69%)	
1	262 (18%)	13 (15%)	
>=2	244 (17%)	14 (16%)	
Any Prior Stone Surgery	381 (41%)	27 (48%)	0.3
Prior Stent	369 (26%)	29 (33%)	0.14
Location Of Largest Stone			0.054
Both	147 (11%)	8 (9.4%)	
Renal	914 (66%)	66 (78%)	
Ureter	330 (24%)	11 (13%)	
Maximum Stone Diameter (mm)	7.0 (5.0, 10.0)	8.0 (6.0, 13.0)	0.068
Maximum Stone Diameter (mm)			<0.001
(0, 5]	361 (26%)	18 (21%)	
(10, 15]	245 (18%)	14 (16%)	
(5, 10]	670 (49%)	36 (42%)	
>15	95 (6.9%)	17 (20%)	
Stones in Multiple Locations	390 (27%)	31 (36%)	0.10
Ureteral Dilatation Performed	291 (21%)	15 (17%)	0.5
Ureteral Access Sheath Used	738 (52%)	59 (69%)	0.002
Procedure Type			0.6
Basket Only	142 (10.0%)	7 (8.0%)	
Laser	1,281 (90%)	80 (92%)	
Ureteral Stent Placed At Time Of Surgery	1,099 (77%)	78 (90%)	0.007
Stent Dwell Time (Days)	7.0 (5.0, 11.0)	9.0 (6.0, 16.0)	<0.001
Stone-Free	774 (56%)	30 (36%)	<0.001
Intra-Operative Complications	6 (0.4%)	1 (1.1%)	0.3
Post-Op CT Scan	223 (16%)	18 (21%)	0.2
Post-Op Ultrasound	1,248 (88%)	76 (87%)	>0.9
Patient Discharged With Alpha-Blockers	883 (62%)	53 (61%)	0.8

¹ n (%); Median (IQR)

² Pearson's Chi-squared test (categorical variables); Wilcoxon rank sum test (continuous variables); Fisher's exact test (intra-operative complications variable on account of low sample size)

HEALTH RELEVANCE: Our findings suggests that a goal directed approach for imaging seems reasonable and development efforts are in progress.

Sources of Funding: Blue Cross and Blue Shield of Michigan

(G2-12) THE BIOCHEMICAL ECOLOGY OF THE UROBIOME AFTER MENOPAUSE - FUNDAMENTAL INSIGHTS AND TRANSLATABLE BIOMARKERS FOR RECURRENT URINARY TRACT INFECTION

Michael L. Neugent¹, Neha V. Hulyalkar¹, Debasish Ghosh², Philippe E. Zimmern³, Vladimir Shulaev², Nicole J. De Nisco^{1,3}

¹Department of Biological Sciences, The University of Texas at Dallas, Richardson, Texas' ²Department of Biological Sciences, The University of North Texas, Denton, Texas' ³Department of Urology, The University of Texas Southwestern Medical Center, Dallas, Texas

INTRODUCTION: Postmenopausal (PM) women are an underserved demographic severely impacted by recurrent UTI (rUTI). In a healthy state, the resident urinary microbiome (urobiome) is thought to provide a protective barrier against UTI and to-date, has been well characterized in health and disease. However, only microbial ecology has been assessed, leaving critical knowledge gaps about how this microbial community interacts with its host ecosystem. Of particular interest, the biochemical ecology, the metabolic landscape of the ecosystem, has not been characterized in tandem with the urobiome. Thus, the relationship between the urobiome microbial ecology, biochemical ecology, and susceptibility to recurrent UTIs remains largely unexplored. This study directly assesses differences in the biochemical environment of the urine associated with rUTI and defines a microbe-metabolite association network of the female urinary microbiome.

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. We then performed integrative analyses on microbiome and metabolomic datasets.

RESULTS: By integrating metagenomic and metabolomic data, we find that distinct metabolites, such as methionine sulfoxide and trimethylamine oxide, are associated with differences in urinary microbial diversity. We observe associations between microbial and biochemical beta diversity, highlighting the potential for metabolite-driven ecological shifts that may influence disease susceptibility. We further identify specific lipid species as biomarkers for active rUTI and recurrence risk. Additionally, we identify a potential role of urinary deoxycholic acid (DCA) as a prognostic indicator for rUTI recurrence suggesting that systemic metabolic processes could influence susceptibility, opening new avenues for personalized management.

Variable	All Patients (n=840)	BKVAH (n=45)	No BKVAH (n=799)	p-value	BK DHAemia (n=291)	No BK DHAemia (n=463)	p-value
Recipient variables							
Median age at transplant (IQR) in years	54 (44, 62)	57 (52, 63)	53 (43, 62)	0.088	54 (43, 63)	53 (44, 62)	0.979
Male recipient sex	488 (58.1%)	30 (66.7%)	399 (56.3%)	0.175	176 (60.5%)	253 (54.6%)	0.115
Median recipient BMI (IQR)	28.5 (24.7, 33.2)	27.4 (23.3, 33.5)	28.6 (24.8, 33.1)	0.366	28.2 (24.6, 33.5)	28.8 (25.0, 32.9)	0.571
Hypertension	736 (87.6%)	27 (60.2%)	612 (89.1%)	0.160	251 (86.3%)	418 (90.3%)	0.090
Diabetes Mellitus	348 (41.4%)	23 (51.1%)	325 (46.6%)	0.158	121 (41.6%)	190 (41%)	0.883
Prior transplant (SOT or SCT)	140 (16.7%)	9 (20.0%)	112 (15.8%)	0.458	51 (17.5%)	70 (15.1%)	0.381
Already receiving IST at time of transplant	132 (15.7%)	6 (13.3%)	107 (15.1%)	0.746	48 (16.5%)	65 (14.0)	0.365
Donor variables							
Male donor sex	450 (53.6%)	26 (57.8%)	375 (52.9%)	0.541	151 (51.9%)	250 (54.0%)	0.425
Median donor BMI (IQR)	27.3 (23.7, 34.6)	27.4 (23.3, 30.3)	27.3 (23.3, 31.6)	0.353	26.9 (23.0, 31.2)	28.5 (24.0, 34.6)	0.063
Donor CDC PHS high risk	224 (26.7%)	13 (28.9%)	191 (26.9%)	0.671	81 (27.8%)	123 (26.6%)	0.614
Deceased donor type	634 (75.5%)	39 (86.7%)	523 (73.8%)	0.061	214 (73.5%)	348 (75.2%)	0.619
Transplant variables							
Median KDPI (IQR)	53% (30.5%, 72%)	48% (35.5%, 66%)	53% (30.8%, 72%)	0.915	49.5% (28%, 69.8%)	53% (31%, 73%)	0.373
Ureteral stent placed	596 (71%)	30 (66.7%)	500 (70.5%)	0.584	192 (66%)	338 (73%)	0.040
Multivisceral transplant	102 (12.1%)	6 (13.3%)	79 (11.1%)	0.653	36 (12.4%)	49 (10.6%)	0.450
Median cold ischemia time (IQR) in minutes	987.5 (345.5, 1379.8)	988 (775, 1296)	957.5 (261, 1378)	0.361	951 (321.2, 1398.5)	979 (326, 1373.5)	0.965
Median warm ischemia time (IQR) in minutes	28 (24, 33)	27 (24, 32)	28 (24, 33)	0.595	28 (23, 32)	28 (24, 33)	0.340
Basiliximab induction	144 (17.1%)	9 (20.0%)	116 (16.4%)	0.525	39 (13.4%)	86 (18.6%)	0.064
Anti-thymoglobulin induction	355 (42.3%)	18 (40%)	296 (41.7%)	0.818	125 (43%)	189 (40.8%)	0.563
Alemtuzumab induction	65 (7.7%)	5 (11.1%)	57 (8.0%)	0.469	26 (8.9%)	36 (7.8%)	0.573
Lymphodepleting induction	416 (49.5%)	23 (51.1%)	349 (49.2%)	0.820	148 (50.9%)	224 (48.4%)	0.496
Tacrolimus maintenance IST	777 (92.5%)	41 (91.1%)	652 (92.0%)	0.840	264 (90.7%)	429 (92.7%)	0.344
Mycophenolic acid maintenance IST	793 (94.4%)	41 (91.1%)	671 (94.9%)	0.322	277 (95.2%)	435 (94.0%)	0.472
Sitrolimus maintenance IST	81 (9.6%)	7 (15.6%)	69 (9.7%)	0.213	33 (11.3%)	43 (9.3%)	0.363
Belatacept maintenance IST	100 (11.9%)	5 (11.1%)	90 (12.7%)	0.757	40 (13.7%)	55 (11.9%)	0.452
Delayed graft function	265 (31.5%)	14 (31.1%)	207 (29.2%)	0.716	86 (29.6%)	135 (29.2%)	0.899
Rejection	139 (16.5%)	13 (28.9%)	99 (14.0%)	0.008	54 (18.6%)	58 (12.5%)	0.024
Ureteral stenosis or obstruction	38 (4.5%)	0 (0%)	33 (4.7%)	0.983	17 (5.8%)	16 (3.5%)	0.121

CONCLUSIONS: We present new associations between the postmenopausal urobiome and urinary metabolites utilizing WGMS sequencing and targeted LC-MS/MS. These associations may provide critical insight as biomarkers of urobiome ecology, which we are now translating into mechanistic study.

FUNDING: This work was supported by the Welch Foundation (AT- 2030-20200401) and the NIH (1R01DK131267-01) to N.J.D. and (1F32DK128975-01A1) to M.L.N. This work was also supported by The Felicia and John Cain Distinguished Chair in Women's Health to P.E.Z.



(G2-14) RATES OF BK VIRUS AND URETERAL COMPLICATIONS IN RENAL TRANSPLANT RECIPIENTS

Bin Ni¹, J. Eric Jelovsek², Cameron Miller³, Kent Weinhold⁴, Barbara Alexander¹

¹Division of Infectious Diseases, Department of Medicine, ²Department of Obstetrics and Gynecology, ³Center for Applied Genomics & Precision Medicine, Department of Medicine, ⁴Duke Immune Profiling Core, Department of Surgery, Duke University School of Medicine, Durham, North Carolina, USA.

Introduction and objective: BK polyoma virus is highly prevalent, acquired in childhood, and thereafter remains latent in the renourinary tract. Reactivation in renal transplant recipients can lead to complications such as ureteral stenosis and BK virus-associated nephropathy (BKVAN). Reported frequencies of these complications vary widely due to differences in BK management and immunosuppressive regimens, with sparse, outdated data on ureteral stenosis. Newer immunosuppressants, such as belatacept, are linked to more frequent and severe BK infections, though findings are mixed. This study aims to update the epidemiology of BK reactivation and complications in renal transplant recipients in order to develop risk prediction models from a large Duke University Hospital (DUH) cohort.

Methods: This retrospective study included all renal transplant recipients (single or multi-organ) at DUH between 1/1/2016 and 12/31/2020. Clinical and demographic data, including BK reactivation and outcomes, were abstracted from records, and donor data were collected from the UNOS STAR database. Variables were compared using simple logistic regression analysis.

Results: 840 patients received renal transplants during the study period, with 754 (89.8%) having complete 2-year follow-up. Of those without full follow-up, 37 (4%) had died, 14 (2%) were lost to follow-up, and 35 (4%) lost their graft. Among patients with complete follow-up, 45 (6%) developed BKVAN (15 probable, 20 presumptive, 10 biopsy-proven), with a median diagnosis time of 161 days (IQR: 103, 284). BK DNAemia occurred in 291 patients (39%), with a median time to DNAemia of 119 days (IQR: 66, 303). 136 (18%) patients had high-level DNAemia ($\geq 10,000$ copies/mL). Ureteral stenosis or obstruction occurred in 33 patients (4%), 17 (52%) of whom also had BK DNAemia, but none had histologically confirmed BK-related ureteral complications.

Conclusions: We observed BKVAN in 6%, BK DNAemia in 39%, and ureteral stenosis/obstruction in 4% of renal transplant recipients. Though half of stenosis cases had BK DNAemia, no direct causal link was established. These data will be further utilized in the development of BK risk prediction models.

Health Relevance: This study updates the incidence of BK reactivation and its complications in renal transplant recipients under contemporary immunosuppressive regimens, offering valuable data to support the development of risk prediction models aimed at improving clinical management and outcomes.

(G2-26) SEX HORMONES CONTRIBUTE MORE TO THE DEVELOPMENT OF DIABETIC BLADDER DYSFUNCTION THAN THE SEVERITY OF HYPERGLYCEMIA IN TYPE 1 DIABETIC AKITA MICE

Michael R. Odom, Francis M. Hughes Jr., Huixia Jin, J. Todd Purves

Department of Urology, Duke University School of Medicine, Durham, NC

Introduction and Objective: Diabetic bladder dysfunction (DBD) presents as a variable range of symptoms from overactive bladder (OAB) to underactive bladder (UAB), and the mechanisms responsible for such variability remain elusive. Current dogma suggests DBD begins as OAB and progresses to UAB as a consequence of poor glycemic control. However, clinical studies show that optimal glycemic control does not impact DBD presentation. One family of compounds that is known to regulate bladder function in other disease states is sex hormones and diabetes dysregulates sex hormone-dependent mechanisms to facilitate pathology in other tissues. Therefore, we hypothesize diabetes dysregulates sex hormone-dependent mechanisms in the bladder to facilitate the development of DBD. Here, we test this hypothesis by determining how the absence of primary sex hormones

via gonadectomy impacts blood glucose and the development of DBD in a mouse model of type 1 diabetes.

Methods: Male and female type 1 diabetic Akita mice on a C57BL/6J background as well as male and female non-diabetic C57BL/6J mice (WT) underwent either a gonadectomy at 8 weeks of age or remained gonadally intact. Blood glucose (BG) was measured weekly from 8-15 weeks of age ($n=9-15$ per group). At 15 weeks of age, awake-restrained cystometry was performed ($n=9-11$ per group).

Results: In gonadally intact mice, both diabetic sexes exhibit higher BG than WT ($p<0.05$), and BG of diabetic males is higher than diabetic females ($p<0.05$). Compared to WT, male diabetics show an increase in void volume and decrease in voiding frequency consistent with signs of UAB ($p<0.05$) while female diabetics show a decrease in void volume and increase in voiding frequency consistent with signs of OAB ($p<0.05$). After a gonadectomy, BG of gonadectomized male diabetics decreases to levels comparable to female diabetics, while BG of gonadectomized female diabetics increases to levels comparable to male diabetics. Surprisingly, despite the inverse trend in the severity of hyperglycemia, gonadectomized groups of male and female diabetics fail to develop signs of DBD as their void volumes and frequencies differ from their gonadally intact groups ($p<0.05$) and become comparable to WT. Gonadectomies do not alter BG and voiding parameters of WT.

Conclusions: Sex hormone-dependent mechanisms regulating bladder function become dysregulated by diabetes and facilitate the development of DBD. Gonadectomies minimize the androgens and estrogens required to activate these mechanisms and prevent the early development of respective UAB and OAB due to diabetes – regardless of the severity of hyperglycemia.

Health Relevance: Sex hormone-dependent mechanisms that are dysregulated by diabetes may serve as novel therapeutic targets to prevent or treat DBD.

(G1-2) BLADDER EPITHELIUM-DERIVED ORGANIDS: A NEW MODEL TO INVESTIGATE THE ROLE OF METABOLISM IN AGING

Adwaita R. Parab,¹ Arnold M. Salazar,¹ Margarita Divenko,² Steven J. Bark,¹ Nagireddy Putluri³, Indira U. Mysorekar¹

¹Department of Medicine, Infectious Diseases, Baylor College of Medicine, Houston, TX, United States ²Department of Medicine, Gastroenterology, Baylor College of Medicine, Houston, TX, United States ³Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, United States

INTRODUCTION AND OBJECTIVE: Aging is a progressive decline in molecular, cellular, and tissue functions leading to chronic conditions disproportionately affecting postmenopausal women. The field of aging lacks consensus on the best model for studying aging-related processes, as current models face significant limitations in balancing feasibility and biological relevance. We propose mouse bladder epithelium-derived organoids (mBEDOs) as a new platform for conducting high-throughput studies for assessing cellular and metabolic dysfunction in bladder aging and advancing targeted interventions via metabolite supplementation.

METHODS: mBEDOs were derived using bladder tissue from young (6-8 weeks) and aged (15-24 months) mice and evaluated for urothelial marker expression through histopathology and immunofluorescence. Aging markers DNA damage, oxidative stress, and aberrant mitochondrial and lysosomal compartments were characterized. 2D mBEDOs were infected with uropathogenic *E. coli* (UPEC) at 1 and 3 hours post infection (hpi). Bone marrow-derived macrophages (BMDMs) were co-cultured with mBEDOs using transwell inserts. LC-MS/MS was performed and differentially altered metabolites were identified and validated using student's t-test. mBEDOs were supplemented with 1mM nicotinamide (NAD) and 25 mM D-Mannose and then examined using previously established markers.

RESULTS: Histopathological analysis revealed multi-layered organoids with lumen, positive for urothelial markers cytokeratin-5 and uroplakin. mBEDOs recapitulated aged bladder phenotypes such as slower growth and differentiation rates, heightened DNA damage, senescence, oxidative stress, increased mitochondria and lysosomes and aberrant antioxidative response. UPEC infection of 2D mBEDOs showed intracellular bacteria with higher levels of bacteria at 3 hpi in aged mBEDOs. BMDMs and mBEDOs



were successfully co-cultured as indicated by cell viability. Metabolomics revealed 20 downregulated (D-Mannose, NAD) and 21 upregulated (adenine, guanine, uracil) metabolites in aged mBEDOs. Treatment with NAD and D-Mannose lowered levels of oxidative stress and mitochondria, and increased expression of antioxidant genes *Nrf2* and *Gclc*.

CONCLUSIONS: We establish mBEDOs as a model to study aging associated cellular and molecular alterations. mBEDOs may be used to test interactions of the epithelial and immune compartments and infectious agents. Metabolite supplementation in mBEDOs improved mitochondrial function.

HEALTH RELEVANCE: mBEDOs are a feasible platform for conducting high throughput metabolic studies and improving aging markers via metabolite supplementation. Our approach may lead to neoteric therapies aimed at revitalizing the lower urinary tract.

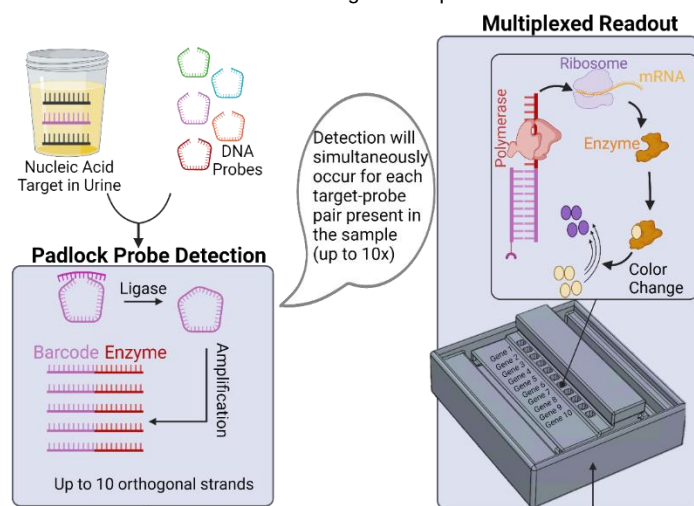
(G1-3) A CELLULAR ATLAS OF HUMAN LOWER URINARY TRACT IN AGING AND DISEASE

Emily Paterson¹, Lari Smith¹, Nikita Malev¹, Jashkaran Gadhvi¹, Samuel Cornelius², Alicia Malewska¹, John Ladin¹, Douglas Strand¹

University of Texas Southwestern Medical Center¹, University of Texas at Dallas²

INTRODUCTION AND OBJECTIVE: Benign and malignant disease of the lower urinary tract (LUT) are common in aging. Fundamental questions regarding the disparate incidence and progression of LUT disease in sex, age, and ethnicity require and understanding of normal. Our objective is to create a cellular atlas of normal adult male and female LUT using single-cell, spatial, and Xenium technologies.

METHODS: Bladder, ureter, urethra, and prostate (from males) were collected fresh from the Southwest Transplant Alliance organ donors and enzymatically digested into a single cell suspension, frozen in OCT, or embedded in paraffin. The 10X Genomics Chromium was used to sequence single-cell RNA and single- nuclear RNA/ ATAC sequencing. Data were integrated and analyzed using Seurat. RNA-scope and immunostaining of anchor genes derived from the scRNA data to localize individual cell types for validation. Spatial transcriptomics was performed using both the 10X Genomics Xenium and the Nanostring GeoMx platforms.



RESULTS: Several new cell types have been discovered in the male and female LUT. Some of these cell types may play central roles in various diseases. Each cell type is catalogued by transcriptional and anatomical profile into the Uberon and Cell Ontology databases. Raw and processed data will be publicly available through the Human BioMolecular Anatomy Project and the GenitoUrinary Development Molecular Anatomy Project.

CONCLUSIONS: Several new cell types have been identified as central to LUT disease. The expansion and transcriptional alteration of an interstitial fibroblast in the periurethral area of the prostate is a new preclinical target in benign prostatic hyperplasia. The identification of antimicrobial club cells in the female urethra is a new preclinical target in urinary tract infections.

Identification of novel fibroblasts could help explain the mechanism in which non-muscle invasive bladder cancer progresses into muscle invasive bladder cancer. The comparison of androgen signaling in seminal vesicle and prostate could help us understand prostate cancer development. This healthy male and female LUT cellular atlas will be instrumental for understanding the cellular basis of disease incidence and progression.

HEALTH RELEVANCE: Generating a resource for cell types and functions of the LUT from different ages will be incredibly useful for the development of therapeutics and strategies against dysfunction and disease in the aging LUT.

(G1-16) TOWARDS RAPID AND SENSITIVE POINT-OF-CARE DIAGNOSTICS FOR URINARY TRACT INFECTIONS

Alexandra Patterson,¹ Andrew Wheeler,² Jiho Seok,¹ Mike Farrell,³ Maria Hadjifrangiskou,⁴ Mark Styczynski¹

¹School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, ²Glocal Access Diagnostics, ³Georgia Tech Research Corporation, ⁴ Department of Pathology, Microbiology and Immunology, Division of Molecular Pathogenesis, Vanderbilt University Medical Center

Introduction and Objective: Despite the 400 million cases yearly, there is a dearth of accurate and convenient diagnostics for urinary tract infections (UTIs). Specifically, the gold-standard UTI diagnostic, urine culture, can take 48 hours to yield results and is prone to false negatives. Accordingly, antibiotics are often prescribed before diagnostic results are received which can lead to unnecessary antibiotic use. Given the proliferative nature of UTI and rapid rise of antibiotic resistance, it is imperative that a diagnostic is developed which can rapidly and sensitively diagnose UTI. To address this need, we are developing a novel point-of-care (POC) platform which can simultaneously detect 20 uropathogenic and antibiotic resistance genes at a sensitivity close to PCR.

Methods: The diagnostic platform will be comprised of five steps. Briefly, 1) when a DNA target is present, the 5' and 3' ends of a reporter probe are circularized via T4 DNA ligase. 2) Once circularized, Phi29 DNA polymerase amplifies the probe. 3) The probes are separated into different reporter regions via a barcode system. 4) *In vitro* transcription and translation (IVTT) reagents transcribe and translate the probes, resulting in expression of a colorimetric enzyme. 5) The enzyme catalyzes a localized color change, which can be directly correlated with the presence of a specific DNA or RNA target. This process will be optimized to occur in less than an hour with little-to-no end-user required steps.

Results: To date, we have completed development of the multiplexed readout. Specifically, we have developed a barcode system (based on base pairing) capable of separating multiplexed probes into different reporter regions. By coupling the barcode system with IVTT, we can sensitively detect captured probes, allowing for a rapid color change visible to the naked-eye at less than 10⁷ copies of probe. Initial proof-of-concept tests to integrate the multiplexed readouts with the padlock detection are underway and we are currently working with manufacturers to build and test prototypes of the final device.

Conclusion: The platform shows promise as an effective POC nucleic acid diagnostic. Future work will focus on applying the platform for UTI. Specifically, A) developing a 20-plex panel of uropathogen and antibiotic resistance genes and B) detection in urine.

Health Relevance: Development of an improved POC UTI diagnostic would enable more accurate diagnosis, leading to better patient outcomes and decreased antibiotic usage.

(G2-32) IMMUNOHISTOCHEMICAL DETECTION OF SEROTONIN PRODUCING NEUROENDOCRINE CELLS AND SEROTONIN RESPONDING INTERSTITIAL CELLS OF CAJAL IN THE ADULT MALE MOUSE AND HUMAN URETHRA

Akhil Pidikiti, Chad Vezina

University of Wisconsin – Department of Urology

Introduction and Objective: Lower Urinary Tract Dysfunction (LUTD), which may include incomplete bladder emptying and more frequent urination,



especially at night, is increasingly common among aging men. Elevated prostate and urethral smooth muscle tone is one cause of male LUTD and obstructs urine passage through the urethra. Alpha adrenergic receptor antagonists are currently approved to target the nerve's role in evoking urethral muscle tone, but other factors may contribute to the condition, making these treatments ineffective for some patients. We recently established that neuroendocrine cells of the female urethra release serotonin in response to bacterial infection, driving urethral contraction by stimulating serotonin receptors on urethral pacemaking cells and activating the calcium-activated chloride channel anoctamin 1 (ANO1). The purpose of this study is to test the hypothesis that male prostate and urethra also harbor serotonin-producing neuroendocrine cells, and pacemaking cells that express serotonin receptors and ANO1.

Methods: We used antibodies against serotonin, the epithelial marker cadherin 1, the pacemaking cell marker cKIT, the smooth muscle marker ACTA2, the serotonin receptor HTR2B, and ANO1 to immunolabel five micron lower urinary tract sections of adult male mice (N= 9). We will use the same antibodies to label prostatic urethra sections from human organ donors. We imaged the mouse tissue sections using a fluorescent microscope to localize proteins to urethral and prostate cells.

Results: We anticipate future results to show that the male urethras also contain serotonin-producing neuroendocrine and serotonin-receptor interstitial cells.

Conclusion: Our results supported our hypothesis and showed that male urethras contained the same serotonin-producing endocrine cells and serotonin-receptor pacemaking cells as the females.

(G2-6) BONE MORPHOGENETIC PROTEIN 5 (BMP5) PLAYS A ROLE IN THE GROWTH OF HUMAN NORMAL AND BPH TISSUE IN PATIENT-DERIVED XENOGRAFT (PDX) MODELS

Alexandra L. Polasko¹, Dalin Zhang¹, Avanti Ramraj¹, Chun-Lung Chiu¹, Fernando J. Garcia-Marques^{2,3}, Abel Bermudez^{2,3}, Kathryn Kapp^{2,3}, Eric Peterson¹, Zhengyuan Qiu¹, Anna S. Pollack⁴, Hongjuan Zhao¹, Jonathan R. Pollack⁴, Sharon J. Pitteri^{2,3}, James D. Brooks^{1,3*}

¹Department of Urology, Stanford University, ²Department of Radiology, Stanford University, ³Canary Center at Stanford for Cancer Early Detection, Stanford University

INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH), characterized by hyperplastic growth of stromal and epithelial cells, often leads to lower urinary tract symptoms in aging men. Transcript levels of the signaling protein bone morphogenetic protein 5 (BMP5) produced by prostate fibroblasts are upregulated 50-fold in human BPH tissues. Our objective was to utilize our novel BPH PDX model to determine the effects of exogenous BMP5 and BMP inhibitors on BPH or N PDX growth and survival.

METHODS: BPH transition or normal (N) peripheral zone prostate tissue cores were obtained from 4 patients and implanted subrenally into RAG2^{-/-}γC^{-/-} male mice (n = 39) supplemented with testosterone. Tissues were grafted for 1 month and then treated over a two-week period, with recombinant BMP5 administered once weekly at 0.2 mg/kg, and the BMP5 inhibitor (K02288, a small molecule inhibitor targeting BMP receptors) given thrice weekly at 0.4 mg/kg. Histopathological changes were visualized by H&E and immunohistochemistry using antibodies against Ki-67, cleaved caspase-3, and pSMAD1/5/8. Markers were quantified with QuPath software and statistical differences were determined using a t-test or ANOVA. Serum prostate-specific antigen (PSA) was measured at the time of explant, and molecular analyses were performed using LC-MS.

RESULTS: N PDX weights showed increases in size after treatment with BMP5 compared to those treated with vehicle. Proliferation in BMP5-treated N PDXs was significantly higher than vehicle-treated PDXs, which was largely driven by epithelial cell growth. Correspondingly, PSA concentrations were elevated in BMP5-treated N PDXs. Molecular profiling revealed protein expression patterns that correlated with IHC results such as decreased apoptosis after BMP5 treatment. BPH PDXs treated with the BMP5 inhibitor (K02288) showed significantly decreased weight compared to vehicle, as well as decreased proliferation and PSA. BPH PDXs treated with BMP5 did increase in weight similar to vehicle controls.

CONCLUSIONS: Addition of BMP5 to N PDXs demonstrated increased growth, proliferation, and serum PSA levels similar to those seen in BPH PDXs. Treatment of BPH PDXs with BMP5 did not significantly increase growth or proliferation, possibly because of high levels of endogenous BMP5 in the BPH PDXs. In contrast, treatment with the BMP inhibitor (K02288) decreased growth in BPH PDXs as well as proliferation and PSA. PDX models can be used to test candidate growth factor pathways. BMP5 treatment of N or BPH PDXs promotes stromal and epithelial cell growth and survival. Our data nominate BMP5 inhibition as a candidate therapeutic target for human BPH.

HEALTH RELEVANCE: This BPH PDX model addresses the need for clinically relevant models of BPH and enables pre-clinical testing of novel treatments. BMP5 inhibitors are a potential novel therapeutic for BPH.

(G2-21) G-PROTEIN COUPLED RECEPTORS AND CALCIUM-ACTIVATED CHLORIDE CHANNELS REGULATE SPONTANEOUS CALCIUM ACTIVITY AND PHASIC SMOOTH MUSCLE CONTRACTIONS IN MOUSE URINARY BLADDER

Jason L. Rengo¹, Hannah J. Fallon¹, Thomas J. Heppner¹, Grant W. Hennig¹, Mark T. Nelson^{1,2}, Gerald M. Herrera¹

¹Department of Pharmacology, Larner College of Medicine, University of Vermont, Burlington, VT; ²Division of Cardiovascular Sciences, University of Manchester, Manchester, UK

Introduction and Objective: Detrusor smooth muscle (DSM) of the bladder exhibits spontaneous Ca²⁺ activity that can propagate 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 (CNS). Calcium-activated chloride channels (CaCl) modulate membrane potential and regulate smooth muscle excitability in the gastrointestinal tract, while G protein-coupled receptors (GqPCR) influence bladder contraction and relaxation. The purpose of this study was to investigate the role of GqPCR and CaCl on TPEs in isolated perfused mouse urinary bladder. We hypothesized GqPCR and CaCl inhibition would reduce Ca²⁺ activity and TPE frequency and amplitude.

Methods: Urinary bladders were isolated from male and female C57Bl/6J mice and B6-GC6f x SMMHC- CreER^{T2}. Ureters were tied off, the bladder was cannulated through the urethra and submerged in an organ bath with physiological saline solution. The bladder was attached to a syringe pump and inline pressure transducer and filled to 80% capacity while recording intraluminal pressure. TPEs were allowed to stabilize for 30 minutes before applying the GqPCR inhibitor YM-254890 (YM) (500nM) or CaCl blocker benzbramarone (10μM). Recordings were analyzed with Spike2 software (Cambridge Electronic Design). Ca²⁺ activity was quantified using a custom "Muscle Action Potential" (MAP) differential extraction protocol (Volumetry G9J).

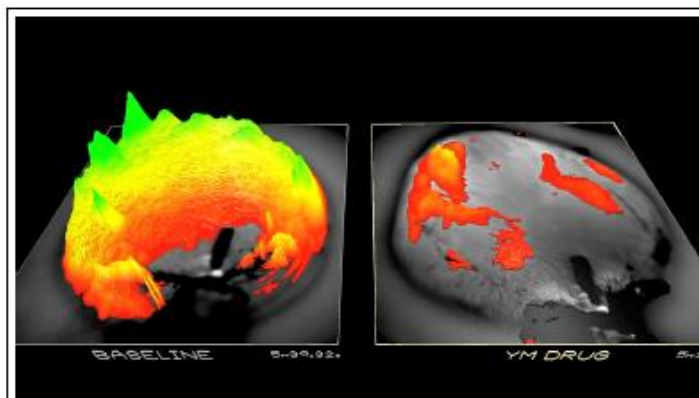


Figure 1. Representative example of accumulated Ca²⁺ events over 5+ minutes before and after inhibition of GqPCR (Seconds of Ca²⁺ Activity Across Bladder Area).



Results: Inhibition of GqPCR signaling decreased mean TPE frequency (88%, $p=0.003$) and amplitude (94%, $p=0.049$) in $n=8$ bladders. Similarly, blocking CaCl_2 resulted in decreases in frequency (76%, $p=0.001$) and amplitude (88%, $p=0.024$) in $n=5$ bladders. Wide field fluorescent microscopy revealed striking reductions in Ca^{2+} activity with both YM ($n=6$) (Fig 1) and benzbromarone ($n=2$).

Conclusions: We found YM and benzbromarone disrupted TPE frequency, amplitude, and Ca^{2+} signaling. This suggests both GqPCRs and CaCl_2 play a role in generating rhythmic DSM contractions. A connection between GqPCRs and CaCl_2 remains to be explored.

Health Relevance: These results highlight the importance of GqPCRs and CaCl_2 in the generation of spontaneous 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.

(G2-23) PERINATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBS) AUGMENT BLADDER SENSITIVITY TO CONTRACTILE STIMULI POTENTIALLY THROUGH RYANODINE RECEPTOR BINDING AND INCREASED BK CHANNEL EXPRESSION

Monica Ridlon, Conner L. Kennedy, Alyssa Lentine, Julia Tlapa, Tamryn Jordan, Kathy Wang, Lindsey Felth Tanaka, Kimberly K. Stietz

Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI.

INTRODUCTION AND OBJECTIVE: BK channels (large-conductance Ca^{2+} -activated K^+ channel) are outwardly rectifying K^+ channels in the detrusor providing negative feedback for myogenic and nerve-evoked contractions through membrane potential maintenance and shaping current rectification. BK channel expression and kinetics, and pressure-dependent myogenic tone, are influenced by RyRs (ryanodine receptors), which can be opened by polychlorinated biphenyls (PCBs). PCBs impact murine urinary voiding physiology including increased intravesical pressure during anesthetized cystometry. We hypothesize PCBs augment contractile activity in part through opening RyRs and increasing BK channel expression in the murine bladder.

METHODS: Female nulliparous mice were dosed with MARBLES PCBs (serum PCB profile of pregnant women enrolled in Markers of Autism Risk in Babies – Learning Early Signs) 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 and purinergic receptors, BK channel, and RyR mRNA abundances. *Ex vivo* tissue baths were performed to measure nerve-evoked and myogenic bladder contraction; nerve-evoked bladder contraction (electrical field stimulation (EFS, 1-50 Hz)), cholinergic neurotransmission (carbachol), purinergic neurotransmission (ATP), and EFS with BK channel opener (NS1021) or closer (paxilline or iberiotoxin). Ongoing work includes observing myogenic contractions when challenged with a BK channel closer and/or ryanodine receptor agonists (caffeine) or antagonists (ryanodine). Analysis utilized one- or two-way ANOVA followed by multiple comparisons tests to analyze PCB exposure group effects and compare to the sex-matched vehicle control group.

RESULTS: PCB exposed mice had sex- and dose-dependent impacts on cholinergic receptors and BK channel subunit mRNA abundance, sensitivity to contractile stimuli, and ability to compensate for BK channel opening and closing. In particular, 1 mg/kg/d male mice compared to male control mice were more sensitive to purinergic or cholinergic stimuli, had decreased cholinergic receptor mRNA, had increased BK channel mRNA, and ryanodine diminished BK channel-mediated myogenic contractions.

CONCLUSIONS: Human-relevant PCBs impact murine urinary voiding physiology in part through augmented myogenic and nerve-evoked contraction. PCBs may open RyRs in the detrusor to influence bladder contractile sensitivity and lead to 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.

(G2-30) OPTICAL INHIBITION WITH TRANSDERMAL LIGHT ILLUMINATION REDUCES BLADDER NOCICEPTIVE BEHAVIORS IN MICE

Gabriella L Robilotto^{1,2}, Rebecca Bornstein³, Daniel Zelmanoff³, Nia A Dufael¹, Ofer Yizhar³, and Aaron D Mickle^{1,2,4,5}

¹Department of Physiological Sciences, University of Florida, Gainesville FL,

²Department of Physiology, Medical College of Wisconsin, Milwaukee, WI,

³Department of Neurobiology, Weizmann Institute of Science, Rehovot,

Israel, ⁴Department of Neurosurgery, Medical College of Wisconsin,

Milwaukee WI, ⁵Department of Urology, Medical College of Wisconsin, Milwaukee WI

INTRODUCTION AND OBJECTIVE: Optogenetic tools are used to better understand neural circuits. However, silencing these pathways remains challenging, as other inhibitory opsins pose difficulties with prolonged inhibition and can cause off-target effects. This is especially challenging when targeting pathways that innervate the bladder. These techniques require surgical implantation of fiber optics or wireless devices to activate opsins in freely moving animals, furthering animal stress. Here, we used a mosquito-derived homolog of mammalian encephalopsin/panopsin protein (OPN3), packaged into PHP.S, which has been used to inhibit neurotransmission *in vivo* with transdermal light illumination. Therefore, we hypothesize that transdermal light-activated PHP.S-OPN3-mScarlet will inhibit neurotransmitter release by primary sensory afferents and suppress bladder nociceptive responses in mice.

METHODS: We injected AAV PHP.S-OPN3-mScarlet intraperitoneally (IP) into neonatal mice postnatal day 3 for broad peripheral nervous system transduction. On day 84, we performed place preference and abdominal von Frey for baseline assessments with and without optical inhibition with infrared LED illumination. A single IP cyclophosphamide injection was administered to induce acute bladder cystitis and behavioral assessments were repeated. The dorsal root ganglia (DRG) and spinal cord tissue were evaluated for PHP.S-OPN3-mScarlet expression.

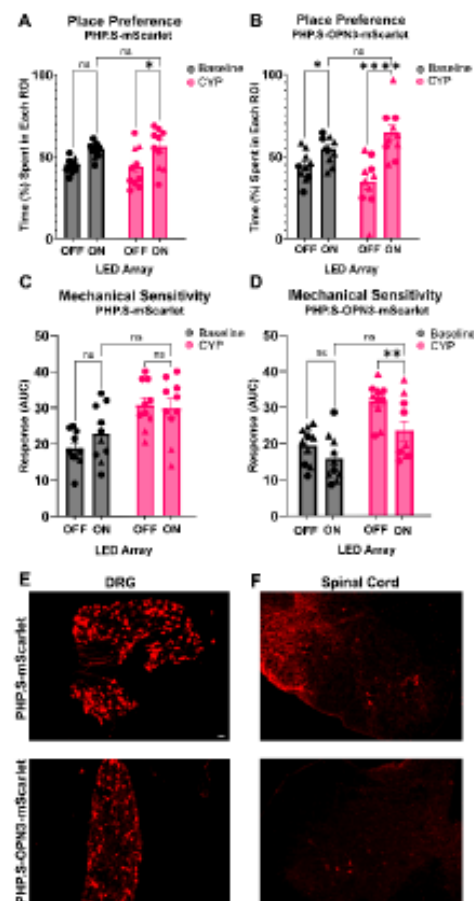


Figure 1. PHP.S-OPN3-mScarlet mice spend more time in LED region after acute cystitis. Place preference graphical representation of the percent time spent in each region before and after cyclophosphamide for PHP.S-mScarlet (A) and PHP.S-OPN3-mScarlet (B). Von Frey mechanical hypersensitivity with and without the LED (C, D). Representative images of mScarlet expression in L6-S1 DRG (E) and in the lumbosacral spinal cord (F). CYP, cyclophosphamide; ROI, region of interest; AUC, area under the curve; DRG, dorsal root ganglia. PHP.S-mScarlet [$n=2$ males (triangles), $n=8$ females (circles)]. PHP.S-OPN3-mScarlet [$n=7$ males (triangles), $n=3$ females (circles)]. Scale bar = 50 μm . $P < 0.05$.



RESULTS: PHP.S-OPN3-mScarlet mice spent more time in the infrared LED-illuminated chamber after acute cystitis (**B**) and had less abdominal sensitivity in response to noxious stimuli upon LED stimulation (**D**). The data suggests some nociceptive suppression with LED illumination. We expected expression of the primary sensory neurons of the L6-S1 DRG and the projections into the dorsal horn of the L4-L6 spinal cord innervating the bladder. We found robust PHP.S- OPN3-mScarlet expression in the L6-S1 DRG (**E**) and minimal expression within the spinal cord (**F**).

CONCLUSIONS: This technique offers a novel optogenetic approach *in vivo* that does not require surgical intervention to manipulate neural pathways. OPN3 may have inhibitory effects on bladder nociception and its expression level may correlate to behavior responses. Further studies are needed to fully understand the bladder pain neural circuits.

HEALTH RELEVANCE: Painful bladder conditions have high public health incidence, especially in women. The optogenetic technique used in this study is an innovative, noninvasive tool that could be used to understand the neural circuits associated with bladder pain.

FUNDING: Rita Allen Foundation and NIHEB031249

(G2-17) DISRUPTING BIOFILMS ON HUMAN KIDNEY STONES: A PATH TOWARD REDUCING POSTOPERATIVE INFECTIOUS COMPLICATIONS

Daniel Massana Roquero^{1,2}, Grace H. Holton^{1,2}, T. Jessie Ge^{1,2}, Zachary Kornberg^{1,2}, Gabriela Rodriguez¹, Vinh La¹, Hubert Lau^{2,3}, Kathleen E. Mach^{1,2}, Joseph C. Liao^{1,2}

¹Department of Urology, Stanford University, Stanford, ²VA Palo Alto Health Care System, Palo Alto, USA

INTRODUCTION AND OBJECTIVE: Pathogenic bacteria are found in almost 40% of stones, where they form biofilms that are protected from systemic antibiotic treatments. Stone surgeries can disperse these bacterial biofilms throughout the urinary tract which can ultimately lead to urinary tract infections or sepsis, the most common and detrimental postoperative complications, respectively. The aim of this study is to determine the antimicrobial efficacy of chitosan, an antimicrobial polymer, in eradicating bacterial biofilms present in the human stones as well as its safety in *in vivo* porcine models.

METHODS: With IRB approval, kidney stone fragments, representing a clinically relevant model of biofilms, were collected from patients undergoing stone surgery. The fragments were incubated for 90 minutes in either saline or chitosan in saline, followed by stone culture and bacterial identification. Scanning electron microscopy (SEM) on culture-positive stones was used to confirm the antimicrobial and antibiofilm effects. In the *in vivo* experiments, anesthetized pigs underwent bladder irrigation with the chitosan-based antimicrobial solution for 90 minutes. Blood was collected before and after irrigation and then, after necropsy, the bladders were harvested for gross examination and histological evaluation.

RESULTS: Out of the 57 stone specimens tested in this cohort, 19 of them had a positive stone culture, predominantly by *Enterococcus faecalis*, *Escherichia coli*, and *Proteus mirabilis*. Interestingly, all 19 patients with positive stone culture received preoperative antibiotic treatments. Regardless of bacterial strain or stone composition, all 19 specimens showed, at least, a 96% bacterial burden reduction after treatment with chitosan. SEM confirmed the biofilm and bacterial integrity disruption. Bladders irrigated with chitosan had no gross abnormalities compared to healthy bladders and we only observed mild urothelial desquamation without other histopathological changes.

CONCLUSIONS: We demonstrated that both Gram-positive and Gram-negative bacteria forming biofilms in human kidney stones can be effectively killed in a short duration treatment. Moreover, we showed the lack of toxicity nor body response to chitosan irrigation *in vivo*. With further clinical study, chitosan could potentially be used as an irrigation adjunct and provide an effective and safe approach to reduce postoperative infectious complications following kidney stone surgeries.

HEALTH RELEVANCE: Kidney stones harbor bacteria that frequently go undetected and are protected from systemic antibiotic treatments. The use

of a broad-spectrum antimicrobial irrigation at the time of surgery could help kill uropathogens and prevent or reduce postoperative infections.

(G1-17) MAGNETIC STONE REMOVAL: FEASIBILITY AND SAFETY IN PORCINE MODELS

Daniel Massana Roquero^{1,2}, T. Jessie Ge^{1,2}, Grace H. Holton^{1,2}, Jacob T. Bryan³, Zachary Kornberg^{1,2}, Kathleen E. Mach^{1,2}, Shan X. Wang³, Joseph C. Liao^{1,2}

¹Department of Urology, Stanford University, Stanford, ²VA Palo Alto Health Care System, Palo Alto, ³Department of Materials Science and Engineering, Stanford University; USA

INTRODUCTION AND OBJECTIVE: Current surgical approaches for kidney stone removal often fail to achieve stone-free status. Recently, we developed MagStone, a medical device to coat stone fragments with a magnetic hydrogel followed by retrieval with a magnetic wire. The objective of this study is to investigate the feasibility of MagStone and the safety of the magnetic hydrogel in a porcine model.

METHODS: With APLAC approval, pigs were divided into acute (n=4) and chronic (n=3) studies. The renal pelvis was accessed retrogradely with a clinical ureteroscope. In the acute studies, renal units underwent stone removal with MagStone (n=4, feasibility), or just received the magnetic hydrogel without stones (n=2, safety). Basketing was conducted to assess trauma associated with ureteroscopy (n=2, control). In the chronic studies, the magnetic hydrogel was instilled in one kidney and left without removal while the other kidney remained untreated as control. Animals were monitored for a week. Blood and urine were collected for CBC, chemistry and urinalysis. Following euthanasia, histological analysis of the kidneys, ureters, and bladder was used to assess toxicity. Magnetic particle imaging (MPI) of those organs and liver, spleen and lung was used to study magnetic hydrogel biodistribution and clearance.

RESULTS: Stones were successfully removed from the kidney confirming that the MagStone device is compatible with ureteroscopy and effective. Perioperative bloodwork and vitals fell within normal thresholds and there were no signs of allergic reactions to the hydrogel. In histopathology, mild inflammation and fibrinous exudate formation were observed in the renal units undergoing stone removal with either the MagStone or the basket. These conditions are typically associated to the trauma caused by the extensive use of ureteroscopic instrumentation. No histopathological abnormalities were detected in the kidneys that simply received the magnetic hydrogel. In the chronic studies, we did not detect any remaining hydrogel in the urinary tract nor systemically indicating that urine flow is the natural mechanism of hydrogel elimination.

CONCLUSIONS: We demonstrated that MagStone can be used during ureteroscopy to remove stone fragments. The peri- and post-operative safety of the magnetic hydrogel was confirmed by the absence of abnormalities in bloodwork, urinalysis, and histology. Also, we demonstrated that residual hydrogel did not become systemic and was cleared from the urinary tract within 1 week by normal urine flow.

HEALTH RELEVANCE: By allowing more effective and efficient stone removal, MagStone could ultimately improve surgical stone free rates and prevent or reduce postoperative complications and stone recurrence.

(G1-9) ADVANCING PRE-CLINICAL THERAPEUTIC EVALUATIONS FOR URINARY DYSFUNCTION: ADAPTING VOID SPOT ASSAY FOR USE IN RATS

Hannah Ruetten^{1,2}, Jadyne Bothe¹, Shannon Lankford¹, Gopal Badlani^{3,4}, and James Koudy Williams¹

¹Wake Forest Institute for Regenerative Medicine, Wake Forest Baptist Medical Center, Winston-Salem, North Carolina; ²Department of Pathology, Section on Comparative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina; ³Department of Urology, Wake Forest Baptist Medical Center Winston-Salem, North Carolina; ⁴Section of Urology, VA Medical Center, Salisbury, North Carolina; USA

INTRODUCTION AND OBJECTIVE: The void spot assay (VSA) is a noninvasive method for evaluating urinary dysfunction in pre-clinical animal



models. It involves placing a rodent in an enclosure lined with absorbent filter paper, allowing the animal to move freely while its micturition is captured. VSA is typically performed on mice due to the difficulty in analyzing larger papers from rat home cages, despite the preference for rat models in surgical intervention pre-clinical testing due to their larger size. This study aims to optimize VSA for rats by testing agents to alter urine color (beets, raspberries, and Phenazopyridine) and comparing post-assay visualization techniques: bright field light (BF), ultraviolet light (UV), and ninhydrin spray (N).

METHODS: At baseline of their respective study 32 male mixed background rats were placed single housed in a cage lined on the bottom by Whatman grade filter paper (27x16cm) for 4 hours (9AM to 1PM EST). Water and food was provided *ad lib* and a Nylabone was provided to discourage paper chewing. After the assay all paper were dried in a biosafety cabinet overnight. BF images were captured with a Nikon D5600 camera. UV images were captured after cutting papers in half using a Darkroom ultraviolet imaging cabinet. After UV imaging papers were sprayed with ninhydrin and again imaged with the Nikon digital camera. All images were converted to black and white for analysis with Void Whizzard.

RESULTS: Attempts to alter urine color failed as rats did not consume enough color-altering agent. UV vs. BF showed significant differences in area. All three groups had significant differences in overall spot count and in the smallest sized spots (0-0.1 cm²). UV vs. N and UV vs. BF showed significant differences in 0.1-0.25 cm² spots, UV vs. N in 0.25-0.5 cm², and N vs. BF in spots 0.5-1 cm².

CONCLUSIONS: Overall BF visualization proved difficult and produced results inconsistent with current mouse VSA visualization standard of UV imaging. N provided an ideal way to highlight urine and image with an ordinary camera. Human fingerprints from pre-assay handling of paper as well as rat footprints interfered with analysis of the smallest sized spots however there were no differences in detection of the larger spots, spot distribution, or overall spot area.

HEALTH RELEVANCE: This study contributes to the development of a standardized VSA protocol for assessing bladder function in both mouse and rat models, ensuring accurate, reliable, and reproducible pre-clinical results.

ACKNOWLEDGEMENTS: Both supported by NIH R25 DK121572; Ruetten supported by NIH T32 OD010957; Research supported by a grant from The Veteran's Association Merit Award. The funding sources had no influence over study design, execution, interpretations, or development of the abstract.

(G1-11) EX VIVO MODEL FOR MEASURING MOUSE URETHRAL PERISTALSIS

Allison L Rundquist^{1,2}, Marcela Ambrogi^{1,2}, Shreya S Nair², Chad Vezina^{1,2}

¹Endocrinology and Reproductive Physiology (ERP) Graduate Training Program, University of Wisconsin-Madison, Madison, WI, 53705, USA.

²Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, 53705, USA.

Introduction and objective: UTIs cost the US healthcare system approximately \$1.6 billion and disproportionately affect women of advancing age. Recent studies have shown the urethra plays an active role in protecting the body from pathogenic invasion by secreting serotonin (5-HT) and driving urethral muscle contraction. We have developed an *ex vivo* model to test the hypothesis that 5-HT accelerates the expulsion of small beads, simulating bacteria, from the proximal to the distal urethra in a coordinated manner (peristalsis).

Methods: The adult female mouse urethra is dissected from the bladder and body wall, secured with dissection pins, and injected with 6 µm fluorescent beads. The urethra is bathed in physiological saline solution (PSS) and time lapse microscopy is used to track bead velocity, distance traveled and directional movement over time under basal conditions and in response to KCl (60 mM) and 5-HT (30 µM) and other drugs.

Results: Fluorescent beads move slowly under basal conditions, suggesting the potential for an autonomous mechanism of urethral peristalsis. KCl appears to increase bead velocity, but beads move without directionality. 5-HT also appears to increase bead velocity, but beads appear to move in a coordinated direction (from proximal to distal urethra).

Conclusion: The *ex vivo* model we created demonstrates a method to visualize, measure, and determine whether urethral contractions are coordinated to encourage particle movement through the mouse urethra. We will use the model to determine the rate and efficiency of urethral peristalsis in response to drugs targeting serotonin receptors, urethral pacemaking cells, and smooth and striated muscle. We will also examine how age, sex, diabetes, and variables affecting UTI risk also affect urethral contractions.

Health Relevance: Coordinating particle movement from proximal to distal urethra is likely beneficial in expelling ascending microbes and protecting the urinary tract against infection. The *ex vivo* model system described here could be used as a screening tool for drugs that coordinate urethral contraction and potentially support UTI treatment and prevention.

(G1-21) THE ROLE OF FIBRO-ADIPOGENIC PROGENITOR CELLS IN AGED FEMALE URETHRA STRIATED MUSCLE AND URINARY INCONTINENCE (UI)

Zhina Sadeghi¹, Amberly Vu¹, Xiaolin Zi¹, Yi Xi Wu¹

¹University of California Irvine, Department of Urology, Orange, CA

INTRODUCTION AND OBJECTIVES: Urethral function declines by roughly 15% per decade and profoundly contributes to the pathogenesis of UI. Previous studies on human and mouse urethras, have consistently demonstrated a significant age-related decline in the urethral striated muscle with a concurrent increase in connective tissue and fibrosis. A key factor contributing to these changes may be fibro-adipogenic progenitor (FAP) cells, which are essential regulators of skeletal muscle homeostasis, including muscle regeneration and extracellular matrix (ECM) remodeling. The dynamic shifts in FAP sub-populations with aging suggest their crucial role in muscle atrophy and fibrosis, implicating them in urethral dysfunction. Therefore, this study aims to provide direct evidence of FAP cell involvement in ECM remodeling within aging urethral striated muscle tissues, potentially uncovering a novel mechanism underlying urethral dysfunction in UI.

METHODS: Urethral tissues were harvested from young and aged C57BL/6 female mice (n = 3/group). Total RNA was extracted from the harvested tissues, followed by next-generation sequencing (RNA-seq) to evaluate the transcriptomic changes. Bioinformatic analysis of the RNA-seq data was conducted using Qiagen CLC BenchWork. One-step real-time reverse transcription-quantitative polymerase chain reactions (RT-qPCR) were performed on selected target genes to validate differentially expressed genes between young and aged urethral tissues. Additionally, RNA scope was used to detect FAP cell markers, PDGFRα and Ly6, as well as FAP cell-derived fibrogenic factors that were differentially expressed between young and aged urethral tissues, as identified by RNA-seq.

RESULTS: Our RNA-seq data revealed many fibrotic and ECM remodeling genes upregulated in the aged mouse group. Of note, ECM remodeling genes derived specifically from FAP cells, such as *Adams5*, *Dpep1*, and *C3*, were upregulated in the aged group. Fibrogenic factors, such as *Ctgf*, *Vcam1*, *Thy1*, *Serpine1*, and *Lif*, have also been upregulated in the aged group from the bulk RNA-seq data. RNA scope demonstrated significant upregulation of FAP-derived factors impacting ECM remodeling (i.e., *Ctgf*) in our aged mice urethra.

CONCLUSIONS: Our data shows upregulation of specific fibrogenic factors in aged mice urethral tissue, which have been previously identified as FAP cell-derived fibrogenic markers in the striated muscle system, suggesting FAP cells may play a crucial role in changing the ECM architecture resulting in urethra dysfunction.

HEALTH RELEVANCE: This research highlights the potential role of FAP cell-derived fibrogenic factors in altering extracellular matrix architecture, contributing to urethral dysfunction, which could inform new therapeutic strategies for age-related urinary incontinence.

FINANCIAL DISCLOSURE: AUA Care Foundation research scholarship 2024-26 awarded to Dr. Sadeghi; UCI Urology departmental funding; UCI Physician Scientist Dean's Office scholarship awarded to Dr. Sadeghi.



(G1-27) IFRD1, A TRANSLATIONAL REGULATOR PLAYS AN ESSENTIAL ROLE IN REGULATION OF ER STRESS AND MAINTENANCE OF BLADDER EPITHELIAL HOMEOSTASIS

Arnold M. Salazar¹, Bisiayo E. Fashemi², Steven J. Bark^{1,3}, Jason C. Mills³, Indira U. Mysorekar^{1,4}

¹Department of Medicine, Section of Infectious Diseases, Baylor College of Medicine, Houston, TX; ²Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO, 63110; ³Department of Medicine, Section of Gastroenterology and Hepatology, Baylor College of Medicine, Houston, TX; ⁴Huffington Center of Aging, Baylor College of Medicine, Houston, TX 77030, USA

Introduction: The urinary bladder primarily stores and releases urine through cycles of distension-contraction. At homeostasis, the urothelium exhibits low turnover rates, but injury prompts rapid regeneration within 72 hours. Also, aging disrupts urothelial homeostasis. While factors involved in urothelial development, injury and disease response are identified, the molecular mechanisms for maintaining homeostasis amid constant stress of exposure to waste and tensile forces of expansion and contraction are not well understood. In this study, we sought to examine the role of interferon-related developmental regulator 1 (IFRD1) in the bladder as this protein has been reported to function in stress-related responses to injury and regeneration in several tissues/organs.

Methodology: Using *Ifrd1*^{-/-} mice and WT littermate controls [C57BL/6, 2 months (young) and 15-18 months (aged)], we performed histopathological, cytological and biochemical analyses to evaluate the cellular and molecular milieu of the bladder and urine such as TEM, qPCR, western blot, SUnSET assay, and IF/H&E staining. We also conducted proteomic and transcriptomic analyses, and void spot assay to determine impact on voiding behavior.

Results: We show that IFRD1 is expressed at homeostasis specifically in the urothelium of young mice. *Ifrd1* loss leads to endolysosomal accumulation and dysregulated uropod localization. It is also associated with increased oxidative stress, DNA damage, cell death/exfoliation, enhanced basal cell proliferation, reduced superficial cell maturation, increased urothelial permeability, and aberrant voiding behavior. Mechanistically, the loss of IFRD1 disrupts protein translation leading to ER stress via PERK (UPR) and increased oxidative stress. Our IP/MS show IFRD1 interacts with mRNA-translation-regulating factors in human urothelial cells and its loss alters global transcriptome status. Finally, we identified that *Ifrd1*^{-/-} urothelial dysfunction mirrored alterations seen in aged bladders.

Conclusion: Our findings indicate IFRD1 is important in preserving urothelial structure and bladder function at homeostasis. It is a key regulator of protein translational machinery and its loss alters urothelial homeostasis resulting in adverse effects on bladder. Also, IFRD1 affects pathways like in aging. Exploring IFRD1 roles provides insights into urothelial homeostasis that are affected in aging/damaged/infected/diseased bladders.

Health relevance: Postmenopausal women with Stress Urinary Incontinence show activation of PERK pathway with no changes in IRE1α or ATF6, with increased apoptosis like in IFRD1 null mice. Also, IFRD1 mRNA levels are altered in human Pelvic Organ Prolapse tissues. These suggest that IFRD1 may serve as a target for further investigation in aging and age-related bladder symptoms/diseases.

(G2-20) FUNCTIONAL MRI ANALYSIS OF OVERACTIVE BLADDER: BRAIN AND BRAINSTEM MECHANISMS AND THE ROLE OF TIBIAL NERVE STIMULATION

Samadifam, Farshad¹; He, Wei¹; Slavik, Nick¹; Budde, Matthew D.^{2,3}; Greenberg Adam S.¹; Hokanson, James A.¹

¹ Joint Department of Biomedical Engineering, Marquette University and Medical College of Wisconsin, Milwaukee, Wisconsin; ² Department of Neurosurgery, Medical College of Wisconsin, Milwaukee, Wisconsin; ³ Clement J. Zablocki Veteran's Affairs Medical Center, Milwaukee, Wisconsin

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 (SAIL, 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.

(G2-19) CHARACTERIZING CHANGES IN BARRINGTON'S NUCLEUS ESTROGEN RECEPTOR-EXPRESSING NEURONS AS A FUNCTION OF AGING

Cassandra Seifert^{1,2}, Anne M.J Verstegen²

¹University of Maryland School of Medicine, Baltimore, MD. ²Department of Medicine, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, USA.

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 bladder's detrusor muscle and relaxation of the external urethral sphincter (EUS). Bar is integral to the neurologic circuit that allows for switches 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 via Bar neurons remains unexplored. This research characterizes proenkephalin (Penk)-expressing neurons, a unique estrogen receptor expressing neuron subpopulation in Bar, and compares quantities of estrogen receptor-alpha (Esr1) positive and negative Penk neurons between male and female mice at different ages. The objective of this research is to uncover the mechanisms by which estrogen signaling in Bar influences female urogynecologic health across different hormonal states.

Methods: We collected brain tissue at the level of Bar from male and female Penk-IRES2-Cre transgenic mice crossed to a reporter mouse line (H2B-mCherry) at 8, 14, and 17.5 weeks of age. Using an antibody directed against the Esr1 receptor and fluorescence imaging, we quantified the red Penk-mCherry labeled neurons contained within Bar and the number of overlapping blue Esr1-expressing neurons.



Results: The total number of Penk+ neurons in Bar increases with age in both male and female mice. Furthermore, the average absolute number of Penk+ neurons expressing Esr1 in Bar increases with age in male and female mice while the absolute number of Penk+ neurons negative for Esr1 expression remains constant. Lastly, the average absolute number of Penk+ neurons expressing Esr1 in Bar is significantly greater in male mice compared to female mice.

Conclusions: Penk+ neurons with the capacity to respond to estrogen signaling via Esr1 increase in population size with age while the number of Penk+ neurons without the capacity to respond to estrogen signaling via Esr1 remains constant, suggesting an association between Esr1 signaling capacity and the maintenance and proliferation of Penk+ neurons of Bar with age.

Health Relevance: This study reveals dynamic changes in estrogen signaling capacity within the neurological control of LUT function and could further implicate estrogen signaling at the level of Bar in altered micturition behavior observed with aging and menopause. Future studies will be directed at quantifying the total number of Esr1-expressing Bar neurons at different ages, including after menopause, and exploring the functional role of Esr1-expressing neurons of Bar.

(G2-22) IMPACT OF STIMULATION LOCATION ON BLADDER FUNCTION FROM TIBIAL NERVE STIMULATION IN THE FEMALE RAT

Nick A. Slavik, James A. Hokanson

Joint Department of Biomedical Engineering, Marquette University and the Medical College of Wisconsin, Milwaukee, WI.

INTRODUCTION AND OBJECTIVE: Stimulation of the tibial nerve near the ankle has been shown to improve bladder function in both animals and people. We are interested in pursuing studies of chronic tibial nerve stimulation in animals, to understand better mechanisms of action. Stimulation at a location proximal to the knee may provide a structural advantage for a chronic implant, but the impact of stimulating the tibial nerve at a more proximal location is unknown. Based on our results we also assessed the impact of sciatic nerve stimulation.

METHODS: To investigate this, 21 healthy female CD rats were anesthetized with urethane (1.2-1.6 g/kg). Bladders were instrumented with a catheter placed in the dome of the bladder. Rats were randomly divided into three groups, with nerve cuffs placed at the distal tibial nerve (dist-TNS, near ankle, the standard approach), the proximal tibial nerve (prox-TNS, just distal to sciatic nerve branching), and at the sciatic nerve (sciatic-TNS, just prior to branching). Following implantation, rats underwent 5 cystometry trials (control). Bladders were filled at a constant rate per animal (2-12 ml/hr) until voiding occurred. Voided and residual volumes were collected and used to calculate bladder capacity and voiding efficiency. Stimulation at all three sites was delivered at an amplitude of three times the threshold of observable motor twitch of the toe for 30 minutes at 10 Hz and 200 μ s pulse width. After stimulation, 5 cystometry trials were conducted (post-stim).

RESULTS: Post-stimulation bladder capacity increased by $20 \pm 7\%$ ($P=0.00035$, $n=7$, t-test) for dist-TNS and $43 \pm 18\%$ ($P=0.00064$ $n=9$, t-test) for prox-TNS compared to pre-stimulation averages. Sciatic TNS did not significantly increase bladder capacity ($P=0.075$, $n=6$, t-test). Voiding efficiencies did not change (dist-TNS: $P=0.25$; prox-TNS: $P=0.26$; sciatic-TNS: $P=0.095$ t-tests) following stimulation. Bladder compliance significantly increased for all stimulation locations (dist-TNS: $26 \pm 16\%$ $P=0.002$; prox-TNS: $26 \pm 13\%$ $P=0.003$; sciatic-TNS: $12 \pm 9\%$ $P=0.023$, t-tests). Prox-TNS compared to dist-TNS showed a significant increase in bladder capacity ($P=0.028$, t-test).

CONCLUSIONS: These results provide evidence that proximal tibial nerve stimulation, at least in our animal model, is as effective as the current clinical approach of stimulating the distal tibial nerve.

HEALTH RELEVANCE: Our findings pose to improve current clinical applications of tibial nerve stimulation and improved health outcomes for patients with overactive bladder.

(G1-24) A CELLULAR ANATOMY OF NORMAL ADULT HUMAN FEMALE URETHRA

Lari Smith, John Lafin, Alicia Malewska, Philippe Zimmern, Ramy Goueli, Douglas Strand

Department of Urology, UT Southwestern Medical Center, Dallas, TX

Introduction and Objectives: Urinary voiding dysfunction and infection are highly prevalent in aging women. A deeper understanding of the cellular composition of female urethra in the non-diseased state could provide insights into the etiology of dysfunction. Single cell RNA sequencing was performed on female urethra from young organ donors to generate a cellular atlas.

Methods: Urethra from female organ donors aged 18-45 ($N=4$) was collected fresh from surgery and enzymatically digested into a single cell suspension. Single cell RNA sequencing was performed using 10x Genomics V3 chemistry. Data were integrated with previously published male prostatic urethra in Seurat for cell type annotation. RNAscope, immunohistochemistry and Xenium spatial transcriptomics were used for validation of cell types using anchor genes derived from the data on sagittal and transverse sections.

Results: The female proximal urethral lumen and dorsal ducts are lined with a transitional epithelium composed of KRT5+/KRT14- basal cells with KRT13+ intermediate epithelia and LCN2+ luminal epithelia. The distal urethra is lined with a squamous epithelium composed of KRT5+/KRT14+ basal cells with KRT13+ intermediate epithelia and KRT4+ luminal epithelia. CHGA+ neuroendocrine epithelia are found in the distal urethra and dorsal ducts. The urethral lumen is lined with a single layer of STC1+ periepithelial fibroblasts proximal to distal. C7+ major interstitial fibroblasts interdigitate amongst a circumferential layer of longitudinal smooth muscle and the striated muscle sphincter. The dorsal meatus is populated with muscle, nerves, blood vessels and MFAP5+ minor interstitial fibroblasts and the squamous epithelium of the vaginal wall is lined with a layer of APOE+ perivaginal fibroblasts.

Conclusions: The identification of specialized fibroblasts surrounding the urethra and anti-bacterial luminal cells lining the entire length of the urethra could yield new insight into the regulation of infection and inflammation.

Health relevance: Urinary tract infections (UTIs) affect 50-60% of women throughout their lifespan, posing a significant burden to the US healthcare system. The identification of the urethra as a barrier to bacterial ascension to bladder will redefine how we think about urinary tract infections.

Financial funding: NIH R01 DK135535, RC2 DK129994

(G2-28) INVESTIGATING NEUROINFLAMMATORY MARKERS IN DORSAL ROOT GANGLIA IN ACUTE CYSTITIS MOUSE MODEL

Sara A. Stuedemann¹ and LaTasha K. Crawford¹

Department of Pathobiological Sciences, University of Wisconsin-Madison School of Veterinary Medicine

INTRODUCTION AND OBJECTIVE: Urologic chronic pelvic pain syndrome (UCPPS) is characterized by persistent pain in the pelvic region and genitalia accompanied by bladder pain and urinary dysfunction without an identifiable cause. Characterization of patients have revealed distinct phenotypic subgroups based on urologic and pain severity profiles. In our model of acute cystitis, mice develop voiding dysfunction and pain, but phenotypes can vary in severity across groups. Bladders are innervated by sensory neurons that have their cell bodies in dorsal root ganglia (DRG). After nerve injury, GABA α alpha subunit 5 (GABRA5) and activating transcription factor 3 (ATF3) upregulate in DRGs and helps maintain chronic pain. In cystitis, the role of GABRA5 and ATF3 in pain and dysfunction is unknown. We hypothesize that cystitis causes an increase in GABRA5 and ATF3 in DRGs.

METHODS: Cystitis was induced via direct instillation of acrolein into the bladder. We performed void spot assay (VSA) to quantify spontaneous voiding behavior at baseline and 24 hours after instillation. Approximately 48 hours after instillation, bladder weights were collected, and mice were euthanized and perfused. Fixed T13-L6 DRGs were dissected and placed in RNAlater. RT-qPCR was used to detect GABRA5 and ATF3 expression in



DRGs with Livak method ($2^{-\Delta\Delta C_q}$) for relative gene expression analysis. Statistical comparisons were made using Mann-Whitney and Spearman correlation analysis in GraphPad Prism.

RESULTS: Cystitis mice developed voiding dysfunction (total urine spots, p -value < 0.0001). Cystitis mice had heavier bladders (p -value < 0.0001). Contrary to our hypothesis, GABRA5 and ATF3 expression was not significantly different. However, two cystitis mice had much higher GABRA5 and ATF3 expression in L6, a bladder innervating DRG. These mice had the largest bladders, and one mouse had the most total urine spots of the cystitis group. To investigate the relationship between gene expression, voiding dysfunction and bladder weights, Spearman correlation analysis was performed. For GABRA5 and bladder weights, increased GABRA5 correlated with larger bladder (correlation coefficient $r = 0.800$, p -value = 0.072), indicating a trend towards positive correlation. A larger n is necessary to make stronger conclusions.

CONCLUSIONS: Acrolein induced cystitis caused significant voiding dysfunction and enlarged bladders. Although, there was no difference between groups in average GABRA5 or ATF3 expression, a subset of mice with the largest bladders and most severe voiding dysfunction had much higher expression of these genes.

HEALTH RELEVANCE: UCPS symptoms change in severity over time and among patients. Identifying genes that play a critical role in driving and perhaps even exasperating UCPS symptoms is crucial in developing impactful treatments.

(G1-6) ENSURING CYTOCOMPATIBILITY AND BIOCOMPATIBILITY OF HIGHLY ELASTIC NANOFIBROUS SHEETS FOR URETHRAL APPLICATION

Sturm R¹, Yiu F¹, Zhong Z², Afshari R², Jimenez R¹, Jung R¹, Cabuang A¹, Annabi N²

¹Departments of Urology and ²Chemical Engineering, University of California Los Angeles

Introduction and Objective: Current autologous tissues used for urethral reconstruction lack the mechanical and structural properties of the multilayered anterior urethra. Our prior work developed a nanofibrous synthetic scaffold with tensile properties mimicking the native urethra. This study objective was to evaluate these candidate materials in vitro and in vivo to assess cytocompatibility and biocompatibility, respectively.

Methods: Nanofibrous scaffold sheets were synthesized by electrospinning two biomaterials, 5% ELP (elastin like peptide)/5% GelMA (gelatin methacryloyl) weight per volume (w/v, 5E5G) or 10% w/v GelMA alone (0E10G). Human bladder- and foreskin-derived cell lines were seeded onto scaffolds in monoculture conditions (1×10^5 cells/mL), and evaluated at day 1 to 7 endpoints. Biocompatibility testing evaluated Sprague-Dawley rats in 4 subcutaneous cohorts: 1) sham, 2) 5E5G, 3) 0E10G and 4) one-ply small intestinal submucosa (SIS, Cook), assessed at day 1 to 56 endpoints.

Results: 5E5G and 0E10G nanofibrous scaffolds demonstrated excellent cell viability from day 1 to 7 across urothelial (UC), smooth muscle (SMC) and fibroblast (Fib) cell lines. Proliferation and spreading of SMCs and Fib was robust, while UCs demonstrated less scaffold adherence and proliferation. Furthermore, addition of ELP enhanced degradation and tissue ingrowth, and minimized acute or chronic inflammation in biocompatibility testing.

Conclusions: Highly elastic engineered GelMA/ELP hybrid scaffolds are non-cytotoxic and biocompatible. Future scaffold modifications are ongoing to optimize parameters for UC proliferation and creating multi-layered 3-dimensional structures for urethral application.

Health relevance: The creation of a layered urethral tubular tissue construct holds critical translational value for patients with urethral disease by providing a potential option for urethroplasty with decreased morbidity versus current surgical procedures.

(G1-25) INVESTIGATING THE MODULATION OF AUTISM SPECTRUM DISORDER AND LOWER URINARY TRACT SYMPTOMS IN A GENE-BY-ENVIRONMENT MODEL UTILIZING SHANK3 MUTANT MICE AND POLYCHLORINATED BIPHENYL EXPOSURE

Lindsey Felth Tanaka, Julia Tlapa, Alyssa Lentine, Conner Kennedy, Monica Ridlon, Tamryn Jordan, Kimberly Keil Stietz

Department of Comparative Biosciences, University of Wisconsin-Madison

Introduction and Objective: Autism spectrum disorder (ASD) is a neurodevelopmental disease with multifactorial etiology characterized by atypical social and repetitive behavior. Lower Urinary Tract Symptoms (LUTS) are reported as a comorbidity in 77% of ASD patients, and increase with ASD severity. Although ASD behavioral deficits may cause LUTS, it is also possible that ASD risk factors alter connectivity in neuronal networks controlling voiding or directly affect the lower urinary tract. Complex gene x environment interactions underlying ASD and LUTS severity are understudied. To begin to address this gap, we investigate the severity of ASD and LUTS phenotypes in a gene x environment interaction mouse model.

Methods: Mutations in Shank3, a post-synaptic scaffolding protein essential for signal transduction and plasticity in glutamatergic synapses, is a genetic risk factor for ASD. Polychlorinated biphenyls (PCBs) are environmental toxicants that are hypothesized ASD risk factors and can alter voiding function in mice. In this gene by environment model, we investigate the interaction of Shank3 mutation and PCB exposure. Juvenile wild type, heterozygous, and homozygous Shank3 mutant mice are dosed daily with an environmentally relevant mixture of PCBs (0, 0.1, or 1.0mg/kg) for 3 weeks (P21-P42). Mice are then assessed for ASD phenotypes using the three-chamber social approach, repetitive grooming, and olfactory habituation tests. Voiding function is assessed using Void Spot Assay (VSA). Brains are collected for future study.

Results: Preliminary data validates ASD behavioral assay characterization, with wild type mice spending more time engaging in social behavior than Shank3 mutants. VSA indicates that Shank3 heterozygous and homozygous male and female mice produce fewer small urine spots compared to wild type vehicle controls. VSA also indicates that in wild type mice, juvenile PCB exposure decreases small urine spots in male mice at the 0.1mg/kg PCB dose versus control. Additional treatment groups are being tested to determine effects of PCBs on these behaviors in each genotype.

Conclusions: Shank3 mutations not only result in altered social behavior as expected, but are also sufficient to alter voiding behavior. Juvenile PCB exposure is also sufficient to alter voiding patterns in mice. This validates the model to assess Shank3 mutation and PCB effects in ongoing gene x environment studies. We hypothesize that if these two risk factors interact, ASD relevant behavioral phenotypes and LUTS severity will be greater in Shank3 mutants exposed to PCBs than in either group alone.

Health Relevance: Understanding the pathophysiology of ASD and its comorbidities, such as LUTS, and modifiable risk factors like toxicant exposures, may help improve ASD treatment options.

(G2-33) EXAMINING LONG-TERM MOLECULAR CHANGES IN DORSAL ROOT GANGLIA OF A MOUSE MODEL OF ACUTE CYSTITIS

Leira G. Torres Rivera^{1,2}, Emily L. Tran¹, Sara A. Stuedemann¹, and LaTasha K. Crawford¹

¹University of Wisconsin – Madison School of Veterinary Medicine,

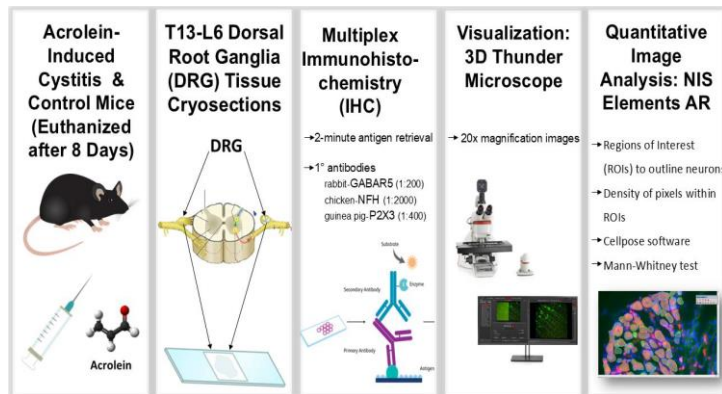
²University of Puerto Rico - Humacao

INTRODUCTION AND OBJECTIVE: Urologic chronic pelvic pain syndrome (UCPPS) comprises interstitial cystitis/bladder pain syndrome and chronic prostatitis, and is characterized by chronic pain in the lower pelvic region and genitalia, along with urinary symptoms. Some patients report secondary effects such as hypersensitivity of the skin, but the underlying



pathophysiological mechanisms are unknown and there are no effective treatments. The alpha5 subunit of GABA-A receptor (GABAR5), Neurofilament Heavy (NFH), and Purinergic Receptor P2X₃ (P2X₃) are upregulated in dorsal root ganglia (DRG) in nerve injury pain, but it is unknown what changes occur in cystitis-induced pain. In our acute cystitis model, phenotypes such as paw hypersensitivity can be observed at 7-8 days, long after resolution of voiding dysfunction. We hypothesized that at 8 days, GABAR5, NFH, and P2X₃ will be upregulated in DRG of cystitis mice compared to control mice.

METHODS:



RESULTS: Acrolein bladders trended toward being heavier than control bladders ($p=0.0571$), suggesting that bladder thickening may persist at 8 days. Pilot DRG staining evaluated the optimal duration for antigen retrieval treatment (AR) and antibody concentrations. Results indicated that 2-min was most effective AR duration for our antibodies. While NFH and P2X₃ worked well in L5 and L6 DRG, GABAR5 was difficult to assess due to high staining background. Cellpose software was introduced to accelerate the cell segmentation process during image analysis. Due to low image quality, L6 was excluded from analysis. For all three markers, there was no significant difference between treatment groups. Results were compromised by poor tissue quality, wherein AR caused tissues to fold or wash off slides; high staining background also impacted analysis.

CONCLUSIONS: We confirmed the effectiveness of our target markers and observed a range of expression patterns in animals that received acrolein. Upcoming experiments will increase the number of L5 and L6 images for a more rigorous analysis.

HEALTH RELEVANCE: If our hypothesis is supported, the results would suggest that acute cystitis causes a long-lasting neuropathy that contributes to hypersensitivity phenotypes, even after urinary symptoms have resolved. Identifying molecular changes that contribute to chronic urologic pain will inform novel and impactful therapies to help UCPPS patients.

(G2-29) ACUTE CYSTITIS INCREASES SENSORY NEURON ACTIVATION IN INTACT DORSAL ROOT GANGLIA

Emily L. Tran, Sara A. Stuedemann, LaTasha K. Crawford

Department of Pathobiological Sciences, University of Wisconsin-Madison
School of Veterinary Medicine

INTRODUCTION AND OBJECTIVE: Urologic chronic pelvic pain (UCPPS), which can stem from cystitis, is often accompanied by somatic pain that is poorly understood and underdiagnosed. In mice, cystitis can produce secondary mechanical hypersensitivity in hind paw skin, mirroring lower limb hypersensitivity seen in humans. The mechanisms underlying this "referred pain" remains unclear. We conducted *ex vivo* Ca²⁺ imaging studies of intact dorsal root ganglia (DRG) to test the hypothesis that cystitis increases neural activity in bladder- and paw-innervating DRGs.

METHODS: Snap25-GCaMP6s-D mice detect neuronal activation via EGFP expression upon Ca²⁺ influx. Female mice were randomly assigned to received acrolein (cystitis, $n = 6$) or 0.9% sterile saline (control, $n = 8$) instilled into the bladder via catheter. At baseline and 24-to-48 hours post-instillation mice underwent void spot assay (VSA) to measure bladder dysfunction and von Frey (vF) to assess mechanical allodynia in hind paws. At 48-hours whole

L3-L6 DRGs were acutely dissected for *ex vivo* Ca²⁺ imaging. Peak amplitudes of GFP fluorescence were recorded before, during, and after 30mM KCl bath application to stimulate sensory neurons. T-stack videos were corrected for physical drift using ImageJ (Fiji) and analyzed using NIS Elements-AR (Nikon). Individual neurons were defined as regions of interest (ROI) and fluorescent signal was measured over time per ROI. Statistical comparisons included Mann-Whitney tests for peak amplitude, and two-way ANOVAs and Sidak's Multiple Comparison's post-test for VSA and vF data.

RESULTS: Expectedly, acute cystitis caused significant increases in microvoids ($p = 0.0004$), indicating bladder dysfunction. Contrasting our previous cystitis studies with other mouse strains, mechanical allodynia varied across mice with no significant difference between groups. Neurons exhibited larger amplitude of KCl responses in L5 ($p < 0.0001$) and L6 ($p < 0.0001$) DRG after cystitis, indicating an increase in Ca²⁺ influx in DRG housing bladder neurons. Notably, though having little to no bladder innervation but strong paw innervation in mice, L3 ($p < 0.0001$) and L4 ($p < 0.0001$) DRG also exhibited larger KCl responses after cystitis, suggestive of increased excitability.

CONCLUSIONS: Our results suggest cystitis may increase the excitability of bystander neurons, including paw afferents. While VSA confirmed bladder dysfunction, hind paw hypersensitivity remains unclear. Future retrograde tracer labeling combined with pharmacologic and genetic Ca²⁺ imaging experiments will confirm if paw afferents indeed increase excitability after cystitis, and help elaborate on specific molecular contributors.

HEALTH RELEVANCE: These studies help provide insight to molecular mechanisms underlying comorbid pain conditions related to UCPPS or referred pain in distant sites of the body.

(G2-8) VALIDATING MOUSE MODELS OF BENIGN PROSTATIC HYPERPLASIA: A PROJECT OVERVIEW

Renee E. Vickman,¹ Petra Popovics,² Nadia A. Lanman,³ and Simon W. Hayward¹

¹Endeavor Health, Evanston, IL; ²Macon and Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA; ³Purdue University, West Lafayette, IN

INTRODUCTION AND OBJECTIVE: Chronic inflammation is associated with benign prostatic hyperplasia (BPH), but supporting data of a causal link are limited. Inadequate characterization of BPH models leaves them subject to scrutiny during the review process, so a direct comparison of mouse models with prostatic inflammation and hyperplasia both to one another and to human tissue is necessary to identify appropriate models for specific research directions. We hypothesize that specific cell states and voiding symptoms that mirror those in human tissues will be found in individual mouse models indicating those aspects of human disease that can be reasonably investigated with a specific model.

METHODS: We assembled a multidisciplinary team comprising a senior expert and early-career investigators with specialized experience in mouse models, pathological analysis, urinary function assessment, scRNA-seq, immune cell profiling, and bioinformatics. Voiding function evaluations using uroflow and scRNA-seq analysis of individual prostate lobes will be applied to six different models of prostatic inflammation and hyperplasia: steroid hormone imbalance, prostate luminal E-cadherin knockout, high-fat diet, Aire-knockout, non-obese diabetic, and prostate-specific IL-1b overexpression. Immune cell profiling will be conducted on T and B cells from mouse models and human BPH tissues. A novel notion of transcriptomic distance will be calculated between mouse and reference human cellular populations.

RESULTS: Our preliminary studies indicate an overlay between major cell types from the Aire-knockout mouse and human prostate. Furthermore, a subpopulation of macrophages determined to accumulate in the human prostate as size increases, TREM2+ macrophages, was also identified in the mouse model of steroid hormone imbalance. We anticipate this work will lead to improved use of current models for BPH research, provide a basis for future R01 applications by each ESI, and lead to future novel pre-clinical studies.

CONCLUSIONS: These comparative cellular and functional studies will provide an accessible database of cellular, pathological, and voiding function information for each BPH model that can be used as a benchmark and expanded by the benign urologic community. The searchable database



will be an invaluable resource for the BPH research community, providing guidance on murine model selection and facilitating molecular and functional queries.

HEALTH RELEVANCE: Benign prostatic hyperplasia is one of the most common symptomatic conditions in aging men. This Center will identify the similarities and differences between mouse models of BPH and the human disease, establishing the most appropriate platforms on which to develop new medical strategies.

(G1-19) DEVELOPMENT OF QUALITATIVE INTERVIEW GUIDES TO ASSESS CAREGIVER AND CLINICIAN NEEDS FOR PARTNERSHIP IN DECISIONS DURING CHILDREN'S URINARY TRACT INFECTION EVALUATION

Rachel L. Wattier¹, Anneka M. Hooft^{1,2}, Sunitha V. Kaiser^{1,3}

¹Department of Pediatrics, University of California San Francisco, San Francisco, CA; ²Department of Emergency Medicine, University of California San Francisco, CA; ³Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA; USA

Introduction and Objective: Patient-centered communication and shared decision-making are associated with more evidence-based care,^{1,2} improved patient/caregiver health-relevant knowledge,^{3,4} and better health outcomes.^{2,5} Prior studies have revealed areas of opportunity to improve caregiver involvement in decision-making when children are evaluated for possible urinary tract infection (UTI).^{6,7} In preparation for a qualitative study on this topic, we developed interview guides to assess caregiver and clinician needs for partnership in decision-making during children's UTI evaluations.

Methods: The decisions we will explore are 1) whether or not to test for UTI, 2) whether to start treatment before confirmation of UTI or wait for urine culture results, and 3) when/how to provide screening and counseling around bladder health. We selected two relevant frameworks to guide interview content development. For preference-sensitive decisions (without one best answer), primarily for caregiver interviews, we developed questions based on the Ottawa Decision Support Framework (ODSF).⁸ For questions about recommended evidence-based practices, primarily for clinician interviews, we segmented decisions into specific evidence-based practices, and developed questions based on the Capability, Opportunity, Motivation – Behavior (COM-B) model.⁹

Results: In Table 1 we show example questions about urine testing, using ODSF for caregiver questions and COM-B for clinician questions. We will use a similar approach for questions about treatment decisions (ODSF and COM-B) and screening and counseling around bladder health (COM-B). We will seek feedback from caregiver advisors and clinician collaborators to optimize

Table 1. Questions related to urine testing decision for caregiver and clinician interviews

Open-Ended Questions	Possible Semi-Structured Probes (linked to concepts within model or framework)	Model or Framework
Caregiver Interview Decision 1: Test for UTI? Let's talk about deciding whether a child should get a urine test to check for a bladder infection. If the child is not toilet trained that may mean using something called a catheter. A catheter is a small tube that goes into the child's bladder to collect the urine. Sometimes this decision is easy, but other times it can be hard, even for doctors and nurses.		
Can you share your experience with this decision?	- How did you feel when making this decision? (conflict, contributing factors) - What do you think made the decision difficult? (conflict, contributing factors)	ODSF
What do you see as the main choices in this situation?	- Of the choices you described, what do you see as the main benefits of those choices? (knowledge) - What do you see as the main downsides of those choices? (knowledge) - Which of these benefits and downsides are most important to you? (values)	ODSF
How do you usually handle a decision like whether your child will have a urine test?	- Some families want their child's doctor or nurse to decide about testing, some may want to decide with the child's doctor or nurse (support & resources, preferred role) - What do you think would help with this decision? (support & resources, facilitators)	ODSF
Clinician Interview Evidence-Based Practice A: Testing Young Children with UTI Symptoms There are certain situations in which guidelines recommend testing children for UTI. The literature shows that younger children are less likely to be tested.		
Can you describe an experience with urine testing in an infant or toddler?	- Can you describe your knowledge of risk factors for UTI? (capability – psychological) - What is your perception of your team members' comfort with collecting urine samples in young children? (capability – physical)	COM-B
What barriers have you faced in obtaining a urine test when you thought it was needed?	- Related to your work environment? (opportunity – physical) - Related to anyone else's expectations? (opportunity – social)	COM-B
Are there other factors that influence your decisions about testing?	- Possible benefits or downsides? (motivation – reflective) - Are there situations in which you routinely do a urine test? (motivation – automatic)	COM-B
Clinician Interview Evidence-Based Practice B: Avoiding Testing in Low Risk Scenarios There are certain situations in which urine testing may be avoidable based on guidelines or evidence. For example, the literature shows that older children frequently have urine testing even if they have symptoms that may be related to other illnesses.		
Can you think of a clinical situation where you or your colleagues may test for UTI even when the risk of UTI is very low?	- What might drive testing in these situations? - Can you describe your knowledge of factors that decrease the likelihood that a child has a UTI? (capability – psychological)	COM-B
What barriers have you faced in avoiding urine testing when you thought the likelihood of UTI was low?	- Related to your work environment? (opportunity – physical) - Related to anyone else's expectations? (opportunity – social)	COM-B
Are there other factors that influence testing when you think likelihood of UTI is low?	- Possible benefits or downsides? (motivation – reflective) - Habits or protocols (motivation – automatic)	COM-B

ODSF: Ottawa Decision Support Framework⁸
COM-B: Capability, Opportunity, Motivation – Behavior Model⁹

clarity and relevance of these questions. We will then conduct semi-structured interviews with caregivers and clinicians using these interview guides.

Conclusions: By incorporating 2 complementary frameworks, we developed questions to explore important dimensions of decision-making for children's UTI evaluation from both caregiver and clinician perspectives.

Health Relevance: This study will lead to an intervention that leverages the benefits of patient-centered communication and shared decision-making to improve care at the time of children's UTI evaluation. Because 1 in 10 emergency department visits by children involve urine testing,¹⁰ there is a large opportunity to positively impact children's health and healthcare experience for their families.

(G1-13) SACRAL NERVE STIMULATION TO MEDIATE BOWEL DYSFUNCTION FOLLOWING SPINAL CORD INJURY

Natasha L. Wilkins, Warren M. Grill

Department of Biomedical Engineering, Duke University, USA

INTRODUCTION AND OBJECTIVE: Traumatic spinal cord injury (SCI) impacts multiple organ systems and has the potential to significantly impact quality of life. In individuals with SCI, bowel dysfunction including chronic constipation, slow colon transit, and incontinence are very common, impacting 80% of the population. Sacral nerve stimulation (SNS) is a minimally invasive, FDA-approved treatment in use for the treatment of bladder and bowel dysfunction in individuals without spinal cord injuries. We plan to test a novel electrical stimulation paradigm of SNS that uses temporal patterns to delineate between continence to treat fecal incontinence and increased colonic motility to treat chronic constipation in a rodent model of SCI.

METHODS: Female and male Sprague Dawley rats with thoracic contusion spinal cord injuries will undergo acute SNS under urethane anesthesia at four weeks post-injury. SNS will be applied with three different stimulation parameters (14 Hz continuous stimulation, 30 seconds of stimulation at 18 Hz followed by 18 seconds without stimulation, and 40 seconds of stimulation at 20 Hz followed by 20 seconds without stimulation) and will be applied with and without rectal distention. During stimulation, changes in rectal and colon pressure, and electromyography of the external anal sphincter will be recorded to measure changes in colonic motility. Following initial stimulation, the rats will undergo a complete spinal transection at T8 to remove supraspinal input, or serial spinal transections below T9 to determine the lumbosacral contribution to SNS induced bowel control.

RESULTS: Per previous studies which examined SNS for bowel function in both awake and anesthetized spinally intact rats and in an ex vivo preparation of the mouse colon, we anticipate that continuous stimulation (at 14 Hz) will result in a decrease in colonic activity with an increase in activity within the external anal sphincter relative to periods without stimulation. It is also anticipated that burst-patterned stimulation (30 seconds of stimulation at 18 Hz followed by 18 seconds without stimulation) will increase the occurrence of colonic motor complexes and decrease activity of the external anal sphincter. T8 transections will likely not alter SNS-induced colonic activity, but lumbosacral transections will ablate SNS-induced responses.

CONCLUSIONS: Burst patterned stimulation applied via SNS is likely to be an impactful intervention for neurogenic bowel dysfunction in SCI rats. This work provides the framework for the application SNS to improve bowel dysfunction in humans with SCI.

HEALTH RELEVANCE: Neurogenic bowel dysfunction negatively impacts quality of life for those living with SCI. This project provides preclinical evidence to support translation into a clinical study that can be used to alleviate bowel dysfunction following SCI.



(G1-28) LONG-TERM IMPACT OF PBS MUTATIONS ON BLADDER, KIDNEY, AND REPRODUCTIVE FUNCTION IN FLNA-C2152R MICE

Justin Wobser, Nathalia Amado, Chaitrali Atre, Binta Mboob, Linda A Baker

Nationwide Children's Hospital, Columbus, Ohio

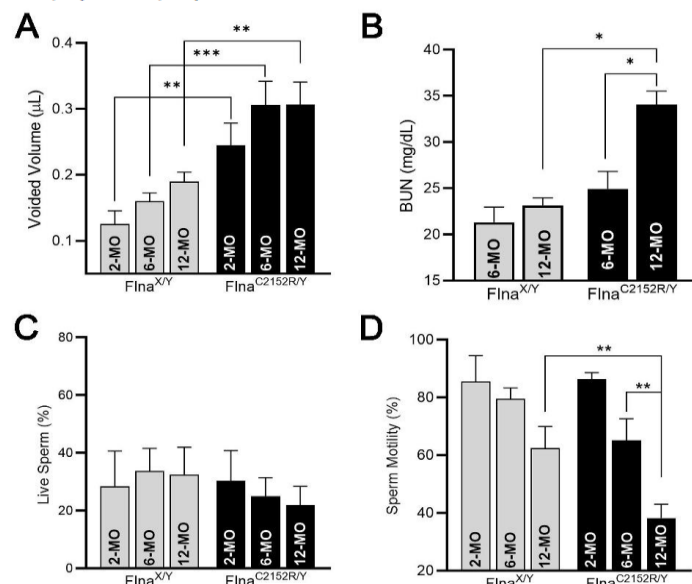
INTRODUCTION AND OBJECTIVE: Filamin A (Flna) is an actin-binding, cytosolic protein highly expressed in the detrusor that mechanotransduces force across the membrane from actomyosin to the extracellular matrix. We previously generated Flna-C2152R mutant mice (F-2152) carrying a human PBS mutation previously identified by our group. F-2152 mice exhibited reduced bladder function at the early adult age of 2 months (MO); however, no kidney or fertility issues were initially observed. This study investigates the impact of aging on bladder and kidney function, as well as fertility, in F-2152 mice.

METHODS: Male offspring (WT [Flna^{+/y}] and F-2152) at 2, 6, and 12 MO were evaluated for bladder and kidney function, as well as sperm viability and motility. For bladder function, a 24-hour metabolic cage measured voided urine volume and frequency (UroVoid system). Blood Urea Nitrogen (BUN) levels were measured to assess kidney function. Live, motile sperm were counted from freshly dissected epididymis. Mann-Whitney tests were used to assess group differences, with $p < 0.05$ considered significant.

RESULTS: Compared to WT, F-2152 mice showed a significant increase in voided volume by 95%, 91%, and 61% at 2, 6, and 12 MO, respectively. Similarly, there was an increase in void intervals by 70%, 107%, and 130% at 2, 6, and 12 MO, respectively, suggesting significant impairment of bladder function with aging. Compared to WT 6 MO mice, a 60% increase in BUN levels was observed in F-2152 mice at 12 MO. Notably, no significant differences in BUN levels were observed in WT mice between 6 and 12 MO, but F-2152 mice showed a 36% BUN increase from 6 to 12 MO, indicating declining kidney function. When compared to WT, sperm parameters showed

a decrease in F-2152 sperm motility by 18% and 39% at 6 and 12 MO, respectively, with no differences observed at 2 MO. Live sperm decreased by 32% at 12 MO in F-2152 mice. Consistent with these findings, a reduction in successful pregnancies was observed when older F-2152 male breeders were used.

Figure 1. Age-related changes in bladder, kidney, and reproductive function in WT (Flna^{+/y}) and Flna-C2152R (F-2152) mutant mice. (A) Voided urine volume measured in WT and Flna^{C2152R/y} mice at 2, 6, and 12 MO by UroVoid. **(B)** Blood Urea Nitrogen (BUN) levels measured in WT and Flna^{C2152R/y} mice at 6 and 12 MO. **(C)** Percentage of live sperm in WT and Flna^{C2152R/y} mice at 2, 6, and 12 MO. **(D)** Sperm motility percentage in WT and Flna^{C2152R/y} mice. Mann Whitney test as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



CONCLUSIONS & HEALTH RELEVANCE: With aging, F-2152 mice manifest progressive deterioration of bladder and kidney function, and sperm quality. These findings highlight the long-term impact of the PBS FLNA mutation on urinary and reproductive health, underscoring the need for further investigation into potential therapeutic interventions.

(G1-18) PERCEIVED BARRIERS TO CARE FOR URINARY TRACT INFECTIONS IN EMERGING ADULTHOOD

Jennifer Yarger, PhD^{a,b}, Anne M. Suskind, MD, MCS, FACS, FPMRS^{c,d}, Ina U. Park MD, MS^{d,e}, Dean Schillinger, MD^{a,f}, Iris Wong^d, Hannah K. Hecht, MPH^d, Cynthia C. Harper, PhD^{a,d}

^aPhilip R. Lee Institute for Health Policy Studies, University of California, San Francisco ^bDepartment of Epidemiology and Biostatistics, University of California, San Francisco ^cDepartment of Urology, University of California, San Francisco; ^dDepartment of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco; ^eDepartment of Family and Community Medicine, University of California, San Francisco ^fDivision of General Internal Medicine, University of California, San Francisco/San Francisco General Hospital

INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) are common, with about one-third of women treated by age 24. However, little is known about barriers to UTI care in emerging adulthood (ages 18- 29). This study examines perceived barriers to UTI care, analyzing differences by social determinants of health.

METHODS: We used survey data from May-August 2024 from a supplementary study to an ongoing cluster randomized trial in a diverse community sample in California and Texas. The sample included 406 individuals aged 19-29, assigned female at birth, and sexually active with male partners. We measured perceived barriers to UTI care using 14 items based on Levesque's model of health care access, dichotomizing responses to indicate agreement. We used descriptive statistics to identify the six most common barriers and multivariable mixed-effects logistic regression to model these barriers by sociodemographics, insurance, and usual source of care.

RESULTS: Participants' average age was 22.6 years, with 56% Hispanic. Nearly one quarter (23%) were uninsured, 34% lacked a usual care source, and 30% had a prior UTI. Perceived barriers to UTI care included worry about bad news (58%) or having a sexually transmitted infection (STI) (40%) and not wanting parents to know about symptoms (44%). Other common barriers were appointment delays (47%), being too busy (40%), and cost (45%). Multivariable analysis showed that worry about bad news was higher among younger participants (aOR 1.72, 95% CI 1.14-2.58), while worry about STIs was higher among Asian/Pacific Islanders (API) (vs. Hispanic) (aOR 2.08, 95% CI 1.08-3.99) and food insecure individuals (aOR 1.65, 95% CI 1.06-2.58). Younger participants were more likely to want privacy from parents (aOR 1.55, 95% CI 1.02-2.34), as were API (aOR 2.42, 95% CI 1.24-4.73) and non-English speakers at home (aOR 1.64, 95% CI 1.01-2.64). Those with no usual care source were more likely to expect appointment delays (aOR 2.01, 95% CI 1.29-3.12), while food insecure participants were more likely to be too busy (aOR 1.68, 95% CI 1.08-2.62). Cost concerns were more common among those who were uninsured (aOR 2.81, 95% CI 1.66-4.75), food insecure (aOR 1.78, 95% CI 1.13-2.81), lacked a usual care source (aOR 1.73, 95% CI 1.10-2.71), and younger (aOR 1.54, 95% CI 1.01-2.35).

CONCLUSIONS: Emerging adults report substantial challenges to UTI care, especially among younger individuals and some underserved groups. Youth-focused interventions, including education and expanded telehealth services, are needed to promote health equity for UTIs.

HEALTH RELEVANCE: Identifying the unique needs of emerging adults is key to developing interventions that improve UTI care access. This can reduce delays in care, minimize pain, and prevent complications from untreated UTIs.



(G1-23) ANALYSIS OF THE BLOOD TRANSCRIPTOME IN HYPERCALCIURIC KIDNEY STONE FORMERS IDENTIFIES POTENTIAL CLUES FOR UNDERLYING PATHOGENESIS

Ajha M. Young¹, Kristina L. Penniston, PhD, RDN², Eva Furrow, VMD, PhD, DACVIM³, Lauren A. Baker, MS, DVM, PhD¹

¹ Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI, ²Department of Urology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, ³Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; USA

INTRODUCTION AND OBJECTIVE: Idiopathic hypercalciuria (IH) is the most common metabolic risk factor associated with kidney stone development. IH is heritable and likely polygenic, though the exact genes that drive IH are unknown. Differentially expressed genes identified from kidney tissues are important for understanding underlying mechanisms of stone disease, but may not be useful as biomarkers for diagnosis or prognosis. The present study used peripheral blood as a “liquid biopsy” to identify RNA biomarkers of kidney stone disease in hypercalciuric stone patients.

METHODS: Cases had documented hypercalciuria (24hr urine calcium ≥ 200 mg/d for women and ≥ 250 mg/d for men), at least one diagnosed calcium stone event, and a 1st degree relative with stone disease. Controls were healthy adults >35 years of age with no personal or family history of stone disease. 3 ml of peripheral blood was collected for RNA extraction. RNA was processed, sequenced, and aligned to the hg38 reference assembly at the University of Wisconsin Gene Expression Center. Principal components analysis (PCA) was used to identify segregating effects due to batch (1 or 2) and/or sex. Low count genes were removed, and differential expression analysis was performed using the DESeq2 R package v.1.40.2 with batch and sex included as covariates. Additional contrasts were performed to identify sex-specific genetic effects in male and female patients vs controls. FDR adjusted p-values <0.05 were considered significant.

RESULTS: Cases (n=23) were 52% female; controls (n=25) were 68% female. Average age of cases was 54.4 and controls 54.0 years. PCA identified segregating effects due to batch and sex. Data analyzed with batch and sex in the design did not reveal differentially expressed genes (DEGs). Likewise, contrasts performed to extract sex-specific effects for males and females did not identify DEGs that met the significance threshold. In females, there were 3 genes with $P_{adj}=0.08$: *ARRDC4*, *NUDT5*, and *UBASH3A*, all of which are associated with ubiquitination pathways that may contribute to calcium homeostasis. The 4th ranked gene identified in females, *ANO5* ($P_{adj}=0.11$), is a mediator of bone homeostasis.

CONCLUSIONS: We were not able to detect large gene expression differences in peripheral blood of hypercalciuric stone formers compared to controls. However, subtle genetic effects were detected in women. Transcriptome analysis of urine samples from the same study participants is underway.

HEALTH RELEVANCE: Peripheral blood may be a relevant source for detecting gene expression differences in kidney stone patients relative to controls, perhaps specifically in women. More research is needed.

(G1-8) NATURALLY INSPIRED ANTIOXIDANT MUCOADHESIVE NANOPARTICLES FOR INTERSTITIAL CYSTITIS TREATMENT

Yuting Zheng,¹ Zhonghong Kong,¹ Felix Yu,² Cristopher Navarro,¹ Renea Sturm,² Nasim Annabi^{1*}

¹ Chemical and Biomolecular Engineering and ² Department of Urology, Division of Pediatric Urology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA

INTRODUCTION AND OBJECTIVE: Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic condition characterized by pelvic pain and increased urinary frequency, with an estimated 3 to 8 million adult U.S. women having symptoms of IC/BPS (Berry 2011). Oxidative stress caused by reactive oxygen species (ROS) plays a role in IC/BPS pathogenesis (Mohammad 2024). Current treatments, including oral medications and bladder instillations, have limited efficacy and side effects. This study developed naturally derived tannic acid (TA)-coated gelatin-based

nanoparticles (GelTA NPs) for IC/BPS treatment, leveraging TA's mucoadhesive properties and antioxidant effects (Gülçin 2010), with sustained release lidocaine for local pain control.

METHODS: NPs were synthesized via a modified solvation method (Kimura 2018) and coated with TA through hydrogen bonding and hydrophobic interactions. Lidocaine was then loaded into the NPs. The size and polydispersity index of the NPs were characterized using dynamic light scattering. Mucoadhesion was confirmed through rheology and atomic force microscopy. *In vitro* cell studies assessed cytotoxicity, while the ROS scavenging activity of the NPs was evaluated using the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay (Roy 2024). *Ex vivo* studies using fresh porcine bladders evaluated mucoadhesion.

RESULTS: The engineered GelTA NPs demonstrated optimal size (144.8 ± 44.37 nm), good homogeneity (PDI: 0.3608 ± 0.1286), and a lidocaine loading efficiency of approximately 40%. The NPs provided sustained lidocaine release for up to 6 days and effectively reduced ROS levels in the NIH 3T3 cells. Lack of cytotoxicity was confirmed with over 95% urothelial cell viability, and mucoadhesion was validated both *in vitro* and *ex vivo*.

CONCLUSIONS: GelTA NPs, characterized as having an excellent cytocompatibility profile, improved sustained bioavailability of lidocaine, and strong mucoadhesion and antioxidant activity. GelTA NPs hold significant potential for broad applications in enhancing mucosal delivery of treatments in the treatment of inflammation and pain.

HEALTH RELEVANCE: The development of lidocaine-loaded GelTA NPs offers a promising approach for improving the treatment of IC/BPS by enhancing drug delivery. This innovation could lead to more effective pain management strategies, ultimately improving the quality of life for patients suffering from this debilitating condition.