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ABSTRACTS

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National Institute of Diabetes and Digestive and Kidney Diseases

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

CAIRIBU 2021 ABSTRACTS

NLRP3-DEPENDENT BARRIER DYSFUNCTION IN DIABETIC MICE IS ASSOCIATED WITH DETRUSOR OVERACTIVITY BUT NOT UNDERACTIVITY

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INTRODUCTION AND OBJECTIVE: Diabetic bladder dysfunction (DBD) has a poorly understood etiology; one known cause is inflammation mediated by the NLRP3 inflammasome in urothelia. Since bladder urothelia normally maintain an impenetrable barrier, and NLRP3 activation causes pyroptosis, a form of programmed necrosis, we hypothesized NLRP3 activation causes barrier dysfunction during diabetes. To investigate our hypothesis, we examined urothelial barrier permeability in type 1 diabetic mice (Akita) +/- NLRP3 at 15 and 30 weeks old. At these respective time points, diabetic mice exhibit detrusor overactivity and underactivity phenotypes yet diabetic NLRP3$^{-/-}$ mice do not develop pathologic phenotypes.

METHODS: Akita and NLRP3$^{-/-}$ mice were crossbred to yield 4 groups of 15 and 30 week old females: control NLRP3$^{+/+}$, diabetic NLRP3$^{+/+}$, control NLRP3$^{-/-}$, and diabetic NLRP3$^{-/-}$. To assess barrier permeability *in vivo*, 150 µl of biotin (1 mg/ml) was instilled intravesically for 30 minutes and bladders were processed for histology (n=3-5 per group). Regions of urothelia that biotin permeated through were measured. For *ex vivo* analysis (n=4-7 per group), bladders were excised and the detrusors were carefully removed from mucosal layers. Urothelial "balloons" were cannulated with catheters, tied proximal to ureters, and infused with 150 ul Evans blue dye (1/mg/ml) while immersed in bathing solution. After 30 minutes, dye in the bathing solution was measured. Additionally, we measured the following barrier gene expression via qPCR (n=6-12 per group): zona ocludin 1 and 2 (ZO1, ZO2), claudin 4 (CL4), uroplakin 1b and 2 (UP1, UP2), and beta catenin (BCT).

RESULTS: At 15 weeks (overactivity), diabetic NLRP3$^{+/+}$ mice displayed higher in vivo (biotin) and ex vivo (Evans blue) dye permeation ($p<0.05$), indicative of barrier dysfunction. Diabetic NLRP3$^{-/-}$ mice did not demonstrate these increases, clearly demonstrating damage was NLRP3-dependent. Urothelia from the diabetic NLRP3$^{+/+}$ mice exhibited significantly ($p<0.05$) decreased expression of ZO1, ZO2, CL4, UP1, and UP2, but not BCT. NLRP3 gene deletion prevented these changes. By 30 weeks (underactivity), no evidence of in vivo or ex vivo barrier dysfunction was present and barrier gene expression was no longer downregulated.

CONCLUSIONS: Increases in urothelial permeability are associated with detrusor overactivity but not underactivity in our model of type 1 diabetes. During the overactivity phase, NLRP3 gene ablation protects diabetic animals from degradation of the urothelial barrier and voiding dysfunction, indicating NLRP3 activation is a causative factor contributing to early DBD development.

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TRAT VIRULENCE FACTOR PROTECTS DRUG-RESISTANT UROPATHOGENIC ESCHERICHIA COLI AGAINST SERUM KILLING

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INTRODUCTION AND OBJECTIVE: TraT protein is a multifunctional virulence factor that is encoded by the majority of conjugative F-like plasmids. These plasmids spread antibiotic resistance genes in clinical enteric pathogens, such as uropathogenic *Escherichia coli* (UPEC) and *Klebsiella pneumoniae*, via direct cell-to-cell gene transfer by conjugation. In model *E. coli*, the TraT protein is a highly-expressed outer membrane lipoprotein that is 244 amino acids long. TraT prevents excessive rounds of plasmid transfer, renders bacteria resistant to a few bacteriophages, and protects against an innate immune response by the host via serum complement killing. The protective effect of TraT within UPEC strains and its mechanism of action are unknown. Therefore, our objectives in this study were (1) to measure the protective effect of TraT in strains of interest and (2) to establish whether purified soluble TraT is sufficient to provide the full protective effect against serum killing.

METHODS: Recombinant TraT33-244 domain was isolated from BL21(DE3) *E. coli* strain using affinity chromatography. Exponentially growing *E. coli* cultures with and without *traT*-encoding plasmids or the purified TraT domain were subjected to different doses of blood serum for 2 hours at 37°C. Viable cells were quantified as colony forming units by plating serial dilutions at 0 and 2 hours after addition of serum.

RESULTS: The *traT*-encoding plasmid provides variable level of protection that depends on serum type, bacterial cells, time, and ratios of bacteria and serum, and can reach several orders of magnitude. We have developed a method of expression and purification of soluble TraT33-244 protein suitable for functional and biochemical studies. By testing this purified extracellular domain in serum resistant assays, we found that it is insufficient to exhibit the full protective action of TraT.

CONCLUSIONS: In this study, we measured the protective effect of *traT*-encoding plasmid F' in model *E. coli* strains and showed that F-like plasmids provide strong but variable serum protection. The extracellular soluble domain of TraT protein was not strongly protective against serum killing when added to bacterial cells in trans. These results suggest that other parts of TraT protein or TraT interactions within the outer membrane are essential to elicit serum resistance. Overall, this study sets the stage for further details that enable investigating the mechanism of how TraT assists UPEC bacteria in evading innate immune responses within the host organism.

INSIGHTS TO PATHOGENIC POTENTIAL: CHARACTERIZING HOW MOBILE GENETIC ELEMENTS CONTRIBUTE TO VIRULENCE IN UROPATHOGENIC *E. COLI*

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INTRODUCTION AND OBJECTIVE: The majority of urinary tract infections are caused by the bacterial species *Escherichia coli*, a diverse microorganism that can colonize the gut as a commensal bacteria or cause disease. Uropathogenic *E. coli* (UPEC), are thought to originate from the gut and migrate to colonize the urinary tract. Despite living in the gut as a commensal, UPEC can carry a range of genes that make them advantageous for causing UTIs. While no discrete gene defines the uropathogenic pathotype, understanding the source of acquired virulence factors might help us to more robustly define UPEC. In this work, we aim to identify how mobile genetic elements contribute to the carriage of virulence factors and antimicrobial resistance genes in uropathogenic *E. coli*. Defining methods of gene acquisition in UPEC might give insights to predicting pathogenic potential.

METHODS: Genomic analyses were carried out to predict prophage, plasmid, and integrative and conjugative element sequences in *E. coli strains* isolated from both asymptomatic and symptomatic patients using open-source software. Virulence factors and antimicrobial resistance genes (AMR) were additionally identified through sequence homology. We cross-correlated the locations of virulence factors and AMR genes with the locations of the mobile genetic elements. We carried out statistical analyses to determine correlation of mobile genetic elements and carriage of such genes.

RESULTS: UPEC carry a plethora of virulence factors and AMR genes regardless of the causing symptoms in the source patient. We show that prophages are not a significant source of virulence factors or AMR genes. Integrative elements and plasmids both carry AMR and virulence factors. Virulence factors carried by mobile genetic elements (MGE) contribute to 10% of all virulence factors identified. AMR genes carried by MGE are most frequently associated with plasmids, constituting up to 30% of the total AMR genes. Interestingly, most of the identified virulence factors and AMR genes were not associated with MGE.

CONCLUSIONS: UPEC exhibit genomic plasticity and frequently carry several virulence factors, without correlation with symptoms. Understanding the sources and mechanisms of the genetic diversity could give insights to the pathogenic potential of a UPEC isolate. While prophage are not significant contributors to carriage of virulence factors or AMR genes, integrative elements and plasmids did carry a significant amount of virulence factors, specifically those related to iron acquisition and the genotoxin, colibactin. This work provides insights into how UPEC might acquire factors that contribute to predicting a strain's pathogenic potential for more accurate diagnostics. Future research will continue to probe for a genetic signature for UPEC.

EFFECT OF METABOLIC SYNDROME ON THE LOWER URINARY TRACT OF MEN ASSESSED ON MRI

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INTRODUCTION AND OBJECTIVE: To quantify lower urinary tract anatomy in men with and without MetS using MRI.

METHODS: This HIPPA-compliant, single-center, retrospective study was performed under a waiver of informed consent. Consecutive prostate MRIs performed between 1/2017 and 5/2021 were evaluated (n=439). Exclusion criteria included: MRI findings of clinically significant prostate cancer (PIRADS 4 or 5; n=222), prior prostate surgery/radiation (n=13), missing IPSS (n=26) or laboratory data for MetS (n=17), and incomplete MRI of the bladder/prostate (n=40). The bladder and prostate were segmented on T2-weighted pelvic MRI. BWV, PVR and PV were quantified from 3D anatomic renderings. Apparent diffusion coefficient (ADC) of the periurethral transition zone was quantified by . LUTS were quantified using the IPSS. Wilcoxon rank sum, chi-square and Fisher's exact tests were used to test for differences in anatomic (BWV, PVR, PV) or functional (ADC) metrics between men with and without MetS and across symptom severity (IPSS mild=0-7, moderate=8-19, severe=20+). Multivariate regression was used to determine predictors for changes in lower urinary tract anatomy and symptoms.

RESULTS: 147 men [median age of 66.5 years (IQR: 62-71) and BMI of 28.8 (IQR: 27.0-31.9)] were included. Men with MetS had a higher BMI (29.6 v 27.8, $p<0.01$) but similar symptom scores, BWV, PVR, PV and ADC ($p=0.33$-0.93) compared to those without MetS. Men with large prostates ($PV>40cm^3$) had higher LUTS (8 vs 5, $p=0.03$), BWV (49.8 vs 44.7 cm^3, $p=0.03$), PVR (63mL vs 40mL, $p=0.05$) and ADC (1403 vs 1338, $p<0.01$). Similarly, men with very large prostates ($PV>80cm^3$) were more symptomatic with higher BWV and PVR ($p=0.03$-0.05). Men with higher symptom scores had a lower QOL ($p<0.001$) and higher BWV and PV ($p<0.001$ and $p=0.01$, respectively).

CONCLUSIONS: Detrusor hypertrophy and increased post-void residual are associated with higher prostate volume, contribute to lower quality of life. There is no clear difference in lower urinary tract anatomy in men without and with metabolic syndrome.

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LOSS OF OSTEOPONTIN FUNCTION ATTENUATES IMMUNE CELL INFILTRATION AND COLLAGEN ACCUMULATION IN A STEROID HORMONE-INDUCED LOWER URINARY TRACT DYSFUNCTION MODEL

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INTRODUCTION AND OBJECTIVE: Inflammatory processes in the prostate have been linked to the development of lower urinary tract symptoms (LUTS) 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; however, this has not been previously demonstrated. Osteopontin (OPN) is a pro-inflammatory cytokine which we found to be associated with LUTS and demonstrated its role in aggravating prostatic inflammation and fibrosis in mice. This study elucidates immune cell infiltration associated with altered estradiol:testosterone ratio and assesses whether osteopontin plays a role in steroid hormone-induced inflammatory responses and fibrosis.

METHODS: 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 2, 6, or 12 weeks later. Collagen I and CD45 (immune cell marker) protein expression was investigated using immunohistochemistry (IHC). Immune cell types are currently being investigated using multiplex fluorescent hybridization chain reaction (HCR). The immune marker genes being targeted by HCR are *Cd68* for monocytes/macrophages, *Prtn3* for neutrophils, *Cd19* for B-cells, and *Cd3d* for T-cells.

RESULTS: WT T+E2 mice displayed a significantly increased number of CD45 positive cells compared to the WT sham mice 2 weeks post-pellet implantation in both the ventral and the dorsal prostate lobes. In contrast, there was no significant increase in immune cell number in OPN-KO mice at 2 weeks. Additionally, the density of collagen I in the ventral prostate significantly increased in WT and decreased in OPN-KO mice 12 weeks post-pellet implantation. The investigation of the numbers of monocytes/macrophages, neutrophils, B-cells, and T-cells are in progress.

CONCLUSIONS: These results suggest that at the 2-week time point there is a significant inflammatory response that accompanies T+E2 treatment in WT but not in OPN-KO mice. Collagen I density is only elevated 12 weeks in the ventral prostate of WT mice, indicating that the early changes in the inflammatory environment do not trigger fibrosis. In contrast, in OPN-KO mice, there is a hindered inflammatory response early on and chronically lower levels of collagen I density. This indicates that OPN deficiency plays a role in inhibiting steroid hormone imbalance-induced inflammatory response and fibrosis.

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 concern that affects up to 40% of the aged population. UAB may involve reduced motor drive (detrusor underactivity, DU) during bladder emptying and/or reduced sensory drive during filling and emptying. Symptoms may include pelvic pressure, urgency, urinary frequency, and/or slow stream, and the management options are often associated with poor quality of life and failure to resolve the symptoms. 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.

RATIONALE: We will use quantitative sensory testing (QST) via catheter electrodes to quantify sensory drive. QST is a diagnostic measure for the functional integrity of peripheral nerves. Current perception threshold (CPT) testing delivers electrical stimulation to activate nerve fibers that evoke sensory perception. CPT tests demonstrated electrophysiological changes in bladder sensory pathways of patients with diabetic DU, as well as overactive bladder. We will leverage the spatial selectivity of electrical stimulation to detect sensory dysfunction in the bladder and urethra. The outcome of these studies will determine the clinical relevance of sensory thresholds, as well as direct the location of our investigational intervention. We will deliver continuous electrical stimulation via catheter electrodes to target selectively reduced sensory feedback from the bladder or urethra. Stimulation location will be determined by the largest percentage change in CPT compared to normative data. Continuous intravesical (IVES) and intraurethral electrical stimulation activate small myelinated primary afferents to initiate reflexive detrusor contractions in rats and cats. IVES also increased voided percentage and improved sensation of bladder filling in persons with DU, while intraurethral stimulation evoked bladder contractions in persons with spinal cord injury. Our investigational stimulation sessions will last up to 1 hour and will be followed by a urinary symptom questionnaire and urodynamic study to quantify voiding function. We expect to observe an acute improvement in bothersome symptoms, as well as increased bladder emptying compared to prior clinic urodynamic study.

CONCLUSIONS: These studies will determine novel pathological CPT reference values to guide tissue specific intervention. This will improve patient selection and establish an individualized approach to neuromodulation in women with UAB.

MOLECULAR DIAGNOSTIC ANALYSIS REVEALS HIGH DIAGNOSTIC RATE IN 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. To define the contribution of point mutations (SNVs) and structural variants (SVs) to the diagnosis of COU, we conducted an exome sequencing (ES) study on 822 COU cases, encompassing three main classes of congenital urinary obstructions: a) Ureteropelvic Junction Obstruction (UPJO; N=338), b) Ureterovesical Junction Obstruction/ megaloureter (UVJO; N=217), and c) COU not otherwise specified (COU-NOS; N=267).

METHODS: We performed molecular diagnostic annotation using the infrastructure from the Analysis Tool for Annotated Variants (ATAV), VarSome, and Diagnosticator with additional manual

curation according to the most recent ACMG recommendations. SNVs were annotated against 383 manually curated genes with associations to COU. We simultaneously ran a genome-wide SV analysis on 461 of the 822 exome cases with existing genotyping data from Illumina DNA arrays.

RESULTS: We detected pathogenic and potentially pathogenic SNVs in 58 cases (7.06% of 822) encompassing 21 UPJO (6.2%), 20 COU-NOS (7.5%), and 17 UVJO (7.8%). We detected large, rare pathogenic and likely pathogenic SV in 19/461 cases (4.1%, 12 losses and 9 gains) distributed uniformly across COU categories. SV analysis using ES data on the entire cohort is ongoing.

CONCLUSIONS: This study shows a high diagnostic yield for COU, with important ramifications for diagnosis, counselling and risk stratification. Incomplete clinical information at birth could lead to overestimation of the diagnostic yield due to inaccurate genotype-phenotype correlations.

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PLEIOTROPY OF CONGENITAL ANOMALIES OF KIDNEY AND URINARY TRACT IN HUMAN 16P11.2 MICRODELETION SYNDROME IS RECAPITULATED IN MOUSE MODELS OF *TBX6* DELETION

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INTRODUCTION AND OBJECTIVE: We previously showed 16p11.2 microdeletion to be a major cause of congenital anomalies of the kidney and urinary tract (CAKUT), and identified *TBX6* as the most likely driver. Whether *TBX6* dose reduction causes the full spectrum of CAKUT seen in 16p11.2 microdeletion has yet to be demonstrated, and consequent mechanisms remain elusive. Here we show the full phenotypic spectrum of CAKUT in *Tbx6* mouse models and identify, *in silico*, downstream signaling pathways and targets that impact development of the genitourinary (GU) tract.

METHODS: We studied a *Tbx6* allelic series using two independent alleles: a null allele and a hypomorphic allele. We conducted detailed phenotypic analysis of these models across developmental stages and performed gene expression, gene-set enrichment analysis (GSEA), and transcription factor binding site (TFBS) prediction from E9.5 tailbud mesenchyme. TFBS predictions were subsequently prioritized by intersection with ATAC-seq of E9.5 tail.

RESULTS: Phenotype analysis recapitulated the full spectrum of CAKUT observed in 16p11.2 patients, with profound upper and lower urinary tract defects (renal hypodysplasia, hydronephrosis, ureter duplication, cloacal septation defects, failed ureter and reproductive duct insertions, urethral and genital tubercle malformations). This indicates an early role of *Tbx6* in GU development. Mechanisms linking *Tbx6* to pleiotropic CAKUT included failed nephric duct insertion and presence of ectopic neural tubes impeding the interaction between the ureteric bud and metanephric mesenchyme. GSEA of differentially expressed genes (DEGs) suggested a critical role of *Tbx6* in somitogenesis, Notch signaling, and Bmp signaling, which in turn play a role in GU development. Validating IHC assays for reporters of Notch and Bmp activity are currently ongoing. TFBS prediction coupled with ATAC-seq-recovered known and novel targets in the *Tbx6* interactome including *Mesp2*, *Ripply2, Fgfr2*, and *Tbx18*, which have been previously identified as monogenic causes of CAKUT when mutated. Results will be validated by qPCR and ChIP-qPCR.

CONCLUSIONS: This study strongly supports a role of *TBX6* in pleiotropic CAKUT, uncovers causal mechanisms, and identifies genes and pathways regulated by *Tbx6*. Involvement of Notch signaling is of particular relevance as mutations of *NOTCH2* and related genes cause Alagille syndrome and spondylocostal dysostosis, characterized by CAKUT and spine defects similar to those observed both in our *Tbx6* mouse models and in patients with 16p11.2 microdeletion. These data implicate loss of *TBX6*-mediated regulation of Notch signaling as critical to the development of CAKUT and spine defects.

SPATIOTEMPORAL PROTEOMIC ANALYSIS OF CLINICAL AND EXPERIMENTAL BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION AND OBJECTIVE: 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 can develop significantly decrease quality of life and place a burden on the healthcare industry, costing over $4 billion in treatment annually. Moreover, it has been estimated that worldwide, BPH may be more lethal than prostate cancer due in part to acute urinary retention. Many BPH associated symptoms are pharmacologically targeted using 5α-reductase inhibitors (5ARIs), but these compounds alter the steroid hormone environment and may work against endogenous mechanisms protecting against further symptomatic development. The need for multiple antibodies for immunohistochemical (IHC) methods can be overcome through utilization of mass spectrometry imaging (MSI). Here, we aim to investigate a hormone-induced mouse model of BPH using MSI to further understand the local steroid hormone environment and identify mechanisms protecting or driving disease progression.

METHODS: C57BL/6 mice (8 weeks of age) were surgically implanted with compressed hormone pellets containing 25 mg testosterone (T) and 2.5 mg 17β-estradiol (E_2) for 4 months. After euthanasia, the urogenital tract was dissected to excise the urethra, prostate lobes and bladder, which were processed for FFPE. After dehydration and embedding in paraffin, transverse sections (12 µM) were cut and mounted onto ITO-coated slides. Slides were heated and re-hydrated to remove the formalin and paraffin before antigen retrieval in 20 mM citric acid buffer. On-tissue digestion and matrix application were performed using a robotic TM sprayer system, then slides were imaged using a Bruker RapifleX MALDI Tissuetyper TOF mass spectrometer.

RESULTS: Though still in progress, we anticipate proteomic differences between control normal tissues and those with BPH to include estrogen regulated pathway proteins and their spatial

distributions, as well as their concentrations. Finally, we aim to distinguish unique protein markers that are present within BPH tissues only that may serve as potential markers for disease progression in the future.

CONCLUSIONS: Through this work, localization of key proteomic players in BPH development and progression will be identified that allow further insights into disease mechanisms. The roles that steroid hormone receptors play in disease formation or suppression will be uncovered and may serve as future pharmacologic targets for improved therapeutic intervention. This research also provides a standardized technique for future use by other urologic investigators.

IDENTIFICATION OF COLLAGEN PRODUCING CELL LINEAGES IN THE INFLAMED MOUSE PROSTATE

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INTRODUCTION AND OBJECTIVE: Prostatic collagen density is a strong predictor of lower urinary tract symptoms (LUTS). Our goal is to identify cell lineages responsible for prostatic collagen synthesis, to facilitate the future goal of pharmacological targeting of collagen production in the prostate.

METHODS: We performed cell fate mapping (lineage analysis) to identify collagen producing cells in the inflamed prostate. A conditional RFP allele was combined with *Myh11-cre*, *Lyz2-cre*, *Cd2-cre*, *Gli1-cre*, *S100A4-cre*, and *Srd5a2-cre* to indelibly mark smooth muscle, myeloid, lymphoid, and prostate fibroblast cells. Mice were infected with uropathogenic *E. coli* UTI89 to drive prostate inflammation and collagen synthesis and pro-collagen and RFP immunostaining was used to identify collagen producing lineage labeled cells.

RESULTS: *E. coli* induced inflammation significantly increases prostatic densities of *Cd2*+ (p=0.0131), *Gli1*+ (p=0.0407), and *S100a4*+ (p=0.0788) 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 strain. Lyz2 RFP+ lineage cells make up approximately 90% of Procol+ cells, S100A4+ cells make up approximately 98%, and *Gli1*+ cells and Cd2+ cells make up approximately 20% of Procol+ cells in the *E. coli* inflamed mouse prostate. We note that the percent contribution of each lineage to total collagen producing cells exceeds 100%.

CONCLUSIONS: We identified *Cd2*+, *Gli1*+, and *S100a4*+ cell lineages as likely contributors to *E. coli* mediated prostatic collagen accumulation in mice and the large relative contributions of each lineage suggests overlap in the cell types identified by cre. We plan to use multiplex RNA detection methods to further characterize collagen producing cells. Supported by NIH U54DK104310, F30DK122686, and the UW-Madison Sophomore Research Award.

PERSISTENT MAST CELL ACTIVITY IN BLADDER PAIN SYNDROME FOLLOWING RECURRENT URINARY TRACT INFECTION

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INTRODUCTION AND OBJECTIVE: Bladder Pain Syndrome (BPS) is a broad-spectrum pelvic pain disorder characterized by pain and at least one underlying lower urinary tract symptom, that affects millions in the US. Despite its prevalence, the underlying etiology is unknown. Current animal models of BPS are insufficient in presenting likely disease progression in patients, as many involve instillation of agents not normally present in the human bladder. Clinically, mast cell (MC) mediators such as histamine are found in the urine of BPS patients, and interestingly, BPS patients often report a history of urinary tract infections (UTIs), a pathology well-described to be associated with MC activity. Based on this, we sought to determine the underlying mechanism linking these clinical observations utilizing our recently developed murine model of BPS.

METHODS: We utilized our previously developed bladder UTI murine model consisting of 3 consecutive E.coli infections separated by 7 days each. In this model, mice display pelvic pain (von Frey) and urodynamic dysfunction (cystometry) 14 days after the final infection. Two MC deficient murine models were used to confirm the role of MCs in our model. In addition, instillation of histamine in the bladder of naïve mice or treatment of infected mice with a histamine receptor antagonist were performed to confirm the specific role of histamine.

RESULTS: In mice subjected to multiple bladder infections, we found prolonged MC activation, based on the presence of degranulating MCs in the lamina propria. Notably, these MCs were adjacent to activated nociceptive nerve fibers, suggesting possible crosstalk. Infected mice lacking MCs were protected from pelvic pain and urodynamic dysfunction. When histamine was instilled in the bladder of naïve mice, both pain and dysfunction developed in a transient receptor potential cation channel subfamily V member 1- (TRPV1) dependent manner. Lastly, when pyrilamine maleate, a histamine receptor antagonist, was administered to infected mice, pelvic pain was reduced.

CONCLUSIONS: Our murine model of BPS recapitulates phenotypes observed in human patients, including prolonged MC activity. In addition, pathology was dependent on nerve mediated signaling of MC derived histamine in the bladder. As our model was based on bladder UTIs, a common bacterial infection in humans, these findings demonstrate a possible etiology of BPS, and define the underlying basis for the observed BPS symptoms. Furthermore, these findings present a potential lasting impact of UTIs on bladder sensation and relevant cell types that may be involved.

CONDITIONAL AND DIFFERENTIAL ABLATION OF *CRKL* SPLICE VARIANTS RECAPITULATES HUMAN CAKUT PHENOTYPES IN MOUSE MODELS OF THE 22Q11.2 MICRODELETION (DIGEORGE) SYNDROME

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INTRODUCTION AND OBJECTIVE: Up to 50% of worldwide cases of pediatric end-stage kidney failure fall within the spectrum of congenital anomalies of the kidney and urinary tract (CAKUT). We previously identified mutations in *CRKL* as key drivers of CAKUT phenotypes in the DiGeorge, or 22q11.2, Syndrome. Motivated by these findings, in the current series of studies we further characterize the expression and developmental necessity of *Crkl* splice variants and isoform balance in the mouse kidney and urinary tract using conditional and global mutants.

METHODS: Implementing the cre-lox system of selective, conditional genetic recombination, 3 *cre* mouse lines (*Six2-cre, Pax2-cre, Hoxb7-cre*) were used to drive the ablation of one (T1) or both (T1+T2) *Crkl* splice variants in the metanephric mesenchyme (MM), both the MM and ureteric bud (UB), or from the UB alone (resp.). F2 progeny were grossly and microscopically phenotyped at 3wks gestation. Mice missing one or both splice variants in the MM were subsequently analyzed for alterations in branched tissue morphogenesis and RNAseq-based differential gene expression at earlier time points, including 13.5dpc and 15.5dpc.

RESULTS: At 3wks gestation, when one or both splice variants were removed from the metanephric mesenchyme (MM), severe renal hypoplasia and glomerular abnormalities were observed. When removed from both the MM and cells derived from the Ureteric Bud (UB), mice exhibited mild renal hypoplasia and anomalies of the ureter and renal pelvis. Lastly, when *cre* was driven by a *Hoxb7* promoter, unilateral and bilateral ureteropelvic dilation and early hydronephrosis were observed only in mutants missing T1. Next, embryonic kidney branched tissue morphogenesis was characterized in mutants where only T1 or both T1 and T2 were missing from MM alone. At 13.5dpc the loss of either T1 only or T1+T2 led to a decrease in tip numbers and branch generations. However, at 15.5dpc, mutants without *Crkl* show *increased* numbers of tips and branch generations, while mutants missing only T1 showed a decrease number of tips and glomeruli. Lastly, bulk RNAseq studies of age- and genotype-matched kidneys reveal more differentially expressed kidney development genes only when both variants are missing, with a smaller repertoire that are differentially expressed when only T1 is missing.

CONCLUSIONS: These data suggest tight regulation and balance of both *Crkl* isoforms during mouse embryonic development with high tissue specificity. Along this line, the diversity of observed phenotypes across all cohorts provides further insights into the inherent pleiotropy of human *CRKL* mutations in the developing kidney and urinary tract.

ELUCIDATING THE ORIGIN AND DEVELOPMENT OF BLADDER RESIDENT MACROPHAGES. COLUMBIA UNIVERSITY OPPORTUNITY POOL PROJECT

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INTRODUCTION AND OBJECTIVE: In the steady state, the myeloid cell compartment is highly heterogenous, 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 phenotypically characterized the murine bladder macrophage populations.

METHODS: To identify the unique bladder macrophage populations, we utilized a multiparameter flow cytometry approach. Bladder macrophages were isolated from the bladders of 1-, 3- and 12-week-old male and female C57BL/6J mice in the steady state.

RESULTS: In C57BL/6J mice at one week of age, we observed 6 macrophage populations (3 F4/80high populations and 3 F4/80+ populations) in male mice and 5 macrophage populations (2 F4/80high populations and 3 F4/80+ populations) in female mice. An increase in the population of the F4/80high macrophages was observed in male mice (4 populations) and female mice (3 populations) at three weeks of age, with no increase in the F4/80+ macrophage populations in either sex. At 12 weeks of age there were 5 populations of F4/80high macrophages in male mice and 4 populations of F4/80high macrophages in female mice. Female mice had 4 populations F4/80+ macrophages at 12 weeks (male mice had 3 populations of F4/80+ macrophages). Analysis of the F4/80high macrophage population in male and female mice had an intermediate expression of MHC class II on the cell surface at 1- and 3-weeks of age, however at 12 weeks of age the F4/80high macrophage population expressed high levels of MHC class II on the cell surface. MHC class II surface expression was low on the F4/80+ macrophage population isolated from the bladders of male and female mice at 1 week of age, however at 3 and 12- weeks of age the F4/80+ macrophages population displayed either a high or low surface expression of MHC class II. Expression of cell surface molecules involved in recruitment, activation and inflammation varied between the macrophage populations, indicating that each of the macrophage populations has a unique function in the bladder.

CONCLUSIONS: The data indicate a dynamic change in the composition of the bladder macrophage populations between sex and age. 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.

DISSECTING THE ROLE OF EPITHELIAL VS. STROMAL ESTROGEN RECEPTOR ALPHA IN LOWER URINARY TRACT DYSFUNCTION

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INTRODUCTION AND OBJECTIVE: Aging men experience lower urinary tract symptoms (LUTS) as a result of pathological changes in the prostate which are attributed to an age-related increase in the ratio of estradiol to testosterone. We previously determined that estrogen receptor-alpha (ERα) plays a key role in translating steroid hormone-imbalance into lower urinary dysfunction (LUTD) in mice, but the specific prostate cell population for this is unknown. We also determined that *in utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a persistent environmental pollutant, exacerbates steroid hormone-induced voiding dysfunction. The objective of this study was to determine the respective roles of epithelial and stromal ERα in the progression of LUTD induced by testosterone and estradiol or their combination with *in utero* TCDD exposure in male mice.

METHODS: ERα$^{lox/lox}$ mice (B6(Cg)-*Esr1*$^{tm4.1Ksk}$) were bred with transgenic mice expressing the Cre enzyme under the control of the Sonic hedgehog (B6.Cg-*Shh* $^{tm1(EGFP/cre)Cjt}$/J) or the Transgelin promoter (B6.Cg-Tg(*Tgln*-cre)1Her/J) to generate mice with ERα deletion in Shh-expressing cells (*Shh*-Cre/ERα$^{lox/lox}$), or Sm22-expressing cells (*Tgln*-Cre/ERα$^{lox/lox}$). The mice were then exposed to TCDD *in utero* or corn oil on E13.5. At six weeks of age mice were either treated with a testosterone (T, 25 mg) and 17β-estradiol (E2, 2.5 mg) or a sham surgery and were euthanized four weeks later. Void Spot Assays (VSAs) were used to assess voiding dysfunction. Bladder masses and volumes were collected.

RESULTS: The loss of epithelial ERα in TCDD/T+E2 treated mice did not significantly affect bladder mass; stromal smooth muscle loss of ERα resulted in a significant decrease in bladder mass. However, epithelial but not stromal ERα deletion reduced the number of void spots in TCDD/T+E2 treated mice. Neither epithelial nor stromal deletion of ERα affected bladder mass or voiding function significantly in corn oil/T+E2-treated mice.

CONCLUSIONS: Stromal ERα may have an increased role in regulating obstruction leading to increased bladder mass but has less of an effect on voiding patterns in TCDD/T+E2-treated mice. In contrast, epithelial ERα may have a major role in developing urinary frequency in this "double-hit" model. We also established that neither epithelial nor stromal deletion of ERα improves urinary function in corn oil/T+E2-treated mice suggesting that the deletion of ERα in multiple cells may be required to prevent compensatory mechanisms. Our future studies will explore pathological changes that drive these phenotypes including proliferation and collagen densities in the bladder, prostate lobes, and the prostatic urethra.

INTERLEUKIN-4 PROMOTES HUMAN PROSTATE FIBROBLAST EXTRACELLULAR MATRIX PROTEIN DEPOSITION

(15) Quentin D'Arcy, Jill A. Macoska

INTRODUCTION AND OBJECTIVE. Pathological deposition of extracellular matrix (ECM) by peri-urethral prostate fibroblasts and myofibroblasts contributes to lower urinary tract fibrosis and consequent urinary voiding dysfunction. Inflammatory mediators, such as chemokines and interleukins, can promote ECM accumulation. IL-4 and IL-13 are abundant in the prostate microenvironment and their cognate receptors are expressed in association with high levels of collagen deposition in periurethral prostate tissue. Moreover, IL-4 andIL-13 signal through a shared axis, suggesting that may coordinately play key roles in the promotion of fibrotic pathology. Based on this data, we hypothesized that the IL-4/IL-13 axis may promote expression of ECM proteins through JAK/STAT activation, thereby contributing to lower urinary tract dysfunction (LUTD).

METHODS: N1 or SFT1 immortalized human prostate fibroblasts were serum-starved for 24 hr then grown in serum-free media with or without 2 hr pre-treatment with antibodies against IL-4Ra or IL-13Ra1, then supplemented with vehicle (PBS or citric acid), IL-4 (40ng/ml), IL-13 (40ng/ml), or TGFB (4ng/ml, as positive control). Cells were then lysed for protein or RNA purification, and subjected to immunoblotting, sircol assays, or qRT-PCR analysis.

RESULTS: Sircol assays demonstrated significantly higher levels of soluble collagens I-V produced by N1 and SFT-1 cells treated with IL-4 ($p<.001$) or IL-13 ($p<.0001$) compared to vehicle-treated cells. qRT-PCR and immunoblot assays showed that IL-4 and IL-13 robustly up-regulated COL1 ($p<.001$) and moderately up-regulated COL3 transcripts and protein ($p<.05$) compared to vehicle-treated cells. Pre-treatment with antibodies against IL-4Ra or IL-13Ra1 ablated the observed IL-4 or IL-13-mediated collagen transcript and protein expression to levels similar to those of vehicle-treated cells ($p<.05$). IL-4 and IL-13 also promoted robust phosphorylation of STAT6, which can induce the expression of the IL-4, IL-13, IL-4Ra and collagen genes.

CONCLUSIONS: IL-4 and IL-13 signal through the JAK/STAT pathway to phosphorylate STAT6, suggesting that the observed IL-4/IL-13 induced expression of high levels of extracellular matrix transcripts and proteins by human prostate fibroblasts likely occurs through JAK/STAT signaling. Several JAK/STAT inhibitors are FDA approved and are in current clinical use to treat human disease. Therefore, further studies that positively link ECM deposition with IL-4/IL-13 mediated JAK/STAT signaling may provide new therapeutic approaches to treat LUTD.

PERFORMANCE OF DIFFERENT URINALYSIS PARAMETERS IN PREDICTING CLINICALLY SIGNIFICANT BACTERIURIA

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INTRODUCTION AND OBJECTIVE: Urinalysis (UA) parameters are often used to determine whether urine cultures should be performed or if treatment is indicated in cases of suspected urinary tract infection. However, there is disagreement over which UA parameters should be used as screening tests for urinary tract infections. Our goal was to compare the performance of different UA parameters in predicting clinically significant bacteriuria.

METHODS: We performed a retrospective review of UA and urine culture data from four sites – Duke University Hospital, Duke Regional Hospital, Duke Raleigh Hospital, and Duke Private Diagnostic Clinics between January 1, 2015 and December 31, 2020. UA and urine cultures were included if they were ordered within 24 hours. Urine culture results were categorized as positive, negative, or mixed. A positive culture with bacterial growth >100,000 colony forming units/mL was considered to be clinically significant bacteriuria. We evaluated UA parameters graphically and with descriptive statistics. Performance characteristics were calculated considering clinically significant bacteriuria as true.

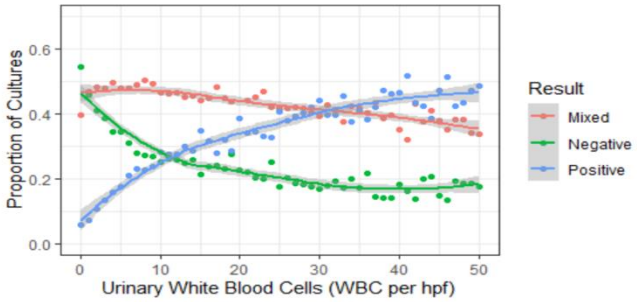
RESULTS: There were 240,195 encounters with UA and urine culture results during the six-year period; 50.7% of these included a microscopic UA. Thirty-eight percent were outpatient encounters; rest were inpatient encounters. Twenty-nine percent were positive, 30.7% were negative and 46.9% were mixed urine cultures. Table 1 shows the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of microscopic UA parameters for clinically significant bacteriuria. WBC count distributions for positive and negative cultures crossed at a value of 10/high powered field (Figure 1).

Table 1: *Diagnostic performance of individual microscopic urinalysis parameters for clinically significant bacteriuria (urine culture with bacterial growth>100,000 colony forming units/mL)*

Leukocyte Esterase	≥Trace	≥1+	≥2+
Sensitivity	0.87	0.78	0.58
Specificity	0.55	0.67	0.83
PPV	0.43	0.48	0.57
NPV	0.91	0.89	0.84
WBC Count/hpf	**>=5**	**>=10**	**>=20**
Sensitivity	0.78	0.61	0.41
Specificity	0.55	0.73	0.86
PPV	0.32	0.38	0.44
NPV	0.90	0.87	0.84
Nitrite	**Positive**		
Sensitivity	0.41		
Specificity	0.95		
PPV	0.75		
NPV	0.80		
Bacteria Count/hpf	**5-50**	**>50**	
Sensitivity	0.92	0.75	
Specificity	0.39	0.75	
PPV	0.39	0.57	
NPV	0.92	0.88	

NPV-Negative Predictive Value, PPV-Positive Predictive Value, hpf-high powered field N may vary by parameter as different UA panels may or may not include all parameters. Also, missing or erroneous results were excluded (e.g., colorimetric interference with nitrite assay for example).

Figure 1: *Relationship between microscopic urinalysis WBC count/hpf and clinically significant bacteriuria, defined as bacterial growth>100,000 colony forming units/mL*



CONCLUSIONS: Trace leukocyte esterase and low-level pyuria had a high NPV and could help physicians rule out clinically significant bacteriuria. No single UA parameter had a high enough PPV to predict clinically significant bacteriuria. Future studies should look at combining UA parameters to improve NPV and PPV, and evaluate the performance of these criteria for clinical diagnosis of urinary tract infection.

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EXOME-WIDE COLLAPSING ANALYSIS REVEALS NOVEL GENES FOR 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 822 COU cases, encompassing three main classes of congenital urinary obstructions: a) Ureteropelvic Junction Obstruction (UPJO; N=338), b) Ureterovesical Junction Obstruction / megaloureter (UVJO; N=217), and c) COU not otherwise specified (COU-NOS; N=267). To investigate the excess burden of rare coding variants on COU, we performed exome-wide collapsing analysis comparing the above 822 cases and 23,958 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 822 COU cases, the top signal was for *ANG*, encoding angiogenin, in the dominant ultrarare model ($P = 3.25 \times 10^{-5}$; OR= 24.40). The signal improved after removal of solved cases, approaching exome-wide significance ($P = 8.28 \times 10^{-6}$; OR= 36.37), and supporting candidacy for this gene. From the subtype analysis, we found the following suggestive signals: *DMTF1* ($P = 1.97 \times 10^{-4}$; OR= infinite) and *BLVRB* ($P = 1.97 \times 10^{-4}$; OR= infinite) for COU-NOS; *KLRD1* ($P = 9.32 \times 10^{-5}$; OR= infinite) and *EXOSC2* ($P = 1.86 \times 10^{-5}$; OR=

infinite) for UPJO; and *MMP15* (P= 6.86 × 10^{-5}; OR= 16.29) for UVJO.

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. Larger cohorts and/or integrated multidisciplinary approaches will be needed to robustly identify and validate novel genes predisposing to COU. (Funding: R01 DK103184, R01 DK115574 and P20 DK116191)

MITOCHONDRIAL COMPLEX 1 REGULATION OF EPITHELIAL BARRIER INTEGRITY VIA NDUFS3 IN PROSTATE EPITHELIAL CELL LINE RWPE-1

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INTRODUCTION AND OBJECTIVE: The NDUFS3 gene encodes a protein subunit that belongs to a part of mitochondrial respiratory chain complex I. Knockdown of NDUFS3 has been reported previously to impair complex I activity in different cell types including the breast cancer cell line, MCF-7, and HeLa cells. NDUFS3 expression is decreased with age in the human prostate and is further decreased in BPH tissues, however the function of NDUFS3 in the prostate is unknown.

METHODS: In vitro cell line assays with benign prostatic epithelial cell line RWPE-1 were utilized to determine the impact of complex 1 inhibition via rotenone and NDUFS3 knockdown on the prostate epithelial barrier. Western blotting and qPCR were used to determine alterations in protein and/or mRNA expression of E-cadherin, occludin and tight junction protein 1 following rotenone stimulation or siRNA knockdown of NDUFS3. FITC diffusion and TEER assays were used to assess epithelial barrier function following knockdown or rotenone stimulation.

RESULTS: Expression levels of epithelial barrier genes was decreased and FITC diffusion was increased following rotenone-induced mitochondrial complex 1 inhibition. Rotenone induced a dramatic decrease in p62 and increased LC3B-11 expression and increased expression of NDUFS3 protein. NDUFS3 knockdown increased p62 and decreased PRDX3.

CONCLUSIONS: Inhibition of mitochondrial complex 1 in prostate epithelial cells dramatically altered autophagy and mitochondrial activity and reduced epithelial barrier integrity. Mitochondrial complex 1 dysfunction could play a role in BPH pathogenesis via reduced epithelial barrier function and subsequent increased prostatic inflammation.

MITOCHONDRIAL DYSFUNCTION CONTRIBUTES TO FIBROSIS IN AGING-ASSOCIATED BENIGN PROSTATIC HYPERPLASIA (BPH)

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is characterized by proliferation, smooth muscle changes, and fibrosis of the prostate. The single greatest risk factor for BPH is age, with 90% of men in their eighties impacted. Many men with BPH will develop lower urinary tract symptoms, which reduce their quality of life as disease severity progresses. Given the multifactorial nature of the disease, treatments have thus far been limited. While aging has been clearly linked to BPH, the molecular mechanisms involved with aging have yet to be fully elucidated. In this study, we specifically examine how mitochondrial dysfunction caused by aging may contribute to fibrosis in BPH.

METHODS: To evaluate how mitochondrial dysfunction may contribute to fibrosis, we used both *in vivo* and *in vitro* models. We examined the complex I protein, NDUFS3 and a mitophagy associated protein, PINK1, via immunohistochemistry in prostate tissue from young (2 months) and old (24 months) C57Bl/6J mice. Additionally, we quantified collagen using picrosirius red as an indicator of prostatic fibrosis. We also assessed loss of complex I function *in vitro* using complex I inhibitor, rotenone, on prostate stromal cells (BHPrS1) and determined collagen gene expression. Complex I rescue experiments using idebenone, a CoQ10 analog, were also tested.

RESULTS: IHC staining of mouse prostate tissue showed decreased levels of NDUSF3, suggesting a decrease in mitochondrial function, specifically associated with complex I of the electron transport chain. Furthermore, PINK1 was also decreased by IHC, suggesting parkin-dependent mitophagy is reduced. qPCR experiments on the rotenone treated BHPrS1 cells revealed increased gene expression for both *Col1a1* and *Col3a1*, suggesting complex I dysfunction can contribute to increased collagen production, and therefore fibrosis. Furthermore, treatment with idebenone was able to rescue this effect.

CONCLUSIONS: Collectively, these *in vivo* and *in vitro* data suggest that mitochondrial dysfunction originating from complex I contributes to the production of collagen, hence the promotion of fibrosis and BPH in men. These data provide new molecular mechanisms and therefore therapeutic targets for the treatment of BPH/LUTS. *U54DK104310 (WAR) and K01AG059899 (TL)*

INTERLEUKIN-4 REPRESSES EXTRINSIC PATHWAY-MEDIATED APOPTOSIS IN HUMAN PROSTATE FIBROBLASTS

(20) **Marissa Sarna-McCarthy**, Jill A. Macoska

INTRODUCTION AND OBJECTIVE: Myofibroblasts, major cellular agents of fibrosis, are resistant to apoptosis, and instead persist, accumulate, and promote pathological deposition of the extracellular matrix (ECM). IL-4, which is abundant in the aging prostate microenvironment, represses Fas-ligand (FasL)-mediated extrinsic apoptotic pathways in Th2 macrophages. IL-4 and IL-13 signal transduction occurs through a shared axis, suggesting that both interleukins may play key roles in myofibroblast resistance to apoptosis and continued persistence. Based on these studies, we hypothesized that the IL-4/IL-13 axis may repress myofibroblast apoptosis in fibrotic tissues, thereby contributing to lower urinary tract dysfunction (LUTD).

METHODS: Primary human prostate fibroblasts were serum-starved for 24 hr then grown in serum-free media with or without 2 hr pre-treatment with pro-apoptotic TRIO cocktail (TNFalpha, Trail, and Fas ligand @50ng/ml each) followed by growth for an additional 2hr or 48hr with or without added IL-4 (40ng/ml) or IL-13 (40ng/ml). Cells were then photographed and/or lysed and subjected to immunoblotting for pro- and cleaved (activated)

caspase 3 or caspase 8, GAPDH or tubulin (loading controls), or FasL receptor.

RESULTS: Primary prostate cells expressed high levels of FasL receptor. When treated with vehicle or IL-4, cells exhibited no caspase cleavage and low levels of cell death. When pre-treated with TRIO followed by supplementation with vehicle, cells exhibited high levels of cell death and caspase cleavage/activation ($p<.0001$) compared to non-TRIO treated. Cells pre-treated with TRIO followed by supplementation with IL-4 demonstrated significantly less caspase 3 ($p<.001$) and caspase 8 ($p<.01$) cleavage/activation and reduced levels of cell death.

CONCLUSIONS: Low concentrations of IL-4 protected primary prostate fibroblasts from undergoing complete programmed cell death in response to activation of extrinsic apoptotic pathways and caspase 8 activation. Prostate fibroblasts abundantly expressed FasL receptor, and further investigation should reveal whether other extrinsic pathway death receptors are similarly expressed. IL-4 also repressed activation of caspase 3, which can be activated by caspase 8 and help induce intrinsic pathway-mediated (mitochondrial) apoptosis. Future studies will elucidate potentially targetable signaling mechanisms coupled to IL-4/IL-13-mediated repression of apoptotic pathways contributing to myofibroblast persistence, pathological ECM deposition, and fibrosis contributing to LUTD.

EXOSOME-INDUCED GENITOURINARY REGENERATION IN A RABBIT MODEL OF MESH EXPOSURE: A PROPOSAL

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INTRODUCTION AND OBJECTIVE: Surgical implantation of polypropylene mesh is a widely accepted and durable treatment for women experiencing stress urinary incontinence and pelvic organ prolapse; however, these surgeries carry a 1-11% risk of mesh exposure in the vagina, bladder, or urethra. Treatment often requires surgical mesh excision. In pilot studies using a porcine model, I have shown that exosome injections (purified exosome product; PEP) incite tissue regeneration and resolve vaginal mesh exposures. The current study aims to: (1) replicate the pilot studies in a rabbit model of vaginal mesh exposure, and evaluate (2) rates of bladder mesh exposure resolution following PEP injection versus sham and (3) the efficacy of PEP as a preventative therapy for mesh exposures.

METHODS: 45 New Zealand white rabbits will be randomly divided into 3 groups: vaginal mesh exposure, bladder mesh exposure, and prevention groups. The vaginal mesh exposure model will be created *via* a previously described transabdominal mesh sacrohysteropexy technique. At 2 weeks, exposures will be injected with 2.5 mL of 20% PEP (reconstituted in 1mL sterile water and 4mL type I bovine collagen) or sham (2mL collagen). For the bladder mesh exposure group, a mesh midurethral sling will be applied with an intentional mesh exposure. At 2 weeks, PEP or collagen will be injected. For the prevention group, the protocol outlined by Moalli and colleagues will be used to create a high-risk-for-exposure (buckled mesh) sacro-hysteropexy model. The mesh-vagina complex will be injected with PEP or collagen at baseline and a vaginal exam under anesthesia performed at 6 and 12 weeks to assess for presence of mesh exposure. Following intervention, all animals will be dosed with 2'-Deoxy-5-ethynyluridine (EdU) to track cellular proliferation. Tissues will be evaluated 6 weeks postoperatively (12 weeks for AIM3). Mesh exposure resolution will be assessed as yes/no for complete exposure resolution or surface area resolution for incomplete resolution. Hematoxylin and eosin and Masson's trichrome stains will be performed. IHC will be used to quantify epithelial thickness, capillary density, macrophage ratio, and cellular proliferation/regenerating proportions (EdU). Each tissue complex will be sampled in triplicate and each slide will be randomly imaged in 4 locations. Evaluation and analysis will be performed blinded.

CONCLUSIONS: This novel study will build upon the prior evidence (n=6, porcine) utilizing an exosome regenerative platform for vaginal mesh exposures. The proposed study will utilize a larger sample size and evaluate the breadth of application to inform clinical translation.

IDENTIFYING GENETIC SUSCEPTIBILITY FACTORS FOR POSTERIOR URETHRAL VALVES (PUV)

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INTRODUCTION AND OBJECTIVE: Posterior urethral valves (PUV) indicate the presence of congenital obstruction at the level of the prostatic urethra (hence present only in males), and it is the most common congenital cause of lower urinary tract obstruction in males. Although affecting only 1 in 4,000 to 7,500 infants, PUV is responsible for up to 17% of pediatric renal failure despite early surgical intervention. The role of genetic factors underlying PUV is less clear; the increased prevalence in patients with Down syndrome suggests that gene dosage might be a critical factor in the etiology of PUV, while the familial occurrence in rare cases suggests that single-gene defects can underlie PUV. Nevertheless, no mutations associated to isolated PUV have been identified so far, providing a unique opportunity for gene discovery.

METHODS: In current work, we dissected the genetic basis of PUV by performing whole genome sequencing (WGS) of 73 PUV trios. Though analysis of WGS still requires substantial efforts we present here results from exome level analysis of these trios. First, we analyzed index cases from PUV trios using IGM ATAV workflow. Briefly, the WGS was aligned hg19 assembly and variants were called using Dragen pipeline within ATAV workflow. We selected high quality rare variants (MAF < 0.01 in gnomad, 1000 genome etc.) to perform downstream collapsing analysis. To reduce the bias of the population stratification we clustered the samples using louvain clustering and each cluster was analyzed separately followed by meta-analysis using Cochran–Mantel–Haenszel *test.* Further, we tested enrichment of de-novo mutation in PUV trios. We extracted high quality rare (0.0001% in 1000 genome) de-novo variants using ATAV workflow and compared with publically available de-novo variants from 31,058 parent-offspring trios.

RESULTS: Using collapsing based method though we did not observe any study-wise significant association ($P<1x10^{-7}$), but several suggestive signals were detected, including: PLCD1 ($P=2.01x10^{-6}$; OR=162); DNAH11 ($P=2.89x10^{-5}$; OR=11); TLK2

(P=5.99x10^{-5}; OR=108). Further, by using denovoanalyzer, we observed a ~2-fold increase in burden of de novo LOF (P=4.1x10^{-3}), while no excess was found for either synonymous or missense DNMs (P>0.1). Using simulation-based statistical test (deNovoWest), while we did not observe excess of synonymous DNMs for any gene as a proof of concept, we did detected suggestive excess burden (P<1x10^{-5}) for damaging DNMs on the following genes: FOXP2, MSLN, COL23A1, DRD4, SKA3, KRT77.

CONCLUSIONS: Altogether, our finding suggest several candidate genes for PUV that can potentially explain genetic basis of PUV.

INCREASED COX-1 EXPRESSION IN BENIGN PROSTATE EPITHELIAL CELLS IS TRIGGERED BY MITOCHONDRIAL DYSFUNCTION

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is an age-related disease associated with chronic prostatic inflammation. Aging is associated with decreases in mitochondrial functional capacity. Increasing metabolic stress may contribute to the release of pro inflammatory mediators potentially contributing to BPH progression or mediation. Cyclooxygenase-1 (COX-1) and cyclooxygenase- 2 (COX-2) are enzymes responsible for converting arachidonic acid to proinflammatory mediators (i.e. prostaglandins and thromboxanes) and are well-established drug targets. Prior studies focused on COX-2 selective blockade with lackluster improvement in BPH symptoms. We recently reported that stromal COX-2 immunostaining was increased with age but not altered in BPH tissues, and epithelial COX-2 had no differences in expression with age variation or BPH tissue type. However, it is unknown whether COX-1 is differentially expressed in response to age or tissue type. Here we looked at COX-1 expression in human tissue and potential pathways that could be involved in regulation of COX-1 and COX-2 within prostate cell lines.

METHODS: The expression of COX-1 was analyzed in 22 clinical BPH specimens by multiplex immunohistochemistry and in two murine models of BPH and lower urinary tract dysfunction. Human prostate epithelial cell line RWPE-1 was treated with TGF-β1 and rotenone (complex I inhibitor) to determine the impact of inflammatory cytokines and mitochondrial dysfunction on COX-1 and COX-2. RWPE-1 cells were transfected with small interfering RNA specific to complex 1 gene NDUFS3.

RESULTS: COX-1 expression was increased in the epithelial cells of BPH specimens compared to young healthy organ donor and in mouse models of BPH and lower urinary tract dysfunction. Cell line assays showed that mitochondrial complex 1 inhibition via rotenone or NDUFS3 knockdown induced an up-regulation of COX-1 and COX-2.

CONCLUSIONS: Our findings suggest COX-1 can be induced in benign prostate epithelial cells in response to mitochondrial complex I inhibition and is elevated in BPH epithelium. COX-1 may play more of a role than previously recognized in the development of age-related benign prostatic disease.

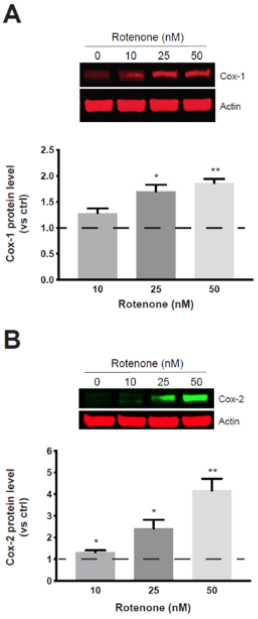

Figure 1. Rotenone dramatically enhances Cox-1 expression and induces Cox-2 in RWPE-1 cells. Elevating Cox-1 and 2 proteins by rotenone is concentration-dependent (*A*, n = 3 and *B*, n = 4). Representative images of increasing Cox-1 and 2 protein blots are shown.

PROSTATIC ANDROGEN RECEPTOR EXPRESSION DECREASES WITH AGE AND MAY BE REGULATED BY DDX3X IN MICE

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INTRODUCTION AND OBJECTIVE: 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. Our previous studies indicated that the RNA helicase, DDX3X, binds to the androgen receptor (AR) mRNA at stress granules and inhibits AR translation, which may lead to a failed response to androgen deprivation therapies. Whereas prostatic AR is primarily localized to the nucleus, DDX3X can be found in in both the cytoplasm and nucleus. Both AR and DDX3X can be found in both stromal and epithelial tissue compartments. We have previously shown that DDX3X regulates AR protein expression in castration resistance however, it is unknown whether DDX3X regulates AR protein translation in normal aging. Here, we evaluated the localization/expression of cytoplasmic DDX3X and nuclear AR in normal aging in the male mouse prostates.

METHODS: We examined the anterior prostate (AP) from 2-month-old (n=9) and 24-month-old (n=7) C57BI/6 mice. AR localization/expression was examined via immunofluorescence and DDX3X was detected via immunohistochemistry. The percentages of AR and DDX3X positivity were determined by Inform software. The mean of optical density (OD) was also determined for DDX3X by Inform software. Through comparison of the cytoplasmic DDX3X and nucleic AR levels among different age groups, we ascertained the relationships between DDX3X/AR and aging.

RESULTS: More prostatic AR was expressed in 2-month-old (young) mice (92.21% positivity), while 24-month-old (old) mice showed reduced AR expression (54.09% positivity). In terms of cytoplasmic DDX3X, similar levels of DDX3X were detected in 2-month-old (46.75% positivity, 0.047 mean OD) and 24-month-old mice (59.74% positivity, 0.055 mean OD). However, in aged but not young mice, DDX3X had an epithelial-cytoplasmic and punctate pattern, consistent with stress granule localization. This punctate pattern is consistent with DDX3X mediated protein translation inhibition, suggesting DDX3X may be involved in decreased prostatic AR protein expression in aged mice.

CONCLUSIONS: Aging is associated with decreased prostatic AR localization/expression and may involve regulation through the DDX3X mediated mechanism.

A NEW MECHANISM LINKING I*N UTERO* ENVIRONMENTAL CHEMICAL EXPOSURE TO PROSTATIC INNERVATION AND URINARY VOIDING DYSFUNCTION

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INTRODUCTION AND OBJECTIVE: Nearly all research into factors causing male lower urinary tract dysfunction (LUTD) has focused on prostatic and bladder changes with advancing age. We recently found that the *in utero* environment exerts a lifelong influence over male voiding function and can sensitize to LUTD later in life. Activation of the aryl hydrocarbon receptor (AHR, a transcription factor that binds numerous environmental contaminants) during LUT development *in utero* permanently increases prostatic noradrenergic axon density and sensitizes prostatic smooth muscle to adrenoreceptor stimulation, leading to excessive prostatic urethral tone throughout life. We hypothesize that fetal AHR activation increases prostatic noradrenergic axon innervation by inducing the expression of a long-noncoding RNA (SlincR) and repressing Sox9. We hypothesize that *SlincR* null mouse fetuses will resist AHR mediated increases in prostatic noradrenergic axon density.

METHODS: We used CRISPR/Cas9 genome editing to generate *SlincR* null mice. Timed-pregnant mice will be dosed with the potent AHR agonist and widespread environmental contaminant, 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD, 25 µg/kg oral maternal dose) or vehicle (5 mL/kg corn oil) on embryonic day E 10.5, before the establishment of differentiated noradrenergic pelvic ganglia neurons. We will collect vehicle and TCDD exposed *SlincR* null and wild type mouse prostates on E 18, after the appearance of tyrosine hydroxylase (TH)+ noradrenergic axons in the developing prostate. We will use *in situ* RNA detection (RNAScope) to visualize and quantify *SlincR lnc*RNA+ cells, RT-PCR to quantify *Sox9* mRNA, and immunostaining to visualize and quantify expression of SOX9+ cells and TH+ noradrenergic axons. We will use Student's T-Test to test for differences between treatment groups and genotypes.
RESULTS: We already found that TCDD increases the number of *SlincR*+ cells in the pelvic ganglia, urethral epithelium, and the testicular and seminal vesicle ducts. We also found that TCDD reduces *Sox9* RNA abundance in the urogenital sinus + pelvic ganglia. We expect to find that *SlincR* null mice are deficient in *SlincR* RNA, resistant to TCDD-mediated decreases in SOX9+ pelvic ganglia cells, and resistant to the TCDD-mediated increase in prostatic density of TH+ axons.

CONCLUSIONS: These findings will be significant because they will identify a potential mechanism linking perturbations in prostatic neuroanatomical development to lifelong hyperactivity of prostatic smooth muscle, increased prostatic urethra tone, and impaired bladder emptying.

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EFFECTS OF EPIDURAL KILOHERTZ FREQUENCY SPINAL CORD STIMULATION ON LOWER URINARY TRACT FUNCTION IN SPINAL CORD TRANSECTED RAT

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INTRODUCTION AND OBJECTIVE: Spinal cord injury (SCI) results in bladder dysfunction, including neurogenic detrusor overactivity (NDO) and detrusor-sphincter dyssynergia (DSD). We recently established the effectiveness of epidural kilohertz frequency spinal cord stimulation (KHF SCS) to modulate bladder function in spinally-intact rats. The purpose of this study was to quantify the effects of KHF SCS on NDO and DSD by quantifying changes in bladder capacity (BC), voiding efficiency (VE), non-voiding contractions (NVC), and external urethral sphincter (EUS) electromyography (EMG) activity following complete spinal transection (SCI_T).

METHODS: Male (n=7) and female (n=10) Sprague-Dawley rats underwent a T10 SCI_T. After four weeks, we performed continuous-fill saline cystometry under urethane anesthesia while measuring bladder pressure, EUS EMG, and voided and residual volumes. A two-contact electrode paddle was placed epidurally over the L6-S1 spinal cord segments, and a nerve cuff electrode was placed on the sensory pudendal nerve. The effects of KHF SCS were measured using three frequencies {1 kHz, 5 kHz, 10 kHz} and three amplitudes {20, 40, 80% of motor threshold (MT)}. Trials were also conducted with no stimulation (intra-block control) and with conventional 10 Hz, 30 Hz, and 50 Hz SCS at 80% of MT; as a control, we stimulated the sensory pudendal nerve at 10 Hz at 80% and 2x EUS reflex threshold (T). Data were analyzed using the Mixed Effects model, where animal and sex were the random components and stimulation parameter as the fixed component; the Tukey HSD test or the Wilcoxon Ranked Sum test were used for post-hoc analysis. A p-value ≤ 0.05 was used to determine statistical significance.

RESULTS: Compared to spinally-intact rats from our prior study (n=14), BC was significantly increased, and VE was significantly

decreased in SCI_T rats (n=17). Phasic bursting of the EUS was not present during voiding in SCIT rats, and there was an increase in tonic EUS activity. KHF SCS at 5 kHz 80% MT significantly decreased the mean BC in SCI_T animals. Several stimulation parameters produced a significant increase in VE including: 1 kHz 40% MT, 1 kHz 80% MT, 10 kHz 80% MT, and 10 Hz 80% MT, as well as sensory pudendal stimulation at 10 Hz 80% T. The number of NVC decreased with SCS at 1 kHz 80% MT. Finally, the duration of tonic EUS EMG activity during filling decreased with SCS at 5 kHz 40% MT, 10 kHz 40% MT, 10 kHz 80% MT, 10 Hz 80% MT, and sensory pudendal stimulation at 10 Hz 2xT.

CONCLUSIONS: Epidural KHF SCS decreased BC, increased VE, decreased the number of NVC, and decreased the duration of tonic EUS EMG activity. These findings suggest that KHF SCS may be a viable approach to restore bladder function after SCI.

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ASSOCIATION OF PRIMARY CARE AND PREVENTIVE VISITS WITH INCONTINENCE CARE AMONG OLDER BREAST CANCER SURVIVORS

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INTRODUCTION AND OBJECTIVE: Breast cancer patients are at particularly high risk for urinary incontinence (UI) due to antiestrogenic genitourinary side effects of cancer treatments, but many patients reduce contact with primary or gynecologic care providers during cancer treatment. We examined whether either the number of visits with primary or gynecologic providers or attendance at a prevention-focused visit (Annual Wellness Visit or AWV) were associated with higher rate of UI care (diagnoses or treatment) among a cohort of older breast cancer survivors.

METHODS: Using administrative (billing) data from a national insurance carrier, we identified Medicare Advantage-enrolled women aged ≥66 diagnosed with and treated for breast cancer. Medicare claims data for 1 year before (for baseline information) and for 2 years after cancer diagnosis date was used to examine care for UI as defined by ICD-9 or ICD-10 diagnostic codes, prescription fills of commonly prescribed UI medications, or UI procedures. Logistic regression models were used to examine the association of UI care with an AWV and number of provider visits in models also adjusted for patient age, race/ethnicity, household income, prior incontinence, Elixhauser comorbidity index score, rural/urban status of zip code, and receipt of a Medicare low-income subsidy.

RESULTS: A total of 5930 patients met the inclusion criteria. Of those, 34.5% underwent 1 or more AWVs after their cancer diagnosis, and 10.9% received UI care. Pre-cancer UI care had the strongest association with post-cancer UI care (Adjusted Odds Ratio 18.6, 95% Confidence Interval 16.2-21.2). Attendance at an AWV was not associated with UI care (AOR 1.13, 95%CI 0.92-1.38). Number of primary care visits (AOR 1.04 (1.02-1.05)) and number of obstetrics and gynecology visits (AOR 1.06 (1.01-1.12)) were associated with higher likelihood of UI care while number of oncology visits was associated with lower likelihood. Residents of rural zip codes (AOR 0.40, (0.18-0.87)) were less likely to be diagnosed/treated. Results for AWV and provider visits were similar in models stratified by pre-cancer UI care.

DISCUSSION: In a Medicare population, a higher number of primary care or gynecology visits was associated with a higher odds of UI care for breast cancer survivors, but attendance at a visit focused on prevention was not. Further research should examine how models of shared and preventive care for cancer survivors might best support incontinence recognition and effective incontinence care.

PROSTATE-SPECIFIC CDH1 HEMIZYGOSITY INDUCES PROSTATE INFLAMMATION AND BLADDER OVERACTIVITY IN AGED MICE

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INTRODUCTION AND OBJECTIVE: Decreased adherens junction protein E-cadherin has been implicated in the development and progression of benign prostatic hyperplasia (BPH). Immunostaining has shown decreased E-cadherin to be associated with inflammation in both BPH and the aged mouse prostate. Homozygous E-cadherin deletion in murine prostate epithelium induced prostate inflammation and bladder overactivity, however, E-cadherin is reduced in BPH, but not completely lost. This study was performed to examine the impact of a reduced E-cadherin expression in combination with advanced age.

METHODS: The PSA-CreERT2 transgenic mouse strain expressing tamoxifen-inducible CreERT2 recombinase driven by a 6-kb human PSA promoter/enhancer was crossed with the B6.129-Cdh1tm2Kem/J mouse to generate bigenic PSA-CreERT2/Cdh1+/- mice. Hemizygous deletion of E-cadherin in the prostate luminal epithelial cells of male mice was induced by transient administration of tamoxifen when mice reached sexual maturity (7 weeks of age) and mice were aged to 24 mos. Immunostaining analyses and cystometry were used to determine the impact of aging and Cdh1 hemizygosity on the prostate and bladder.

RESULTS: Aged Cdh1+/- mice exhibited increased prostatic inflammation, stromal hyperplasia and bladder overactivity compared to age-matched controls.

CONCLUSIONS: Aged mice with prostate-specific hemizygous loss of E-cadherin developed prostatic defects and lower urinary tract symptoms similar to BPH/LUTS. This suggests that decreased E-cadherin expression may synergize with aging may to induce prostatic inflammation and bladder overactivity.

RACIAL DISPARITY IN MEN WITH BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a disease associated with aging, with >210 million cases worldwide; nearly all men will encounter some clinical lower urinary tract symptoms (LUTS) in their lifetime. African American (AA) men have a higher incidence of BPH with increased incidence of non-surgical treatment failure, larger prostates at time of surgery, and surgery occurring at a younger age. This study examines the changes in estrogen receptors alpha and beta (ERα, ERβ) and steroid metabolism genes due to race, age, location, and disease.

METHODS: To evaluate the impact of race on BPH, we examined prostate tissue from 78 men obtained from surgical specimens in the biorepositories at UT Southwestern and the University of Pittsburgh. We had 21 normal transition zone controls, and 57 BPH samples stratified based on self-identified race (AA vs. CA). Using multispectral quantitative multiplex IHC, we examined the steroid hormone-related protein expression of ERα, ERβ, CYP7B1, AKR1C1, COX2, and E-cadherin on one FFPE tissue section. Using InForm® software, we spectrally unmixed each fluorophore and quantified optical density for each protein of interest. In addition to spectral unmixing, we also performed cell and tissue segmentation to examine protein localization. Comparisons between normal prostate and BPH and CA and AA groups were analyzed using a two-tailed student's t-test for continuous variables and two-tailed fisher's exact test for proportions. Statistical analysis was performed using GraphPad Prism.

RESULTS: Examining the racial difference between the proteins of interest, we see significant upregulation of ERα, ERβ, AKR1C1, and CYP7B1 in AA men compared to CA. Looking at changes as a function of age, we see no significant difference. However, when we separated based upon race and disease, we found significant differences in AKR1C1 and CYP7B1. AA men overexpress these two steroid enzyme genes in both BPH and normal above that of CA men. Interestingly, while there is a difference in expression of all the markers between BPH and normal in CA men, this change does not exist or is opposite in AA men between BPH and normal. Additionally, we stratified based on biorepository location to see if environmental factors accounted for these differences; there was a significant upregulation of ERα and ERβ in the Texas cohort, but the alterations based on race remained the same.

CONCLUSIONS: Our study shows a racial difference in steroid metabolism enzymes affecting the expression of ERα and ERβ between normal and BPH. While more in-depth analysis remains, our study suggests that underlying racial differences in protein expression may complicate targeting the estrogen pathway in treating BPH.

POLYCHLORINATED BIPHENYLS (PCBS) SHOW LIMITED EFFECTS ON VOIDING PHYSIOLOGY IN ADULT-EXPOSED FEMALE MICE

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INTRODUCTION AND OBJECTIVE: The continued presence of polychlorinated biphenyls (PCBs) in the environment makes understanding their effects on human health of great importance. While PCBs are known to target the brain, less is known about their effects on other organs. Recent evidence suggests that exposure to PCBs has a significant effect on bladder volume, nerve fiber density, and immune cell abundance in developmentally exposed mouse offspring. However, PCB effects on the adult female dam are unknown. Our goal was to test the hypothesis that PCB exposure leads to changes in bladder function in female mice exposed in adulthood.

METHODS: C57Bl/6J wild type female mice were dosed daily with peanut butter containing either 0, 0.1, 1, or 6 mg/kg MARBLES PCB mixture. Dosing initiated two weeks prior to mating and continued throughout gestation and lactation. Once offspring were weaned, dams underwent void spot assays to measure the total number of voids, the total area of voids, percentage of voids in the center of the VSA paper, and urine spot size distribution. Following VSA, mice underwent uroflowmetry to measure the flow rate of voids, and then anesthetized cystometry to measure voiding interval and bladder pressure. Bladder volume, bladder mass and body mass were measured in a subset of mice not undergoing cystometry.

RESULTS: PCBs did not affect the ability of adult female mice to get pregnant, litter size, or offspring body mass. When assessing the anatomical characteristics of the bladders of adult female dams exposed to PCBs, there were no significant differences in bladder volume or mass. When assessing voiding function in the bladders of adults exposed to PCBs, differences were only observed in the VSA where the number of void spots measuring 2-3 cm were significantly greater in the 1 mg/kg group than the control group. There was no significant difference found in the total area of voids, number of voids or percentage of voids in the center between the dosage groups.

CONCLUSIONS: Overall, PCB exposure at the doses used to elicit physiological changes in offspring, had limited effects on bladder metrics and voiding physiology in female mice exposed as adults. Changes were limited to the 1 mg/kg dose group, indicating that other dosages may either be insufficient to produce an effect or may show competing effects within the mammalian urinary tract. Considering that female mice typically void in large (4+ centimeter diameter) spots, PCBs appear to change this dynamic by increasing the number of 2-3 centimeter spots without changing overall urine area. This suggests that adult exposure to PCBs in female mice may lead to smaller volume voids.

METABOLOME- AND GENOME-WIDE ASSOCIATION TO DISCOVER GENETIC DRIVERS OF CALCIUM OXALATE KIDNEY STONE DISEASE IN A DOG MODEL

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INTRODUCTION AND OBJECTIVE: The metabolic complexity of calcium oxalate (CaOx) kidney stone disease is a barrier to identifying heritable risk factors, as imprecise phenotyping loses genetic associations to statistical noise. Greater than 95% of patients have at least one underlying metabolic abnormality including hypercalciuria, hypocitraturia, hyperoxaluria, low urine

pH, or low urine volume, each with their own genetic drivers. Spontaneous dog models of CaOx stone formation have unique genetic structures, namely reduced genetic diversity within breeds, that improves statistical power to detect genetic associations. The objective of this study was to leverage a Miniature Schnauzer dog model to identify genetic drivers of metabolism that influence CaOx stone development.

METHODS: Plasma metabolomics profiles (836 metabolites from 98 pathways) were generated from 23 CaOx case and 20 control Miniature Schnauzer dogs. Differential concentration analysis was performed using MetaboAnalyst software v5.0, which applies multiple univariate, multivariate, and machine learning analysis methods. Significance was defined as FDR<0.05. A linear model genome wide association study (GWAS) was performed using PLINK v1.9 for metabolites consistently identified as important features in MetaboAnalyst analyses. A Bonferroni correction defined genome-wide significance (P<2.8E-09).

RESULTS: Though no metabolite concentration met the FDR criteria for significance, 9 were chosen for GWAS based on t-test results, partial least square discriminant analysis (PLS-DA) and random forest classification, as those most successful at stratifying cases from controls. GWAS identified genetic loci for each metabolite, but none met genome-wide significance (Table 1).

Table 1. *GWAS results for top metabolites associated with CaOx stones*

Metabolite	Pathway	Link to CaOx Stones	T-test P	FDR	GWAS Region (Chr:Position)	GWAS P	Gene
7-methylguanine	Purine Metabolism, Guanine containing	Downregulated in rodent models of CaOx	2.9E-03	0.24	35:11088620-11741863	2.2E-06	*TMEM170B, ATRP, HIVEP1, EDN1*
10-undecenoate (11:1n1)	Medium Chain Fatty Acid	Unknown	1.3E-03	0.24	17:22944696-23641286, 22:50766021-50767582	5.95E-06, 6.09E-06	*CLIP4, ALK*; intergenic
dihomo-linoleoylcarnitine (C20:2)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Unknown	1.8E-03	0.24	27:35412959-35471771	1.6E-06	intergenic
N-delta-acetylornithine	Urea cycle; Arginine and Proline Metabolism	Unknown	3.2E-03	0.24	16:27036665-27037871	7.8E-06	*FGFR1*
hydroxyproline	Urea cycle; Arginine and Proline Metabolism	Major source of oxalate production	2.6E-03	0.24	7:18021002	2.6E-06	*FAM129A*
(16 or 17)-methylstearate (a19:0 or i19:0)	Fatty Acid, Branched	Unknown	1.8E-02	0.36	3:11675370	3.1E-05	intergenic
12,13-DiHOME	Fatty Acid, Dihydroxy	Unknown	2.6E-03	0.24	29:37583170-38139511	2.8E-05	*TRIQK*
pentadecanoate (15:0)	Long Chain Saturated Fatty Acid	Unknown	1.6E-04	0.13	24:10531327-10549765	1.7E-05	intergenic
malate	TCA Cycle	Increases citraturia	4.1E-03	0.24	13:23498223-25130364	4.0E-05	*LRATD2*

CONCLUSIONS: Top features from metabolomics analysis included metabolites previously implicated in CaOx stone risk, such as hydroxyproline (induces hyperoxaluria) and malate (increases citraturia). Five of 9 top metabolites are fatty acids, suggesting that fatty acid metabolism might be deranged in CaOx stone disease. Notable gene associations include FGFR1, which has a distinct role in phosphate and calcium transport in the kidney and EDN1, a CaOx candidate gene. Given the small sample size and failure to meet thresholds for multiple comparisons, validation cohorts are needed to distinguish true from spurious associations.

DEVELOPING A URINARY INCONTINENCE CARE PATHWAY: ENVIRONMENTAL SCAN OF PRIMARY CARE CLINICS

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INTRODUCTION AND OBJECTIVE: While nearly 50% of adult women report at least one episode of urinary incontinence (UI), most never receive treatment. Furthermore, screening for UI is a Medicare quality measure; however, no guidelines exist within primary care professional societies for its diagnosis and management. Recommendations from urology and urogynecology professional societies for UI include urinalysis and post void residual tests for the initial diagnostic workup and behavioral modifications, pelvic floor physical therapy, medications, and surgical procedures in the treatment plans. We are designing and then testing a guideline-based UI care pathway that integrates primary and specialty care. To understand the context for this intervention, we conducted an environmental scan with key informant interviews to assess the availability of technology and other key pathway resources in the primary care setting.

METHODS: Clinic managers from all primary care centers within the Medical College of Wisconsin network were invited to participate. Environmental scan interviews were conducted in 2021 by two trained medical students using a checklist developed by the multidisciplinary team. The checklist covered clinic structure, availability of diagnostic tools (screening questionnaires, bladder ultrasound, urinalysis), and clinic resources. If the clinic manager did not know the answer to a question, a lead nurse, medical assistant, or other clinic staff was consulted.

RESULTS: Of 24 total primary care clinics, interviews were completed with 21 managers - 13 in-person and eight virtual. The sites included three internal medicine, five family medicine, and 11 combined clinics in urban, suburban, and rural settings. Clinic size varied between two-26 providers. UI screening intake forms were utilized by 14/21 (67%) clinics – 12 via paper and two electronic. While many clinics had point of care urinalysis capabilities (17/21, 81%), most did not have a working bladder ultrasound (14/21, 67%) or on-site pelvic floor physical therapy (18/21, 86%).

CONCLUSIONS: An environmental scan of primary care clinics revealed several barriers to providing guideline-recommended UI screening, diagnosis, and treatment. Streamlining our incontinence care pathway resources may better assist primary care providers in the diagnosis and first-line treatment of UI and, ultimately, improve the integration of primary and specialty care.

INHIBITION OF MITOCHONDRIAL COMPLEX I INFLUENCES THE EXPRESSION OF INFLAMMATORY MEDIATORS IN A HUMAN PROSTATIC STROMAL CELL LINE

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are extremely common in aging men. BPH is characterized by fibrosis and inflammation, however their role in BPH etiology is not well understood. Mitochondrial dysfunction is frequently associated with age-related diseases and stromal inflammation. We sought here to determine if disruption of mitochondrial complex 1 in prostate stromal cells could influence the expression of genes associated with fibrosis and inflammation. Our further objective was to see if this process could be reversed by administration of Nicotinamide mononucleotide (NMN) , which restores NAD+ balance.

METHODS: The prostate stromal cell line BHPrS1 was treated with rotenone to induce mitochondrial damage and then treated with NMN, to reverse the mitochondrial damage by increasing NAD+ biosynthesis. qRTPCR was utilized to determine the mRNA expression levels of markers associated with inflammation (COX-1, IL-6, IL-8) and fibrosis (collagen 1A1) in cells treated with rotenone and/or NMN. Immunofluorescence staining for smooth muscle marker calponin was performed to visualize the influence of rotenone on stromal cell differentiation.

RESULTS: COX-1 and IL-6 mRNA levels were increased, while IL-8 was decreased in response to rotenone treatment in BHPrS1 cells. While collagen 1A1 levels were altered, the results were not consistent. Calponin expression was decreased.

CONCLUSIONS: Disruption of mitochondrial complex 1 induced an increase in inflammatory mediators COX-1 and IL-6 in BHPrS1 cells, suggesting that mitochondrial damage could induce an inflammatory response in prostate stromal cells. Furthermore, smooth muscle marker calponin was decreased by rotenone, suggesting that mitochondrial damage could contribute to stromal fibrosis in the prostate. The variable influence of rotenone stimulation on collagen levels and on the cytokines is something that must be further explored. It may be that collagen deposition is not part of the acute response to mitochondrial disruption. Taken together, these results suggest that mitochondrial disruption may play a role in BPH pathogenesis and that restoring NAD+ balance could have therapeutic potential for BPH treatment.

STEROID HORMONE IMBALANCE STIMULATES OSTEOPONTIN EXPRESSION AND INFLAMMATION IN THE MOUSE PROSTATE

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INTRODUCTION AND OBJECTIVE: Inflammatory processes in the prostate are linked to the development of lower urinary tract symptoms (LUTS) in men. Steroid hormone imbalance is a major age-associated factor driving LUTS, but its relationship to inflammation is not fully understood. Our previous studies identified that osteopontin (OPN), a pro-inflammatory cytokine, is abundant in the prostate of men with LUTS and its secretion is stimulated by inflammation. This study investigates whether an increase in estradiol: testosterone ratio drives inflammation in the prostate and whether OPN is involved in this process.

METHODS: Male wildtype C57BL/6J (WT) or Spp1tm1Blh/J (OPN-KO) mice were surgically implanted with slow-releasing subcutaneous pellets containing 25 mg testosterone (T) and 2.5 mg estradiol (E2) (T+E2). Mice were euthanized two or six weeks later. The protein expression of OPN, and inflammatory markers, cyclooxygenase-2 (COX2) and nuclear factor kappa B (NF-κB), was detected by immunohistochemistry. Tissues were imaged with a Mantra 2 Quantitative Pathology Workstation (Akoya Biosciences) using a 40x objective. Four representative images were taken per tissue. Optical density (OD) and nuclear and cytoplasmic positivity was calculated using the InForm software.

RESULTS: T+E2 treated mice showed a two-fold elevation in OPN expression in the ventral lobe (VP) of the prostate two weeks after implantation ($P<0.001$). Contrasting this, we found no change in prostatic OPN levels at week 6. Our preliminary results show that there was an increase in both cytoplasmic and nuclear staining of NF-κB in prostate epithelial cells in response to T+E2 treatment. The analysis of NF-κB and COX2 expression continue to be assessed.

CONCLUSIONS: These results indicate that the combination of testosterone and estradiol stimulates osteopontin expression, specifically in the ventral prostate lobe. Our preliminary data also identifies that steroid hormone imbalance leads to increased inflammatory signaling in the prostate. The complete analysis of our dataset will reveal whether the loss of OPN alters prostatic inflammation as identified by a change in cytoplasmic COX2 or nuclear NF-κB expression. Our future studies will identify further pro-inflammatory pathways that regulate immune cell infiltration and whether the increased pro-inflammatory profile drives epithelial or stromal proliferation in the prostate.

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

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INTRODUCTION AND OBJECTIVE: Development is an especially critical window during which environmental contaminants can induce lasting effects on the offspring, yet the role of environmental contaminants in shaping bladder structure and function is not completely understood. Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants with known impacts on the central nervous system and have been implicated as risk factors for autism – a condition which often has comorbid lower urinary tract symptoms (LUTS). Therefore, we test the hypothesis that exposure to PCBs at human relevant concentrations during development, leads to voiding abnormalities once mice reach adulthood.

METHODS: Adult C57Bl/6J female mice were dosed with a mixture of PCBs which mimics the top congeners and proportions found in women at risk of having a child with autism. Mice were dosed daily via ingestion in peanut butter with either 0, 0.1, 1 or 6mg/kg PCB for two weeks prior to mating, through gestation and lactation. Developmentally exposed offspring were weaned at 3 weeks of

age and at 6-8 weeks of age underwent void spot assay (VSA), uroflowmetry, anesthetized cystometry or *ex vivo* bladder bath contractility assays.

RESULTS: PCB effects on voiding were sex- and dose-dependent. VSA showed a significant increase in number of the smallest diameter urine spots (0-0.1 cm) produced by male mice of the 0.1 and 6 mg/kg PCB dose group vs. vehicle control, while this increase was seen in female mice of all PCB dose groups. Uroflowmetry revealed a significant decrease in urine stream rating only in male mice at the 0.1 mg/kg PCB dose group compared to control. Cystometry revealed decreased intervoid interval in female mice of the 0.1 and 6 mg/kg PCB group vs. vehicle control. While in male mice the maximum pressure was higher in the 0.1 and 1mg/kg PCB groups compared to control. PCBs also altered bladder sensitivity to cholinergic and purinergic stimulation. In male bladder, PCBs at all doses increased sensitivity to carbachol. However, in female bladder, PCBs increased sensitivity to carbachol only at the 0.1mg/kg PCB dose. In response to purinergic agonist, ATP, PCBs at the 1mg/kg dose increase contractility relative to vehicle control in both male and female bladder.

CONCLUSIONS: Together these results indicate sex- and dose-dependent effects of PCBs on voiding parameters which ultimately produce more frequent voids or small drop voids – parameters which recapitulate lower urinary tract symptoms in humans who suffer from urgency, frequency or difficulty emptying the bladder. Effects on bladder contractility may be due to differences in cholinergic and purinergic pathway stimulation, which is an area of future study.

COLLAGEN CONTENT IS INCREASED IN LICHEN SCLEROSUS

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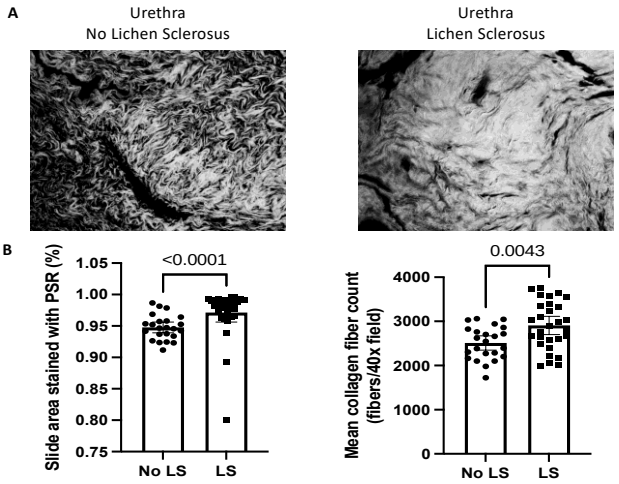
INTRODUCTION AND OBJECTIVE: Lichen sclerosus (LS) is a chronic inflammatory disease of the genital epithelium. It causes severe fibrosis of the vulva and foreskin and in affected men is associated with severe urethral stricture disease refractory to conventional treatment. The mechanisms responsible for fibrosis in LS are unknown. Other fibrotic diseases display increased collagen content and decreased fiber width reflective of increased collagen cross linking. We hypothesize that this pattern is recapitulated in LS.

METHODS: Collagen was stained with picrosirius red (PSR) in human urethral, vulvar, and foreskin specimens with and without LS. An observer blinded to LS status and tissue type acquired three 20x stromal images from each specimen. Collagen content was quantified as the mean proportion of image area stained using Image J. Mean collagen fiber width, length, and count were obtained using CT-FIRE automated image analysis software.

RESULTS: Collagen content and fiber count was significantly higher in all LS specimens (vulva n=9, urethra n=7, foreskin n=7) compared to all non-LS specimens (vulva n=9, urethra n=7, foreskin n=13) (Figure). Collagen fiber width and length were unchanged with respect to LS status.

Figure. *Comparison of collagen content and fiber count in human vulva, urethra, and foreskin with and without LS. (A) Representative fluorescent microscopy images (20x) of PSR stained urethral collagen demonstrating increased collagen content and density in LS compared to non-LS tissues. (B) Comparison of total collagen content defined as proportion of slide area stained with PSR and mean collagen fiber count with respect to LS status. Brackets indicate pairwise comparisons with p values listed above. LS = lichen sclerosus, PSR = picrosirius red.*



CONCLUSIONS: Total collagen content and fiber count is increased in LS tissues compared to non-LS tissues. While the relative differences in collagen content were modest these data suggest that increased collagen deposition is a distinguishing characteristic of LS. We found that collagen fiber width and length were similar with respect to tissue type and LS status. However, the high density of collagen fibers may have precluded proper curvelet fit and prevented accurate assessment of fiber characteristics. To address we plan to use texture analysis to quantify differences in collagen organization in LS tissues.

WORLD PROFESSIONAL ASSOCIATION FOR TRANSGENDER HEALTH (WPATH) GUIDELINE COMPLIANCE

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INTRODUCTION AND OBJECTIVE: Although likely underestimated, the prevalence of gender dysphoria ranges from 0.5 to 1.3% of the population. Currently, the transgender community in the United States is approaching 1,000,000 in number and rapidly growing. As gender dysphoria and associated conditions become increasingly recognized, the request for transgender surgeries will continue to increase. Prior to undergoing surgical treatment for gender dysphoria, the World Professional Association for Transgender Health (WPATH) maintains guidelines regarding hormonal management, psychiatric care and outward patient appearances. We sought to determine WPATH compliance rates for patients undergoing gender affirmation surgeries before and after the establishment of a comprehensive transgender health clinic in an academic medical center.

METHODS: Electronic medical records were queried for patients >17 years of age with the diagnosis of gender dysphoria from March 2015 to May 2021. Charts were reviewed for gender affirmation procedures performed at our institution including facial/neck aesthetic, chest reconstruction ("top") or genital ("bottom") surgeries. Patient age, types of surgical procedures and proper preoperative documentation according to 2012 WPATH guidelines were recorded.

RESULTS: The diagnosis of gender dysphoria was identified in 2,492 patients. A total of 141 individuals underwent 150 gender affirmation procedures during the study period including three facial/neck aesthetic, 65 "top" and 82 "bottom" surgeries. Mean patient age was 28.9 years (range 18 – 71). WPATH guideline non-compliance significantly decreased from 56% (27/48) to 22% (22/102) following establishment of a comprehensive transgender health clinic (p = 0.00004). Failure to comply with preoperative guidelines was due to lack of documented mental health provider release in 47/49 (96%) cases.

CONCLUSIONS: The establishment of a comprehensive transgender health clinic significantly improved WPATH compliance for patients undergoing surgical treatment of gender dysphoria. Non-compliance was almost exclusively secondary to lack of documented release from mental health providers.

MITOCHONDRIAL DYSFUNCTION IN THE AGING ER-BETA KNOCKOUT MOUSE PROSTATE

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INTRODUCTION AND OBJECTIVE: Estrogen receptor beta (ERβ) is expressed in benign prostatic hyperplasia tissues and is thought to play a tumor suppressive role in the prostate. ERβ has also been shown to regulate mitochondrial respiration in adipose tissues. Since preliminary data suggests that BPH tissues are characterized by mitochondrial dysfunction, we sought to determine whether ERβ could also regulate mitochondrial respiration in the prostate using an ERβ-knockout mouse model.

METHODS: Mice with conventional genome-wide deletion of ERβ were generated on a C57/BL6 background and examined at 2 mos and 16 mos of age for differences in mitochondrial respiration compared to age-matched wild type controls. Urine void spot assays were performed in both control and ERβ knockout mice at 16 mos of age immediately prior to mitochondrial respiration assays. Mitochondrial function was measured via oxygen consumption rate using fresh tissue.

RESULTS: Mitochondrial function tests showed deficient mitochondrial activity in the prostate of ERβ knockout animals compared to wild type age-matched controls in both the absence (state 4) and presence (state 3) of ADP for Complex I. This decrease in both basal respiration and maximal capacity for respiration of the mitochondrial tissue in animals lacking ERβ expression suggests a loss of number or function of mitochondria in the prostate compared to age-matched controls. Void spot assays were inconclusive, perhaps due to the small number of animals in the study.

CONCLUSIONS: Overall, loss of ERβ in the mouse prostate appeared to inhibit mitochondrial activity in aged ERβ knockout mice. Future studies will focus on whether ERβ deficiency contributes to bladder voiding dysfunction and will explore the potential for ERβ-selective ligands as potential BPH therapy targeting mitochondrial function.

CHARACTERIZATION OF FGF-5 EXPRESSION IN PROSTATIC TISSUE

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INTRODUCTION AND OBJECTIVE: Fibroblast growth factor 5 (FGF-5) is a secreted protein that acts as a ligand for one of several fibroblast growth factor receptors (FGFRs) to activate downstream signaling pathways, such as Ras/MAPK and PI3K/AKT. FGF-5 is known to act as a growth factor, stimulating both cellular proliferation and angiogenesis. However, there is currently little data on which tissues express FGF-5 protein, and data in the prostate is limited to gene presence. Our aim for this project is to characterize the expression of FGF-5 at the protein level within both normal and diseased prostate tissue.

METHODS: Two tissue microarrays containing human prostate tissue from a total of 392 patients were utilized for this study, with duplicate cores for each patient. Tissue samples included benign prostate tissue, benign prostatic hyperplasia (BPH), high-grade prostatic intraepithelial neoplasia (HGPIN), as well as other disease states. A slide-mounted section of each TMA was multiplexed for FGF-5 (LS-B10340) using Bajoran Purple, E-cadherin (Roche 790-4497) using DAB, and Androgen Receptor (SP242 #M5420) using Discovery Silver. Cores were imaged using the Perkin-Elmer Vectra system, and segmented using Inform. Data analysis was performed using GraphPad Prism 9.2.0.

RESULTS: By analyzing benign tissue core samples from both TMAs, we found that FGF-5 protein is present in both stromal and epithelial cells. Using Inform software to analyze cellular segmentation, we found that in the stroma FGF-5 is mostly expressed in putative fibroblasts and smooth muscle cells; in the epithelium, expression is highest in putative luminal and basal cells. When comparing FGF-5 expression at the subcellular level using Inform software, we found that expression is significantly higher in the nucleus for both stromal and epithelial cells ($p<0.0001$ for all analyses), with expression in the epithelial cell nucleus being significantly higher than in stromal cell nuclei ($p<0.0001$). We also observed increased epithelial FGF-5 expression compared to stromal cells in benign tissue ($p<0.0001$), BPH tissue ($p<0.0001$) and HGPIN ($p<0.0001$) by multiple comparison analysis as well as one-way ANOVA.

CONCLUSIONS: Our study found that FGF-5 localization is expressed differentially throughout different normal and disease states of the prostate. In addition, these results provide support for

further in-depth studies of FGF-5 within the prostate and prostate diseases.

POLYCHLORINATED BIPHENYLS AFFECT VOIDING PARAMETERS IN ADULT-EXPOSED MALE MICE

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INTRODUCTION AND OBJECTIVE: Bladder dysfunction and lower urinary tract symptoms (LUTS) can greatly affect a person's quality of life. LUTS are multifactorial, with variation in age, progression, and severity. Adult male mouse voiding function can be influenced by their environment. Polychlorinated biphenyls (PCBs) are persistent environmental toxicants that can affect human health at varying levels of exposure. PCBs effects on adult male mouse voiding function, however, is unknown. Our aim is to test the hypothesis that PCB exposure leads to changes in bladder function in adult male mice.

METHODS: C57Bl/6J wild type male mice were dosed daily with peanut butter mixed with the 0 or 1mg/kg MARBLES PCB mixture. Dosing began at 10 weeks of age and continued for 60 days. Adult male mice then underwent void spot assay (VSA), uroflowmetry, and cystometry to assess voiding function. Following cystometry, testes and prostate were collected.

RESULTS: VSA analysis is still in progress. Uroflowmetry revealed a significant difference in flow rate in the 1 mg/kg MARBLES PCB dose group, with the rate being higher than the other dose group. There were no significant differences in cystometry parameters. In the ventral lobe of the prostate, the 1 mg/kg daily dose group has an elevated mass. The anterior and dorsal lobes showed no differences. Testes mass also showed no significant differences.

CONCLUSIONS: PCBs have slight effects on voiding in male mice exposed in adulthood. With a difference in flow rate, it is possible that at the 1 mg/kg PCB exposure the bladder and/or urethra have altered contractile properties. This will be further studied with our VSA analysis as well as future studies of bladder and urethra bath contractility assays. The ventral prostate with its elevated mass could be contributing to the voiding changes in the adult male mice. This will be further studied with various immunohistochemistry analyses.

CD44 EXPRESSION IS DECREASED IN LICHEN SCLEROSUS

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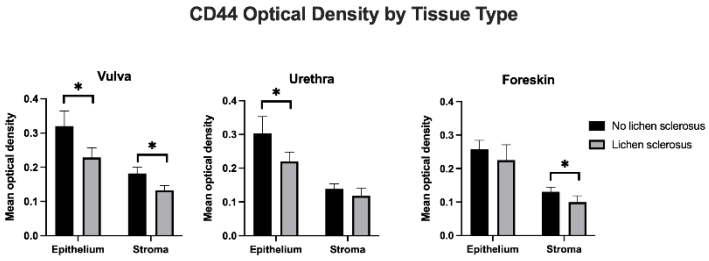
INTRODUCTION AND OBJECTIVE: Lichen sclerosus (LS) is a chronic inflammatory disease of the genital epithelium. It causes severe fibrosis of the vulva and foreskin and in affected men is associated with severe urethral stricture disease refractory to conventional treatment. Decreased cell surface receptor CD44 expression has been reported in LS and implicated in two potential underlying mechanisms. The first is impaired epithelial barrier function. The second is stromal accumulation of hyaluronic acid (HA) resulting from decreased epithelial sequestration. Stromal HA is known to drive chronic inflammation and fibrosis in other diseases. Therefore, we hypothesize that epithelial CD44 expression is decreased and HA abundance increased in human LS tissues compared to tissues unaffected by LS.

METHODS: Human vulvar, urethral, and foreskin tissues were obtained after either circumcision, urethroplasty (male patients) or vulvar biopsy (female patients) and reviewed with a genitourinary pathologist to determine LS status. CD44 expression and HA abundance were quantified using immunohistochemistry. Three 20x regions of interest (ROIs) were selected per slide in a blinded fashion. Vectra and In Form image analysis software was used to segment tissues into epithelial and stromal compartments and quantify staining as mean optical density (OD) in each ROI which were compared using the Student's T-test with respect to tissue type and LS status.

RESULTS: CD44 and HA staining was compared between tissue sections from 23 LS patients (vulva n=9, urethra n=7, foreskin n=7) and 29 non-LS patients (vulva n=9, urethra n=7, foreskin n=13). In vulva, CD44 OD was significantly reduced in both epithelium and stroma. In urethra, epithelial CD44 OD was significantly reduced and stromal expression unchanged. In foreskin epithelial CD44 OD was similar and stromal OD reduced (Figure). HA abundance was similar in all tissues with respect to LS status.

Figure. *Epithelial and stromal CD44 expression quantified by mean optical density (OD) in lichen sclerosus and non-lichen sclerosus vulvar, urethral, and foreskin tissues. * indicates significantly different mean OD (p<0.05).*



CONCLUSIONS: LS tissues displayed a consistent trend of decreased CD44 expression with similar HA abundance. This finding favors loss of epithelial barrier function protecting the underlying stroma from urinary chemical irritants as a potential mechanism in the pathogenesis of LS.

ASSOCIATIONS OF SOCIODEMOGRAPHIC FACTORS AND OBESITY WITH URINARY RISK FACTORS FOR KIDNEY STONE DISEASE

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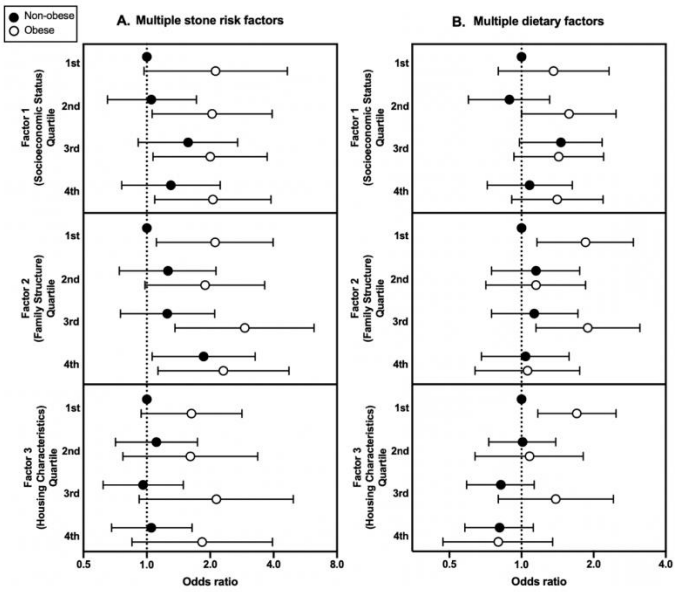
INTRODUCTION AND OBJECTIVE: Obesity is associated with kidney stone disease (KSD), but it is unknown whether this association differs by sociodemographic factors. We aimed to assess the extent to which obesity and sociodemographic factors jointly contribute to urinary risk factors for KSD.

METHODS: We performed a retrospective analysis of adult patients with KSD evaluated with 24-hour urine collections (2001-2020). We obtained neighborhood sociodemographic data and performed a principal component analysis (PCA), which identified three linearly independent factors. We then assessed associations between these factors and individual 24-hour urine measurements through linear regression, as well as groupings of 24-hour urine results, namely stone risk factors and dietary factors, through multivariable logistic regression. Finally, we assessed multiplicative interactions testing effect modification by obesity, and performed analyses stratified by obesity.

RESULTS: We identified 1,264 patients meeting study criteria. Factors retained on PCA represented socioeconomic status (SES), family structure, and housing characteristics. On linear regression, we detected a significant inverse correlation between SES and 24-hour urine sodium (p=0.0002). On multivariable logistic regression, obesity was associated with increased odds of multiple stone risk factors (OR: 1.61; 95% CI: 1.15-2.26) and multiple dietary factors (OR: 1.33; 95% CI: 1.06-1.67). We did not observe significant and consistent multiplicative interactions between obesity and quartiles of SES, family structure, and housing characteristics; analyses stratified by obesity supported these findings (Figure).

Figure. *Odds of (A) multiple stone risk factors and (B) multiple dietary factors by obesity and quartiles of neighborhood factors.*



CONCLUSIONS: Obesity was associated with the presence of multiple stone risk factors and multiple dietary factors; however, the strength and magnitude of these associations did not vary significantly by SES, family structure, and housing characteristics.

FRAILTY IS INCREASED IN AGING MICE WITH LOWER URINARY TRACT DYSFUNCTION

(43) Teresa T. Liu[1], Alexis E. Adrian[1], Laura E. Pascal[2], Scott R. Bauer[3], Jules B. Panksepp[1], Donald B. DeFranco[2], William A. Ricke[1]

[1]University of Wisconsin – Madison, [2]University of Pittsburgh, [3]University of California – San Francisco

INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) with lower urinary tract symptoms (LUTS) is a disease of aging men that leads to a substantial decrease in quality of life, increased morbidity, and is associated with an increased mortality risk. In patients with BPH/LUTS, frailty may increase the risk of disease progression and serious adverse events. A clinical frailty assessment has been developed for use in mice that exhibits similar features as that established in humans. In this study, we examine young and aging male mice for changes in frailty and urinary function. We hypothesize that the frailty index is increased in aging mice with lower urinary tract dysfunction (LUTD).

METHODS: To assess the degree of frailty in young and aged mice, we assessed eight-week-old and two-year-old C57Bl/6J mice using a 31 item frailty assessment. Forelimb grip strength, one measure in the frailty assessment, was quantified using a grip strength meter. Menace reflex, another item assessed in the frailty index, and associated anxiety were examined using an open field test. LUTD was assessed using void spot assays and analyzed with the Void Whizzard software.

RESULTS: As previously described, aging mice develop LUTD when compared to young mice. Assessment of the same mice showed a significant increase in frailty among the aging compared to the young mice. Notably, significant changes with aging included a loss of whiskers, alopecia, malocclusions, loss of forelimb grip strength, and loss of menace reflex. Quantification of grip strength showed a significant decrease in force (Newtons) in aging mice compared to young mice. Menace/anxiety as measured by open field testing showed a decrease in anxiety with aging as compared to young.

CONCLUSIONS: Our study shows that a frailty index calculated using a 31 item survey distinguishes aged from young mice. In addition, frailty in aged mice correlates with alterations in urinary function. Thus, mice provide an appropriate model to determine whether interventions that prolong healthy aging (i.e., limiting frailty) will likewise reduce LUTD and enhance the quality of life in older adult males.

THE ROLE OF URETHRAL NEUROENDOCRINE CELLS IN URINARY TRACT INFECTION

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) occur in 50−60% of adult women in the United States. We are investigating a potential mechanism of innate immunity to combat

ascending urinary tract infections. The mechanism includes multiple cell types, including: neuroendocrine cells (synaptophysin-positive cells), which are concentrated in urethral epithelium and synthesize serotonin, and urethral smooth muscle myocytes which, when activated, contract to expel ascending microbes. The objectives of this study are to test the hypothesis that lipopolysaccharide (LPS), a component of the bacterial cell wall, drives urethral smooth muscle contraction in a serotonin receptor HTR2B-dependent fashion. We will also test the hypothesis that the presence of neuroendocrine cells in mice affords protection against infection by ascending *E. coli* UTI89.

METHODS: Contractility of isolated adult female mouse urethras will be evaluated in response to graded concentrations of LPS, serotonin, and BW 723C86 (an agonist of the serotonin receptor HTR2B). Contractility assays will be conducted in the presence of guanethidine, a drug that inhibits neurotransmitter release from axons, to test the hypothesis that LPS and serotonin mediate their actions in an axon-independent fashion. We will create mice that are deficient in urethral neuroendocrine cells by breeding *Shh-cre* with a conditional null allele of achaete-schete homolog 1 (*Ascl1*), a gene required for neuroendocrine cell differentiation. We will instill *E. coli* UTI89 into the caudal urethra of *Ascl1* conditional null and wild type control mice via a catheter to initiate an ascending infection. We will test whether mutant mice have more *E. coli* (CFUs) and more inflammation (CD45+ cells) in urethra and bladder than infected wild type control mice. We will test whether treatment with the *selective serotonin reuptake inhibitor* (*SSRI*) fluoxetine, which prevents reuptake of secreted serotonin in the lower urinary tract and elsewhere, increases sensitivity to LPS-mediated contraction *in vitro* and reduces severity of infection from instilled *E. coli in vivo*.

RESULTS: We expected that LPS, serotonin and BW 723C86 will drive urethral smooth muscle contraction. We expect *Ascl1* conditional null mice will experience more severe *E. coli* infection than control mice. We predict fluoxetine treatment will increase serotonin-driven muscle contraction *in vitro* and reduce severity of LUT infection and inflammation *in vivo*.

CONCLUSIONS: We expect to establish a key role for urethral neuroendocrine cells in protecting against ascending *E. coli* infection.

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DEVELOPMENT OF CRISPR-CAS9 MEDIATED PROTEIN KNOCKOUT CELLS AS A TOOL AND RESOURCE FOR PROSTATIC RESEARCH

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INTRODUCTION AND OBJECTIVE: Since its discovery in 2012, 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 wide variety of downstream applications including insertion of new DNA sequences, removal of sequences, and knockout (KO) of protein expression. Here, we introduce a method combining stable CRISPR-cas9 expression and transient puromycin resistance to allow for development of cell lines with multiple KO proteins.

METHODS: Utilizing Lentiviral hCMV-Blast cas9 Nuclease, BHPrS1 (prostate stromal cell), BPH1 (prostate epithelial cell), and other prostate cells were transduced overnight. The following morning, complete growth medium was added to quench the viral infection. Cells were allowed to recover for 24 hours, and then media was removed and replaced with media containing blasticidin for selection. We selected one subclone from each parental cell line for transfection. Pre-designed synthetic guide RNAs (sgRNAs) were purchased for genes of interest, including ER-α, ER-β, and FGF-5. Cells were co-transfected with varying concentrations of sgRNA and a plasmid containing both GFP and puromycin resistance. Cells were monitored for GFP expression and treated with puromycin for selection once GFP was observed. After 48 hours of puromycin treatment, media was refreshed, and cells were allowed to recover. Following puromycin selection, we seeded cells into 96-well plates to isolate clonal populations. Gene expression was verified first using Western blot and qRT-PCR.

RESULTS: Using our method described above, we were able to successfully generate multiple blasticidin-resistant cas9-expressing prostate cell lines, including BHPrS1 and BPH1, which can be used in combination with sgRNAs to yield KO cells for a protein or combination of proteins of interest.

CONCLUSIONS: We present here a method for generating prostate cell lines that stably express cas9 endonuclease, while only transiently expressing resistance to puromycin during transfection with sgRNAs. This allows for multiple successive rounds of sgRNA transfection and clonal selection; allowing researchers to generate double, triple, and potentially even quadruple (or more) KO cell lines with identical genetic modifications and complete ablation of target proteins.

HYPOSPADIAS OCCURS IN THE PENIS OF MICE AND HUMANS WITH *RBFOX2* GENE VARIATION

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INTRODUCTION AND OBJECTIVE: Despite the frequency of hypospadias (1:125 live male births), often the etiology is not known. The Lamb Laboratory previously determined that copy number variation (CNV) in *RBFOX2* (RNA Binding Fox-1 Homolog 2) is associated with clinical cases of upper and lower tract genitourinary anomalies, including hypospadias. *RBFOX2* encodes an RNA binding protein that regulates mRNA splicing. The hypothesis that altered mRNA splicing impacts penis development was tested, specifically the mesenchymal to epithelial transition critical for penile uretrha formation, resulting in hypospadias.

METHODS: To identify developmental pathways regulated by RBFOX2 in the genitourinary system, *Rbfox2* haploinsufficient and null mouse models were developed, and penises were collected for RNA-seq, *in situ* hybridization, and micro-CT analysis of urethral development. Transcriptomic data was compared to published direct target transcripts of RBFOX2 in human cell lines.

In situ hybridization together with re-analysis of previously published scRNA-seq datasets were employed to define the anatomical regions of *Rbfox2*-specific expression in the developing mouse penis [Amato & Yao 2021 PNAS, Armfield & Cohn 2021 Dev Biol].

RESULTS: Spatial analysis revealed *Rbfox2* expression is initially ubiquitous around before penile sex determination (E14.5), but after E15.5 *Rbfox2* becomes restricted to the penile urethral mesenchyme. Differential gene expression analysis demonstrated complete *Rbfox2* loss causes mis-expression of gene products associated with pluripotency and differentiation. In some cases dysregulation was the product of differential transcript usage.

CONCLUSIONS: Alteraitons in *RBFOX2* gene dosage clinically correlate with upper and lower tract genitourinary anomalies, specifically hypospadias. Data from human cell lines and mouse models indicate the phenotype seems to be partially the product of an RBFOX2 role in directing the mesenchymal to epithelial transition.

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K_V7 CHANNELS IN RAT URINARY BLADDER SMOOTH MUSCLE: EXPRESSION AND ROLE IN EXCITABILITY AND CONTRACTILITY

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INTRODUCTION AND OBJECTIVE: Voltage-gated K^+ (K_V) type 7 channels ($K_V7.1$–$K_V7.5$/KCNQ1-5) regulate various body functions and display organ or cell type specific expression profile. Our group has initiated studies aimed at elucidating the roles of K_V7 channels in urinary bladder smooth muscle (UBSM) focusing primarily on the guinea pig animal model where all K_V7 channels are expressed and contribute to function. Given potential interspecies differences and translational value of the rat model in pre-clinical drug discovery, we are now investigating the role of K_V7 channels in this species. We hypothesize that K_V7 channels are expressed in rat UBSM, and their activation attenuates UBSM excitability and contractility.

METHODS: UBSM tissue strips without mucosa were obtained from adult male Sprague Dawley rats. UBSM homogenates were used in Western blots to detect K_V7 channel subtypes. UBSM strip contractility recordings were conducted under isometric tension conditions. Single UBSM cells were freshly isolated using enzymatic treatment for immunocytochemistry and amphotericin-B perforated current-clamp (I=0) electrophysiology.

RESULTS: Retigabine ($K_V7.2$-$K_V7.5$ activator), ICA-069673 (selective $K_V7.2$/$K_V7.3$ activator, also active on $K_V7.4$), and ML213 (preferential $K_V7.2$ and $K_V7.4$ activator) all reduced spontaneous phasic, 20 mM K^+-induced, 1 µM carbachol-induced, and electrical field stimulated UBSM contractions in a concentration-dependent manner (EC_{50} values and max inhibition for amplitude, force, and frequency: 1.1 to 111 µM, 40 to 100%, n=4-13). The three activators (each tested at 10 µM) also induced membrane potential hyperpolarization in single DSM cells (Δ -7.6 to -13.8 mV, each n=5 and $p<0.05$), whereas the $K_V7.1$-$K_V7.5$ blocker XE991 (10 µM) caused depolarization (Δ +8.7 mV, n=12, $p<0.01$). In the presence of XE991, retigabine, ICA-069673, and ML213 failed to alter the membrane potential. Western blot experiments detected $K_V7.4$ and $K_V7.5$ channel proteins in UBSM and control brain tissue. Immunocytochemistry revealed that freshly isolated single UBSM cells expressed both alpha smooth muscle actin and $K_V7.4$ channel proteins.

CONCLUSIONS: Rat UBSM expresses K_V7 channels, particularly the $K_V7.4$ subtype. Pharmacological activation of UBSM K_V7 channels reduces single UBSM cell excitability via membrane potential hyperpolarization that in turn decreases UBSM contractility. In rats – just as previously reported for guinea pigs – K_V7 channels regulate UBSM function. Future studies are needed to address the role of individual K_V7 channel subtypes in rat and, more importantly, human UBSM.

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ACTINOTIGNUM SCHAALII: A COMMENSAL BACTERIA OF THE PEDIATRIC URINARY MICROBIOME

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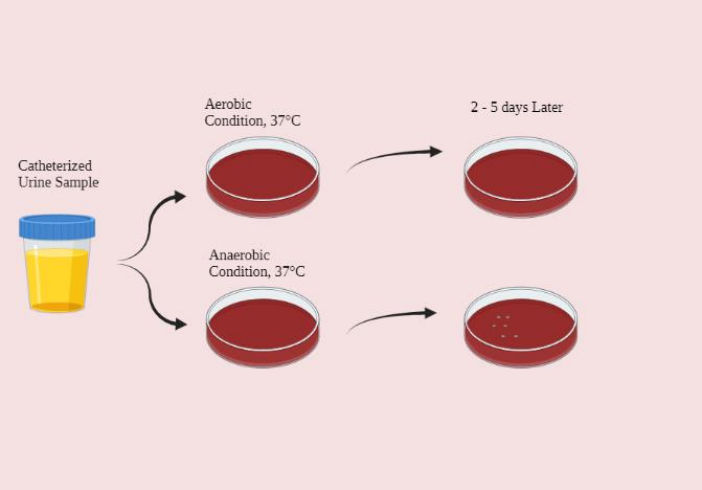
INTRODUCTION AND OBJECTIVE: Recent significant interest has arisen in exploring the urinary microbiome. While a variety of methods exist including 16s rRNA sequencing, expanded quantitative urine culture (EQUC) is a relatively new sensitive culture method that can be applied when standard urine culture fails to detect fastidious uropathogens in symptomatic patients. It has also been used with 16s rRNA sequencing to prove the existence of a urinary microbiome. To date, the pediatric urinary microbiome of asymptomatic healthy infants has not been characterized using expanded quantitative urine culture (EQUC) techniques.

METHODS: IRB approval was obtained to collect sterile catheterized urine samples from healthy infants less than 12 months of age undergoing an elective circumcision under general anesthesia. Exclusion criteria included prior history of UTI, genitourinary anomaly, urethral catheterization or receiving preoperative antibiotics. Urine aliquots were plated using a modified EQUC protocol consisting of 5% Sheep's Blood and Brucella agars, incubated anaerobically and aerobically at 5% at CO2 for 2 to 5 days. Speciation of facultative and obligate anaerobic bacterial isolates were verified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF).

RESULTS: To date, thirteen parents consented to participate in this study. Seven (53%) patients were White and six (47%) were Black. The mean age at the time of surgery was 268 days old (range 183 to 348 days). Five out of thirteen urine samples grew at least a single organism with EQUC. Only one patient grew multiple species. Using MALDI-TOF, a total of three species were recorded: *Clostridium sordelli* in one patient, *Peptoniphilus harei* in another patient, and *Actinotignum schaalii* in four patients (31%). No patient experienced a complication as a result of this study.

CONCLUSIONS: *Actinotignum schaalii* is a recently classified gram-positive facultative anaerobe that has been described as both a commensal member of the urinary microbiota and an emerging urinary pathogen due to improved detection with MALTI-

TOF. Detection of this species is difficult because of its fastidious nature, need for blood-enriched agar media and incubation under 5% CO2 or an anaerobic atmosphere. Using EQUC and MALTI-TOF, we found this species in 31% of our patients. This is the first time the presence of this species has been reported in healthy infants suggesting its presence in the urinary microbiome starts from infancy.



EFFECTS OF ORAL ADMINISTRATION OF A HISTAMINE 1-RECEPTOR ANTAGONIST ON BLADDER HYPERSENSITIVITY AND LOCAL MAST CELL TRYPTASE OVEREXPRESSION IN A RAT MODEL OF PROSTATIC INFLAMMATION

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INTRODUCTION AND OBJECTIVE: Animal models of prostatic inflammation (PI) reportedly exhibit bladder overactivity via prostate-to-bladder cross-organ afferent sensitization through activation of the pelvic nerve. However, the underlying mechanisms for PI-induced afferent sensitization are not fully elucidated. Histamine released from mast cells activated by tissue inflammation has been implicated as an important mediator causing pain and itch sensation, and inhibition of histamine H1 receptors is reportedly effective for the treatment of pain and other bladder symptoms in patients with chronic prostatitis/chronic pelvic pain syndrome. In addition, mast cell tryptase (MCT) has been shown to be a sensitive and specific marker for the presence of mast cells in tissues. Thus, the purpose of this study was to examine the effect of oral administration of a histamine 1-receptor (HT1R) antagonist on bladder hypersensitivity and local MCT expression in a rat model of PI.

METHODS: Male SD rats were divided into three groups: (1) Control group; rats without PI and with oral administration of an HT1R antagonist (desloratadine), (2) Placebo group; PI rats with oral administration of vehicle, (3) Treatment group; PI rats with oral administration of desloratadine. First, 5% formalin was injected into each ventral lobe of the prostate to induce PI in Placebo and Treatment groups. Oral treatment was performed for 14 days after PI induction. Then, awake cystometry (CMG) was performed, and tissues were harvested from non-CMG rats for histological and molecular analyses.

RESULTS: The treatment reduced PI-induced lower urinary tract dysfunction (LUTD) evident as longer intercontraction intervals and greater bladder voiding efficiency (Table 1) in association with reduced MCT in the prostate as well as reduced expressions of HTR1 in the bladder mucosa, prostate and L6-S1 dorsal root ganglia (DRG), and TRPV1 in L6-S1 DRG (Figure 1).

CONCLUSIONS: The histamine-1 receptor expressed in the bladder, prostate and sensory pathways could be an effective target for the treatment of irritative LUTD induced by prostatic inflammation.

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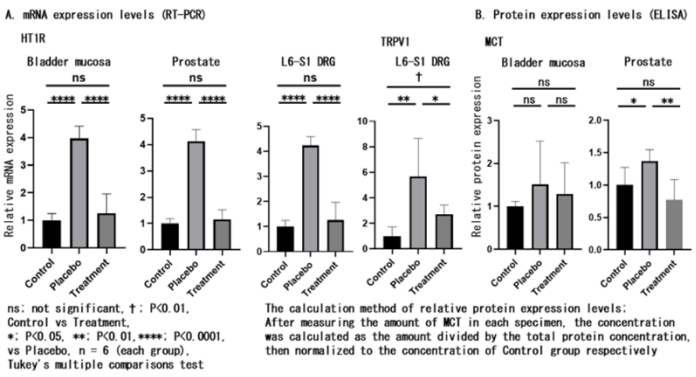
Table 1. Measurements of cystometric parameters

	ICI (s)	BP (cmH_2O)	TP (cmH_2O)	MVP (cmH_2O)	NVC	VV (ml)	PVR (ml)	BVE (%)
Control	907 ± 81*	6.3 ± 1.1	14.2 ± 1.3	35.7 ± 2.5	0.3 ± 0.2	0.59 ± 0.05*	0.05 ± 0.01**	92.5 ± 1.7**
Placebo	720 ± 112	7.8 ± 0.6	13.9 ± 1.5	36.3 ± 3.5	0.6 ± 0.4	0.38 ± 0.03	0.28 ± 0.06	58.9 ± 7.7
Treatment	956 ± 133**	6.5 ± 0.3	11.6 ± 1.1	32.9 ± 2.3	0.1 ± 0.1	0.71 ± 0.15	0.05 ± 0.03**	94.3 ± 2.2**

****: P < 0.0001, ***: P < 0.001, **: P < 0.01, *: P < 0.05,
vs Placebo, (n = 6 in each group), Tukey's multiple comparisons test

- ICI: Intercontraction intervals
- BP: Baseline pressure
- TP: Threshold pressure
- MVP: Maximal voiding pressure
- NVC: Non voiding contractions (number/micturition)
- VV: Voided volume
- PVR: Post voided residual volume
- BVE: Bladder voiding efficiency

Fig. 1



ns: not significant, †: P<0.01,
Control vs Treatment,
*: P<0.05, **: P<0.01, ****: P<0.0001,
vs Placebo, n = 6 (each group),
Tukey's multiple comparisons test

The calculation method of relative protein expression levels:
After measuring the amount of MCT in each specimen, the concentration was calculated as the amount divided by the total protein concentration, then normalized to the concentration of Control group respectively

SPATIAL TRANSCRIPTOMICS REVEALS A TRANSITION FROM A PROSTATE LUMINAL TO CLUB-LIKE CELL STATE IN 5-ALPHA REDUCTASE INHIBITOR TREATED BPH PATIENTS

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INTRODUCTION AND OBJECTIVE: Understanding how prostate luminal cells adapt and survive in a low androgen environment is central to the clinical treatment of benign and malignant disease. 5-alpha reductase inhibitors (5ARIs) block the conversion of testosterone to dihydrotestosterone (DHT) and are used to treat Benign Prostatic Hyperplasia (BPH). 5ARI treatment reduces tissue DHT levels and causes involution of prostate glands. However, the histologic response to 5ARIs is heterogeneous and lower urinary tract symptoms in treated BPH patients often persist or worsen, leading to surgical intervention. We examined

transcriptional changes accompanying 5ARI treatment to determine the cellular mechanisms of treatment resistance.

METHODS: Mass spectrometry was performed to measure 5ARI drug and hormone levels in prostate tissue from BPH patients. Prostates from untreated and 5ARI treated men were selected for Visium spatial transcriptomics. Immunostaining and in situ hybridization were used to confirm gene expression changes.

RESULTS: We observed that patients with detectable levels of 5ARI drug display reduced DHT and increased testosterone. In 5ARI treated patients, regions of normal prostate glandular architecture can often be seen adjacent to regions with smaller atrophied glands, defined as histologically-resistant and -responsive, respectively. Spatial transcriptomics performed on histologically-resistant vs. -responsive regions revealed a urethral club-like molecular signature in small atrophied glands. Based on the degree of club-like gene expression, distinct prostate luminal cell states could be identified that represented a transition from a secretory luminal to a club-like state. Prostate luminal to club-like transition was accompanied by a decrease in gland size, reduced luminal infoldings, increased activation of the NF-κB signaling pathway and reduced androgen receptor mediated signaling.

CONCLUSIONS: We conclude that 5ARI treatment results in small atrophied glands lined by cells with a club-like gene signature. Our results indicate that prostate luminal cells gradually acquire this club-like gene signature in response to 5ARI treatment. Further research is needed to determine whether inhibition of androgen signaling or inflammation associated with 5ARI treatment is responsible for this adaptation.