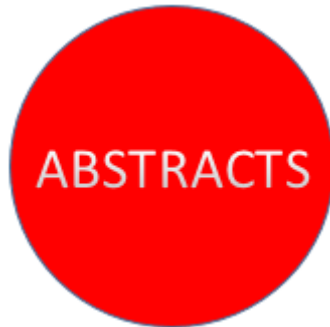


“Targeting Aging in the Lower Urinary Tract”

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1.

Mitochondrial dysfunction is a potential driver of fibrosis in benign prostatic hyperplasia (BPH) as a result of aging

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Background: Benign prostatic hyperplasia (BPH) is associated with proliferation, smooth muscle dysfunction, and fibrosis of the prostate. The greatest risk factor for BPH is age, with 90% of men in their eighties impacted. Many men with BPH develop bothersome lower urinary tract symptoms (LUTS). Given the multifactorial nature of BPH/LUTS, treatments have been unsuccessful for many patients. The molecular mechanisms underlying the aging prostate are not fully elucidated.

Mitochondrial dysfunction is a hallmark of aging, and this study aims to characterize the connection between mitochondrial dysfunction, fibrosis, and aging. **Methods:** Both mouse and cell line models were used. Picosirius red staining quantified collagen bundles as a marker of fibrosis.

Immunohistochemistry was done for the complex I protein, NDUFS3, and a mitophagy protein, PINK1, in prostate tissue from young (2 months) and old (24 months) C57Bl/6J mice. Human prostate stromal cells were treated with the complex I inhibitor, rotenone, to model mitochondrial dysfunction. Protein and gene expression changes were examined using mass spectrometry and RT-qPCR. **Results:** IHC staining showed decreased levels of NDUSF3, suggesting reduced oxidative phosphorylation (OXPHOS) function. PINK1 was also decreased, indicating a reduction mitophagy. qPCR experiments on rotenone treated stromal cells revealed increased gene expression for both *Coll1a1* and *Col3a1*, suggesting complex I dysfunction can contribute to increased collagen production. Protein changes further confirmed mitochondrial dysfunction. **Discussion:** Combined, this *in vivo* and *in vitro* data suggests that mitochondrial dysfunction, potentially originating from complex I of OXPHOS, is contributing to fibrosis in models of BPH/LUTS.

2.

Collaborative Evaluation of a Consortium of NIDDK-Funded Programs Focused on Benign Urologic Diseases and Disorders: Preliminary Results from the CAIRIBU Progress Survey

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INTRODUCTION: In 2020, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) funded a U24 “Interactions Core” to facilitate collaboration between Centers and Programs within the community known as CAIRIBU, *Collaborating for the Advancement of Interdisciplinary Research in Benign Urology*. CAIRIBU is an umbrella organization of NIDDK-funded investigators devoted exclusively to investigating benign genitourinary (GU) diseases and disorders. CAIRIBU investigators and trainees represent the George M. O’Brien (U54) Cooperative Urology Research Centers, P20 Exploratory Centers for Interdisciplinary Research in Benign Urology, and the K12 Career Development Programs focused on benign urology epidemiologic research (KUroEpi) and basic science research (KURe). The broad objectives of the CAIRIBU Interactions Core are to build and foster a community within CAIRIBU that promotes: (1) information- and resource- sharing, (2) collaborative research activity both within and outside of CAIRIBU that advances benign GU science, and (3) training the next generation of leaders in benign GU research. National Institutes of Health (NIH) cooperative agreement mechanisms (U-grants) involve the coordination of several grants and/or resources and involve substantial NIH staff involvement. As a percentage of research project grant activity codes, NIDDK funding for all U-grants (excepting U01 grants) significantly increased from 2010 to 2020. However, there is very little published research on evaluation of these large research initiatives.

METHODS: To understand the value and utility of the CAIRIBU U24 Interactions Core, we developed a 7-step plan for evaluating our efforts; steps include: (1) identify the mission, (2) identify the objectives, (3) develop logic models that reflect these objectives, (4) define the outcomes to be measured, (5) present the preliminary evaluation plan to stakeholders, (6) determine the specific measures, data sources, and timeline, and (7) finalize the plan. As one piece of our comprehensive evaluation plan, we developed the CAIRIBU Progress Survey, originally conceived as a triennial instrument, which we disseminated to all CAIRIBU investigators and trainees. Survey goals are to collect data on personal, professional, and scientific accomplishments as well as information about numbers of grants applied for and awarded. A pilot survey was disseminated in November 2021 with a follow-up in March 2022.

RESULTS: Survey results were obtained from 25% of potential CAIRIBU investigators and trainees in 11/2021 and improved to 47% in 03/2022. Results will provide baseline measures for future data collection and comparisons. Lessons learned included the need to define and target the specific pool of investigators and trainees from data are needed each time and the need for a standardized survey process to ensure that data are collected uniformly in each iteration. CAIRIBU investigator feedback included suggestion to reduce survey frequency (e.g., ≥ 6 months between surveys) and a request to

provide individuals' responses to earlier surveys so that accomplishments within each recall period are most accurate.

CONCLUSION: Data from the CAIRIBU Progress Survey will help the CAIRIBU U24 Interactions Core redirect efforts and change course as needed and restructure internal processes to be most effective. Additionally, results will add to other data we collect during ongoing evaluation efforts that demonstrate overall impact and utility of the NIDDK U24 grant program in benign urology.

The role of Urethral Neuroendocrine cells in Urinary Tract Infection

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Background/Introduction:

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.

Hypothesis:

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 to which they are electrically coupled, to expel bacteria from the urinary tract.

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.

4.

Immune cell single-cell RNA sequencing analyses suggest a role for age-associated T cell subset in symptomatic Benign Prostatic Hyperplasia

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Age-related immune dysfunction impacts a variety of age-associated chronic conditions. Benign Prostatic Hyperplasia (BPH) is among the most common age-associated conditions in men. Likewise, prostatic immune cell infiltration is frequently observed with aging coincident with BPH nodules; however, a relationship between age-related immune changes and BPH has not been defined. We seek to define immune cell types and their associations with BPH clinical symptoms. Prostate immune cell analysis revealed a T lymphocyte-dominant leukocyte phenotype with a relative shift in T cell subset proportions in BPH compared to normal prostates from young men. Interaction analyses suggest enhanced ligand-receptor interactions among CD8⁺ T cells and macrophages. Macrophage gene expression reveals mixed M1 and M2-associated inflammatory profiles indicating a mixed inflammatory microenvironment, which may contribute to progressive non-resolving BPH inflammation. Notably, we identify a CD8⁺ Granzyme K high, Granzyme B low T cell subset previously associated with an aging immune system which positively correlated with IPSS. Overall, these data demonstrate a dominant T cell inflammatory response in prostate tissue and suggest a link between CD8⁺ T cell subsets and symptomatic BPH.

5.

Identification of Cell Types that Contribute to Fibrotic Disease in the Prostate

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Lower urinary tract symptoms (LUTS) are increasingly prevalent in men of advancing age and include weak or intermittent urine stream, incomplete bladder emptying and more frequent urinary voiding especially at night. Historically, prostatic enlargement was considered the most common cause of LUTS. However, recent studies show several contributing factors to LUTS. Excessive collagen accumulation driven by prostatic inflammation is a strong predictor of LUTS. The purpose of this experiment is to identify which cells and their daughter cells (cell lineages), produce collagen during prostatic inflammation. Six genetically edited mouse lines were used:

Myh11-cre, *Lyz2-cre*, *CD2-cre*, *S100a4-cre*, *Srd5a2-cre*, *Gli1-cre*. To trace potential collagen-producing cell types, each mouse line harbored the Red Fluorescent Protein (RFP),

uniquely marking one of six cell lineages of interest: smooth muscle cells (*Myh11*), bone marrow derived myeloid cells (*Lyz2*), lymphoid cells (*CD2*), or a type of fibroblast (*S100A4*, *Srd5a2* or *Gli1*).

Immunostaining was conducted on inflamed mouse prostate tissue to identify lineages that increased in abundance and produced collagen. It was determined that *Lyz2*, *S100a4*, *Gli1*, and *CD2* lineage-

labeled cells increased in abundance and produced collagen during inflammation, while cells derived from the *Srd5a2* and *Myh11* lineages did not. The contribution of

lineage-labeled cells to total collagen-producing cells was independently calculated for each mouse strain: *CD2* lineage-labeled cells contributed approximately 27% of collagen-producing

cells, *S100a4* contributed approximately 76%, and *Gli1* and *Lyz2* each contributed approximately 70%.

All the lineages combined contributed to over 100% of the collagen-producing cells, indicating potential overlap of RFP expression among lineages.

6.

Title:

Sensory Testing in Models of Cystitis and Neuropathic Pain Reveals Partially-conserved Neural Substrates for “Referred” Somatic Pain.

Authors:

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Abstract:

Up to 75% of people with urologic chronic pelvic pain syndrome experience comorbid pain outside the urinary tract, yet there is little understanding of why comorbid pain is so common or what the underlying mechanisms are. Somatic pain syndromes in patients are mirrored in rodent models, where hypersensitivity of the hindpaw skin is a consistent yet poorly-understood “referred pain” phenotype of irritant-induced cystitis. We used a battery of *in vivo*, modality-specific assays of sensation to gain a more precise understanding of the neural correlates of hindpaw pain. Cystitis-associated phenotypes were compared to the spared nerve injury (SNI) model of neuropathic pain, which produces pan-modality secondary pain in the hindpaw. After cystitis, mice exhibited modality-specific hypersensitivity to static mechanical stimuli in the von Frey test that was spatially restricted to the sural dermatome of the glabrous hindpaw skin. Interestingly, this spatial pattern is a well-established feature of the SNI phenotype due to the neuroanatomy of the uninjured “spared” sural nerve, and its origin from the L5 DRG in mice. Dual tracer studies targeting the bladder and the sural dermatome of the paw confirmed that bladder afferents and paw afferents can co-populate the L5 DRG, in contrast to textbook descriptions of bladder sensory neuroanatomy. Thus, the neural mediators of static mechanical allodynia appear conserved across the two types of pain, along with a spatial distribution that suggests common anatomic substrates. These data likewise provide functional evidence of a distinct role of sensory neuron subtypes in “referred pain” mechanisms associated with cystitis.

7.

The Role of Immune Cells in *Toxoplasma gondii*-Induced Prostatic Hyperplasia

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Our lab has recently used *Toxoplasma gondii* infection to develop a novel mouse model that mimics most of the key features of human benign prostatic hyperplasia (BPH). BPH is a common condition affecting 50% of men by 50 years of age. It is typically associated with frequent urination, urgency, straining, and nocturia. BPH presents histologically with hyperplasia and the formation of small, ringlike structures called microglands that can form larger, well-defined epithelial nodules. These microgland structures are characteristic of advanced, highly-symptomatic BPH. Another common feature observed in BPH patients is inflammation with an expansion of T cell infiltrates. A strong T helper 1 (Th1) response occurs early during BPH, but the tissue microenvironment transitions to a milieu of cytokines and inflammatory cell types involved in cell proliferation as BPH progresses. However, the role of these cytokines in BPH progression remains unclear. **Our central hypothesis is that strong *Toxoplasma gondii*-induced IFN γ responses promote microglandular hyperplasia in prostatic inflammation.** We have shown IFN γ -producing Th1 and CD8+ T cell responses in prostate hyperplasia corresponding to persistent *Toxoplasma gondii* infection. The production of IFN γ is known to promote polarization of macrophages that release cytokines like IL-1 and IL-6 that drive cell proliferation and are prevalent in human BPH. Our objective is to use our innovative mouse model to determine the contribution of immune cells to *Toxoplasma gondii*-induced inflammation in the prostate and how these cells and subsequent cytokine changes promote microglandular hyperplasia.

8.

Assessment of Bladder Biomechanics Using MRI

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Background: Existing methods to evaluate the lower urinary tract (LUT) are invasive and provide limited anatomical and functional information. Studies have demonstrated that magnetic resonance imaging (MRI) can provide dynamic, high-fidelity 3D images of the bladder. The purpose of this study is to implement an MRI urodynamics protocol for the comprehensive assessment of bladder biomechanics during voiding.

Methods: In this IRB-approved, HIPAA-compliant study, 5 men with no history of LUTS and 1 patient with benign prostatic hyperplasia (BPH) were recruited. MRI scans were performed on a clinical 3T scanner using a modified DISCO Flex acquisition sequence. Bladder wall and lumen were segmented to create 3D anatomic models. Bladder neck displacement, bladder wall thickness, post void residual, total voided volume, and volumetric flow rate were quantified from these models. Bladder neck angle (BNA) was measured at resting pre-void, resting post-void and maximum flow phases.

Results: Subjects with highest flow rates display the highest variations in BNA between resting and maximum flow phases. Bladder neck displacement behaves similarly to the BNA in all subjects that voided more than 150cc and ranges between 3-25mm. As flow rate increases the bladder neck descends towards the lower pelvis to potentially assist funneling of the bladder neck, favoring urine flow upon reaching maximum flow rate. As flow rate decreases, the bladder retracts to resting position as void ends.

Conclusion: Developed a novel, non-invasive, MRI methodology that allows anatomical and functional characterization of bladder biomechanics during any voiding event, providing a more complete assessment of the LUT during voiding.

9.

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Impact of CYP7B1 Disruption in Mouse Models of Urinary Dysfunction

Background: Benign prostatic hyperplasia (BPH) is an aging disease characterized by prostate enlargement, and causes increased urinary urgency and retention. Estrogen receptors alpha (ER α) and beta (ER β) have been shown to play a role in the prostate, with alpha acting as a driver of dysfunction, and beta playing a protective role (Nicholson et al., 2015). In mouse models of lower urinary tract dysfunction (LUTD), ER α has been implicated in disease development. Because ER α and ER β play opposing roles, the differential activation of the receptors could alter BPH treatment. The steroid enzyme CYP7B1 is responsible for the catabolism of 3 β -adiol, a ligand for ER β .

Purpose: The purpose of this study is to determine the significance of CYP7B1 on LUTD, and determine whether inactivation of its expressing gene has effect on urinary function. We hypothesize that the loss of CYP7B1 would increase ER β ligands, increase ER β activation, and decrease urinary dysfunction.

Methods: A mouse model developed to simulate the hormonal environment of aging men has been developed in the Ricke lab, utilizing subcutaneously implanted testosterone (T) and 17 β estradiol (E2) pellets. These pellets contain 25 mg and 2.5 mg of steroid, respectively, and are meant to simulate decreasing T with maintained E2 levels, leading to an overall decrease in the T:E2 ratio with age (Nicholson et al., 2012). Wild type mice age 8 weeks with the CYP7B1 gene knocked out were subcutaneously embedded with these steroid pellets, and compared with wild type mice containing wild-type CYP7B1 genes also implanted with steroid. Sham mice with both the wild-type and KO CYP7B1 genes were also observed. Urinary function was measured through void spot analysis (VSA), in which mice were placed onto a paper sheet and withheld water for periods of 4 hours. Urine spots were counted at the end of the 4-hour period. As the CYP7B1 gene was a global KO, body mass was also examined to determine potential systemic alterations. After 4 weeks, mice were euthanized, and bladder size and volume were measured.

Results: The results of this experiment showed no difference in bladder size and volume between CYP7B1 KO mice and mice with the wild type gene. There was, however, a significant difference between the urine retention of the CYP7B1 KO mice and mice with the wild type gene. This result indicates that while knocking out CYP7B1 does not eliminate the overall progression of LUTD, with increased bladder size and volume remaining consistent in both groupings, symptoms of LUTD were greatly reduced, as KO mice were able to eliminate urine all at once. This was indicative through decreased number of void spots compared to the mice with wild type CYP7B1 genes. Body masses between the KO and wild-type mice did not experience a significant difference, indicating the loss of the CYP7B1 gene in KO mice showed no apparent deleterious alterations.

Conclusion: KO mice treated with T+E2 did not display the same droplet voiding of that of the wild-type treated mice. However, because the KO mice are still exhibiting bladder and prostate changes similar to wild-type treated mice, this suggests that ER β only alleviates the symptoms of urinary dysfunction, and that the steroid hormones were still having a morphologic effect on the

lower urinary tract. The extent that CYP7B1 is altering ER β has not been assessed, but these results could point to an alternative, effective treatment for urinary symptoms, greatly increasing the quality of life of these individuals.

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10.

Title: The Analysis of Urethral Biomechanics During Voiding Using MRI

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Background: Dynamic imaging during voiding using Magnetic Resonance Imaging (MRI) allows for non-invasive assessment of the urethra anatomy and biomechanics. This study aims to implement an MRI-based urodynamics protocol for the comprehensive assessment of LUT biomechanics during voiding.

Methods: In this IRB-approved, HIPAA-compliant study, 4 healthy men were recruited. All scans were completed on a clinical 3T scanner (Premier, GE Healthcare, Waukesha, WI), using the dynamic sequence, 3D Differential Subsampling with Cartesian Ordering (DISCO) Flex. 15 minutes before the scan, 1/3 of a single weighted dose (0.1 mmol/kg) of gadolinium-based contrast was hand injected intravenously into the subjects who were equipped with a condom catheter to void in the scanner. MR images were imported into Mimics (Materialise, Leuven, Belgium), where the urethra and prostate were segmented at each time point during the voiding event to create discrete 3D Renderings. These renderings were used to calculate urethral length and volume, post-void ureteral residual (PVUR), prostate volume (PV), prostatic urethral angle (PUA), and internal and external urethral sphincter (IUS & EUS) diameters.

Results: All subjects were able to void in the scanner, and anatomy and biomechanics were successfully analyzed. The average PV, length, volume, and PVUR equaled 31.15cc, 22.7cm, 14.2cc, and 4.4cc, respectively. Penile and prostatic urethras always had a greater diameter than the membranous urethra. IUS did not correlate with flow rate ($R^2=0.14$), while the EUS did ($R^2=0.87$).

Conclusion: This non-invasive, comprehensive MRI protocol is able to evaluate urethra anatomy, function, and biomechanics throughout the voiding event in a safe, accurate, and reproducible way.

11.

DEVELOPMENTAL EXPOSURE TO ENVIRONMENTAL CONTAMINANTS, POLYCHLORINATED BIPHENYLS, IMPACT VOIDING PARAMETERS IN YOUNG ADULT MICE

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Polychlorinated biphenyls (PCBs) are environmental contaminants with known impacts on the brain. PCB effects on other organs such as bladder are unknown. We test the hypothesis that developmental exposure to PCBs at human relevant concentrations, leads to voiding abnormalities in adult mice. Female mice were dosed daily for two weeks prior to mating, through gestation and lactation with a PCB mixture (0, 0.1, 1, or 6 mg/kg) mimicking proportions found in pregnant women. Offspring 6-8 weeks of age underwent void spot assay (VSA), uroflowmetry, anesthetized cystometry and bladder contractility assays. PCBs increase the number of small urine spots (0-0.1 cm) during VSA in male mice of the 0.1 and 6 mg/kg dose group vs. control, and in all female PCB dose groups. Uroflowmetry revealed a decrease in urine stream rating only in male mice at the 0.1 mg/kg PCB dose group vs. control. Cystometry revealed decreased intervoid interval in female mice of the 0.1 and 6 mg/kg PCB groups, but higher maximum pressure in male mice of the 0.1 and 1mg/kg PCB groups vs. controls. PCBs altered bladder contractility. PCBs at all doses increased sensitivity to electrical field and cholinergic stimuli in male bladder. In female bladder PCBs decreased sensitivity to electrical field stimulation in the 1mg/kg group, but increased sensitivity to carbachol at the 0.1mg/kg and 6mg/kg dose vs. control. These results indicate sex- and dose-dependent effects of PCBs on voiding which ultimately produce more small/frequent voids – parameters which recapitulate lower urinary tract symptoms in humans.

12.

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Chronic pelvic pain conditions such as interstitial cystitis/bladder pain syndrome (IC/BPS) remain clinical and mechanistic enigmas. Microglia are resident immune cells of the central nervous system (CNS) that respond to changes in the gut microbiome, and studies have linked microglial activation to acute and chronic pain in a variety of models, including pelvic pain. We have previously reported that mice deficient for the lipase acyloxyacyl hydrolase (AOAH) develop pelvic allodynia and exhibit symptoms, comorbidities, and gut dysbiosis mimicking IC/BPS. Here, we assessed the role of AOAH in microglial activation and pelvic pain. RNAseq analyses using the ARCHS⁴ database and confocal microscopy revealed that AOAH is highly expressed in wild type microglia but at low levels in astrocytes, suggesting a functional role for AOAH in microglia. Pharmacologic ablation of CNS microglia with PLX5622 resulted in decreased pelvic allodynia in AOAH-deficient mice and resurgence of pelvic pain upon drug washout. Skeletal analyses revealed that AOAH-deficient mice have an activated microglia morphology in the medial prefrontal cortex and paraventricular nucleus, brain regions associated with pain modulation. Because microglia express Toll-like receptors and respond to microbial components, we also examine the potential role of dysbiosis in microglial activation. Consistent with our hypothesis of microglia activation by leakage of gut microbes, we observed increased serum endotoxins in AOAH-deficient mice and increased activation of cultured BV2 microglial cells by stool of AOAH-deficient mice. Together, these findings demonstrate a role for AOAH in microglial modulation of pelvic pain and thus identify a novel therapeutic target for IC/BPS.

13.

PCB Exposure Causes Changes in Axonal Growth at Specific Timepoints in Cultured Dorsal Root Ganglia

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Abstract

Lower urinary tract symptoms (LUTS) are prevalent in the aging population and in individuals with neurodevelopmental disorders. Although LUTS etiology is complex, exposure to environmental toxicants may play a role. Polychlorinated biphenyls (PCBs) are persistent environmental toxicants found in human tissues, and developmental exposure can alter voiding function in mice. PCBs alter neuronal morphology in the central nervous system, but their impact in the peripheral nervous system is largely unknown. Since peripheral nerve innervation of the bladder may influence LUTS, the present study assesses whether PCB exposure affects axon growth in dorsal root ganglia (DRG). DRG were extracted from male C57Bl/6j mice and cultured for 2 or 4 days with 0, 1 pM, 1 nM or 1 μ M MARBLES PCB mix. DRG were fixed, immunostained with antibodies targeting beta-III tubulin (a pan-neuronal marker to label all axons) and calcitonin gene-related peptides (to label sensory DRG) and imaged. Both axon and tip number were significantly increased in DRG cultured for 2 days in 1 nM MARBLES PCB vs vehicle control. This effect was no longer present in DRG cultured for 4 days. No difference was found in the number of CGRP+ cells among dosage groups. Thus, PCBs may alter early phases of axonal growth in DRGs. Whether this results in altered peripheral innervation of the bladder remains to be determined but implies that environmental chemicals can alter axon growth in the peripheral nervous system. Investigation into the molecular pathways underlying PCB effects on axon growth is ongoing.

14.

Optimizing a MALDI-MSI workflow for detection of proteins in mouse prostate tissue

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Abstract: Benign prostatic hyperplasia (BPH) affects the majority of the aging male population. Though BPH is non-malignant in nature, the associated lower urinary tract symptoms (LUTS) that develop can significantly decrease quality of life and place a burden on the healthcare industry, costing over \$4 billion in treatment annually. Current basic research methods using mouse models utilize immunohistochemical (IHC) techniques for targeted analyses; unfortunately, each protein of interest needs a unique antibody for identification and quantification can vary widely. The need for multiple antibodies for immunohistochemical (IHC) methods can be overcome through utilization of mass spectrometry imaging (MSI), as MSI allows for detection of dozens of proteins within a single experimental run. Here, we focused upon designing a workflow to analyze prostate lobes from mouse models of BPH using MSI. Fixed tissues of mouse anterior (AP), ventral (VP), and dorsolateral (DLP) prostate lobes were sectioned multiple times at a variety of thickness levels to initially determine optimal tissue thickness for MALDI-MSI analysis. Antigen retrieval buffer of 10mM citric acid with or without Triton-X was tested to determine optimal retrieval for MSI. On-tissue digestion of proteins and matrix application was performed based on previously utilized protocols. An initial raster size of 100µm during early protocol optimization steps was used for MALDI-MSI and was decreased to 50µm once these other variables were determined. Taken together, these methods aim to aid researchers in further understanding of proteins and pathways contributing to disease progression as well as improve quantification efforts.

15.

A Role For A Long Non-Coding RNA, *SlincR*, In Mouse Development

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Introduction:

TCDD is an environmental contaminant and agonist of the aryl hydrocarbon receptor, a transcription factor which can influence urinary tract morphogenesis, function, and development. Additionally, TCDD toxicity occurs on a wide spectrum and can also include craniofacial malformations, cardiovascular toxicity, and endocrine disruption. In zebrafish, TCDD toxicity is mediated by a long-noncoding RNA, *SlincR*, which has mouse and human orthologs. The goal of this study is to determine the role of *SlincR* in mouse development and TCDD toxicity.

Methods:

We used CRISPR/Cas9 genome editing to excise exon 3 of the mouse *SlincR* gene to create an allele with a predicted reduction in activity (*SlincR*^{Δex3}). In one arm of the study, body composition and organ weights were compared in 8-10 -week-old male *SlincR*^{+/+} (control) and *SlincR*^{Δex3 Δex3} mice. In the second arm, *SlincR*^{+/+} and *SlincR*^{Δex3 Δex3} mouse fetuses were exposed *in utero* to TCDD (25 μg/kg maternal dose, PO) or corn oil (5 ml/kg) on gestation day 10 and evaluated on gestation day 18.

Results:

Thymuses, testes, seminal vesicles, and body mass index were greater, but anogenital distances were smaller, in *SlincR*^{Δex3 Δex3} than *SlincR*^{+/+} adult male mice. TCDD induced cleft palate, and changes in kidney and heart weight were not statistically significant between groups.

Conclusion

SlincR appears to be required for normal development of the male mouse but does not appear to mediate the endpoints of TCDD toxicity examined in this study. Supported by NIH grants RO1ES001332 and T32ES007015 and the UW-Madison Graduate Research Scholar Fellowship.

16.

Title: In Vitro Validation of a Real-time 3D MRI Urodynamics Protocol

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Introduction: Lower urinary track symptoms (LUTS) occur frequently as individuals age. Patients with LUTS are evaluated through multi-channel urodynamic studies, however these tests are invasive and indirect. Non-invasive methods of imaging the bladder show promise, but their use has been limited. This study utilizes a non-invasive 3D MRI protocol to assess the voiding performance of an in vitro bladder model and is validated with high-speed optical imaging.

Methods: An anatomically realistic in vitro bladder model was fabricated from 3D MRI data obtained from a healthy volunteer. A ¼” diameter tube was attached to represent the urethra and used to fill the model with water. Data were acquired using a 3D DISCO Flex MRI protocol to analyze the model deformation during the voiding cycle. In vitro MRI data were compared to images obtained from high-speed cameras and percent deformation rate was calculated for both techniques.

Results: The calculated rate of deformation was 6.2 and 5.3% deformation per second for the 3D MRI and optical cases, respectively. There was a 15% difference in the two deformation rates relative to the phantom camera deformation rate. Qualitative analysis of both methods suggests similar patterns of deformation.

Conclusion: 3D MRI urodynamics was able to capture real-time displacement of an in vitro model of the bladder during voiding. Deformation during voiding was validated using optical imaging. This will help improve novel MRI-based methods to image the bladder voiding process in vivo.

17.

Developmental exposure to the environmental toxicant, polychlorinated biphenyls, leads to increases in mouse bladder volume, nerve density, and mast cells

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Abstract: Environmental contaminants are risk factors for several disorders, yet their contribution to bladder dysfunction is understudied. Polychlorinated biphenyls (PCBs) influence nervous and immune systems, yet whether PCBs act on these pathways to disrupt bladder function is a gap in understanding the molecular underpinnings of bladder dysfunction. Therefore, we tested the hypothesis that developmental PCB exposure in mice results in alterations to bladder innervation and inflammation. Female mice were dosed through gestation and lactation with an environmentally relevant mixture of PCBs. Offspring were collected at 4 or 6 weeks of age. Without signs of overt toxicity, effects of PCBs on bladder morphology were sex- and dose-dependent. Bladder volume was increased in the highest dose group compared to control in male offspring at 4 and 6 weeks of age, and female offspring at 6 weeks of age. Overall suburothelial nerve density was increased in the highest dose group compared to control in male offspring at 4 weeks. This phenotype may in part be driven by increases in sensory nerves; CGRP+ sensory nerves were also increased between the lowest and highest dose group males at 4 weeks. Testing at 6 weeks is ongoing. Inflammation can influence nerve density. Mast cell density correlated with nerve density for the highest dose group males at 4 weeks of age, and mast cell quantities were increased in this group compared to controls at 6 weeks of age. PCB induced changes in mast cell and nerve densities support future study of the functional consequences of these pathways.

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18.

A NEW Approach for Characterizing Mouse Urinary Pathophysiologies

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Abstract

Void spot assay (VSA) is a cost-effective method for evaluating mouse urination phenotypes. VSA has been used to differentiate voiding behaviors between experimental groups, but not as a diagnostic assay. To build toward this goal, we used the VSA to define urination patterns of male mice with diabetic diuresis (BTBR.Cg-Lepob/WiscJ mice), irritative urinary dysfunction (E. coli UT189 urinary tract infection), and obstructive urinary dysfunction (testosterone and estradiol slow-release implants) compared to their respective controls. Many studies compare individual VSA endpoints (urine spot size, quantity, or distribution) between experimental groups. Here, we consider all endpoints collectively to establish VSA phenomes of mice with three different etiologies of voiding dysfunction. We created an approach (normalized endpoint work through (NEW)) to normalize VSA outputs to control mice, and then applied principal components analysis and hierarchical clustering to 12 equally weighted, normalized, scaled, and zero-centered VSA outcomes collected from each mouse (the VSA phenome). This approach accurately groups mice based on voiding dysfunction etiology. We then used principal components analysis and hierarchical clustering to show that within a test group of aged mice (>24 m old) some develop a phenotype that groups with the obstructive or a diabetic diuresis VSA phenotype while others develop a unique phenotype that does not cluster with that of diabetic, infected, or obstructed mice. These findings support continued use of VSA to identify specific urinary phenotypes in mice. The VSA is a beneficial quick and cheap test used to identify urinary dysfunction in research studies.

19.

Title

Simulating Bladder Voiding Using Real-Time MRI-Based Computational Fluid Dynamics: A Pilot Study

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Introduction

Multi-channel urodynamic studies are used to assess bladder voiding. However, they are invasive and provide insufficient information on biomechanics, anatomical, and functional information. MRI-based computational fluid dynamics (CFD) has largely focused on studying cardiovascular flows. We hypothesize that MRI-based CFD can be used to study urodynamics in bladder voiding.

Methods

Two healthy male subjects were recruited following an IRB-approved HIPAA-compliant protocol. The subjects were scanned on a 3T scanner. Gadolinium-based contrast was injected into the subjects prior to scanning. As the subjects voided in the scanner, 3D real-time MR images were acquired. The bladders were semiautomatically segmented. We developed a mapping algorithm that processes the surfaces of the bladder wall, so they all have the same surface topologies. This enabled wall-driven CFD simulations which numerically solves the conservation equations inside the voiding bladder.

Results

Subject-specific CFD simulations were successfully executed using real-time 3D MR images. The simulations provided pressures and velocities of the urine in the bladder during voiding. For Subject 1, $Q_{\max} = 19.23$ mL/s, $P_{\det} Q_{\max} = 38.45$ cmH₂O, and for Subject 2, $Q_{\max} = 15.12$ mL/s, $P_{\det} Q_{\max} = 9.13$ cmH₂O. For Subject 1, BOOI = -0.01, BCI = 134.6, and for Subject 2, BOOI = -21.11, BCI = 84.73.

Discussion

MRI visualized the deformation of bladder during voiding and CFD visualized/quantified urodynamics. This was the first time BOOI and BCI was calculated using 3D real-time MRI. Based on calculations, Subject 1 has no obstruction and normal contractility while Subject 2 has no obstruction and impaired contractility.

20.

The role of osteopontin in steroid hormone-induced immune cell infiltration of the prostate

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Inflammatory processes in the prostate have been linked to the development of lower urinary tract symptoms in men. The age-related increase in estradiol:testosterone ratio leading to hormonal imbalance is likely a key factor in triggering inflammation in the prostate. This study elucidates immune cell infiltration associated with alterations of the estradiol:testosterone ratio and assesses whether osteopontin (OPN), a pro-inflammatory cytokine, plays a role in steroid hormone-induced inflammatory responses. Male C57BL/6J (WT) or *Spp1*^{tm1Blh}/J (OPN-KO) mice were surgically implanted with slow-releasing subcutaneous pellets containing 25 mg testosterone (T) and 2.5 mg estradiol (E2). Mice were euthanized two weeks later, and ventral (VP) and dorsal prostate (DP) tissue was collected. We quantified immune cells with immunohistochemical staining of CD45. In addition, we analyzed several immune cell subsets using fluorescent *in-situ* hybridization, toluidine blue staining, and hematoxylin and eosin staining. Our study suggests that OPN plays a role in mediating steroid hormone imbalance-induced inflammation. CD45+ cell counts were reduced in OPN-KO mice, indicating that OPN exacerbates inflammation in response to steroid hormone treatment. Additionally, elevated counts of *Cd68*+ cells in WT mice, but not in OPN-KO mice, indicate that OPN is required for the recruitment of monocytes/macrophages in steroid hormone imbalance. This suggests that OPN-targeting therapies may be beneficial for LUTS patients by inhibiting steroid hormone-induced inflammation.

21.

**POLYCHLORINATED BIPHENYLS AFFECT PROLIFERATING PROSTATE CELLS IN
ADULT EXPOSED MALE MICE**

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Abstract

Lower urinary tract dysfunction (LUTD) can greatly affect a person's quality of life, especially in aging populations. In men, LUTD can be attributed to obstruction caused by changes in the prostate. Polychlorinated biphenyls (PCBs) are persistent environmental toxicants that affect human health at varying levels of exposure. PCB exposure to male mice later in adulthood and its effects in the prostate are unknown. We seek to test the hypothesis that adult male mice exposed to PCBs changes prostate morphology. C57Bl/6J wild type male mice were dosed daily with peanut butter mixed with the 0 or 1 mg/kg MARBLES PCB mixture. Dosing began at 10 weeks of age and continued for 60 days. After the dosing regimen was completed, uroflowmetry was conducted. Mice were then euthanized, and prostates were weighed, micro-dissected by lobe, fixed, and prepared for immunohistochemistry. Uroflowmetry showed a significant increase in flow rate in the 1 mg/kg dose group compared to the vehicle control. In the ventral lobe of the prostate, absolute and relative mass was significantly increased in the 1 mg/kg group compared to the vehicle control. In the anterior prostate, there was a significant decrease in Ki67+ cells in the total number of cells compared to the vehicle control. There were no significant differences in the number of caspase positive cells or in smooth muscle thickness. This data shows that PCB exposure into adulthood can affect voiding function and prostate histology. Future experiments aim to repeat the same stains performed on the anterior lobe on the ventral and dorsal lobes of the prostate.

22.

***Toxoplasma gondii* infection induces lower urinary tract symptoms (LUTS) in mice and correlates to BPH-LUTS incidence and epithelial nodule formation in men**

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Background: BPH-LUTS is characterized by urinary frequency, urgency, incomplete emptying, and pelvic pain. Histological features of BPH include inflammation and epithelial nodule formations harboring microglandular hyperplasia. Systemic infection with the common parasite *Toxoplasma gondii* induces both inflammation and microglandular hyperplasia in mice. In this study we characterize LUTS in *T. gondii*-induced prostatic hyperplasia in mice and identify *T. gondii* infection incidence in men.

Methods: We infected CBA/j, CD-1, and C57Bk6/j i.p. with *T. gondii* tachyzoites. Mice were analyzed weekly from 14 days post infection (d.p.i), until sacrifice at 60 d.p.i. using Void Spot Analysis and Void Whizzard software. Mice were isolated in cages lined with chromatography paper for two, two-hour increments, and the paper was imaged using UV transillumination. We assessed human blood samples from patients treated for BPH and age-matched donor controls (age range 45-70; average 60 and 56) for *T. gondii* seropositivity by ELISA.

Results: Infected mice exhibited significantly more small urine spots (less than 0.5 cm area) than control mice. These mice exhibit prostatic inflammation, reactive hyperplasia, and microglandular hyperplasia. Men with BPH had a *T. gondii* positivity rate of 38% versus 9% in the controls. Within the BPH group, 9/9 prostates from seropositive men exhibited large epithelial nodules with microglandular hyperplasia versus 7/21 in seronegative patients.

Conclusions: Systemic *T. gondii* infection induces inflammation and microglandular hyperplasia in mouse prostates associated with increased voiding phenotype. Seropositivity to *T. gondii* is associated with increased BPH incidence in men and is associated with inflammation and the formation of epithelial nodules with microglandular hyperplasia.

Development and Utilization of CRISPR-cas9 Mediated Protein Knockout Cell Lines as a Resource for Translational Prostate Research

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INTRODUCTION AND OBJECTIVE: CRISPR-cas9 has become a staple technique amongst molecular biologists. Using the cas9 endonuclease, precise DNA strand breaks can be introduced to allow for a variety of downstream applications including insertion of new DNA sequences and knockout (KO) of protein expression. We introduce a method combining stable cas9 expression and transient puromycin resistance to allow for development of cell lines with multiple KO proteins.

METHODS: Utilizing Lentiviral hCMV-Blast cas9 Nuclease, BPH-1 (prostate epithelial cell line), BHPs-1 (prostate stromal cell line), and other prostate cells were transduced overnight. Complete growth medium was then added to quench the viral infection, and blasticidin was added for selection. Cells were co-transfected synthetic guide RNA (sgRNA) and a plasmid containing both GFP and puromycin resistance. Puromycin was used for selection for 48 hours once GFP was observed. Cells were seeded into 96-well plates to isolate clonal populations. Gene KO was verified using immunofluorescence (FGF-5) or Western blot, and qRT-PCR.

RESULTS: We were able to successfully generate multiple blasticidin-resistant cas9-expressing prostate cell lines. We were also able to confirm successful KO of FGF-5 in PC3 cells using immunofluorescence.

CONCLUSIONS AND FUTURE DIRECTIONS: We present a method for generating cell lines that stably express cas9, while transiently expressing puromycin resistance, allowing for multiple successive rounds of sgRNA transfection and clonal selection. We plan to further assess the phenotypic effects of targeted protein KO in prostate cancer cells to determine how target proteins contribute to the development of castration resistance and development of metastases. We also plan to use LC-MS/MS analysis to determine how targeted KO may affect the proteome.

24.

An Evaluation of Short-Term Treatment with Raloxifene as an Interventional Therapy for BPH/LUTS

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INTRODUCTION AND OBJECTIVE: Benign Prostatic Hyperplasia (BPH) is a common condition among aging men, often resulting in the development of Lower Urinary Tract Symptoms (LUTS). Previously, we have shown that co-treatment with Raloxifene prevented development of voiding dysfunction in the T+E₂ mouse model of BPH/LUTS. As BPH is often diagnosed following the onset of LUTS and is a progressive disease, we are now evaluating the potential of Raloxifene as an interventional therapeutic.

METHODS: Male 24-month-old and 8-week-old C57Bl6/J mice underwent either sham surgery or surgery to implant pelleted T+E₂. Voiding was monitored using weekly void spot assays (VSAs) for one month. Following monitoring, Raloxifene or Vehicle (PBS + 1% DMSO) was administered for 5 days via IP injection. A final VSA was performed the day following the last injection. Animals were then euthanized, and their urogenital tracts (UGTs) were collected, dissected, and massed.

RESULTS: Hemiprostate mass was significantly decreased in 24-month-old T+E₂ + Raloxifene treated animals compared to vehicle controls (p=0.0018). The 24-month-old T+E₂ Raloxifene treated animals also saw a decrease in average number of void spots from 200 pre-treatment to 100 post treatment. In contrast, 24-month-old sham + Raloxifene animals saw a decrease from an average of 200 void spots to 160 post treatment. No significant effect was observed in 8-week-old animals following Raloxifene treatment.

CONCLUSIONS AND FUTURE DIRECTIONS: This pilot study found that 5-day treatment with Raloxifene significantly decreases hemiprostate mass in T+E₂ treated aged male mice. Animals also experienced a large reduction in number of void spots, indicative of resolving urinary dysfunction. For future studies, we plan to follow up on aged male mice following short term Raloxifene to assess how long the ameliorative affects last. We also plan to assess different dosing strategies for the 8-week-old animals.

25.

The expression of prostatic androgen receptor shows lobe-specific changes during aging in mouse

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The androgen receptor (AR) is a nuclear receptor transcription factor that can be activated by the androgens, testosterone (T) and dihydrotestosterone (DHT). As men age, the serum levels of T decreases, while the level of estrogen (E₂) increases. Studies suggest that the prostate diseases, such as benign prostate hyperplasia (BPH) and prostate cancer, are closely linked to hormones/receptor changes and aging. However, these detailed cellular and molecular mechanisms remain unclear. Since it has been shown that male mice have a similar drop of T during aging as men do, the study of hormones/receptor imbalance in mice would be helpful to human urologic research. Here, we evaluated the expression of nuclear AR in the anterior prostate (AP), ventral prostate (VP), and dorsal lateral prostate (DLP) from 2-month-old (young) and 24-month-old (old) C57BL/6 mice, using immunofluorescence (IF) and immunohistochemistry (IHC). In the AP, more prostatic AR was expressed in young mice (n=9), while old mice (n=7) showed reduced AR expression. Similarly, young mice (n=19) showed a higher positivity score in the VP, compared with old mice (n=14). However, no significant difference in AR expression was observed between young and old mice in the DLP. These results suggest aging is associated with decreased prostatic AR expression in mouse AP and VP.



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