



CAIRIBU MEETING ABSTRACTS BOOKLET

Each year, CAIRIBU-affiliated trainees and early-stage investigators are invited to submit abstracts for presentation as posters at the annual meeting. The abstracts submitted for the 2022 CAIRIBU Meeting are printed here to encourage learning from each other, networking, and collaborative discussion. Abstracts are printed as they were submitted, without proofreading or standardized headings within the body of the abstract. Please note that some data are unpublished. Therefore, **treat ALL abstracts and the findings reported herein with the appropriate discretion and confidentiality.** The 2022 abstract guidelines and evaluation criteria are below.

2022 CAIRIBU MEETING ABSTRACT GUIDELINES

Collaborating for the Advancement of
Interdisciplinary Research in Benign Urology



General abstract guidelines and evaluation criteria

- Abstract submitters and presenters must be trainees or early-stage investigators (per NIH definition)
- All authors named in the abstract must have agreed to its submission for presentation
- It is the responsibility of the submitting author to ensure the abstract is in perfect order with no spelling or grammatical errors as abstracts will not be corrected
- Submitted abstracts will be evaluated individually by peer reviewers based on these criteria:
 - Context and interest: Question or hypothesis is clearly stated, key evidence leading to question or hypothesis is clearly articulated, scientific/clinical significance is cited
 - Experimental design and data presentation: Experimental approach and methods are clearly described, data is concisely presented and prioritized to include most relevant findings
 - Evaluation and analysis of data: Results are concisely described, conclusions are clearly noted without over- or under-statement, future work to alleviate any remaining questions is cited, figures and/or tables (if used) are helpful to illustrate findings
 - Written communication: Title is informative, abstract is written well and presented in logical order without irrelevant information, scientific nomenclature is correctly used
- Abstracts of works “in progress” are acceptable
- An author may submit up to 3 abstracts as first author/presenter

Preparation of abstracts

- **Deadline.** Abstracts are due by 11:59 p.m. (Central Time) on October 19, 2022. Upload abstracts [HERE](#) as a Word doc or PDF. Sorry – no extensions.
- **Size.** Abstract is limited to 2,330 characters not including spaces. Character count includes body of abstract, tables, and graphics (i.e., NOT the title, authors' names or affiliations). Tables account for 225 characters per table. Graphics also account for 225 characters each.
- **Title.** Should clearly define the topic and contain no abbreviations.
- **Authors.** List the primary author's full name followed by other authors' names. Spell out the names of all authors using full first name, middle initial, and last name. List each author's affiliation as in the example on the next page.
- **Body of abstract.** The abstract should be informative and detailed.
 - Body must contain 4 separate paragraphs: Introduction and Objective, Methods, Results, and Conclusions
 - It is not acceptable to state that “results will be discussed;” specific outcomes are necessary, including for abstracts that describe studies still in progress
 - Major new findings should be clearly articulated
 - Standard abbreviation may be used after spelling out the full term the first time it is used followed by the abbreviation in parenthesis
 - Graphs and/or tables may be used; character count is as stated above
 - Proprietary names of drugs are not allowed; use generic names



#1 - AMPLIFY: Amplifying Sensation in Underactive Bladder (Work in Progress)

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INTRODUCTION AND OBJECTIVE: Incomplete emptying due to underactive bladder (UAB) is a poorly understood health condition that affects up to 40% of the aged population. Bothersome UAB symptoms for which patients seek care include pelvic pressure, urinary frequency, feelings of incomplete emptying, and/or slow stream. However, current management options are often associated with failure to resolve symptoms and poor quality of life. There is a need to clarify the pathophysiological mechanisms underlying UAB to improve outcomes. This study will quantify and amplify sensory nerve drive in adult women with UAB to establish a prognostic marker for rationally guided treatment.

METHODS: An abridged CASUS questionnaire was administered before and 7 days after the study visit measuring urinary frequency, bladder sensation, voiding effort, urinary flow, feelings during emptying, and quality of life. Higher scores indicate greater severity of lower urinary tract symptoms. The functional integrity of bladder and urethral sensory nerve fibers was assessed using current perception threshold (CPT) testing. CPT values were established using a forced choice paradigm by the method of levels. Continuous electrical stimulation was delivered via catheter electrode to target selectively the reduced sensory feedback from the bladder or urethra. Urodynamic studies (UDS) were performed after the stimulation session (15 min for urethra or 30 min for bladder) to assess bladder sensation and emptying function.

RESULTS: Data has been collected in 10 participants. Compared to normative asymptomatic women, the current amplitude to elicit sensation was $349 \pm 189\%$ higher in the bladder and $3,208 \pm 3,324\%$ higher in the urethra. Compared to their pre-stimulation clinic UDS, first sensation in cystometry decreased by 113 ml, max cystometric capacity decreased by 166 ml, pressure flow voiding efficiency increased by 11%, max flow rate increased by 6 ml/s, and max detrusor pressure increased by 7 cmH₂O. Compared to baseline, the average total CASUS score decreased by 9 points seven days after the study visit (54 ± 12 vs 45 ± 9). The subscale measuring frequency decreased by 4 (53 ± 16 vs 49 ± 11), effort decreased by 10 (51 ± 24 vs 41 ± 23), flow decreased by 15 (48 ± 17 vs 33 ± 8), emptying decreased by 8 (50 ± 21 vs 42 ± 21), and quality of life decreased by 18 (72 ± 14 vs 54 ± 21).

CONCLUSIONS: CPT testing confirmed poor sensation in the urethra and bladder in participants with symptoms of an UAB. Early findings suggest targeted electrical stimulation can acutely improve bladder symptoms and function. Future analyses will compare the effects of bladder or urethral stimulation, and future studies will determine if multiple stimulation sessions provide greater benefit.

Funding: K01 DK120632 (EJG)

#2 - The role of Urethral Neuroendocrine cells in Urinary Tract Infection

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Introduction and Objective: Urinary tract infections (UTIs) are the most common infections in United States, affecting 50–60% of adult women and commanding an annual cost of \$1.6 billion. Emergence of antibiotic resistant bacteria is a concern and necessitates new medical therapies for managing UTIs. Neuroendocrine cells represent a rare population of non-neuronal serotonin producing cells in the lower urinary tract. We hypothesize that bacteria stimulate

serotonin secretion from these cells, thereby activating a serotonin receptor on urethral pacemaking cells, and causing them to initiate contraction of smooth muscle myocytes (via ANO1 channel) to which they are electrically coupled, to expel bacteria from the urinary tract. Thus, the objectives of this study are to test that urethral neuroendocrine cell derived serotonin (non-neuronal synthesis) protect against UPEC colonization and that serotonin mediates urethral contraction via HTR2B.

Methods: We infected wild type and tryptophan hydroxylase 1 (*Tph1*) null adult female mice (deficient in non-neuronal serotonin biosynthesis) with UTI89 uropathogenic *E. coli*. We counted *E. coli* (CFUs) in urethra and bladder. We measured contractile responses of uninfected adult female mouse urethra to graded concentrations of serotonin and SB204741 (HTR2B antagonist).

Results: *Tph1* null female mice develop more severe bladder infections than wild type mice, serotonin drives urethral contraction, and serotonin-induced contractions are inhibited by SB204741.

Conclusion/Discussion: Our results are consistent with a critical protective role of non-neuronal serotonin in UTI, through a mechanism involving HTR2B.

#3 - Testing aryl hydrocarbon receptor (AhR) modulation via 2-(1H-Indol-3-ylcarbonyl)-4-Thiazolecarboxylic acid methyl ester (ITE) as a treatment for antigen-induced Experimental autoimmune prostatitis in mice

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Chronic prostatitis (CP) affects 10-15% of males in the United States and is the most common and poorly understood form of prostatitis. The presence of prostate-specific auto-reactive T cells found in CP patient populations, in tandem with increased memory T, T-helper (Th)-1, Th17, and Th22 cell populations in peripheral blood samples, supports the hypothesis that CP is an autoimmune disease. In search of potential therapies for CP, we note that recent studies using mouse models of colitis and irritable bowel syndrome demonstrate a therapeutic benefit of shortacting agonist of the orphan receptor and transcription actor, aryl hydrocarbon receptor (AhR), in autoimmune disease.

In this study, we used a mouse model of autoimmune prostatitis to test the hypothesis that ITE will reduce prostatic inflammation, collagen accumulation, and voiding dysfunction.

Whole prostates were collected from freshly euthanized adult male rats (Sprague Dawley (SD) rats aged 12-60 weeks) and were homogenized in phosphate buffered saline and centrifuged. The supernatant was passed through a 0.2-micron filter, mixed with equal volume TiterMax® Gold adjuvant and diluted to a concentration of 10ng protein/ml). The prostate antigen (PaG) was injected subcutaneously into 8–9-week-old adult male C57BL/6J mice (50 microliters into the base of the tail and 50 microliters into the shoulder). Control mice were injected subcutaneously with TiterMax® adjuvant as described. Seven days post injection, mice were euthanized, prostates collected, dehydrated, cleared and infiltrated with paraffin, cut into sections and stained with hematoxylin and eosin for histological analysis. We observed mononuclear cell infiltration in the dorsal and ventral prostate of PaG but not vehicle treated mice. We will use immunostaining to test whether PaG increases CD45+ cell densities and decreases the thickness of ACTA2+ periductal smooth muscle. We will use various urodynamic testing assays, such as cystometry (CMG), void spot (VSA), and uroflowmetry to test whether PaG treated mice void differently than vehicle control treated mice. We will test whether daily IP injections of ITE, beginning the same day as prostate antigen injection and continuing through the study endpoint, reduces PaG-mediated prostate inflammation and voiding dysfunction.



#4 - The Response of Prostatic and Urethral Neuroendocrine cells to Prostatic Inflammation

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Male lower urinary tract symptoms may include increased urgency and frequency to urinate, nocturia and negative impacts on the quality of life. One cause of male LUTS includes prostate smooth muscle dysfunction. Alpha adrenoreceptors on prostatic smooth muscle myocytes contribute to prostate muscle dysfunction, but some men are resistant to alpha adrenoreceptor blockers, raising the possibility that adrenoceptor independent mechanisms also contribute. We recently found that neuroendocrine cells in the female mouse urethra appear to drive urethral contraction in response to uropathogenic *E. coli* infection. Here, we test the hypothesis that neuroendocrine cells in the male prostate and urethra also drive prostatic muscle contraction in response to inflammation. In female mice, urethral neuroendocrine cells synthesize serotonin and release it upon exposure to uropathogenic *E. coli*. Serotonin appears to activate the serotonin receptor HTR2B on interstitial cells of Cajal, driving contraction of smooth muscle myocytes to which they are coupled by an ANO1 dependent mechanism. We will use immunohistochemistry to test whether male mouse prostatic neuroendocrine cells also synthesize serotonin, and test whether cKIT⁺ interstitial cells of Cajal in the male prostate contain HTR2B and ANO1. We will use wire myography to test whether lipopolysaccharide, a membrane component of gram-negative bacteria, serotonin and HTR2B agonists drive a contractile response in adult male C57BL/6J mouse prostate and whether this response is resistant to alpha blockers. We expect to reveal a new pathway for inflammation mediated, and alpha adrenoreceptor independent muscle contraction that may be responsible for prostatic smooth muscle dysfunction and male LUTS.

#5 - Cellular senescence in aged urothelium leads to NLRP3/Gasdermin/IL-1 β -driven pyroptotic epithelial cell shedding and increased susceptibility to urinary tract infection

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Aging contributes to increased risk for chronic and severe diseases mainly as consequences of increased susceptibility to infection, decreased ability to maintain homeostasis, inefficiency in combatting stress, and decreased regenerative capacity. In the lower urinary tract, aging increases the risk of developing urinary incontinence and urinary tract infection (UTI). However, mechanisms underlying these conditions and how the urinary tract mucosa is affected by aging and related immune changes and what mechanistic role these changes might play in the development of UTIs and their recurrence are unclear. Our objectives were to delineate the cellular and molecular milieu of the bladder in the context of aging and UPEC-induced UTIs, identify core mechanistic underpinnings, and evaluate treatments to ameliorate age-associated dysfunction.

We performed histopathological analyses of young (8-12wks) and aged (18-24 months) female mice and induced a UTI in a subset, and treated aged mice with D-mannose for 14 days. Impact of age, UTI, and D-mannose on cellular and molecular changes in the bladder and urine was evaluated using urinalysis, cytological analysis and cytokine quantitation (ELISA). Bladders were collected for transmission electron microscopy (TEM), qPCR, Western blot, enzyme activity assay, and immunohistochemical/cytochemical staining, as well as for transcriptomic analysis.

Cytological and immunological assays revealed notable changes in urothelial cell biology including increased vesicle congestion, dysfunctional autophagy, lysosome, and mitochondria; heightened oxidative stress; diminished antioxidative response; and intensified immune response in aged bladders. We also found increased inflammation, senescence-associated secretory phenotype, and DNA fragmentation (γ -H2AX), and spontaneous pyroptotic cell death via activation of Nlrp3 inflammasome and GasderminD in the aged urothelium. We noted age-dependent and microbiome-independent development of tertiary lymphoid structures in bladder stroma and demonstrated that aged bladder supports increased bacterial persistence and spontaneous recurrence of infection. Finally, we found evidence of beneficial effects of D-mannose in the aging bladder by promoting autophagic flux and reducing pyroptotic epithelial shedding.

Overall, our findings show that aging alone induces cellular and molecular alterations in the aging bladder. We found that chronic inflammation due to aging can initiate and enhance cellular senescence of the urothelium, which can be further aggravated by bacterial infection and in turn promote greater susceptibility to rUTIs. Finally, our study suggests that D-mannose supplementation serves as a senotherapeutic to alleviate urothelial dysfunction caused by aging.

#6 – Loss of Thrombospondin-1 in Macrophages as a Promoter of Benign Prostatic Hyperplasia

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) affects the vast majority of aging men and is associated with chronic inflammation. Recent work indicates that systemic targeting of inflammatory pathways reduces hyperplasia in the prostate, but a mechanistic understanding of how inflammation contributes to BPH is lacking. Thrombospondin-1 (TSP1) is a secreted glycoprotein with anti-angiogenic, anti-inflammatory, and extracellular matrix remodeling functions. TSP1 induces TGF β activation and expression decreases in BPH versus normal tissues. These studies were conducted to determine if immune cells contribute to TSP1 loss in BPH.

METHODS: Single-cell mRNA-sequencing studies of CD45⁺ immune cells were conducted on the transition zone of 10 small (<40 grams) and 10 large (>90 grams) human prostates. Analysis of differentially expressed genes between groups and evaluation of primary cells were completed. TSP1 expression was evaluated with immunofluorescence (IF) and ELISA, and either TSP1 siRNA or LSKL peptides were used to decrease expression or inhibit TGF β activation function of this protein, respectively.

RESULTS: A subpopulation of macrophages (M ϕ s) identified as HLADR+CD16⁺ accumulate in large versus small prostate tissues and their abundance positively correlates with patient symptoms. TSP1 is significantly downregulated between leukocytes from large versus small prostates. Immunofluorescence staining indicates that TSP1 is primarily secreted by BPH M ϕ s, and HLADR+CD16⁺ M ϕ s express lower TSP1 than HLADR+CD16⁻ M ϕ s. *In vitro* analysis of TSP1 function in THP-1 cells revealed that pro-inflammatory M1 M ϕ s secrete less TSP1 than anti-inflammatory M2 M ϕ s. Additionally, inhibition of TSP1-mediated activation of TGF β using LSKL peptides induced higher secretion of pro-inflammatory cytokines IL-1b and IL-6 versus control peptides.

CONCLUSIONS: TSP1 is secreted by BPH M ϕ s but downregulated during prostate expansion. A subpopulation of M ϕ s with low TSP1 expression accumulates as prostate size increases and symptoms worsen, which may exacerbate inflammation and result in the loss of TGF β -mediated regulation of stromal/epithelial proliferation. Ongoing work is pursuing novel therapeutic strategies that target chronic inflammation in BPH.



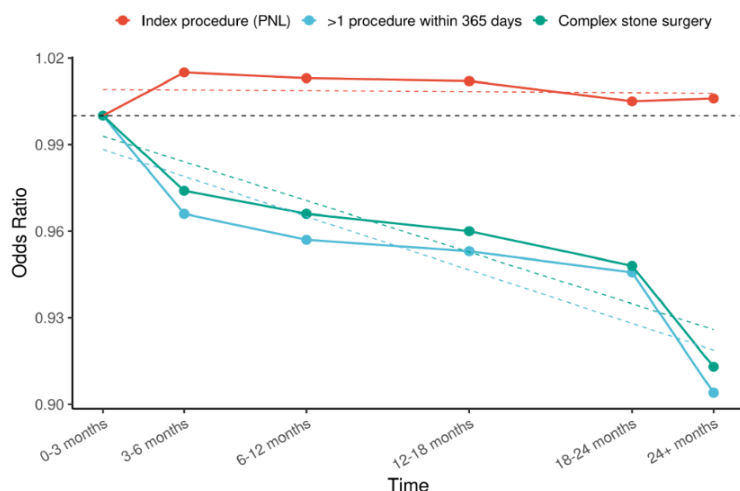
#7 - Longer surgical wait times after visit to urology clinic is paradoxically associated with decreasing odds of eventual complex kidney stone surgery

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Introduction and objective: Kidney stones are known to grow over time and delays between emergency department (ED) visits and surgical care are associated with more complex surgical approaches for stone treatment. As such, the objective of this study is to investigate the effect of the time interval from urology clinic visit to surgery on the eventual surgical approach for patients with nephrolithiasis.

Methods: A longitudinal study using a national database, the AUA Quality (AQUA) Registry, was undertaken. Adult patients (age ≥ 18) having a diagnosis of nephrolithiasis and undergoing at least one urologic stone procedure were identified. These patients were then followed for at least 365 days following their index procedure to determine the total quantity of kidney stone procedures. Any patients with fewer than 365 days of follow-up were excluded. Our outcomes were defined as percutaneous nephrolithotomy (PNL) vs. non-PNL index procedure, undergoing more than 1 urologic procedure within 365 days, and receipt of complex surgery (PNL as index procedure or > 1 procedure within 365 days). Odds ratios for each outcome were measured using a multivariate logistic model with surgical wait time as predictor controlling for age, gender, race, insurance, social deprivation index, obesity, diabetes, and flomax prescription.

Results: A total of 137,477 patients underwent at least one urologic stone procedure. Of these, 6,497 (4.7%) patients underwent PNL as their initial procedure and 40,968 (29.8%) patients underwent more than 1 procedure within 365 days. Stratifying patients by index procedure type revealed higher odds of undergoing PNL for any wait time (ref: 0-3 months) but little change was observed as wait times increased (slope = 0.0005, $P = 0.29$). Odds of undergoing multiple procedures (slope = -0.0035) and receipt of complex stone surgery (slope = -0.0031) both showed a decreased trend as wait times increased ($P < 0.001$).



Conclusions: Increased surgical wait times is generally expected to lead to worse outcomes due to disease progression in the patient without prompt treatment. Paradoxically, we show that an increasing time interval from urologic clinic visits to surgical treatment associates with lower odds of complex surgery. Therefore, time from diagnosis of stone disease to evaluation by a urologist may be the rate limiting step in the time course of stone disease treatment. Reducing delays between stone diagnosis and urology consultation may reduce previously recognized disparities in the eventual need for complex surgical treatment for kidney stone disease.

Source of Funding: K12DK111028

#8 - Compound 48/80 causes the release of Urothelium-Derived Mediators to Induce Urinary Bladder Smooth Muscle Contractions

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Introduction and Objective: The urothelium serves as an active barrier to prevent pathogens and chemical stimuli within the urine from influencing urinary bladder function. However, certain pathogens can activate inflammatory signaling pathways that lead to urothelial cell damage, dysregulation of adenosine triphosphate (ATP) release, and neural activation. This is evident in urinary tract infections, interstitial cystitis, pelvic pain, and painful bladder syndrome. Current animal models used to induce interstitial cystitis or bacterial infection implicate mast cell degranulation as a leading cause of hypersensitivity that induces lower urinary tract symptoms. Thus, we hypothesized that phasic contractions caused by the mast cell activator compound 48/80 were due to mast cell degranulation and the subsequent release of prostaglandin E2 from the urothelium. Based on this hypothesis, we also examined the effects of compound 48/80 on urinary bladder smooth muscle (UBSM) strips from non-diseased mast cell deficient and C57BL/6 mice to establish a physiological mechanism by which mast degranulation can cause smooth muscle excitability.

Methods: Based on this hypothesis, we also examined the effects of compound 48/80 on urinary bladder smooth muscle (UBSM) strips from non-diseased c-KitW-sh/W-sh mast cell deficient and C57BL/6 mice via isometric contractility. UBSM strips were incubated with vehicle or the following drugs prior to exposure to compound 48/80: the nonsteroidal anti-inflammatory and cyclooxygenase (COX) inhibitor indomethacin (5 μ M); the prostaglandin EP1 receptor antagonist SC51089 (10 μ M); the non-selective P2 purinergic antagonist PPADS (100 μ M); and the Gq/11 signaling inhibitor YM254890 (1 μ M).

Results: In UBSM strips from C57BL/6 and c-KitW-sh/W-sh mice, compound 48/80 caused both an increase in basal UBSM tone as well as an increase in phasic contractions. Removal of the urothelium significantly reduced the amplitude and occurrence of phasic contractions in both strains. SC51089 had no effect on phasic contractions induced by compound 48/80. However, indomethacin, PPADS and YM254890 significantly reduced phasic contractions.

Conclusions: Our data suggest that the basic secretagogue compound 48/80 is increasing phasic contractions via activation of urothelial cell signaling that is causing the release of ATP and activation of a cyclooxygenase-mediated pathway to increase the synthesis of pro-contractile mediators.

#9 - Identification of collagen producing cell lineages in the inflamed mouse prostate

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INTRODUCTION AND OBJECTIVE: Prostate fibrosis, triggered by inflammation and characterized by excess collagen accumulation in the prostate, is a strong predictor of lower urinary tract symptoms (LUTS). Our objective is to identify cells responsible for collagen synthesis, to facilitate the future goal of pharmacological targeting of collagen synthesis in the prostate.

METHODS: In many fibrotic diseases, collagen producing cells are activated from phenotypically distinct progenitors. To trace collagen producing cells in the inflamed mouse prostate back to the progenitor cells from which they arose, cre/lox recombination gene editing was used to drive Red Fluorescent Protein (RFP) expression in specific cell types and their daughter cells. We generated mouse lines carrying an inducible RFP allele combined with a cre allele directed



to one of six cell types linked to fibrosis in other organ systems: smooth muscle cells (*Myh11-cre*), myeloid cells (*Lyz2-cre*), lymphoid cells (*Cd2-cre*), pericytes and perivascular stromal cells (*Gli1-cre*), fibroblasts (*S100a4-cre*), and prostatic fibroblasts (*Srd5a2-cre*). Mice were infected with uropathogenic *E. coli* UTI89 to drive prostate inflammation and collagen synthesis. Pro-collagen and RFP immunostaining was used to identify collagen producing lineage labeled cells. Hybridized chain reaction (HCR), a RNA labeling method, was used in combination with a pro-collagen antibody, as an independent means to validate collagen producing cells.

RESULTS: *E. coli* infection significantly increases prostatic densities of *S100a4*+ ($p=0.0065$), *Lyz2*+ ($p=0.0063$), *Gli1*+ ($p=0.0058$), and *Cd2*+ ($p=0.00071$) cell lineages without increasing *Myh11*+ and *Srd5a2*+ cell lineages. The contribution of lineage labeled cells to total collagen producing cells was independently calculated for each mouse line. *S100a4*+ lineage cells make up approximately 86% of Pro-collagen+ cells in the inflamed mouse prostate, while *Lyz2*+ and *Gli1*+ lineage cells each make up 70%, and *Cd2*+ cells make up 27%. We note that the percent contribution of each lineage to total collagen producing cells in the prostate exceeds 100%, indicating potential overlap of cell lineages. Preliminary HCR results reveal a population of Pro-collagen+ cells coexpressing *Lyz2* and *S100A4* mRNAs.

CONCLUSIONS: We have determined the cell lineage contributing to *E. coli* mediated prostatic collagen accumulation is marked by *Lyz2* and *S100A4*. From this data along with ongoing research, we hypothesize a bone marrow derived myeloid cell is recruited to the inflamed mouse prostate where it differentiates and produces collagen.

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* Both are first authors

#10 - *SlincR*: A New Modulator For Inflammation In The Reproductive Tract

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Introduction and Objective: Inflammation is implicated in several benign urological diseases, including prostate inflammation and urinary tract infection. These diseases are ubiquitous and deleterious to one's quality of life. There is considerable interest in understanding inflammation mechanisms and strategies to modulate them. *SlincR* is a long non-coding RNA with unresolved function, but several studies indicate a potential role in T cell differentiation and inflammation. Genetic polymorphisms in the human *SLINC*R have been linked to a variety of soft tissue cancers in which T cell signaling plays a role. *SlincR* is also implicated in the toxicity of persistent environmental contaminants which bind the aryl hydrocarbon receptor and cause immunosuppression. *SlincR* interacts with the T cell antigen receptor, required for T-cells to recognize foreign antigens, and is predicted to interact with *Elavl1*, a gene required for T cell differentiation. Therefore, we hypothesize that *SlincR* may function as an immunosuppressor in the lower urinary tract and elsewhere and we predict it will be possible to pharmacologically manipulate *SlincR* abundance as a therapeutic strategy to control inflammation.

Methods: We used CRISPR/Cas9 genome editing to excise exon 3 of the mouse *SlincR* gene (*SlincR*^{ΔExc3}) to create a null allele and confirmed germline transmission in C57BL/6J mice. 6-8 week old wild type control and *SlincR*^{ΔExc3/ΔExc3} male mice were immunized subcutaneously with rat prostate antigen to drive experimental autoimmune prostatitis (EAP). Urinary voiding behaviors were measured 1 week later by the void spot assay and cystometry. Prostates were collected 1 week later. We will use immunostaining to test whether *SlincR*^{ΔExc3/ΔExc3} mice mount a more severe inflammatory response (more prostatic CD45+ leukocytes) than wild

type control mice and we will use cell sorting to quantify T regulatory and T helper T cells. In a second experiment, a transurethral catheter was placed in 8-10 week old wild type control and *SlincR*^{ΔExc3/ΔExc3} female mice to deliver uropathogenic *E. coli* UTI89 and cause UTI. Urinary bacterial colony forming units were measured at fixed intervals to test whether *SlincR*^{ΔExc3/ΔExc3} clear the infection more quickly than wild type mice. We will collect bladders from these mice and use cell sorting to quantify T regulatory and T helper cells.

Expected and observed Results: We observed a decrease in bacterial colonies collected from *SlincR*^{ΔExc3/ΔExc3} mice and an increase in void spots in EAP *SlincR*^{ΔExc3/ΔExc3} mice.

Conclusions: Our preliminary studies reveal a potential role for *SlincR* in mediating biologically relevant immune responses in the lower urinary tract of male and female mice.

#11 - Defining the Infant Urobiome

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INTRODUCTION AND OBJECTIVE: The human urinary tract harbors a complex microbial community termed the urobiome. Although changes in the urobiome have been associated with a variety of benign urologic diseases, there remain key unknowns about how the human urobiome develops over time. To begin to bridge this gap, we sought to define the structure of the pediatric urobiome in infants younger than 1 year old.

METHODS: Urine was collected via transurethral catheterization from infant males (n=50, median age 0.6 years old) immediately prior to undergoing circumcision in an operating room. Urine volumes ranging from 0.4mL-28mL were collected. Urine was sent for DNA extraction and sequencing to UCSD Microbiome Core. Following PCR amplification of the V4 hypervariable region of the 16S rRNA, Illumina paired-end sequencing was performed. Resulting reads were analyzed using the DADA2 program. Negative controls included extraction blanks, hospital saline, and saline flushed through a catheter. Furthermore, we designed an extended culture protocol to broadly capture culturable bacteria using limited urine volume using blood agar and Brucella agar under aerobic and anaerobic conditions.

RESULTS: A consistent microbial composition was observed via 16S rRNA sequencing, with specific taxa identified in all samples. We compared urobiome diversity based on available metadata, including preterm delivery, caesarean section, and prior antibiotic exposure. We identified that 64% of urine samples contained culturable bacteria using extended culture. The most common phylogenetic family detected by extended culture was *Actinomycetaceae*, with *Actinotignum schaalii* being identified in 9 samples. Whole genome sequencing revealed *A. schaalii* isolates were unique strains to each patient.

CONCLUSIONS: Infants under the age of 1 year have a detectable and diverse urinary microbiome. This study begins to inform our understanding of the development of the urobiome beginning in infancy.

#12 - Histamine causes endothelium-independent dilation of mouse bladder feed arterioles

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Introduction and objective: In addition to being highly vascularized, the urinary bladder vasculature has unique properties that help to maintain blood flow during voiding and filling. Unlike other vessels of similar size, bladder arterioles do not contract to increases



in intraluminal pressure. Prolonged decreases in bladder blood flow are linked to many types of bladder dysfunction, including painful bladder syndrome and interstitial cystitis. Although the vasculature has an important role in bladder dysfunction, the mechanisms regulating vasculature diameter in the bladder are still widely unknown. We tested the hypothesis that histamine, an important pro-inflammatory compound released from mast cells in the bladder wall, also alters bladder arteriolar diameter.

Methods: We euthanized and dissected the urinary bladder from 10-14 weeks old C57BL/6J mice. Mouse bladder feeder arterioles are isolated and then cannulated in a pressure myograph system. This allows us to keep the arteriole at constant pressure inside while exposing the arterioles to any desired drugs or environment.

Results: Concentration-response curves to histamine (1 nM – 10 μ M) were performed in vessels constricted with the thromboxane mimetic U-46619 (10 nM). Histamine caused a concentration dependent vasodilation that was inhibited by the histamine H2 receptor antagonist cimetidine (10 μ M) but not the H1 receptor antagonist fexofenadine (10 μ M). Histamine-dependent vasodilation was not inhibited by the NOS inhibitor L-NAME (200 μ M), the inward rectifier K⁺ channel blocker BaCl₂ (100 μ M), or endothelial denudation, suggesting the dilation was endothelium-independent. Alternatively, increasing extracellular K⁺ (35 mM) or exposure to the KV channel blocker XE-991 (10 μ M), and the non-selective K⁺ channel blocker 4-Aminopyridine (3 mM) attenuated dilations to histamine.

Conclusion: These findings suggest the H2 receptors in bladder vascular smooth muscle, and not the endothelium, mediate histamine-induced dilation in bladder arterioles. Since H2 receptors are coupled to G α -S subunit, these findings also suggest that histamine dilation results from an increase in cAMP that may hyperpolarize smooth muscle cells via protein kinase A-mediated activation of smooth muscle KV channels.

Supported by NIH K01-DK103840 (NRT), R01-DK119615 (NRT), and P20-127554 (NRT)

#13 - Mechanism of Increased Detrusor Contractility Induced by Mast Cell Activator Compound 48/80 in Murine Urinary Bladder

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HYPOTHESIS/AIMS OF STUDY: A balance between stiffness and compliance is essential to normal bladder function. Inflammatory processes, such as mast cell activation, can affect the integrity of the bladder wall and lead to increased stiffness. The resultant bladder wall remodeling ultimately leads to many lower urinary tract symptoms. Compound 48/80 is a basic secretagogue that rapidly degranulates mast cells and brings about release of multiple inflammatory mediators including prostaglandins, and matrix metalloproteases (MMPs). These mediators alter urinary bladder smooth muscle contractility as well as wall compliance. In this study, we explored if and how compound 48/80 alters urinary bladder smooth muscle contractility and wall compliance.

STUDY DESIGN: Whole mouse bladders were dissected and mounted in the custom designed Pentaplanar Reflected Image Macroscopy (PRIM) System for simultaneous measurement of transient pressure events, bladder volume, intravesical pressure, wall stress and wall stretch during *ex vivo* bladder filling. Using the recorded intravesical pressures, infusion volumes, image data, we next calculated the bladder wall compliance (defined as the stress versus stretch relationship of the bladder wall). To test our hypothesis, bladders were exposed to either broad spectrum MMP inhibitor Doxycycline (20 μ M) or Cyclooxygenase inhibitor Indomethacin (10 μ M) prior to the addition of vehicle or the mast cell activator compound 48/80 (10 μ g/ml).

RESULTS: Compound 48/80 alone significantly increased the amplitude and leading slope of transient contractions while increasing bladder wall compliance. The broad spectrum MMP inhibitor doxycycline prevented the increase in compliance caused

by compound 48/80; however, the amplitude and leading slope of transient contractions was unaffected. Interestingly, the non-selective cyclooxygenase inhibitor indomethacin prevented all effects of compound 48/80 implying that the mechanism through which compound 48/80 acts is two-fold and parallel.

CONCLUSIONS: Our study discovered that the effects of Compound 48/80 on the urinary bladder are multi-faceted, in that it rapidly activates mast cells to release of prostaglandins, which then increase bladder smooth muscle contractility and activate MMP-mediated increases in bladder wall compliance. Thus, bladders can show signs of overactivity while actually increasing in compliance. Furthermore, changes in compliance and urinary bladder contractility can occur simultaneously and independently. This pathway paves the way for reframing our knowledge on treatments offered for bladder dysfunction that is accompanied by inflammation.

#14 - Alteration of intracellular collagen 1 distribution in prostate fibroblasts by beta-sitosterol, the major component of prostate health supplements

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University of Massachusetts Boston

Introduction and Objectives: Saw Palmetto is marketed as an effective "natural" product for ameliorating BPH and LUTD symptoms by both decreasing prostate volume and easing urinary incontinence. The main active ingredient of these supplements is β -sitosterol, a phytosterol shown to function as an androgen receptor blocker. While millions of men are taking these supplements for their reported benefits, the function of β -sitosterol in prostate fibroblasts, and its long-term effects on prostate health, are poorly categorized. Because β -sitosterol reportedly activates known collagen synthesis pathways, we hypothesized that a potential long-term consequence of β -sitosterol usage may be increased collagen synthesis by human prostate fibroblasts and consequent prostatic fibrosis.

Methods. N1 human prostate fibroblasts were grown in serum-free media with or without increasing levels of β -sitosterol (10 μ M – 50 μ M). N1 cells were similarly treated with the canonical pro-fibrotic, TGF β (4ng/mL), as a positive control. Levels of collagen production were measured between treated and non-treated cells via western blot and qRT-PCR. Morphologic differences were also categorized through cell morphology images captured at t = 0hr, t = 24hr, and t = 48hr post-treatment.

Results. Western blot studies demonstrated significantly higher levels of intracellular collagen I produced by N1 cells treated with β -sitosterol (p<.05) and TGF β (p <.05) compared to vehicle-treated cells. Whereas β -sitosterol induced expression of collagen1a2, TGF β induced that of collagen1a1 components of the collagen 1 protein. Interestingly, qRT-PCR showed similar levels of COL1/3 transcripts in β -sitosteroltreated cells compared to vehicle-treated cells and significantly lower levels of COL1/3 transcripts compared to TGF β -treated cells (p < .05). Cell morphology analysis showed the accumulation of "inclusions" throughout the cytoplasm in β -sitosterol-treated cells which were not present in vehicle- or TGF β -treated cells.

Conclusions. Initial results indicate that β -sitosterol treatment induces an increase in intracellular collagen 1, though qRT-PCR results do not demonstrate a concomitant increase in COL1A1 transcript levels. Cell morphology also shows bright inclusions in the cell cytoplasm that are not seen in vehicle or TGF β -treated cells. Future work will compare COL1A2 transcript levels in β -sitosterol- and TGF β -treated cells, include analysis of additional cell lines and primary prostate fibroblast cells, and examine whether β -sitosterol impairs exocytic trafficking through measurement of extracellular collagen as well as co-localization of collagen within COPII vesicles.



#15 - An *In vitro* Evaluation of Thermal Injury Risk in Popdusting using Thulium Fiber Laser

Junqin Chen and **Pei Zhong**

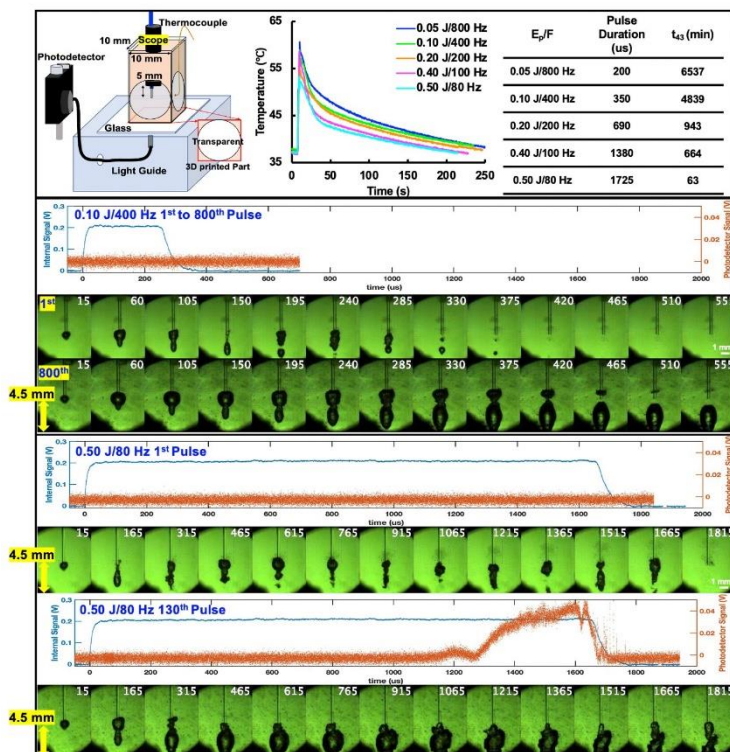
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INTRODUCTION AND OBJECTIVE: Thermal damage is a significant clinical concern during popdusting of kidney stone patients when high power lasers are used in a confined renal environment. In this work, we evaluate the effects of pulse energy (E_p) and frequency (F) of thulium fiber laser (TFL) on the thermal injury risk in an *in vitro* kidney model.

METHODS: To mimic a calyx environment, a 3D-printed model with a spherical chamber of 5 mm in radius was embedded into a cuvette (10 x 10 x 40 mm, L x W x H) filled with degassed water at 37 °C. A TFL system (TLR-50W, IPG Photonics) with 200 μ m fiber was used to deliver a total laser energy of 80 J under 40 W power with various combinations of E_p (0.05 – 0.50 J) and F (80 to 800 Hz, see Fig. 1) without irrigation. The fiber tip was placed 4.5 mm away from the cuvette wall, i.e., the tissue boundary. The temperature changes in the chamber were recorded by thermocouples placed 5 mm away from the fiber tip at a sampling rate of 0.2 s and the concomitant bubble dynamics were captured by high-speed video imaging. In addition, experiments were performed with the bottom surface of the cuvette replaced by a fused quartz glass slide so that any laser energy reaching the “tissue boundary” could be detected using a light guide, connected to a InGaAs photodetector (PDA10D, Thorlabs).

RESULTS: For the same total laser energy delivered, the combination of low E_p and high F caused higher overall temperature rises in the model and thus reaching the thermal dose of tissue injury ($t_{43} = 120$ min) more rapidly than high E_p and low F (Fig. 1). Results from high-speed imaging show that the bubble collapse time was prolonged with elevated fluid temperatures during popdusting under all laser settings. In particular, at high E_p and low F, such as 0.50 J/80 Hz, the elongated bubbles created a long vapor tunnel, extending to the “tissue” boundary directly facing to the fiber tip. In contrast, at low E_p and high F, such as 0.1J/400 Hz, the elongated bubble was smaller and irregular with a high tendency to break up, and thus all the laser energies were absorbed in the fluid, leading to higher global temperature rise in the kidney model.

FIGURE 1:



CONCLUSIONS: Without irrigation, thermal tissue injury during TFL treatment may be caused by two distinctly different mechanisms: (1) the global heat conduction in the intrarenal fluid due to laser absorption at low E_p and high F, and (2) local laser irradiation of the tissue directly facing the fiber tip due to the significantly elongated vapor tunnel produced under high E_p and low F.

#16 - Developmental exposure to the environmental toxicant, polychlorinated biphenyls, alters voiding physiology, bladder volume, nerve density, and TRPV1 agonism in mice

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Environmental contaminants are risk factors for several disorders, yet their ability to influence lower urinary tract (LUT) function is understudied. Polychlorinated biphenyls (PCBs) can influence the nervous and immune systems, yet connection between PCBs and bladder function is a current gap in the etiology of LUT dysfunction. Using a PCB profile that is relevant to human health, we study the effects on voiding physiology of developmentally exposed mouse models, when sensitivity of tissue generation and repair may predispose later in life adverse outcomes. TRPV1, a calcium channel that mediates neurological signaling during bladder distention, has not been studied with PCB exposure.

Nulliparous mice were dosed through gestation and lactation with an environmentally relevant mixture of PCBs at 0, 0.1, 1, or 6 mg/kg/d. Offspring were tested at 4, 6, or 12 weeks. Body mass, bladder mass, bladder volume, and epithelial thickness were tested at 4 or 6 weeks for overt toxicity, bladder obstruction, or decreased barrier function. Voiding physiology tests were performed at 6 or 12 weeks using void spot assays, uroflowmetry, and anesthetized cystometry. 12 week offspring were also infused with 30uM capsaicin after saline cystometry to agonize TRPV1. Immunohistochemistry was performed on bladders from 4 or 6 week offspring using B-tubulin III for pan-nerve staining and CGRP for sensory nerve staining to measure nerve density at the suburothelium.

At 4 weeks, there were no observed changes indicating overt toxicity, bladder obstruction, or decreased barrier function. Bladder volume was increased in 6 mg/kg/d males at 4 and 6 weeks, and 6 mg/kg/d females at 6 weeks. Effects on voiding physiology were sex- and dose- dependent generally presenting as frequent, small, drop-like voids. Overall nerve density was increased in 6 mg/kg/d males at 4 weeks and testing at 6 weeks is ongoing. Sensory nerve density was increased between 0.1 and 6 mg/kg/d males at 4 weeks and testing at 6 weeks is planned. Voiding physiology at 12 weeks was modified by capsaicin by both male and female mice in a sex- and dose-dependent manner.

Developmental exposure to PCBs in mice causes changes to mouse voiding physiology, suburothelial nerve densities, and neurological signaling involving TRPV1. Altered voiding responses to TRPV1 agonism, and changes to nerve densities, indicate that developmental exposure to PCBs may alter the plasticity of bladder innervation or modulation of neurological signaling from the bladder.

Supported by NIH awards R00ES029537, T32ES007015, and U54DK104310

#17 - Alterations in estrogen metabolism alleviates the development of steroid hormone induced lower urinary tract dysfunction

Teresa T. Liu, **Lauren Hackner**, **Donald B. DeFranco**, **William A. Ricke**

Background: Benign prostatic hyperplasia is a multifactorial disease that, with a combination of smooth muscle dysfunction, prostatic proliferation, and fibrosis, can lead to lower urinary tract symptoms. Regardless of the underlying manifestation of the disease, age is the single greatest risk factor for the development of BPH/LUTS. Steroidogenesis in the prostate is not limited to the metabolism of



testosterone (T) to dihydrotestosterone (DHT); these androgen ligands can be further metabolized into estrogen receptor alpha and beta (ER α and ER β) ligands. One function of aging in men is the decrease in circulating levels of T while levels of estrogens (E) remain the same or increases, which contributes not only to a decreased T:E ratio but also alter the ER α :ER β activation. In this study, we use a genetic knockout of a steroid hormone enzyme, CYP7B1, that is critical to the metabolism of 3 β -diol, a high-affinity ligand for ER β , to examine the initiation and progression of lower urinary tract dysfunction (LUTD). We hypothesize that the loss of CYP7B1 increases the accumulation of ER β ligands leading to decreased proliferation and resolution of LUTD.

Methods: Using wild-type and CYP7B1 knockout mice, in a C57Bl6/129S mixed background, we examined the development of LUTD using the steroid-hormone induced model of LUTD. We measured the induction of LUTD upon steroid hormone implantation weekly using void spot assays (VSA). Mice were euthanized after four weeks, and tissues were collected for immunohistochemistry and LC-MS.

Results: The B6/129S mixed background mice responded to T+E2 hormones as previously characterized in C57Bl6 wild-type mice. The CYP7B1 wild-type mice with steroid hormones showed a significant increase in urine spot counts suggesting an increase in LUTD. The knockout mice, under the same conditions, showed a significant decrease in void spot counts, suggesting an alleviation of voiding dysfunction characteristic of this model. Interestingly, upon euthanasia of these animals at four weeks post implantation, the knockout mice still presented with urine retention as measured by bladder volume.

Conclusion: Although the loss of CYP7B1 in this mouse model showed an improvement in voiding function as measured by VSA, these mice still developed bladder and prostate changes similar to that of wild-type mice. These results could indicate the importance of ER β activation on alleviating the voiding dysfunction but not the cellular changes within the prostate and bladder.

#18 - Investigation of Lower Urinary Tract Biomechanics Using Dynamic MRI and Computational Fluid Dynamics

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Introduction/Background: Voiding physiology is most studied with video-urodynamics. Its limitations include inability to simultaneously measure dynamics changes in pressure at in the bladder and at multiple positions along the urethra. Also, it lacks spatial and temporal resolution for the volumetric assessment of dynamic changes in urethral anatomy during voiding. Here, we used dynamic magnetic resonance imaging (MRI) to obtain dynamic, high-fidelity 3D images of bladder and lower urinary tract during voiding. Additionally we coupled MRI and computational fluid dynamics to characterize biomechanics of voiding in a young healthy man.

Methods/Materials: MRI was performed on a healthy young man using a clinical 3T scanner according to an IRB- approved protocol, acquiring bladder images every 1.5 s during voiding. 3D anatomic models of the bladder and urethra were created with MIMICS (Materialise, Leuven, Belgium) and analyzed using a novel algorithm for CFD. This uses computational software to simulate fluid behavior under specified constraints of geometry and flow and enables calculation of voided volume, residual, flow, pressures and wall shear stress.

Results: **Figure 1** shows a sagittal reconstruction of the change in bladder shape during the voiding cycle (A) and angle of the open bladder neck at maximum flow. **Figure 1.C** shows the urine flow curve and bladder pressures. Wall shear stress (WSS), pressures and fluid velocities along the length of the urethra during maximum flow were also obtained from the CFD (**Figure 2**). Most notably, we noted a striking change in PUA during voiding. PUA becomes less

acute as flow increases, being straightest at peak flow rate (**Figure 2d**).

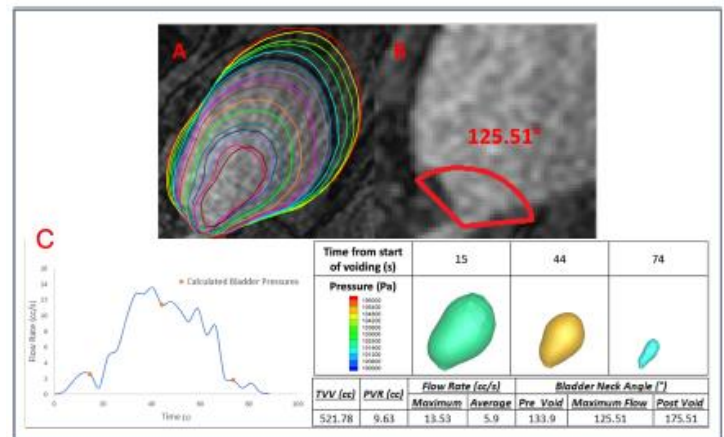


Figure 1: A) Sagittal contour lines of the bladder lumen during voiding. B) Bladder neck angle at maximum flow. C) (Left) Urine flow curve determined by time-dependent changes in calculated bladder volume. Timepoints of calculated bladder pressure are indicated by red dots. (Right) Color-coded bladder pressures at three times during voiding.

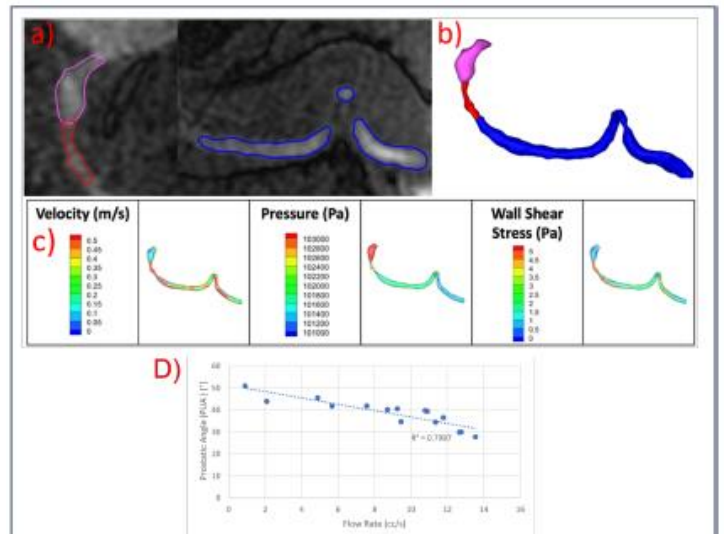


Figure 2: a) Segmentation of the prostatic (purple), membranous (red) and penile (blue) urethras during voiding. b) 3D reconstruction of the urethral lumen. c) Color coded representation of Pressure, WSS and fluid velocity along the course of the urethra during voiding. d) PUA vs flow rate.

Conclusions: Dynamic MRI and CFD is a non-invasive alternative to video-urodynamics that provides both superior anatomic detail and a more comprehensive functional assessment of lower urinary tract biomechanics during voiding.

#19 - Targeted metabolomics of aged bladders reveals a key role for Irg1 in modulating inflammatory responses

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Aging is a multifactorial progressive decline in molecular, cellular, and tissue functions. Recently, we reported that aging promotes chronic inflammation or “inflammaging” and epithelial senescence in mouse and human bladders. Additionally, we demonstrated an increase in oxidative stress coupled with an unresponsive antioxidant mechanism in aged urothelium promoting increased NLRP3 inflammasome activation, pyroptotic urothelial cell death and



exfoliation into the urine. Here, we hypothesize that these alterations in inflammaging and senescence are a result of metabolic dysfunction. Dysregulated metabolism (including mitochondrial metabolism) is a hallmark of aging and a comprehensive understanding of metabolites and their signaling will pave the way for targeted interventions in the aging bladder.

We performed liquid chromatography and mass spectrometry (LC-MS/MS) on bladders from aged (22 months) and young (6-8 weeks) C57BL/6 female mice. LC-MS data were processed, log2 transformed, and normalized. Differentially altered metabolites were determined using the Benjamini-Hochberg method and their significance were validated using student t-test. qPCR analysis for highly altered pathways was performed in aged and young cohorts, Irg1-/- young mice lacking the Immune-Responsive Gene 1 (Irg1) an itaconate producing enzyme in the tricarboxylic acid (TCA) cycle, and dimethyl itaconate (DMI)-treated aged mice.

LC-MS data revealed >150 differential metabolites significantly altered in the aged compared to young bladder. Preliminary analyses demonstrated key mitochondrial metabolic pathways significantly impacted with age include the TCA cycle. We found that aged bladders have decreased Irg1 compared with young. We show that itaconate is required for the activation of the anti-inflammatory transcription factor Nrf2 to increase the expression of antioxidant and anti-inflammatory pathways. Irg1-/- mice have reduced Nrf2 activation and increased inflammatory markers such as NLRP3 inflammasome and IL-1 β as well as increased epithelial exfoliation. Treatment with DMI activated Nrf2 in the bladder, and significantly lowered oxidative stress, cell exfoliation, and inflammatory index. Our findings highlight Irg1 as a significant enzyme in the TCA cycle that can regulate age-associated inflammatory responses. Loss of Irg1/itaconate metabolism results in a 'premature' aging phenotype. However, treatment with DMI reverses these changes in the bladder further solidifying the role of itaconate in epithelial homeostasis and limiting inflammation in the aging bladder. Future studies targeting inflammaging should consider using itaconate as a stand-alone supplement or in combination with other aging interventions.

#20 - Identification of key molecular players in the development of Radiation Cystitis through RNA sequencing

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Introduction and objectives: Pelvic cancer survivors that were treated with radiation therapy are at risk for developing radiation (hemorrhagic) cystitis (RC) up to 15 years after completion of radiation therapy. Patients with RC suffer from lower urinary tract symptoms, including frequency, nocturia, pelvic pain and incontinence. At an advanced stage, patients can develop hematuria, which can become life-threatening if bleeding cannot be controlled. To date, no safe and reliable therapy exists to treat RC, in part due to the unavailability of bladder biopsies. Taking biopsies of an already fragile bladder is unethical. The objective of this study is to use our established preclinical model to gain insight in the molecular pathways that drive radiation-induced tissue changes in the bladder.

Methods: Female C57BL/6 mice received a single dose of 40 Gy using the SARRP irradiator. Bladder physiology was assessed using void spot assay. Bladders were harvested at different time points (1 week, 4 weeks, 3 months and 6 months) after irradiation, RNA was harvested, and mRNA sequencing was performed at paired-end 150bp on the Illumina NovaSeq6000 with a target of 30 million reads per sample. Following RNA sequencing, thorough bioinformatics analysis was performed. Findings of the RNA sequencing were validated using qPCR analysis.

Results: At 1-week post-irradiation, altered gene expression was detected in genes involved in DNA damage response, apoptosis, and transcriptional regulation. By 6-months post-irradiation, significant changes in gene expression were observed in vascular

health, wound healing, inflammation, and apoptosis. Genes significantly up- or downregulated in these pathways are listed in table 1. Affected pathways include the Wnt signaling, p53, JAK-STAT, and PI3K-Akt pathways. These findings are currently being validated in vitro in human urothelial cells (SV-HUC-1), and in vivo in bladder tissues from our preclinical model.

Table 1: Gene expression changes during different phases of RC

	Early RC		Late RC		
	DNA Damage/Apoptosis	Transcriptional Regulation	Wound Healing	Inflammation	Vascular Health
Up-regulated genes	Rpm	Etv4	F7	IL12b	Col22a1
	Cdkn1	Wnt10a	F10	IL17	Itgax
	Cong1	Wnt6		IL18	Cxcr2
	Gtse1	Ctnna2		H2-Aa	
	Polk			H2Ab1	
	Bax			H2-Eb1	
Down-regulated genes	Igf3	Sox7	Pecam1	Pecam1	Pecam1
			Wnt10b	Col21a	Lrg1
				Col21c	Cldn5
				Itgax	Tek

Conclusions: The outcome of these studies will have a tremendous impact on the field. This is the first study to determine the molecular changes in the bladder in response to radiation treatment. We hereby identify the physiological changes that drive the progression of radiation cystitis which will aid in identifying potential therapeutic targets.

#21 - A Cost and Time Analysis of Participant Recruitment and Urine Sample Collection through Social Media Optimization

Prasun Sharma¹, **Sarah N. Bartolone**², Elijah Ward², Kenneth M. Peters^{1,2}, Joseph Janicki, Nitya Abraham³, Melissa Laudano³, Christopher Smith⁴, Bernadette M.M. Zwaans^{1,2}, Michael B. Chancellor^{1,2}

¹Oakland University William Beaumont School of Medicine, Rochester, MI; ²Beaumont Health, Royal Oak, MI; ³Albert Einstein College of Medicine, New York, NY; ⁴Baylor College of Medicine, Houston, TX

INTRODUCTION AND OBJECTIVE: Clinical research studies can be expensive and time consuming. One reason is the difficulty of recruiting sufficient study participants in a short time frame. We hypothesized that research studies using online recruitment and engaging patients via social media channels have the potential to reach a large population in a small timeframe, at a reasonable cost. This is an especially appropriate adaptation during COVID-19, with limited hospital and clinic access. To our knowledge, no prior study has performed a detailed cost analysis of the use of online recruitment of clinical sample collection. We sought to address this knowledge gap by comparing cost and efficiency of the recruitment of urine samples online compared to recruitment of urine samples at clinical research sites.

METHODS: We performed a retrospective cost analysis of a cohort study comparing cost per sample and time per sample for both online and clinically recruited participants. The retrospective review was conducted from August 2020 to September 2021. During this time, cost data was collected based on patient surveys, urine sample analyses, invoices, and budget spreadsheets. The data was subsequently subjected to statistical analysis. This study was performed with full institutional IRB approval.

RESULTS: Each sample collection kit contained 3 urine cups, 1 for the IC sample and 2 for control samples. Out of the 3576 (1192 IC + 2384 control) total sample cups mailed, 1,211 (677 control) samples were returned, with a return rate of 44.8% for IC samples and 28.4% for controls. Comparatively, the two clinical sites collected 305 samples in the same period. Men and women of all age groups (18+) and ethnicities enrolled in the study, representing all 50 states. Although the initial startup cost of online recruitment



was higher, cost per sample for online recruited was found to be \$147.06 compared to \$398.14 for clinic sample.

	Online Recruited (\$)	Clinic Recruited (\$)
Total Samples	1,111	105
Total Cost	1,639,004.62	3,214,512.00
Cost Per Sample	147.06	306.14
Supplies	30,000.00	4,000.00
Salary & Wages	41,400.00	54,000.00
Prize Money	25,000.00	25,000.00
Web Development	15,000.00	0
Google Ads	2,000.00	0
Social Media Promotion	4,000.00	0
Shipping	18,000.00	180

Fig 1: Study cost comparison and demographics data. A) Total and cost per sample comparison between online recruited against clinic recruited urine samples. B) Geographical distribution of urine samples received from 50 states.



CONCLUSIONS: We conducted a nationwide, contactless, urine sample drive through online participant recruitment, in the midst of the COVID-19 pandemic. Results were compared with the samples collected in traditional clinical setting. Online patient recruitment can be utilized to collect urine sample rapidly, efficiently and at a cost per sample that was 37% of in an in-person clinic and without risk of COVID-19 exposure.

#22 - Conducting Urology Research during COVID-19: Social Media-Based Crowdsourcing of Urine Samples for Biomarker Development

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INTRODUCTION: Conducting research during the COVID-19 pandemic remains a challenge for the entire urologic community. We hereby report our effort to use social media-based crowdsourcing methodology to recruit participants from the entire United States, including Alaska and Hawaii. We developed a novel research model that can engage multiple stakeholders and allows for fast and broad participation for urine biomarker development towards a diagnostic tool for Interstitial Cystitis (IC).

METHODS: We partnered with a patient advocacy group, the Interstitial Cystitis Association (ICA), to aid in recruitment efforts. A website with study information and links to the HIPAA-compliant enrollment questionnaire was created. The study was advertised through Google, and Beaumont's and ICA's social media using Twitter, emails, YouTube videos, Facebook posts, websites, and TikTok. Enrolled participants were shipped at-home collection kits containing urine cups with preservative, a prepaid return mailing envelope, instructions, and survey materials.

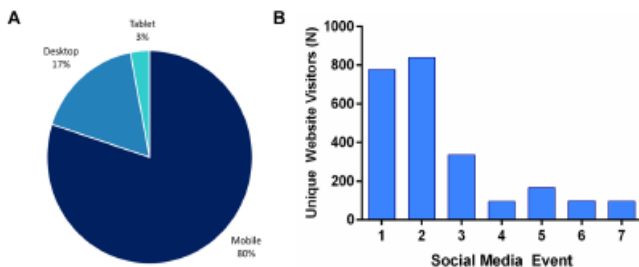


Fig 1: Study participant recruitment by device type and referral method. A) Devices used by study participants to register for the study. B) Number of participants registered.

RESULTS: National public participation was strong, and enrollment was closed within three months. Men and women of all age groups and ethnicities enrolled in the study, representing all 50 United States. Currently, 1,211 crowdsourced samples have been returned,

including 677 asymptomatic control samples. Surprisingly, most participants accessed the website via mobile devices, as opposed to via desktop computer or tablet. Potential participants were referred to the website primarily by direct links (i.e. email links), with other referring sites being Facebook, Google Advertisements, the ICA's website, and Beaumont Hospital's website.

CONCLUSION: Following COVID-19 safety guidelines, we conducted a nationwide crowdsourced collection of urine samples. Social media and web-based recruitment tools were used to recruit participants. Having a website user-friendly on mobile devices was essential. Nontraditional recruitment methods, such as TikTok videos, encouraged study participation. Utilizing various social media platforms, it is possible to crowdsource urine samples from across the United States quickly, economically, and most importantly during the COVID-19 pandemic, safely.

#23 - Towards a Genetic Signature: Genomic Analysis of Escherichia coli from Asymptomatic and Symptomatic Bacteriuria Patients

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Introduction and Objectives: Urinary tract infections (UTI) afflict over 50% of women over the course of their lifetime. While uropathogenic *Escherichia coli* (UPEC) causes >75% of reported UTIs, UPEC lacks a specific molecular signature. In the clinical setting, this poses a significant dilemma when patients present with asymptomatic bacteriuria (ASB), in which significant numbers of *E. coli* is found in the urine of patients, but without the associated symptoms of UTI. Both ASB and UTI-causing *E. coli* carry similar types of virulence factors, and – to date – no genomic signature exists to tell these two *E. coli* types apart. We hypothesize a genomic signature for UPEC might lie in subtle changes in the core genome, rather than as a discrete, traditional virulence factor.

Methods: In this study, we sequenced approximately 800 *E. coli* strains from the urine of symptomatic and asymptomatic patients. We utilized a bioinformatic pipeline to characterize strains outside of the traditional virulence factors, hypothesizing that a signature lies not in the carriage of distinct virulence genes, but rather in discrete polymorphisms that change tropism and pathogenic potential. We utilized phylogenomic analyses, feature annotation, microbial Genome-Wide Association studies, as well as machine learning methodology to detect potential loci associated with the pathotype in an unbiased manner.

Results: We sequenced a cohort of approximately 800 *E. coli* isolates, comprising of ~22% from ASB cases. We show that these strains are highly diverse, as they show an open pangenome, and we were able to identify strains from all phylogenetic clades. These strains additionally carry a variety of virulence associated factors with no correlation of the development of symptoms. Using GWAS, we identified that tRNA loci, particularly for lysine, valine, and glycine, were significantly associated for differentiating ASB and UTI strains.

Conclusions: We aim to identify a genomic signature associated with true UPEC strains, that can differentiate these isolates from strains causing ASB. Using bioinformatics techniques, we show the diverse nature of *E. coli* from bacteriuria patients. We identified tRNA loci as areas of interest that were significantly associated with differentiating the strains. These findings provide supporting evidence for our hypothesis where a genomic signature might lie within changes in the core genome. Future directions include validation of these findings using orthogonal machine learning approaches, and following up using molecular genetics. We hope the validation of these studies will have implications in the diagnosis and differentiation of UTIs and ASB.



#24 - Urinary bladder microRNAs mediate the severity of experimental cystitis

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Interstitial cystitis (IC), also called painful bladder syndrome (PBS), affects 3 to 8 million women and 1 to 4 million men in the U.S., a rate 2 to 5 times greater in women than that observed for men, yet its cause and pathogenesis remain unclear. The symptoms include suprapubic pain related to bladder filling, accompanied by urgency, frequency, and discomfort in the bladder. Autoimmunity has been suspected to play a role in the etiology and pathophysiology of IC. The differential expression of microRNAs (miRs) in naïve, activated and effector immune cells suggest that miRs also serve as critical immunoregulators of IC. Here, we investigated the role of an altered set of the urinary bladder (UBs), and miRs in the pathogenesis of IC. To achieve the goal, we used cyclophosphamide (CYP) induced experimental IC, which provides an excellent model and develops a phenotype comparable to clinical IC with functional and histological alterations confined to the urinary UBs. In this study, we noticed differential changes in the UB miRs expression in IC mice as compared to naïve control mice. Microarray analysis showed that 107 miRNAs from the UB had a 1.5-fold greater difference in expression of the IC than the control. Among them, we observed upregulation of a set of miRs that includes miRs-34a/b/c and miR-21a from the bladder were determined by reverse-transcription polymerase chain reaction (RT-PCR) analysis. These miRNAs alter cell proliferation, migration, and many inflammatory signaling pathways. Further, inhibiting these miRs alters detrusor smooth muscle (DSM) function in IC. We also noticed significant infiltration of macrophages, neutrophils, and mast cells in IC as compared to the control. The histological UB inflammatory scores are also elevated in IC as compared to the control. The mechanisms associated with IC induction might be due to the alterations of immune cells by CYP induction, which modulate specific miRs that increase the local inflammatory response in the bladder. The present study discovers an altered set of new UBs miRs to identify mechanisms underlying the pathogenesis of IC and may provide a potential biomarker and/or therapy for the treatment of IC.

(Supported in part by NIH grant R01 AI140405)

#25 - Urethral lichen sclerosis tissue displays a loss of basal epithelial markers

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INTRODUCTION AND OBJECTIVE: Lichen sclerosis (LS) is a chronic inflammatory disease of the genital epithelium that results in fibrosis and severe urethral stricture disease in affected men. Disordered epithelial differentiation, has been identified as a feature of other fibrotic disorders such as idiopathic pulmonary and renal fibrosis. Keratin 5 (KRT5) and p63 have been reliably established as basal cell markers in urothelium. Therefore, we hypothesized that urethral epithelium affected by LS would have decreased basal expression of KRT5 and p63.

METHODS: Formalin fixed urethral tissue from patients with and without LS after either urethral biopsy or urethroplasty were identified from a prospectively maintained database. Slides were independently reviewed by a genitourinary pathologist (WH) to determine LS status. Multiplexed fluorescent immunohistochemistry was used with primary antibodies directed against KRT 5, CDH1, p63, and DAPI. Three epithelial regions of interest were imaged per specimen. Images were processed in ImageJ and then imported into QuPath software for analysis. Urethral epithelium was segmented into basal and luminal compartments and the cell detection function used to count the number of nucleated cells positive for each primary antibody within each of these compartments which was expressed as relative abundance (number of positive cells/number of nucleated

cells). Relative abundances were compared with respect to each marker and basal/luminal compartment using a Mann-Whitney U test

RESULTS: We analyzed 14 urethral specimens (n=7 LS, n=7 non-LS). The relative abundance of KRT5 and p63 positive cells were similar with respect to epithelial versus basal compartment and LS status. However, we noted a change in patterning such that LS tissues displayed a shift of KRT5 and p63 expression from the basal to the luminal epithelium. To quantify this, we compared the ratio of basal to luminal expression KRT5 and p63 expression within each specimen and dichotomized this as greater or less than 1. We found that LS tissues were significantly more likely to have luminal predominant expression of p63 and KRT5. (p=0.017) (Figure 1).

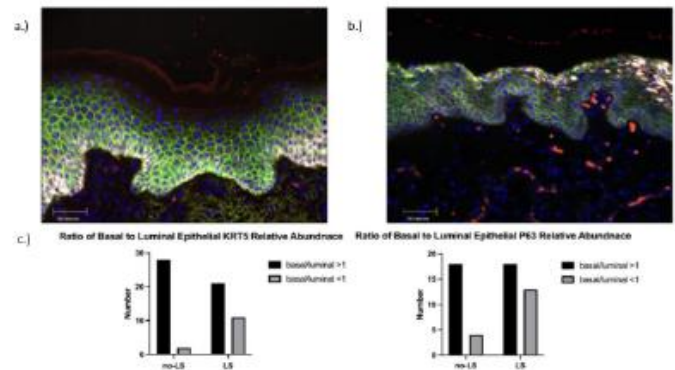


Figure 1. Multiplexed fluorescent immunohistochemistry of urethra in Non-LS (A) and LS (B) tissue. Blue staining represents DAPI, green staining represents CDH1, white staining represents KRT5, and red staining represents p63. Relative abundance of KRT5 and P63 was dichotomized based on ratio of basal/luminal expression into greater or less than one. LS tissues were significantly more likely to have luminal predominant expression of both KRT5 and P63 (P=0.016)(c)

CONCLUSIONS: We identify a change in pattern of expected basal epithelial markers (KRT5 and p63) in LS tissues. Loss of epithelial differentiation is a feature of other chronic inflammatory diseases and may be implicated in the propagation of profound urethral fibrosis seen in LS.

#26 - Urine Microbiota Analysis and microbiota GWAS in Children with Urinary Tract Infections and Vesicoureteral Reflux

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Introduction and Objective: Vesicoureteral reflux (VUR), the retrograde flow of urine from the bladder into the ureters and kidney, accounts for 25-30% of pediatric ESRD worldwide. Urinary tract infections (UTI) are often associated with VUR. Urinary dysbiosis has been implicated in pathologic states such as nephrolithiasis, bladder cancer, and recurrent infections. Reports describing the urinary microbiota in kidney transplant patients showed reduced bacterial diversity compared to healthy individuals. Our objective is to examine host genetic - microbiota interactions in UTI and VUR, patients

Methods: We performed a microbiota analysis based on 16S rRNA gene sequencing from 325 urine samples of children with urinary tract infections with or without VUR from the Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) and Careful Urinary Tract Infection Evaluation (CUTIE) studies' cohorts, and a microbiota GWAS (mGWAS) in 278 of them, with genomic DNA available for genotyping.

Results: We found a significant decrease in urine microbiota alpha diversity with VUR (P=3x10⁻⁸), lack of toilet training (P=4x10⁻⁴), and younger age (P=1x10⁻³). Our mGWAS identified genome-wide significant associations with zero- truncated relative abundance of



Pseudomonas on chr10 ($P=2 \times 10^{-9}$) and *Clostridia* on Chr3 ($P=5 \times 10^{-8}$), and a suggestive association with *Bacillales* ($P=3 \times 10^{-7}$) on the same locus as with *Pseudomonas*. Secondary analysis in female participants showed a suggestive association with *Gammaproteobacteria* on Chr11 ($P=6 \times 10^{-8}$). The top SNPs in these loci were on or near genes associated with immune surveillance, inflammation, and genitourinary tract development and disease (CXCL12, ROBO1, WNT11).

Conclusions: We report the first combined urine microbiota analysis and mGWAS in children with UTI and VUR, showing associations of bacterial taxa's relative abundances with clinical variables and human host genetic factors.

suggests FP agonists may be effective therapies to treat existing underactive bladder dysfunction.

Funding: NIH-K12 DK100024; NIH-RO1 DK117890

#28 - Spatiotemporal proteomic analysis as a compliment to immunohistochemistry

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Introduction and Objective: Benign prostatic hyperplasia (BPH) is an age-associated disease that can cause lower urinary tract symptoms (LUTS) in men. The development and progression of the disease are multifactorial, and the underlying mechanisms are not yet fully understood. It is, however, known that changes in the steroid environment as men age are a contributing factor. Mouse models recapitulate this change and result in lower urinary tract dysfunction. Given the complex nature of the disease, novel tools and technology are necessary for identifying new biomarkers and targetable pathways.

Methods: C57BL/6J mice (8 weeks of age) were implanted with compressed testosterone (25 mg) and 17 β -estradiol (2.5 mg) pellets for eight weeks. Tissues were collected then formalin-fixed, and paraffin-embedded (FFPE). Tissues were sectioned (5 μ m) and mounted onto either ITO-coated or glass slides. Slides were rehydrated prior to antigen retrieval in a 10 mM citrate buffer. For mass spectrometry analysis, on-tissue tryptic digestion and α -Cyano-4-hydroxycinnamic acid (CHCA) matrix application were performed using a robotic TM sprayer system. Slides were analyzed using a Bruker Rapiflex MALDI TissueTyper TOF Mass Spectrometer. For immunohistochemistry (IHC), serial sections were rehydrated prior to antigen retrieval in Tris-EDTA buffer. Using the OPAL Multiplex kit, we stained for e-cadherin and estrogen receptor beta.

Results: This project focuses on optimizing MALDI-MSI for mouse prostate using multiplexed-IHC as a validation tool. Thus far, optimization has demonstrated that 5 μ m thick sections are sufficient for adequate protein identification, compared to the more traditional 12 μ m sections. Additionally, using a decloaker at 95°C for 15 minutes, provided a robust signal for MALDI-MSI. Going forward, further adjustments will be made to improve spatial resolution and adjust matrix types for targets of interest.

Our current protocol has identified more than 30 proteins that we will discuss related to steroid hormones, mitochondrial function, and fibrosis; all of which are critical processes of interest in BPH/LUTS. Initial data supports MALDI-MSI as a robust method for identifying spatial and quantitative data about proteins of interest in a higher throughput manner than traditional methods. Continued validation with IHC will show that MALDI-MSI is an excellent tool for examining protein expression while accounting for the heterogeneity associated with BPH and LUTD.

Conclusions: Development and optimization of a MALDI-MSI method for benign prostate disease models using FFPE tissues for future targeted analyses will increase the efficiency of protein identification.

U54DK104310 (WAR), 1R01DK127081 (WAR), KO1AG059899 (TL)
The RapiFlex is a shared instrument purchased with the support of the S10OD025084 grant

#29 - ATP2C1 is a gene candidate for Interstitial cystitis/bladder pain syndrome

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Introduction and Objective: Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a chronic pain disorder causing symptoms of urinary frequency, urgency and bladder discomfort or pain. Although this condition affects a large population, little is known about its etiology.

#27 - Detrusor contractility via FP receptor activation is increased in diabetic mice with bladder underactivity

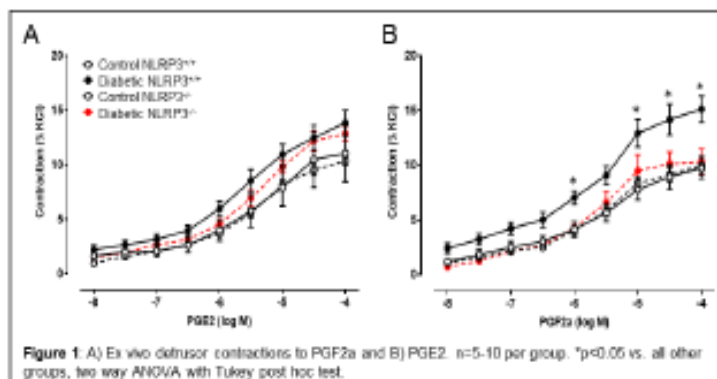
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Introduction and Objective: Diabetic bladder dysfunction, affecting >50% of diabetics, is driven by bladder inflammation triggered by the NLRP3 inflammasome. Prostaglandin (PG) production in the bladder is dysregulated by inflammation but the inflammatory mechanisms responsible during diabetes, and how this disease affects PG-mediated bladder contractility, are unknown. Here, using the Akita genetic mouse model of Type 1 diabetes that develops bladder underactivity crossbred with NLRP3 null mice, we investigated how NLRP3-driven inflammation impacts PG release and bladder contractility.

Methods: Type 1 diabetic Akita mice were crossbred with NLRP3 $^{-/-}$ mice to yield: control/NLRP3 $^{+/+}$, diabetic/NLRP3 $^{+/+}$, control/NLRP3 $^{-/-}$, and diabetic/NLRP3 $^{-/-}$ mice. Females were aged to 30 weeks when bladder underactivity is apparent. To measure ex vivo PG release, strips of isolated mucosa and isolated detrusors were stretched to release PGE2 and PGF2 α , which were quantified using ELISAs ($n=4$). To assess ex vivo smooth muscle contractility, contractile force of intact bladder strips in response to PGE2 and PGF2 α +/- FP receptor antagonist AL8810, were measured.

Results: Diabetes doubled PGE2 released from the mucosa while reducing release from the detrusor by 67%. Surprisingly, no changes in PGE2 mediated bladder contractility were noted. In contrast, diabetes did not change PGF2 α release in either the mucosa or the detrusor. However, intact bladder strips from diabetics demonstrated a 33% increase in PGF2 α -mediated contractility which was abolished by AL8810. Diabetics lacking NLRP3, and therefore NLRP3-mediated inflammation, did not exhibit dysregulated PG release or PG-mediated bladder contractility.



Conclusions: Diabetes inversely affects PGE2 release from bladder mucosa and detrusor while not altering PGE2-induced contractions. Surprisingly, release of PGF2 α was unaffected by diabetes although contractility to PGF2 α , working through the FP receptor, was increased. This suggests that diabetes causes a change in FP receptor expression or signaling. These changes were driven by NLRP3-induced inflammation. The increased sensitivity to PGF2 α



Methods: We performed genetic analyses of whole exome sequencing (ES) on a total of 109 individuals with IC/BPS.

Results: One family had a previously reported SIX5 variant (ENST00000317578.6:c.472G>A, p.Ala158Thr), consistent with Branchiootorenal syndrome 2 (BOR2). A likely pathogenic heterozygous variant in ATP2A2 (ENST00000539276.2:c.235G>A, p.Glu79Lys) was identified in another proband, indicating possible Darier-White disease. Two private heterozygous variants were identified in ATP2C1 (ENST00000393221.4:c.2358A>T, p.Glu786Asp (VUS/Likely Pathogenic) and ENST00000393221.4:c.989C>G, p.Thr330Ser (Likely Pathogenic)), indicative of Hailey-Hailey Disease. Both Darier-White and Hailey-Hailey have specific therapies. SKAT analysis found a trend towards increased burden of rare ATP2C1 variants in the IC/BPS cases vs a control cohort (p=0.03, OR=6.76), though did not survive Bonferroni correction.

Conclusions: Our data suggest that some individuals with IC/BPS may have unrecognized Mendelian syndromes. Comprehensive phenotyping and genotyping aids in understanding the range of diagnoses in our population-based IC/BPS cohort. Conversely, ATP2C1, ATP2A2, and SIX5 may be candidate genes for IC/BPS. Further evaluation with larger numbers is needed. Genetically screening individuals with IC/BPS may be useful in diagnosing and treating this painful disorder due to its heterogeneous nature.

#30 - Defining the bladder resident macrophage populations in the steady state and in response to an immunological challenge

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Introduction and Objective: In the steady state, the myeloid cell compartment is highly heterogeneous, and contains cells of different origins and functions. These cells include macrophages and dendritic cells, and each play important roles in tissue maintenance, including development, homeostasis, immunity and repair following tissue injury. Each tissue has a unique macrophage population, and we have phenotypically characterized the murine bladder macrophage populations in the steady state and in response to an immunological challenge.

Methods: To identify the unique bladder macrophage populations, we utilized a 33-parameter flow cytometry approach. Macrophages were isolated from the bladders of 8-week-old male and female C57BL/6J mice in the steady state and in response to an immunological insult.

Results: Immune organs and non-immune organs including the bladder contain a heterogeneous population of macrophages in the steady state. Murine tissues' macrophages can be generally identified using the F4/80 and CD11b cell surface marker, allowing us to phenotypically characterize the resident macrophage population. For adult mice in the steady state, the resident bladder macrophage population is heterogeneous, and most bladder resident macrophages have high surface MHC class II expression, unlike other tissue macrophages (spleen and peritoneal cavity) that typically have low MHC class II expression. Analysis of the F4/80high resident macrophage populations demonstrated 4 populations in female mice and 5 populations in male mice, with 90% of the cells expressing high MHC class II surface expression. The F4/80high resident macrophage populations in male and female mice had a bimodal, high or low, expression of Cx3cr1. In the steady state, surface expression of cell surface molecules involved in recruitment, activation and inflammation varied between the macrophage populations, indicating that each population has a unique function in the bladder. Following an immunological challenge, a urinary tract infection, the F4/80high resident macrophage populations expressed elevated levels of MHC class II surface expression and additional activation markers. We observed an increase in the recruitment of

neutrophils and monocytes, with the recruited monocyte population transitioning into a macrophage phenotype.

Conclusion: The data indicates a dynamic change in the composition of the bladder macrophage population in the steady state and following an immunological challenge. This would suggest each population plays a role in bladder homeostasis and immunity. Future studies will be directed towards elucidating the functions of each of the identified macrophage populations.

#31 - Macrophage differentiation in the prostate in a mouse model of steroid hormone imbalance

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Introduction and Objectives: Benign prostatic hyperplasia (BPH) refers to the non-malignant growth of the prostate, that leads to various symptoms collectively known as Lower Urinary Tract Symptoms (LUTS). The prevalence of BPH/LUTS increases linearly over time in men over 40, making it one of the most prevalent diseases in aging men. Apart from proliferation, several other pathological changes are associated with BPH, including smooth muscle dysfunction, inflammation, and fibrosis. An imbalance in steroid hormones is thought to play an influential role in the etiology of BPH but previous studies have conflicting results on how steroid hormone dysregulation may drive inflammation in the prostate. Using a mouse model of steroid hormone imbalance, we have previously demonstrated that estradiol and testosterone (T+E2), induces the migration of macrophages to the lumen (luminal macrophages) as well as increases the number of macrophages in the tissue. We also identified that luminal macrophages accumulated lipid droplets and differentiated into foam cells. Our current study aims to determine what macrophage subtype(s) make up the luminal and the tissue macrophage populations which will help us to understand their function in steroid hormone imbalance.

Methods: Mice were subcutaneously implanted with pellets containing 25 mg testosterone (T) and 2.5 mg estradiol (E2) to male C57BL/6J mice and prostates were collected after two or six weeks. Paraffin-embedded tissues will be subjected to immunohistochemistry (IHC) using antibodies against iNOS (M1 marker) or Arginase I. (M2 marker). Tissue samples will be analyzed using a Mantra II. Pathological Workstation and InForm software. Student t-tests and one-way ANOVAs or their non-parametric equivalent will be used for statistical analysis. The data will be presented as mean $\pm$ SD. Statistical significance will be determined by p-values <0.05.

Results: Experiments and analyses are currently in progress. Based on our preliminary observations we expect that both macrophage populations will be predominantly of M2 subtypes.

Conclusions: Determining whether the M1 pro-inflammatory or the M2 anti-inflammatory (but often pro-fibrotic) macrophage populations are dominant will help us to identify how macrophages contribute to disease progression. Our results will also advise our future experiments targeting the depletion of specific macrophage subsets to determine their impact on urinary function.



#32 - The taxonomic ecology and functional potential of the urobiome is shaped by recurrent urinary tract infection and estrogen in postmenopausal women

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INTRODUCTION AND OBJECTIVE: Urinary tract infection (UTI) is one of the most common bacterial infections observed in humans and often develops into recurrent UTI (rUTI). Postmenopausal women are an underserved demographic group who are severely affected by rUTI with recurrence rates approaching 50%. In this population, rUTI can persist for years, reducing quality of life and imposing a significant healthcare burden. The female urinary microbiome (urobiome) has been identified as a key component of the urogenital environment. However, compositional and functional changes in the urobiome underlying rUTI in postmenopausal women are not well understood. We sought to perform a multi-omic survey of the postmenopausal urobiome in a controlled crosssectional cohort designed to capture the cyclic nature of rUTI.

METHODS: We curated a controlled cross-sectional cohort of postmenopausal women (n=75) and performed whole genome metagenomic sequencing to survey the urobiome in rUTI patients and healthy comparators. Estrogens were also extracted from of urine, and analyzed by targeted LC-MS/MS. We used both non-parametric and Bayesian analytical approaches to robustly identify taxonomic enrichment and associations with urinary estrogens. We further used functional metagenomic profiling and patient-derived isolate phenotyping to identify microbial metabolic pathways, antimicrobial resistance genes (ARGs), and clinically relevant antimicrobial resistance phenotypes enriched between disease-states.

RESULTS: Our results suggest that a history of rUTI strongly shapes the taxonomic and functional ecology of the urobiome. We further find that a putative protective bacterial population may exist within the urobiome of women who do not experience UTI and use estrogen hormone therapy (EHT). Integrating quantitative metabolite profiling of urinary estrogens with WGMS, we observed robust associations between urobiome composition and urinary estrogen concentrations, suggesting that EHT strongly alters the taxonomic composition of the female urobiome, an observation which merits further mechanistic study. Our data suggest distinct metabolic and ARG signatures of the urobiome associated with rUTI status and history.

CONCLUSIONS: Taken together, our data suggests that rUTI history and estrogen use strongly shape the functional and taxonomic composition of the urobiome in postmenopausal women. Further research in relevant model systems is needed to validate and determine the mechanisms behind our observations. Our work provides a robust basis focused on representing postmenopausal women.

#33 - Urinary Glycosaminoglycans are Associated with Recurrent UTI and Urobiome Ecology in Postmenopausal Women

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INTRODUCTION AND OBJECTIVE: The composition of urinary glycosaminoglycans (GAGs) is not fully understood. The luminal urothelial GAG layer is likely an important interface between the human host and the resident urobiome, which has been shown to be altered in postmenopausal (PM) women with history of recurrent urinary tract infection (rUTI). We sought to profile the urinary GAG distribution and associate urinary GAGs with the ecology of the resident urobiome in a controlled cohort of PM women designed to study rUTI.

METHODS: Clean catch midstream urine was collected from a controlled cohort of PM women (n=75) who passed a set of exclusion criteria for an ongoing study of rUTI. GAGs were extracted from urine via macromolecular filtration and then enzymatically digested into constituent disaccharides for chondroitin Sulfate (CS), heparin Sulfate (HS), and hyaluronic Acid (HA) following a published protocol. The liberated disaccharides were analyzed and quantified via targeted liquid chromatography-mass spectrometry (LCMS/MS). For whole genome metagenomics, DNA was purified from matched urine and prepared libraries were sequenced on an Illumina NextSeq 500 and analyzed for taxonomic enrichment as described previously.

RESULTS: We find that CS is the major urinary GAG in PM women and women who were experiencing an active UTI exhibited significantly higher urinary CS compared to those with no UTI. Lastly, we find that two urobiome bacterial species, *Atopobium vaginae* and *Blautia obeum* exhibit significant negative associations with urinary CS.

CONCLUSION: Our findings of increased urinary CS during active UTI may be a sign of tissue damage during infection. We find negative associations between bacterial species known to be associated with vaginal dysbiosis and urinary CS levels. These associations may be indicators of bladder dysbiosis and alterations of urothelial GAGs. Mechanistic studies are needed to understand these findings in the context of rUTI pathology.

#34 – Subdued Limits Bacterial Infections and Calcium Oxalate (CaOx) Crystal Aggregation in *Drosophila* Renal Tubules

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Introduction and Objective: Anoctamins (ANO) are activated by intracellular Ca²⁺ and function as phospholipid scramblases and/or Cl⁻ channels. ANO4 is mutated in miniature schnauzers with CaOx stones (GWAS), and ANO4 protein in urinary extracellular vesicles is decreased in human CaOx stone formers. In *Drosophila* Malpighian tubules (MT), subdued (Ano4-homolog) functions as Ca²⁺-activated Cl⁻ channel, scramblase, and plays a role in host defense against gram-negative bacteria. Increasing evidence shows the participation of bacteria in urinary stone formation. CaOx crystal formation is increased when uropathogenic *E. coli* (UPEC) is transurethrally inoculated in mice. Herein, we used a *Drosophila*



avatar to investigate whether *subdued* changes CaOx crystallization and if crystallization relies on bacterial infection.

Methods: Uro:Gal4 flies were crossed with UAS:*subdued*-RNAi flies to knockdown (KD) *subdued* in MT principal cells or with W1118 flies to generate normal controls (WT). F1 generation underwent CaOx crystallization in association or not with UPEC infection. *In vivo*, flies were fed on diet supplemented with 20mM sodium Ox (NaOx) + UPEC:eGFP for 4 days. For *ex vivo* assays, dissected MTs were submerged in solution containing 10mM NaOx+ UPEC:eGFP for 90 minutes. *Subdued* expression was quantified by qPCR. Crystal number, size and presence of UPEC within MT were assessed by polarized light and fluorescence microscopy.

Results: *subdued*-KD in principal cells reduced *subdued* mRNA levels by 30% in MT. Crystal number or size was not change by *subdued*-KD after prolonged NaOx feeding. However, when UPEC is introduced in the NaOx diet, *subdued*-KD flies exhibited a massive presence of bacteria in MT lumen and produced enlarged CaOx crystals compared to WT flies (Figure). Similar augmented presence of UPEC in *subdued*-KD MTs was observed during *ex vivo* assays. Short-term exposure to NaOx+UPEC resulted in increased number of crystals in *subdued*-KD MTs compared to WT MTs, but no difference was observed in crystal size.

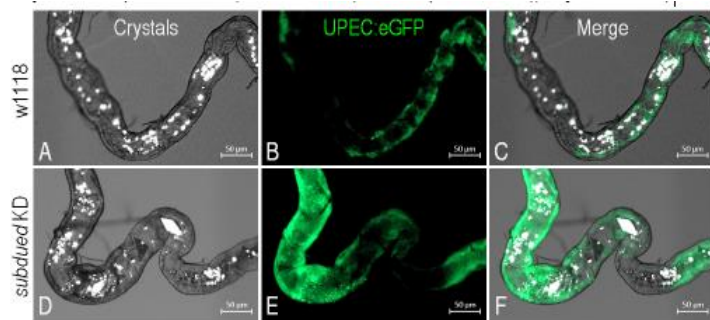


Figure. 4 days of NaOx and UPEC feeding. Controls (w1118 = Uro:Gal4 x w1118) and *subdued* knockdown (Uro:Gal4 x *subdued*-RNAi) *Drosophila* Malpighian tubules viewed with polarized light for CaOx crystals (A, D: birefringence) or fluorescence (B, E) to visualize UPEC:eGFP and merged images (C, F).

Conclusions: *subdued* limits CaOx crystals formation and aggregation through reducing bacterial invasion in the MT lumen. These *Drosophila* results suggest a role for Ano4 in bacterial-related human, dog and mouse kidney stone formation. Future experiments will determine whether other functional aspects of *subdued* affect CaOx crystallization.

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#35 – Location-Specific Elasticity of Murine Bladder During Ex-Vivo Filling

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INTRODUCTION AND OBJECTIVE: Mechanical analysis of the bladder during filling gives insight to the health and function of the tissue. Mechanical models of the bladder often approximate the organ as a spherical pressure vessel; however, the bladder is more accurately represented by an ellipsoid than a sphere. More accurate modeling of bladder geometry throughout filling is necessary to better understand the organ's unique tissue mechanics. The aim of this study was to show that the bladder has location-specific elastic stretch behavior via precise 3D models of the organ's shape during filling experiments.

METHODS: Urinary bladders from male and female mice (appx. 3 months age) were dissected and mounted on our novel Pentaplanar Reflected Image Macroscopy (PRIM) system for simultaneous video recording and bladder pressure measure at 10 Hz. Bladders were

then filled from empty to 25mmHg pressure. A novel method was developed for recreating 3D geometry/meshing of the organ from PRIM video data. The data processing method is highly automated and open sourced. This 3D reconstruction of the bladder throughout the filling experiment provides a 4D (space and time) vectorized representation of the organ from empty to full capacity. Paired geometry and pressure measures were then used to evaluate stress-stretch behavior in the lateral region (i.e., equatorial line) and in the dome. Student's t-test was used for statistical comparisons.

RESULTS: Accurate 3D reconstructions from the experimental data were obtained. Accuracy was validated by comparing volume measures between automated reconstruction and manual segmentation at points throughout filling. In the mechanical analysis, the bladder was found to have higher stretch in the lateral/equatorial direction than the trigone-to-dome direction. Bladder volume at high pressures was found to be significantly lower in females compared to males. Female bladders also showed lower stresses and stretches at high pressures.

CONCLUSIONS: This study shows that precise geometrical modeling of bladder filling can reveal the complex mechanical behavior of the organ. Mechanical behavior of the tissue wall varies with location.

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#36 - On-tissue imaging of steroid hormones in mouse prostate using MALDI post-ionization

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Introduction and Objectives. Benign prostatic hyperplasia (BPH) impacts the majority of the aging male population, with associated lower urinary tract symptoms (LUTS) costing the healthcare industry around \$4 billion annually. Steroid hormone imbalances are implicated in the development and progression of BPH/LUTS, with many treatment strategies targeting enzymes that metabolize these hormones. Multiple imaging strategies exist to collect spatiotemporal information within tissues, such as immunohistochemistry (IHC) and mass spectrometry imaging (MSI), yet these techniques are only applicable to proteins. Recently, a novel post-ionization technique was developed in tandem with matrix-assisted laser desorption/ionization MS (MALDI-MS) to allow for increased detection of compounds with poor ionization efficiency, such as lipids and steroid hormones. Here, we set out to examine the utility of this technique for spatiotemporal localization of steroid hormones in rodent prostate tissues.

Methods. Prior to on-tissue analysis, standards of 17 β -estradiol, androstenedione, 5 α -androstane-3 α ,17 β -diol and 5 α -androstane-3 α ,17 α -diol were mixed with 2,5-dihydroxybenzoic acid (DHB) matrix and spotted to test limit of detection. All tissues were flash frozen using liquid nitrogen and embedded in gelatin. Tissue sections of 10 μ m were cut using a cryostat and set onto ITO-coated slides. Slide images for future reference of tissue position were obtained prior to DHB matrix application using a robotic TM sprayer. MALDI-2 spectra were acquired using a Bruker timsTOF Flex with MALDI-2 capabilities.

Results. Initial experiments using 1 μ g of steroid hormone standards (17 β -estradiol, androstenedione, and 5 α -androstane-3 α ,17 β -diol) show drastic improvements in steroid detection, as the protonated peak for β -estradiol (273.18 m/z) was detected using MALDI-2 yet undetected with MALDI alone. Additional hormone adducts were detected for β -estradiol, indicating good overall coverage and detection of steroid hormones using post-ionization. Limits of detection are currently being evaluated for these steroids, and on-tissue imaging will soon follow.

Conclusions. MALDI-2 imaging provides a novel approach for obtaining spatial information of steroid hormones in prostate tissue,



allowing us to determine how these hormone levels directly impact prostatic disease formation and progression.

#37 - Large-scale exome sequencing analysis implicates *FOXQ1*, *FOXI2*, *EXOSC2*, and *MMP15* as candidate genes for human congenital obstructive uropathy

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INTRODUCTION AND OBJECTIVE: Congenital obstructive uropathy (COU) is the most frequent urinary tract anomaly occurring in up to 2% of pregnancies, constituting a leading cause of pediatric chronic kidney disease. The genetic causes of COU are not well understood and remain to be deciphered.

METHODS: We conducted an exome sequencing (ES) study on 880 COU cases, encompassing three main classes of congenital urinary obstructions: a) Ureteropelvic Junction Obstruction (UPJO; N=331), b) Ureterovesical Junction Obstruction / megaloureter (UVJO; N=188), and c) COU not otherwise specified (COU-NOS; N=361). To investigate the excess burden of rare coding variants on COU, we performed exomewide collapsing analysis comparing the above 880 cases and 16,135 population controls with ES data using 4 main genetic models (3 dominant and one recessive). Analyses were conducted on the entire dataset and then again after removal of cases harboring diagnostic/pathogenic Mendelian mutations and structural variants. Study-wise exome-wide significant threshold was set at 1.25×10^{-6} .

RESULTS: In the analysis on the entire cohort of 880 COU cases, the top signal was for *FOXQ1* in the dominant ultrarare model from European population ($P = 8.49 \times 10^{-6}$; OR= infinite). The signal improved after removal of solved cases, exceeding exome-wide significance ($P = 7.64 \times 10^{-7}$; OR= infinite), and supporting candidacy for this gene. From the subtype analysis, we found the following suggestive signals: *EXOSC2* ($P = 2.96 \times 10^{-5}$; OR= infinite) and *FOXI2* ($P = 5.52 \times 10^{-5}$; OR= 82.63) for UPJO; *MMP15* ($P = 2.56 \times 10^{-5}$; OR=21.93) for UVJO. These genes are highly expressed in the developing mouse urinary tract are intolerant to deleterious genetic variations.

CONCLUSIONS: These findings expand and deepen our understanding of the genetic underpinning of COU, identify novel candidate genes, and highlight the high genetic heterogeneity of disease. We are currently pursuing these findings using human and mouse derived urinary tract enriched organoid systems.

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#38 - Recurrent bladder infections drive persistent pain via sensory nerve sprouting and sensitization

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Introduction/Objectives Urinary tract infections (UTIs) account for almost 25% of all infections in women and a large fraction recur (rUTIs). rUTI patients often complain of chronic pelvic pain accompanied by voiding symptoms such as frequency, yet the standard of care remains prophylactic antibiotics even in the absence of bacteriuria. Previously, we described a mouse model of rUTI consisting of 3 once-weekly E.coli bladder infections leading to increased void frequency 14 days after the last infection, when urine cultures are negative. We have since found these mice also display increased pelvic sensitivity after abdominal probing. Analysis of their bladder revealed significant sensory nerve growth in the lamina propria, a finding we also observed in bladder biopsies from rUTI patients. Based on this, we sought to (1) confirm that multiple infections are required for nerve growth/sensitization and (2) identify the causal factor leading to nerve growth/sensitization and assess whether this could be therapeutically targeted in our rUTI model. We hypothesize that nerve growth factor (NGF) may play a role as elevated levels are found rUTI patients and NGF is known to mediate nerve growth/sensitization in other ailments.

Methods Using our rUTI mouse model, we assessed pelvic sensitivity using von Frey probing of the abdomen 14 days after 1, 2, or 3 once-weekly bladder instillations of E.coli in female mice. Saline was used as control. Immunofluorescence microscopy and 3D modeling was used to assess nerve growth in infected and control mice 14 days after final infection, with Substance P used to label sensory nerves. NGF levels were determined by ELISA of the bladder of each mouse. A tropomyosin receptor kinase A (TrkA) antagonist (GW-441756; given either during or after rUTI) was used to confirm the role of NGF in nerve growth/sensitization and assess the therapeutic potential of blocking NGF-TrkA signaling in rUTI mice.

Results We found that 3 total infections were required for nerve growth/sensitization. In addition, rUTI mice displayed elevated bladder NGF compared to control mice, suggesting its role in pathology. Confirming this, continuous inhibition of TrkA during each infection prevented nerve growth and development of pelvic sensitivity in mice. Lastly, blocking TrkA 14 days after last infection reversed pelvic sensitivity in rUTI mice, demonstrating the role of NGF in prolonging sensitization.

Conclusions These studies reveal NGF mediates nerve growth and sensitization after multiple bladder infections in mice. In addition, these results present NGF as a potential therapeutic target for relief in rUTI patients. Future work will assess possible roles of other neurotrophins during rUTI.

#39 - Machine Learning Model to Predict Likelihood of Spontaneous Ureteral Stone Passage

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Introduction & Objective: Treatment options for patients with a ureteral stone are a trial of passage or surgical intervention. Prior studies have attempted to identify factors that predict the likelihood of spontaneous passage, such as stone area and location, but this remains difficult. Successful prediction of spontaneous passage can help avoid the risks of unnecessary surgery or shorten the period of severe symptoms in those that will ultimately require surgical intervention. Our goal was to create a machine learning model incorporating patient clinical and imaging characteristics to accurately predict the likelihood of ureteral stone passage.

Methods: We performed a retrospective cohort study of pediatric and adult patients presenting with ureteral stones on CT scan. Chart



review was performed to ascertain patient clinical and imaging characteristics and to determine the primary outcome of spontaneous stone passage. A random forest model was built using these characteristics to predict spontaneous ureteral stone passage.

Results: 103 pediatric (median age 14 years, 56% female) and 153 adult (median age 57 years, 35.9% female) patients with confirmed ureteral stones were identified. Spontaneous passage occurred in 54% of pediatric and 44.4% of adult patients. Separate models were created for pediatric and adult patients since different features were important for prediction in each cohort. The pediatric model had an

accuracy of 70% (95%CI 67-74%) and the adult model 63% (95%CI 58-66%). Stone area was the most important feature in both models (Figure 2).

Conclusions: We created a machine learning model that predicted ureteral stone passage based on clinical and imaging features with 63-70% accuracy. Our long-term aim is to create a deep learning model that incorporates clinical characteristics and automated segmentation of CT imaging to accurately predict the likelihood of ureteral stone passage without the need for human abstraction and input. Our hope is that this will allow for better individualized care through early identification of patients who will have a successfully trial of passage versus those that require surgical intervention.

#40 – Novel Immune Defense Cells of the Kidney Epithelia

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) are one of the most common bacterial infections worldwide, affecting infant boys, older men, and most prominently, women of all ages. It is predominately caused by uropathogenic *E. coli* and can lead to urosepsis and complications such as kidney failure if untreated. We have found a major role for kidney epithelia, specifically the intercalated cells (IC) that are activated in defense against bacteria. Given the heterogeneity of kidney cell types, we hypothesize that there could be other cells that respond during a UTI.

METHODS: We performed single cell RNA-seq (scRNAseq) using 10x Genomics of whole kidneys from mice that developed UTI at 12hrs post inoculation and from mice without infection. To study the response of the collecting duct epithelia, we performed scRNAseq of IC from lipopolysaccharide (LPS)-induced mice at 0, 8 and 24hrs using a novel mRNA profiling method, pooled library amplification for transcriptome expression (PLATE)-seq. We used nTnG;Atp6v1b1-Cre reporter mice that activated GFP in IC, and subsequently sorted GFP+ cells for PLATE-seq.

RESULTS: scRNAseq across 16 samples from 10 mice (5 UTI-Control pairs) yielded 64,760 cells for downstream analyses. Clustering analysis resulted in populations of all major kidney cell types, as well as rare populations, i.e., pelvic transitional epithelial cells and double-positive (DP) collecting duct cells, which have markers of both intercalated cells and principal cells. By gene set enrichment analysis of upregulated genes, we found that these DP cells activate gene sets involved in cell killing and innate immune responses at 12 hours post UTI. DP cells were also found to be highly transcriptionally active in response to infection in a cross analysis using our dataset from nascent 4-thiouracil-labeled RNAs purified from Atp6v1b1+ cells. scRNAseq on Atp6v1b1+ population after LPS induction (525 total cells) further revealed time-dependent activation of immune responses. We found that these IC/DP cells increased *Umod* expression in both UTI (1.57-fold change; $p_{adj}=1.01E-02$) and LPS (1.7-fold change; $p_{adj}=1.05E-06$) scRNAseq analyses. *Umod*, known to have innate immune properties, is commonly expressed in the loop of Henle and distal tubule, but may have a novel bacterial defense role in the IC/DP cell population.

CONCLUSIONS: We found a novel immune defense role of the double positive collecting duct cells in response to UTI. These transcriptionally active cells induce genes involved in cytotoxic and innate immune responses and can provide new insights into the epithelial defense of the kidney.



Figure 1. Receiver Operating Curves (ROC) for pediatric (A) and adult (B) models

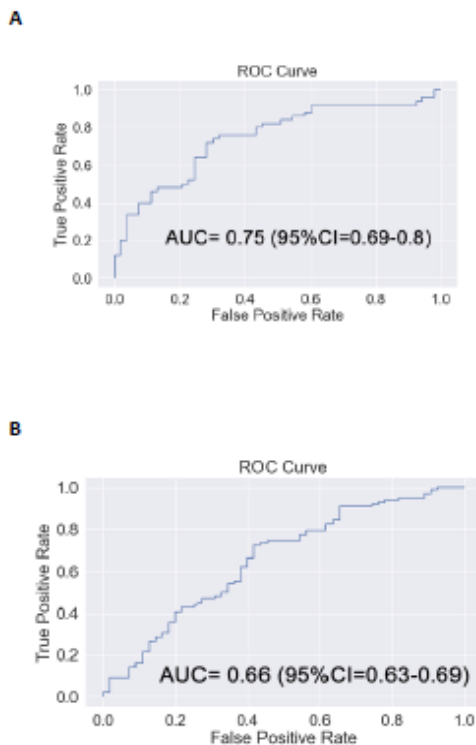
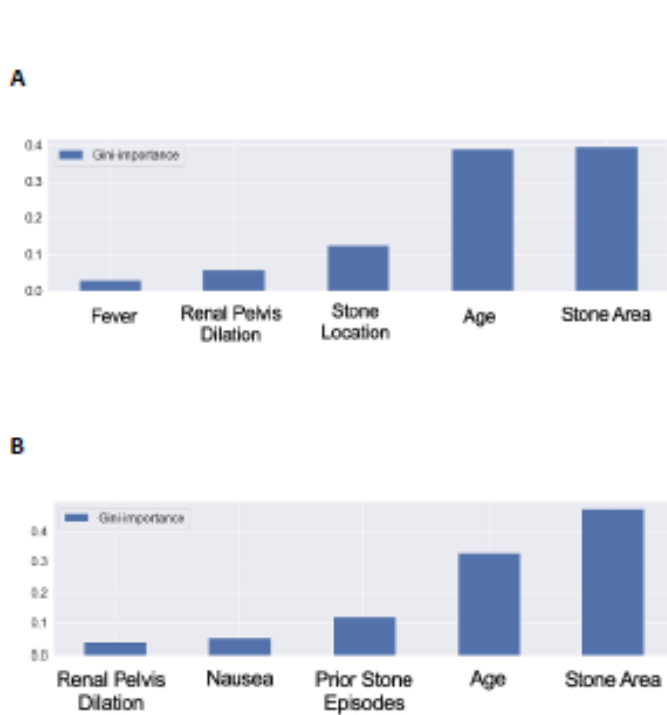


Figure 2. Feature Importance for pediatric (A) and adult (B) models





#41 - Identification and Mechanism of the Collagen Producing Cell in the Development of Lower Urinary Tract Dysfunction

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Introduction/Objectives: Lower urinary tract dysfunction (LUTD) is nearly ubiquitous in aging men¹. Prostatic inflammation is linked to prostatic collagen accumulation, and both have been linked to LUTD severity, rate of progression, or treatment resistance^{2,3}. Myofibroblasts, which are differentiated fibroblasts essential for wound healing are currently accepted to be the prominent collagen producing cell throughout the human body^{4,5,6}. This is true for most organ systems, but our recent studies have shown a lack of myofibroblast activity in the inflamed human prostate. This finding has created a need for further investigation into the mechanisms leading to collagen deposition. Our objective is to identify the collagen producing cell and its mechanism of collagen production in the human prostate. Transforming growth factor 1 (Tgfb1), a central mediator of fibrosis, has been shown to have many roles in the development of fibrotic diseases^{7,8}. Identifying TGFB1 as a main contributor to collagen accumulation in the human prostate can lead to new targets for treatment in men with LUTD. We hypothesize that prostate inflammation causes myeloid cells to home to the prostate and synthesize/secrete TGFB1, which triggers the synthesis of collagen.

Methods: Using known primary antibody markers for myofibroblasts (Smooth muscle actin (SMA) and collagen type I alpha 1 (COL1A1)), immunohistochemistry (IHC) was performed on formalin fixed paraffin-embedded inflamed prostate sections of three separate species (human, mice, and canine) to visualize the presence or absence of myofibroblasts. For prostatic inflammation mouse models, we instilled uropathogenic *Escherichia coli* (UPEC) via a catheter as previously described². All three species are tested for inflammation based on the presence of bone marrow derived immune cells (CD45+) and epithelial morphology. RNA in situ hybridization (RNAscope) with probes against Lysozyme-M (LY2Z), a marker of myeloid cells, and COL1A1 was performed as described by the manufacturer (Advanced Cell Diagnostics) on formalin fixed paraffin-embedded high grade-inflammation or low grade-inflammation human prostate tissues. RNAscope allows visualization of single RNA molecules in individual cells. We will additionally use RNAscope with probes against LY2Z, COL1A1, and TGFB1 in human prostate sections with high-grade inflammation or low-grade inflammation to determine if 1.) TGFB1 is upregulated in the high-grade inflammation tissue when compared to the low and 2.) If the infiltrating LY2Z+ cells are producing TGFB1. We will use cell counts to determine both.

Results: In our IHC stains for SMA and COL1A1, all three stains (human, mouse, and canine inflamed prostate tissue) showed that all collagen producing cells present were negative for SMA (Figure 1).

ABSTRACT TRUNCATED AT 2,400 CHARACTERS
PER ABSTRACT PREPARATION GUIDELINES

#42 – Impact of Bladder Filling Rate on Cystometric Variables in Anesthetized Rats

Wei He¹, **James Hokanson¹**

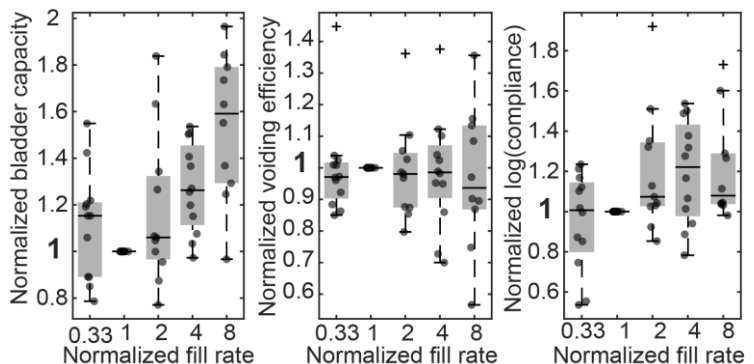
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INTRODUCTION AND OBJECTIVE: Clinically “fast” filling of the bladder is believed to increase the likelihood of eliciting detrusor overactivity, although evidence of this is generally lacking. In animal models, generally little thought is given to the impact of fill rates on outcomes. In our efforts to create a testing suite to compare and contrast animal models, we sought to first test the impact of fill rate on cystometric parameters.

METHODS: Female rats (CD, n = 12) were anesthetized with urethane (1.2 g/kg), followed by single-fill saline cystometry. Our baseline rate varied, and historically has been chosen/adjusted such

that a control trial lasts approximately 7 minutes. After stabilization 1 – 6 sets (median 3) were run at 1/3x, 1x, 2x, 4x, and 8x of this baseline rate with the order randomized. Voided volume (VV) and residual volume (RV) were measured to calculate bladder capacity (BC) and voiding efficiency (VE). Compliance was calculated as the inverse of the slope (fit using Thiel-Sen regression) from 30% to 70% of the filling phase, then deskewed (log transformed) prior to normalizing. Outputs were fit to normalized rates ≥ 1 using linear regression.

RESULTS: The median base fill rate was 4 ml/hr (0.067 ml/min, range 1-12 ml/hr). From previous work we expected that faster fill rates would reduce bladder capacity, instead rates $> 1x$ generally showed increasing bladder capacity (slope=0.063, $P<0.001$, $R^2=0.33$). No trend was observed with voiding efficiency ($P=0.61$). Bladder compliance increased with increasing fill rate (slope=0.03, $P=0.013$, $R^2=0.09$).



CONCLUSIONS: Contrary to expectations, faster fill rates led to increasing bladder capacities. Although these rates are almost certainly super-physiologic, these types of tests are relevant for comparing results across studies and for getting a better understanding of how the system functions. For example, at higher fill rates electrical stimulation may be less able to inhibit the bladder if the fast fill rate is already bringing the bladder closer to a “maximum capacity.”

#43 - Incidence and risk factors for prostate cancer diagnosis at holmium laser enucleation of the prostate

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INTRODUCTION AND OBJECTIVE: Incidental prostate cancer (IPCa) is commonly detected after holmium laser enucleation of the prostate (HoLEP) with published rates varying widely from 7-23%. Pre-operative factors including increased age, PSA, PSA density, and smaller prostate volume have been variably associated with IPCa detection. As a larger volume of prostate tissue is removed during HoLEP and submitted for pathologic evaluation we aim to test the hypothesis that the proportion of enucleated tissue examined by pathology is associated with IPCa detection.

METHODS: Consecutive patients undergoing HoLEP from 2018-2022 were included and comprehensive clinical data abstracted from a prospectively maintained database. Patients with known diagnosis of prostate cancer prior to HoLEP were excluded (n=31). IPCa was defined as a diagnosis of PCa on pathologic examination of the HoLEP specimen. Patients with and without IPCa were compared with respect to pre-operative clinical variables using Chi-square and Wilcoxon rank sum tests. Association of clinical variables with diagnosis of IPCa were assessed using univariate and multivariable logistic regression.

RESULTS: We included 913 patients in the analysis. Of these, 183 (20%) had IPCa. The majority of cancers were cT1a (84%) and grade group 1-2 (87%). Patients diagnosed with IPCa were significantly older, had lower estimated prostate volumes, higher PSA density,



were less likely to have a negative preoperative prostate biopsy, and had a higher proportion of prostate surgical specimen examined by a pathologist (67 vs 52%, $p < 0.001$) (Table).

Table

Variable	All Patients (n=913)	No Cancer (n=730)	Incidental Prostate Cancer (n=183)	P
Median patient age (IQR)	71(65,76)	71(65,76)	72(67,76)	0.011
Preoperative Imaging, n (%)				0.899
MRI	217(23.77)	174(23.84)	43(23.50)	
CT	569(62.32)	453(62.05)	116(63.39)	
TRUS	60(6.57)	50(6.85)	10(5.46)	
None	67(7.34)	53(7.26)	14(7.65)	
Median preoperative prostate volume (cc) (IQR)	100.78(68.32,150.96)	104(72,153)	90.25(59,136.15)	0.001
Median pre-op PSA (IQR)	3.56(1.81,6.36)	3.585(1.79,6.39)	3.44(1.902,6.26)	0.456
Median pre-op PSA density (IQR)	0.340(0.202,0.618)	0.323(0.189,0.602)	0.380(0.253,0.738)	0.006
Negative pre-HOLEP biopsy obtained, no. (%)	278(30.37)	239(32.65)	39(21.31)	0.003
TRUS	193(69)	163(68.20)	30(76.9)	
Fusion	32(12)	29(12.13)	3(7.69)	
Not reported	53(19)	47(19.67)	6(15.38)	
Tobacco use, no. (%)				0.512
Never	447(48.96)	364(49.86)	83(45.36)	
Current	67(7.34)	53(7.26)	14(7.65)	
Former	388(42.50)	303(41.51)	85(46.45)	
Not Reported	11(1.20)	10(1.37)	1(0.55)	
5-ARI				0.059
Current	372(40.74)	308(42.19)	64(34.97)	
Former	46(5.04)	40(5.48)	6(3.28)	
None	495(54.22)	382(52.33)	113(61.75)	
Family history PCa, no. (%)				0.292
Yes	168(18.40)	127(17.40)	41(22.40)	
No	563(61.66)	455(62.33)	108(59.02)	
Not reported	182(19.93)	148(20.27)	34(18.58)	
HOLEP surgical specimen, median, g. (IQR)				
Submitted specimen weight	52(28.81)	54(30.84)	40(19.66)	0.001
Pathology examined weight	20(14.30)	21(14.30)	18(14.26)	0.045
Number of cassettes examined	14(10.21)	14(10.21)	13(10.21)	0.199
Proportion of surgical specimen examined by pathology				
Percentage of surgical specimen examined	52(43.26,73.68)	52(42.70,73.68)	66.67(46.62,100)	<0.001
Cassettes used/OR specimen weight	0.2831(0.2115,0.4)	0.2727(0.2,0.375)	0.333(0.25,0.5)	<0.001

CONCLUSIONS: We identified a 20% rate of IPCa at the time of HoLEP at our institution. Our study validates in a large, modern cohort previously associated preoperative factors associated with IPCa diagnosis (age, PSA density, preoperative biopsy status). In addition, we identify the proportion of surgical specimen examined by pathology as a novel associated predictive factor which may account for the variability in IPCa detection between HOELP centers.

#44 – Prevalence of Kidney Stone Disease Among American Indian Adults in the Strong Heart Family Study

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INTRODUCTION AND OBJECTIVE: The epidemiology of kidney stone disease (KSD) has not been well described in American Indian communities. We studied the risk of prevalent KSD in the Strong Heart Family Study, a large prospective cohort of American Indian adults.

METHODS: Strong Heart Family Study participants are members of American Indian tribes from Arizona, Oklahoma, and North/South Dakota. Self-reported history of KSD was available from the Phase VI visit (2014-2018). Differences in characteristics of participants with and without KSD were determined using chi-square tests for

categorical variables and independent ttests for continuous variables. Modified Poisson regression with robust variance estimation was used to examine associations of participant characteristics with prevalent KSD.

RESULTS: Among 2,069 participants from the Phase VI visit (mean age 51.6 years, 789 [38.1%] male), 133 (6.4%) had a self-reported history of KSD. No significant difference in KSD prevalence was observed by sex (5.8% for males vs. 6.8% for females, $p=0.4$). Prevalence ratio estimates derived through modified Poisson regression are shown in Table 1. Residence in Oklahoma or Arizona was associated with greater KSD prevalence, compared to North/South Dakota. Annual household income $< \$20,000$ was also associated with greater KSD prevalence, compared to $\geq \$20,000$. Several established risk factors for KSD in the U.S. (obesity, diabetes, and hypertension) were not significantly associated with prevalent KSD in this study.

Table 1. Multivariate modified Poisson regression model assessing risk of prevalent KSD.

Characteristic	Prevalence ratio	95% confidence interval
Age		
26-45 years	Reference	Reference
46-65 years	1.44	0.88 to 2.37
>65 years	1.75	0.95 to 3.21
Sex		
Male	Reference	Reference
Female	0.84	0.55 to 1.27
Center		
North/South Dakota	Reference	Reference
Oklahoma	1.53	1.00 to 2.34
Arizona	2.75	1.52 to 4.96
Annual household income		
$\geq \$20,000$	Reference	Reference
$< \$20,000$	1.65	1.13 to 2.41
Obesity	0.94	0.63 to 1.40
Diabetes	1.24	0.80 to 1.94
Hypertension	1.23	0.77 to 1.94
Estimated glomerular filtration rate		
≥ 90 mL/min/1.73m ²	Reference	Reference
< 90 mL/min/1.73m ²	0.86	0.53 to 1.40

CONCLUSIONS: In a large cohort of American Indian adults, residence in Oklahoma or Arizona as well as lower income were significantly associated with greater KSD prevalence. These findings suggest that there are meaningful socioeconomic risk factors for KSD in American Indian communities which require further investigation.

#45 - Computational model of bladder function and dysfunction: a preliminary study

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Introduction and Objectives: A person's bladder health is closely tied to the overall quality of life. An unhealthy bladder may exhibit one or more symptoms caused by one or more conditions, such as overactive bladder, bladder outlet obstruction, and urinary tract infection. Of particular interest are symptoms related to bladder compliance, such as incontinence and intermittent flow of urine. Currently, there is lack of a methodology to quantify the difference in compliance between functional and dysfunctional bladders from experimental data. This preliminary study aims to address this gap by combining experiments with modeling. Objectives of this study are, (1) to present a mechanistic model of the urinary bladder, (2) to



detail a methodology to analyze experimental data and identify modeling parameters, (3) to propose a metric to quantify the difference in compliance between functional and dysfunctional bladders. We hypothesize that the power density of bladder pressure trace follows a power law distribution with a negative exponent α , which could be used as a metric to quantify bladder function/dysfunction from cystometry data.

Methods: We model the dynamics of the bladder with equations based on current understanding of physiology and nerve signaling. The methodology and hypothesis are tested using model simulations as well as experimental data from animal models. Cystometry data was obtained for healthy young rats and an old rat with age-related underactive bladder. Power spectra of bladder pressure time series are calculated for each dataset. Probability density function for components within each spectrum is determined. Power law exponent, α is then determined for each case.

Results: The mechanistic model captures several key aspects of the physiology of the bladder, such as spontaneous fill and void cycles. Material property for young animal's bladder tissue is determined from cystometry data. Analysis of cystometry data reveals different power law exponents for data from young and old animals. α for filling phase data from young animals are in the range 1.51-1.52, whereas α for data from old animal is less than 1.5. We anticipate seeing similar results for the analysis with model simulations.

Conclusion: This preliminary study suggests that the power densities of the pressure traces come from different distributions for young and old animal bladders. The methodology presented is able to differentiate between the data. Power law exponent can be used as a metric to quantify the differences in the bladder pressure traces for functional and dysfunctional bladders.

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