Annual CAIRIBU Meeting: December 3-4, 2020

CROSSING THE DISCIPLINARY DIVIDE

** Abstracts by CAIRIBU Center/Program (author & title)

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2	**Derek Ho, PhD** *(also a postdoctoral fellow, Duke University P20 Center)*	The major contribution of cavitation to stone damage in dusting mode during laser lithotripsy
3	**James Hokanson, PhD**	Sacral neuromodulation in rats: parameters and pathways
4	**Maryellen Kelly, DNP, CPNP, MHSc**	Utility estimation for neurogenic bowel dysfunction in the general population
5	**Michael Odom, PhD**	NLRP3-dependent mechanisms downregulate genes controlling urothelial barrier function in diabetic mice
6	**Casey Steadman, PhD**	Effect of epidural kilohertz frequency spinal cord stimulation on lower urinary tract function in a rat spinal cord transection model
7	**Tatyana Sysoeva, PhD**	Interactions of commensal bladder lactobacilli with urinary pathogens

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9	**LaTasha Crawford, PhD** *(presented by **Emily Tran** and listed under "Tran" in printed abstracts)*	Genetic tools that target mechanoreceptors can be used to label bladder afferents
10	**Matthew Grimes, MD**	Decreased CD44 expression is associated with increased hyaluronic acid abundance in human lichen sclerosus
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14	**David Bayne, MD, MPH**	Influence of food insecurity on stone burden and velocity of stone recurrence after surgical intervention
15	**Eva Raphael, MD, MPH** *(pending K12 Scholar for 2021)*	Trends and patient characteristics of urine infections caused by extended-spectrum beta-lactamase- producing Escherichia coli in 3 clinical settings of a San Francisco healthcare system, 2014-2020

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* Denotes research previously completed as part of the **Mayo Clinic Summer Undergraduate Research Program** (NIDDK R25) in Nephrology & Urology *(PI, Michael Romero, PhD)*

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Thank you to the members of the 2020 CAIRIBU Abstracts and Poster Session Committee for their leadership in organizing this session:

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** Printed Abstracts by Presenter's Last Name (alphabetical)

BETAINE-DEPENDENT RE-METHYLATION ABNORMALITIES ARE ASSOCIATED WITH INCREASED HOMOCYSTEINE AND DECREASED ENDOTHELIAL NITRIC OXIDE SYNTHASE EXPRESSION IN POLYCYSTIC KIDNEY DISEASE

(38) Alexis Adrian[1], Gizem Yilmaz[2], Yasin Goksu[2], Ivan Vuckovic[3], Slobodan Macura[3], Fouad Chebib[2], Peter C. Harris[2], Alfonso Eirin[2], Amir Lerman[4], Lilach O. Lerman[2], Vicente E. Torres[2], and Maria V. Irazabal[2]

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INTRODUCTION AND OBJECTIVE: Polycystic kidney disease (PKD) is a significant cause of end stage renal disease and vascular abnormalities are the most significant non-cystic complication contributing to disease severity. Endothelial dysfunction (ED) develops early on and precedes vascular abnormalities, but the underlying mechanism is unknown. High homocysteine (Hcy) levels are associated with ED, likely resulting from a reduction in nitric oxide (NO) bioavailability. Previous studies have found increased levels of Hcy in association with decreased NO availability in patients with PKD, but the mechanisms leading to increased Hcy remain to be elucidated. We hypothesized that abnormalities in Hcy metabolism are responsible for the increased levels, leading to Hcy-induced ED in PKD.

METHODS: Kidneys were harvested, frozen in liquid nitrogen, or preserved in formalin for metabolomics analyses and ex-vivo studies from 4-week-old PCK and Sprague-Dawley (SD) control rats (n=12 each). Twenty-four-hour urine and terminal blood samples were collected for metabolite analysis, via 1HNMR or ELISA, and chemistries. Endothelial nitric oxide synthase (eNOS) was assessed by double immunofluorescence staining for CD31/eNOS.

RESULTS: PCK rats maintained renal function, evaluated by serum creatinine and blood urea nitrogen, but had significantly higher kidney weight/body weight ratios and cystic indices.

Plasma Hcy levels were elevated in PCK rats; betaine, a methyl donor to Hcy, was elevated in the PCK urine but decreased in the tissue. The increased betaine excretion was also correlated to the increased plasma Hcy levels. PCK rats also showed higher levels of glutathione and no significant difference in vitamin B12 or folate, suggesting the transsulfuration and folate-dependent remethylation pathways were an unlikely source of a defect. The increase in Hcy was also associated with a decrease in eNOS immunoreactivity, likely decreasing nitric oxide bioavailability.

CONCLUSION: Early PKD is associated with elevation in Hcy, likely related to betaine-dependent re-methylation abnormalities, and decreased eNOS immunoreactivity. These findings provide novel insights into Hcy-induced ED in PKD and suggest candidate markers that may be useful to assess vascular and renal disease severity early on.

GENETIC ASSOCIATION PATTERNS ARE SHARED BETWEEN BLOOD IONIZED CALCIUM, URINARY CALCIUM, AND RISK OF CALCIUM OXALATE URINARY STONES IN A DOG MODEL

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INTRODUCTION AND OBJECTIVE: Kidney stones are common, chronic, and excruciatingly painful. Calcium oxalate (CaOx) stones are the most common urinary stone, and risk of development is heritable. Confirmed genetic drivers of CaOx stone formation could be used to develop targeted therapy to prevent recurrence, but genetic discovery has been limited by disease complexity. CaOx stones are also common and heritable in dogs, and reduced genetic diversity within breeds increases power to detect genetic associations with disease. Existing genetic studies in humans and dogs have largely focused on the trait of stone disease. However, many abnormalities can lead to CaOx stones, and treating all stone formers as one group may reduce power to detect risk variants. In this study, we focus on genetic drivers of calcium homeostasis as it relates to CaOx stones in the dog model. Our objective was to identify patterns of genetic associations that are shared under three conditions: calcium measured in blood, calcium measured in urine, and CaOx stone formation.

METHODS: >390,000 single nucleotide polymorphism (SNP) genotypes were available from 249 Miniature Schnauzer dogs. A genome-wide association analysis (GWAS) was performed separately for 3 conditions with sex as a covariate: CaOx stone diagnosis (101 cases, 148 controls), urine calcium to creatinine ratio (92 dogs), and blood ionized calcium (82 dogs). SNP effect size estimates from each GWAS were used with the multivariate adaptive shrinkage (mash) algorithm to evaluate for shared patterns of association across the three conditions. Significance was determined using local false sign rate (lfsr), which is analogous to false discovery rate. SNPs with an lfsr <0.05 in all 3 conditions were considered significant. The UCSC Genome Browser was used to evaluate significant loci for the presence of genes or noncoding transcripts.

RESULTS: We identified 175 SNPs that were significantly associated with all 3 conditions. Significant loci highlighted 31 candidate genes. The top locus contained 65 SNPs with lfsr<0.05 and spanned ~2 megabases on chromosome 9. This region contained 6 genes, including KCNJ16, which encodes a subunit of a potassium channel highly expressed in the basolateral membrane of the distal renal tubule. This gene regulates pH and electrolyte balance, and knockout mice develop a metabolic acidosis and hypercalciuria.

CONCLUSIONS: Multiple genetic loci are associated with calcium levels in blood and urine, as well as CaOx stone risk. Whole genome sequencing data will be used to interrogate significant regions for variants within or near candidate genes. This analysis will also be extended to other non-calcium variables that are relevant to CaOx stones.

ASSOCIATION OF FRAILTY WITH CLINICAL BENIGN PROSTATIC HYPERPLASIA PROGRESSION AND SERIOUS ADVERSE DRUG EVENTS: THE MEDICAL THERAPY OF PROSTATIC SYMPTOMS (MTOPS) STUDY

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is a common histologic diagnosis among older men that can cause bothersome lower urinary tract symptoms (LUTS) which are increasingly treated with medications. Frailty may shift the balance of benefit and harms of medical therapy for BPH. Our objective was to assess the associations between frailty and clinical BPH progression or serious adverse drug events (ADE).

METHODS: We analyzed data from the Medical Therapy of Prostatic Symptoms (MTOPS) Study, a randomized placebo-controlled trial testing the effect of doxazosin, finasteride, or combination therapy on clinical BPH progression defined as an increase of ≥4 in the American Urological Association symptom score (AUASS), acute urinary retention, urinary incontinence, renal insufficiency, or recurrent urinary tract infection. Participants were 3047 men, age 50-89 years, with moderate-to-severe LUTS, reduced urinary flow rate, and no history of intervention for BPH, hypotension, or elevated PSA. We created a frailty index (range: 0-1) using 69 items collected at baseline and categorized men as fit (≤0.1), less fit (0.1-<0.25), or frail (≥0.25). The 2 primary outcomes were time to first progression event or ADE requiring hospitalization as defined in the original trial. Cox proportional hazards regression models were adjusted for age, demographics, intervention arm, and measures of obstruction.

RESULTS: Among MTOPS participants, 28% were fit, 58% were less fit, and 14% were frail. During follow-up (mean 4.5 years), the incidence rate of clinical BPH progression per 100 person-years was 2.2 among fit, 3.0 among less fit (HR =1.28, 95% CI 0.98, 1.67, P=0.07), and 4.1 among frail men (HR=1.60, 95% CI 1.13, 2.26, P=0.01) with no evidence effect modification by intervention arm (P-interaction=0.64). Among men randomized to placebo, the ADE incidence rate per 100 person-years was 4.3 for fit men versus 9.4 for frail men (HR=2.94, 95% CI 1.81, 4.79, P<0.0001). Among men randomized to combination therapy, the ADE incidence rate was 3.4 for fit men versus 12.7 for frail men (HR=5.98, 95% CI 3.76, 9.52, P<0.0001).

FIGURE 1. Kaplan-Meier curve for A) clinical BPH progression, and B) serious adverse drug event, stratified by frailty status.
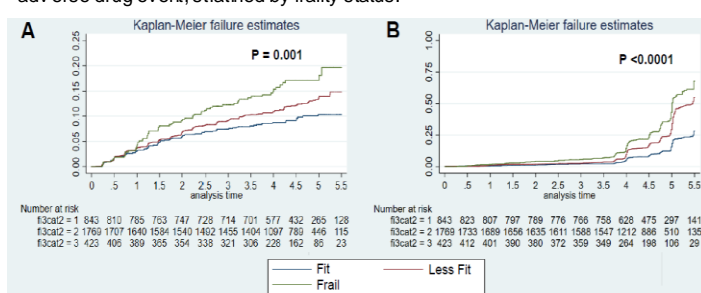
CONCLUSIONS: Frailty is independently associated with greater risk of both clinical BPH progression and serious ADE among men randomized to placebo or medical therapy. The decision to initiate medical therapy for BPH among frail men should therefore include a discussion of both benefits and risks via shared decision making.

INFLUENCE OF INCOME AND FOOD INSECURITY ON STONE BURDEN AT PRESENTATION FOR UROLOGIC SURGERY

(14) **David Bayne**[1], Cameron Hicks[1], Manuel Armas-Phan[2], Sudarshan Srirangapatanam[3], Tom Chi[1],

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INTRODUCTION: Although patients from communities of low socioeconomic status (SES) are known to present with larger kidney stones, it remains unclear how low SES contributes to disparate kidney stone burden. It is possible that patients of low SES face obstacles to purchasing healthy, stone preventative diets. We sought to investigate how income and food insecurity influence stone burden.

METHODS: A review of prospectively collected data using the UCSF ReSKU (Registry of Stones of the Kidney and Ureter) database was conducted. Inclusion criteria were patients who underwent one or more interventions for a single stone episode and had preoperative imaging prior to surgical intervention. Patient data was linked to publicly available United States Department of Agriculture (USDA) census tract food insecurity and income data. Food insecure areas were defined as census tracts greater than 1 mile from a supermarket in urban communities and 10 miles in rural communities.

RESULTS: 332 patients had imaging and USDA data available for review. Lower census tract median family income as a linear variable was a significant predictor of increased stone burden at presentation (p < 0.001). Patients from food insecure areas (37mm vs 47mm, p = 0.066), low income areas (37mm vs 42mm p = 0.190), and areas that were both food insecure and low income (38mm vs 61mm, p = 0.103) all had higher stone burden at presentation, although these differences were not statistically significant.

CONCLUSION: This study confirms previous findings that residing in communities with lower median household income predicts increased stone burden at presentation to surgical care. This study also suggests that income may influence stone burden at presentation more so than neighborhood food insecurity. Additional studies with larger patient cohorts are necessary to better understand the relationship between income, food insecurity, and stone burden.

CYTOCHROME BD IS REQUIRED FOR UROPATHOGENIC ESCHERICHIA COLI PATHOGENESIS AND BIOFILM DEVELOPMENT

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INTRODUCTION AND OBJECTIVE: Uropathogenic Escherichia coli (UPEC) is the most common cause of urinary tract infection (UTI). During UTI, UPEC forms multicellular bacterial communities known as biofilms on the bladder epithelium, within urothelial cells, and on indwelling catheters. During biofilm development, bacteria secrete a structured extracellular matrix that protects cells from external threats, including immune defenses and antibiotics. As such, biofilms hamper our ability to treat UTI and are a major contributor to recurrent infection. In this work we investigate how bacterial respiration contributes to UPEC pathogenesis and the formation of robust biofilms capable of withstanding antibiotic treatment. Defining mechanism that contribute to biofilm development will aid in the development of anti-biofilm therapeutics for the treatment of UTI.

METHODS: RT-qPCR and microscopy were used to measure the expression of respiratory operons in biofilms and during bladder infection. To determine the role of respiration in UPEC pathogenesis, we created genetic deletions of each respiratory oxidase in a well-characterized cystitis isolate and used a murine model of UTI to assess the ability of each strain to colonize the bladder. We used in vitro biofilm assays to investigate the ability of respiratory mutants to form biofilms and withstand treatment by antibiotics. We used biochemical approaches to investigate mechanisms by which respiratory oxidases contribute to biofilm antibiotic tolerance.

RESULTS: Although UPEC heterogeneously expresses respiratory operons in biofilms, only one respiratory enzyme, cytochrome bd, is necessary for pathogenesis and biofilm formation. Loss of cytochrome bd reduces bladder colonization during acute UTI. Cytochrome bd deficient biofilms have reduced extracellular matrix synthesis and are unable to form robust biofilm communities. Interestingly, loss of cytochrome bd increases antibiotic sensitivity in a biofilm specific manner by altering extracellular matrix production and enhancing cellular uptake of antibiotics.

CONCLUSIONS: Despite encoding a flexible respiratory chain, UPEC requires cytochrome bd for biofilm formation and bladder colonization. Without cytochrome bd, UPEC virulence is impaired and bacteria are unable to withstand antibiotic treatment. This work provides mechanistic insights into biofilm development and will serve as the basis of future work evaluating the potential of small molecule inhibitors of cytochrome bd as preventative therapies and adjuvants to antibiotics.

DECODING STROMAL HETEROGENEITY ACROSS BPH PHENOTYPES

(39) **Diya Binoy Joseph**[1], Gervaise H Henry[1], Alicia Malewska[1], Jeffrey C Reese[2], Ryan C Hutchinson[1], Claus G Roehrborn[1], Douglas W Strand[1]

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INTRODUCTION AND OBJECTIVE: Benign Prostatic Hyperplasia (BPH) is a non-malignant enlargement of the prostate that occurs with aging and is associated with Lower Urinary Tract Symptoms (LUTS). Therapeutic options often fail, necessitating surgical resection of the prostate. The phenotypic and cellular heterogeneity of BPH is thought to contribute to treatment resistance. BPH patients present with multiple nodules grouped around the prostatic urethra in the transition zone. The composition of these nodules vary with some being solely comprised of stromal cells and others containing a mixture of stromal and epithelial cells. In addition, some patients present with a band of fibrotic tissue around the prostatic urethra that we term as peri-urethral fibrosis. Here, we describe stromal cell heterogeneity in the normal human prostate and across the different BPH phenotypes.

METHODS: We used an unbiased single cell RNA-sequencing (scRNA-seq) approach to obtain transcriptomes of stromal cells from normal prostates and prostates from BPH patients who underwent simple prostatectomy. Cell clusters identified from scRNA-seq were validated in situ using immunohistochemistry and RNA in situ hybridization.

RESULTS: We found that the stromal composition of the normal prostate consists of two major fibroblast populations, a prostate smooth muscle cell type, a vascular smooth muscle cell type and pericytes. One fibroblast sub-type, marked by expression of MFAP4, is abundant around the prostatic urethra and in the interstitial space between prostate glands. The second fibroblast population, marked by expression of APOD, is found closely associated with the secretory epithelium of the prostate and is absent from the spaces between prostate glands. The MFAP4+ fibroblast subtype extends into the bladder and represents a lower urinary tract fibroblast whereas APOD+ fibroblasts are restricted to the prostate. MFAP4+ fibroblasts are present within stromal and glandular nodules from BPH patients and are abundant in peri-urethral fibrosis. APOD+ fibroblasts are absent from stromal nodules and regions of peri-urethral fibrosis. Desmin expressing smooth muscle cells are largely absent from regions of peri-urethral fibrosis. Wisps of smooth muscle are present in stromal nodules while glandular nodules are packed with Desmin expressing cells.

CONCLUSIONS: Our results highlight the identity and anatomical location of stromal cell types in the normal human prostate and across BPH phenotypes. We expect that a molecular understanding of stromal cell types in the prostate will aid in a better understanding of the etiology of BPH and provide new therapeutic opportunities to reduce lower urinary tract symptoms.

VAGINAL INTRACELLULAR RESERVOIRS IN RECURRENT URINARY TRACT INFECTION

(23) **Brannon, J.R.**[1], Dunigan, T.L.[1], Beebout, C.J.[1], Ross, T., Wiebe, M.A.[1], Reynolds, W.S.[2], and Hadjifrangiskou, M.[1,2,3]

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INTRODUCTION AND BACKGROUND: Uropathogenic Escherichia coli is the primary causative agent of urinary tract infections (UTIs) and account for the majority of antibiotic prescriptions. In this light, outlining factors that allow for E. coli to reside within human reservoirs that contribute to recurrent UTIs is of importance. Due to the shorter urethra length and perineal distance, UTIs predominately occur in woman. Several studies demonstrate vaginal colonization by E. coli precedes UTI, and the vagina is a likely reservoir for recurrent infections. Approximately 30-50% of women, experience recurrent UTIs. Recurrent UTIs can originate from either: 1) the re-emergence of E. coli that invaded the bladder urothelium and form intracellular reservoirs, thus evading antibiotic treatment or 2) the re-accession of E. coli from the intestinal reservoir across the perineum, vaginal introitus, and

to the urethra. Previous studies have demonstrated that prior to UTI, E. coli adheres to vaginal epithelial cells. However, the full extent to which E. coli interact with vaginal cells, as it transverses the perineal space, remains largely unknown in both mouse models and humans with acute or chronic UTIs. We have begun to assess the full extent to which E. coli colonizes the vaginal epithelium.

METHODS: We use a gentamicin-based protection assay to detect intracellular E. coli. As a membrane impermeable antibiotic, gentamicin, is unable to reach and kill intracellular bacteria. We further confirm the presence of intracellular bacteria with confocal laser scanning microscopy and transmission electron microscopy. We test the ability of E. coli to invade vaginal cells in an immortalized cell line model, two mouse models of UTI, a mouse model of E. coli vaginal colonization, and in clinical samples.

RESULTS: Here, we show prototypical and clinical E. coli isolates adhere to vaginal cells. Additionally, we find that E. coli invades vaginal cells in: in an immortalized cell line model, in vaginally and transurethral inoculated mice, as well as, vaginal cells from clinical samples from women with a history recurrent UTI. Furthermore, a combination of scanning electron microscopy and pharmacological experiments demonstrate this invasion occurs through a zipper-like mechanism.

CONCLUSIONS: Our results demonstrate that uropathogenic E. coli invades vaginal cells where it may reside safely from neutrophils, antibiotics, and away from the competition of the host's microbiota. We propose that uropathogenic E. coli invasion of vaginal cells may serve as vaginal intracellular reservoirs that re-seed the occurrence of recurrent infections in woman.

RARE KNOWN PATHOGENIC VARIANTS FOR UROGENITAL DISORDERS IN 129 EXOMES FROM SEVERE IC/BPS PATIENTS

(27) **Catherine A Brownstein**[1], Elicia Estrella[1], Shira Rockowitz[2], Richard Yu[3], Ali G Gharavi[4], Louis M. Kunkel[1]

[1]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA; [2]Department of Bioinformatics, Boston Children's Hospital, Boston, MA; [3]Department of Urology, Boston Children's Hospital, Boston, MA; [4]O'Brien Urology Research Center, Columbia University, New York, NY

INTRODUCTION AND OBJECTIVE: Interstitial cystitis, also called "Bladder Pain Syndrome" (IC/BPS) is an understudied but major subset of bladder dysfunction. We have collected biological samples and phenotypic information on over 400 families with severe and/or early onset IC/BPS over the past two decades. Our hypothesis is that genetics plays a major role in IC/BPS, which is discoverable by combining rich phenotypic data with next generation sequencing.

METHODS: We have conducted pilot genetic analyses of whole exome sequencing data on a total of 129 IC/BPS cases. DNA from blood was extracted using standard protocols and sequenced at the Broad Institute on the Illumina platform. Variants were analyzed using the Genomic Learning System at Boston Children's Hospital (Boston, MA) and Codified Genomics (San Diego, CA). CNVKit (San Francisco, CA) was utilized for analyzing copy number variations from exome data.

RESULTS: We observed multiple variants of interest across the cases, including some predicted pathogenic variants based on the ACMG guidelines for diagnostic analysis of sequence data.

Notably, in three patients from two families, we found a previously reported pathogenic variant in SIX5, a heterozygous c.472G>A change producing a missense variant p.A158T. This same variant is known to be associated with branchiootorenal syndrome (BOR2). This variant is rare in gnomAD (checked July 27, 2020), with 26 instances of A158T in 232,976 alleles. With 2/130 in the IC/BPS cohort of unrelated cases, the OR~100.

An analysis of copy number variations in 98 patients detected a 16p11.2 deletion (N=1) and a 16p11.2 duplication (N=1). 16p11.2 CNVs are associated with congenital anomalies of the kidney and urinary tract (CAKUT). In contrast, no 16p11.2 deletions or duplications were identified in 21,000 controls, suggesting an OR =∞.

CONCLUSIONS: Prior data suggest that some patients with complex urologic disorders have unrecognized Mendelian syndromes. That may also be the case here, with genes and CNV intervals for BOR2 and CAKUT syndrome identified. While the patients in our cohort do not have documented diagnoses of BOR2 syndrome or CAKUT, it is possible that there are mild structural anomalies that eluded detection and will be identified upon further clinical review. As an O'Brien opportunity pool project, an additional 100 exomes will be analyzed to extend and replicate these findings.

NEUROPSYCHIATRIC COMORBIDITIES SELF-REPORTED BY INTERSTITIAL CYSTITIS PATIENTS ON AN ONLINE PEER HEALTH NETWORK

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INTRODUCTION AND OBJECTIVE: Studies have found high prevalence rates of depression and anxiety in Interstitial cystitis (IC) cohorts, and research suggests these comorbidities exacerbate IC/BPS symptoms. So far, the research conducted on neuropsychiatric comorbidities has been limited to small samples. Big data through online platforms can help us with comorbidity mapping. Specifically, we hypothesized that IC patients have a higher probability of reporting anxiety and depression as comorbidities than other patient groups on an online peer health network. Inspire, a digital peer health platform, has over 200 communities and 2 million members. Over 21,000 patients list having IC as a condition.

METHODS: To test our hypotheses, we compared the rates of selected neuropsychiatric disorders in patients who listed IC as a condition against the rates in patients who listed any health condition besides IC.

RESULTS: As expected, depression is frequently declared by Inspire patients who report having IC (15.8%). This is greater than the overall active Inspire patient population who listed any condition (other than IC) and also listed depression (14.2%) as compared by Fisher Exact Test (p < 0.00001). Anxiety is also more frequently declared by Inspire members with IC (2.4%) than those without IC (1.2%, p < 0.00001). In contrast, the IC population does not differ from the rest of Inspire in listing bipolar disorder (2.4% vs 2.7%, p =0.115).

CONCLUSIONS: Our neuropsychiatric comorbidity mapping confirms previous studies' findings of high rates of depression and anxiety with IC. Since the usage of online platforms for epidemiological purposes is relatively new, we need a better understanding of a) how patients who join these digital communities differ from those who do not and b) how patients who report diagnoses on their profiles differ from those who list only some or no conditions. We are conducting follow-up surveys with two demographically distinct IC cohorts to assess replicability of findings.

ANDROGEN PLAY CRITICAL ROLES IN EPITHELIUM PROLIFERATION IN BPH NOT NORMAL ADJACENT

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BACKGROUND: The androgen receptor (AR) is expressed by both stromal and epithelial cells in the prostate, and plays a critical role in normal development and homeostasis as well as prostate pathogenesis. Our preliminary data showed that stromal AR signaling was essential for the proliferation of epithelial cells in BPH, but not in normal adjacent prostate (NAP). However, the underlying mechanisms of this phenomenon are still unknown.

METHOD: Human prostate specimens obtained from BPH patients undergoing simple prostatectomy for symptomatic BPH. Patient derived explants (PDE) and stromal cell cultures from BPH and NAP tissues were established and utilized to evaluate the impact of androgens. Proliferation, cytokines and androgen-responsive genes were quantified in clinical BPH specimens and paired normal prostatic specimens via immunohistochemistry, RNA-Seq and qPCR.

RESULTS: PDEs derived from BPH and paired NAP tissues were able to maintain their original architecture and AR signaling in culture for at least 4 days. Androgen could induce epithelial proliferation in BPH, but not NAP explants. Stromal cells derived from BPH tissues secreted higher levels of CCL family proteins (CCL8, CCL7, CCL11, CCL13 and CCL28), CXCL proteins (CXCL6, CXCL12), interleukins (IL6, IL7 and IL32), and growth factors than those derived from the paired NAP tissues. RNA sequencing identified several cytokines and growth factors which were up-regulated after androgen stimulation in BPH, but not NAP. These results were confirmed by qPCR.

CONCLUSIONS: Androgens could increase epithelial proliferation in BPH but not NAP in the PDE model. Androgens were shown to influence the expression of several genes including CXC and interleukins in BPH stromal cells, but not NAP stromal cells. Our results suggest that androgen signaling in BPH stromal cells is dysregulated and could contribute to prostatic epithelial growth and provide a strong foundation to elucidate the mechanisms of androgen-dependent stromal regulation of epithelial cell growth in BPH.

CLAUDIN 1 IS DOWN-REGULATED IN THE AGING PROSTATE AND ASSOCIATED WITH INCREASED INFLAMMATION IN BPH

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BACKGROUND: Benign prostatic hyperplasia (BPH) is an age-related disease that is frequently associated with chronic prostatic inflammation. In our previous studies, we detected the presence of PSA protein in the stroma of BPH nodules and down-regulation of junction proteins E-cadherin and claudin 1. Transmission electron microscopy (TEM) imaging showed a decrease in tight junctions suggesting the luminal epithelial barrier in BPH tissues may be altered. Recent in vitro studies showed that stimulation of benign prostate epithelial cell lines with TGFβ1 induced a decrease in claudin 1 expression suggesting that inflammation might be associated with alterations in the prostate epithelial barrier. This study explored the potential associations between aging and loss of junction proteins and the presence of inflammatory cells in prostate tissue specimens from young healthy donors and aged BPH patients.

METHOD: Immunostaining of serial prostate sections from BPH patients and healthy young donors was performed for claudin 1, CD4, CD8, CD20 and CD68. H-Scores and the number of inflammatory cells were calculated for the same area in donor, normal adjacent prostate to BPH (NAP) and BPH specimens. Quantification and statistical correlation analyses were performed.

RESULTS: Down-regulation of junction protein claudin 1 was associated with increasing age and inflammation in NAP and BPH compared to young healthy donor prostate.

CONCLUSION: These findings suggest that aging is associated with down-regulation of claudin 1 and claudin 1 is further decreased in BPH. Claudin 1 down-regulation was associated with increased infiltration of inflammatory cells in both NAP and BPH tissues. Claudin down-regulation in the aging prostate could contribute to increased prostatic inflammation, subsequently contributing to BPH pathogenesis.

GENDER, RACE, AND ETHNICITY AMONG STONE FORMERS AT ACADEMIC MEDICAL CENTERS IN BIRMINGHAM, ALABAMA AND DALLAS, TEXAS

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INTRODUCTION AND OBJECTIVE: While nephrolithiasis is more common in men, the gender ratio is decreasing. Significant increases in stone prevalence have been reported for Black and

Hispanic patients in the U.S., but the gender-specific contribution to this trend is unclear. We sought to evaluate the distribution of stone formers by gender, race, and ethnicity in contemporary cohorts.

METHODS: Patients with a nephrolithiasis diagnosis (ICD-10 code N20) at two large academic medical centers from 1/2015-6/2020 were stratified by location: University of Alabama at Birmingham Medical Center (Birmingham cohort) or University of Texas Southwestern Medical Center/Parkland Memorial Hospital (Dallas cohort). Gender, race and ethnicity were determined from the electronic medical records at each center. Differences in gender distribution among Black/White and Hispanic/non-Hispanic stone formers were assessed using Fisher's exact test.

RESULTS: Numbers of patients with available gender, race, and ethnicity data are displayed in **Table 1**. In the Birmingham and Dallas cohorts, 47% and 49% of stone formers were female, respectively. The Birmingham cohort had a greater percentage of Black stone formers than the Dallas cohort (21% vs. 16%), whereas the Dallas cohort had a greater percentage of Hispanic stone formers than the Birmingham cohort (38% vs. 1%). In both cohorts, the majority of Black and Hispanic stone formers were female (56% and 52%, respectively, for Birmingham; 56% and 53%, respectively, for Dallas). In contrast, fewer White and non-Hispanic stone formers were female (44% and 47%, respectively, for Birmingham; 48% and 47%, respectively, for Dallas). In both cohorts, the percentage of women was significantly greater among Black stone formers compared to White stone formers (p<0.001). In the Dallas cohort, the percentage of women was significantly greater among Hispanic stone formers compared to non-Hispanic stone formers (p<0.001); this relationship did not meet conventional levels of statistical significance in the Birmingham cohort (p=0.06).

Table 1: Distribution of stone formers at academic medical centers in Birmingham, Alabama and Dallas, Texas from 1/2015-6/2020 based on gender, race, and ethnicity. P-values correspond to the difference in percentage of women for Black vs. White and Hispanic vs. non-Hispanic stone formers.

		Birmingham	p	Dallas	p
Race	Black female	2,992 (12.0%)	<0.001	4,398 (9.0%)	<0.001
	Black male	2,356 (9.4%)		3,422 (7.0%)	
	White female	8,653 (34.6%)		19,530 (40.2%)	
	White male	11,004 (44.0%)		21,282 (43.8%)	
	Total	25,005		48,632	
Ethnicity	Hispanic female	178 (0.7%)	0.06	10,451 (20.0%)	<0.001
	Hispanic male	164 (0.7%)		9,448 (18.1%)	
	Non-Hispanic female	11,401 (46.1%)		15,013 (28.8%)	
	Non-Hispanic male	12,999 (52.5%)		17,244 (33.1%)	
	Total	24,742		52,156	

CONCLUSIONS: At academic medical centers in two large cities, the majority of Black and Hispanic stone formers are female, whereas the majority of White and non-Hispanic stone formers are male, demonstrating a significant difference in gender distribution. Nephrolithiasis in Black and Hispanic women may be driving increases in prevalence reported for Black and Hispanic individuals.

THE DECISION MAKER: THE ROLE OF IFRD1 IN UROTHELIAL PLASTICITY AND REGENERATION

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The bladder urothelium forms a highly specialized watertight barrier to urinary wastes. The urothelium offers an unusual example of tissue regeneration: although urothelial cells do not rapidly turn over under physiological conditions, they have an impressive capacity to regenerate tissue upon injury. Even more remarkable, depending on the modality of injury (sterile, infectious) there appear to be two distinctive modes of urothelial regeneration. We have previously shown that in response to a urinary tract infection (UTI), the urothelial stem cell niche becomes activated and induces rapid restoration of the urothelium, whereas, regeneration following sterile injury does not involve stem cell activity. However, the key driver(s) of mode of regeneration choice has yet to be elucidated.

To better understand the regulatory pathways important for tissue regenerative response, we performed large unbiased RNA-Seq and proteomics analyses. We identified interferon-related developmental regulator 1 (IFRD1), a transcriptional co-regulator, as a gene that is rapidly activated upon the induction of a UTI. Ifrd1 has been shown recently to be important for paligenosis, a process differentiated cells use to reenter the cell cycle to regenerate lost tissue.

Interestingly, we observe that even in the absence of injury, loss of Ifrd1 results in gross urothelial defects: excess vesicular congestion in terminally differentiated cells including aberrant accumulation of mitochondria and abnormal endoplasmic reticulum (ER). Furthermore, we show that Ifrd1 affects localization and trafficking of uroplakins, tetraspanin proteins that constitute organized urothelial plaques, and which dimerize in the ER and assemble into heterotetramer in the Golgi and trans-Golgi network (TGN), where they undergo chain-specific glycosylation and proteolytic processing and eventual degradation. Loss of Ifrd1 results in dysfunctional uroplakin ER-> Golgi translocation and aberrant accumulation in ER. Proteomic analyses revealed a significant increase in the unfolded protein response (UPR) and stress. In agreement with this, we note an increase in the ER chaperone, Bip and increased phosphorylation of eIF2α, the critical translation initiation factor that quenches global, cellular mRNA translation and can ultimately trigger apoptosis. Indeed, induction of injury in the Ifrd1-/- mouse results in massive epithelial exfoliation into the urine and dysregulated recruitment of progenitor cells for regeneration.

Ongoing work is elucidating the molecular underpinnings of this response. In sum, we suggest IFRD1 plays a role in the decision-making matrix of urothelial regeneration and that IFRD1 plays a role in urothelial plasticity.

EPITHELIAL ESTROGEN RECEPTOR-ALPHA IS INVOLVED IN THE DEVELOPMENT OF LOWER URINARY TRACT DYSFUNCTION

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INTRODUCTION AND OBJECTIVE: A shift in hormone levels naturally occurs in aging men and contributes to the development of lower urinary tract dysfunction (LUTD). We previously determined that in utero and lactational exposure to the persistent environmental toxicant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), invokes susceptibility to voiding dysfunction in the testosterone (T) and 17β-estradiol (E2) model of LUTD. It was further discovered that estrogen receptor-alpha (ER-α) was necessary for the development of LUTD in C57Bl/6 male mice. The objective of this study was to determine if epithelial ER-α was vital for the development of LUTD.

METHODS: To determine the specific role of estrogen receptor-alpha (ER-α) in prostatic epithelium we used epithelial-specific cre (B6.Cg-Shh tm1(EGFP/cre)Cit/J) mice crossed to ER-α floxed mice, which deleted prostatic epithelium ER-α. These mice were exposed in utero to TCDD at embryonic day 13.5 (or corn oil), then in adulthood at six weeks were treated with T (25mg) and E2 (2.5mg) or sham using slow release subcutaneous implants. To assess voiding behavior, we utilized void spot assays (VSA) each week for four weeks following hormone treatment. Mice were necropsied after the 4th VSA to measure the bladder volume, bladder mass and prostate mass.

RESULTS: We observed decreased bladder mass and volume in the epithelial ER-α knockout in comparison to wild type for the TCDD and hormone treated groups along with differences in prostate lobe sizes. There was also a marked difference in voiding patterns, in which the TCDD and hormone treated wild type exhibited a higher spot count than the ER-α knockout under the same treatment conditions.

CONCLUSION: The findings of this study suggest that epithelial ER-α plays a key role in the development of lower urinary tract dysfunction (LUTD) in this 2-hit model of LUTD. Future work will incorporate mass spectrometry and proteomics to investigate changes in protein abundance specific to the prostatic tissues. Furthermore, to determine the role of ER-α in other cell types, namely the stroma, future work will use stromal-specific smooth muscle cre: (B6.Cg-Tg(Tgln-cre)1Her/J) mice under the same treatment conditions. We hope to find a novel molecular target that may be used for the development of therapeutics to treat diseases such as benign prostatic hyperplasia.

VOIDING AND MUSCLE CONTRACTILITY DYSFUNCTION IN A NOVEL MODEL OF DETRUSOR UNDERACTIVITY

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INTRODUCTION AND OBJECTIVE: Detrusor underactivity (DUA) is an understudied health concern with inadequate clinical management. The limited availability of animal models that exhibit the pathophysiology of DUA impedes the development of new therapeutic approaches. The current studies characterized voiding function of an obese prone (OP) rat model of DUA and measured the contractility of detrusor and urethral tissues.

METHODS: Eight-week old female OP and obese resistant (OR) rats were fed a 60% fat diet from 9 weeks old to end of study. At 45-65 weeks old, rats were anesthetized with urethane (1.2 g/kg sc) for acute urodynamic studies. A catheter was inserted into the bladder dome to infuse saline and measure pressure. A bipolar paddle electrode was placed between the pubic symphysis and the EUS to record EMG signals. After voiding function testing, rats were euthanized and full thickness longitudinal bladder strips and urethral rings (proximal and middle) were harvested and mounted in chambers (DMT, Denmark). Viability was assessed with potassium chloride (120mM). Both bladder strips and urethral rings underwent escalating concentration response curves to carbachol ($10^{-8}M$ to $10^{-5}M$). Urethral rings were then exposed to caffeine ($4 \times 10^{-2}M$) for 10 min. Bladder strips also underwent electric field stimulation (EFS) with or without atropine ($10^{-7}M$) or alpha,beta-methylene ATP ($10^{-6}M$), whereas EFS in urethral rings was in the presence or absence of dantrolene ($10^{-5}M$).

RESULTS: Voiding efficiency decreased by 31% in OP rats compared to OR rats (95% CI = -41, -22, p < 0.0001). EUS activity during filling increased by 4.6 µV (95% CI = 2.4, 6.8, p = 0.0011) and EUS bursting time decreased by 1.45 s (95% CI = -2.57, -0.317, p = 0.0175) in OP rats. In addition, pressure at volume threshold increased in OP rats by 6.8 cmH2O (95% CI = 2.2, 11, p = 0.0067). Compared to OR rats, OP rats had decreased contraction strength on exposure to carbachol in the bladder (p = 0.0002) and proximal urethra (p = 0.0004), whereas OP rats had increased contraction strength in the middle urethra (p = 0.002). Following EFS, OP rats had increased contractility at 32 Hz in both the proximal (p = 0.006) and middle (p = 0.047) urethra. Contractility was also increased with caffeine in the middle urethra of OP rats (p = 0.006).

CONCLUSIONS: These results suggest that impaired cholinergic-dependent contractions of the bladder may contribute to poor voiding function in OP rats. In addition, the middle urethra of OP rats appears to compensate for poor bladder contractility by increasing its activity and responsiveness. This urethral compensation may indicate an attempt to stimulate the pudendo-vesical reflex to promote bladder activation.

DECREASED CD44 EXPRESSION IS ASSOCIATED WITH INCREASED HYALURONIC ACID ABUNDANCE IN HUMAN LICHEN SCLEROSUS

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INTRODUCTION AND OBJECTIVE: Lichen sclerosus (LS) is a poorly understood chronic inflammatory disease of the genital skin. In affected men, LS is associated with severe urethral stricture disease refractory to conventional surgical treatment. Stromal accumulation of hyaluronic acid (HA) is a distinguishing feature of LS, and HA is known to drive chronic inflammation and fibrosis in other diseases. CD44 is the principal cell surface receptor involved

in HA degradation and has been previously suggested to be downregulated in LS. We postulate that decreased epithelial CD44 expression in LS causes decreased sequestration of HA thereby leading to stromal accumulation of HA and propagation of inflammation and fibrosis. In this experiment, we will compare CD44 expression and HA abundance in human LS and control tissues to test the specific hypothesis that epithelial CD44 expression is lower and stromal HA abundance higher in human LS.

METHODS: The experimental group includes 30 patients with pathologic diagnosis of LS after circumcision or urethroplasty (male patients) or vulvar biopsy (female patients). The control group includes 30 patients without LS after the same procedures. We will use a multiplexed IHC approach to detect CD44 and HA expression, and use Vectra imaging system to scan the slides and quantify CD44 and HA abundance in three histologic compartments: epidermal, inflammatory, and stromal. CD44 expression and HA abundance will be measured as mean optical density in six regions of interest (ROIs) per slide (2 ROIs per compartment). LS and control patients will be compared with respect to each histologic compartment using the Student's T-test.

EXPECTED RESULTS: Our expected results are summarized in the Table as predicted differences in LS patients relative to controls. We anticipate LS patients will display: 1) significantly decreased CD44 expression, 2) increased HA abundance in the stromal compartment, and 3) increased CD44 expression and HA abundance in the inflammatory compartment.

CONCLUSIONS: We expect our quantitative approach to demonstrate downregulation of epidermal CD44 expression and increased stromal HA accumulation in human LS. These data will provide a foundation to support the overarching hypothesis that CD44 dysregulation drives HA accumulation and propagation of inflammation and fibrosis characteristic of LS.

Table: Expected CD44 expression and HA abundance in the three compartments of genital skin of lichen sclerosus patients relative to control patients

	Epithelial	Stromal	Inflammatory
CD44 expression	Decreased	Increased/ unchanged	Increased
HA abundance	Decreased/ unchanged	Increased	Increased

MITOCHONDRIAL DYSFUNCTION IN BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION AND OBJECTIVE: Aging remains the biggest risk factor for BPH/LUTS, yet little is known about its exact effect on the lower urinary tract, disease initiation and progression, and response to treatment. Age-dependent decline in mitochondrial function is a significant contributing factor to the development of many diseases and conditions in the elderly but has not been investigated in the prostate. Mitochondrial dysfunction is characterized by a loss of electron transport chain (ETC) efficiency leading to a decreased production of ATP as well as excessive leaking of electrons and generation of reactive oxygen species. It commonly leads to an increase in mitochondrial mass in an adaptive response to overcome diminished mitochondrial bioenergetics and a decrease in the elimination of damaged mitochondria through the process of mitophagy. In collaboration with the O'Brien Center at the University of Wisconsin-Madison, we have set out to explore the potential role of mitochondria dysregulation in prostatic inflammation and lower urinary tract dysfunction.

METHODS: The RWPE-1 human prostatic cell line was used to examine the effects of the ETC complex I inhibitor, rotenone, on various markers of autophagy and inflammation by Western blot analysis and in situ analysis of mitophagy using a proximity ligation assay. Mitochondrial markers were examined in mouse, rat and human prostate tissue by immunohistochemical staining/

RESULTS: Our studies suggest that mitochondrial homeostasis may be disrupted in BPH specimens. Furthermore, preliminary data suggest that electron transport ETC complex I may be selectively impaired in a rodent aging/BPH model. Acute pharmacological inhibition of ETC complex I activity in vitro by the pesticide, rotenone, in human prostate epithelial cells and in rats have also identified specific disruptions in prostatic mitochondria homeostasis (e.g. altered mitophagy).

CONCLUSIONS: Considering the importance of mitochondria in aging diseases, tissue homeostasis, and inflammation, mitochondria dysregulation is likely a critical step in BPH pathogenesis and will be the focus of ongoing studies in cell culture, animal models and human tissue.

THE MAJOR CONTRIBUTION OF CAVITATION TO STONE DAMAGE IN DUSTING MODE DURING LASER LITHOTRIPSY

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INTRODUCTION AND OBJECTIVE: During Holmium (Ho):YAG laser lithotripsy (LL), rapid vaporization of fluid at the tip of the laser fiber results in the formation of a vapor bubble that improves laser energy transmission to the stone. The contribution of cavitation to stone damage in LL, particularly by the bubble collapse, has been largely neglected. Here, we investigate the contribution of the LL-generated bubble collapse to stone damage during dusting treatment.

METHODS: Artificial stone samples (BegoStone, Bego USA) were polished, soaked for 24 hours, and treated using the dusting mode (0.2 J, 20 Hz) from a clinical Ho:YAG lithotripter (H Solvo 35-watt laser, Dornier MedTech) in water and in air to differentiate the effect of cavitation from photothermal ablation. The laser fiber was oriented perpendicularly to the sample surface at standoff distances (SD) of either 0 or 0.5 mm from the stone. In addition, we devised a novel counter-plate setup (**Fig. 1a**) during 0.5 mm SD treatment in water to minimize the damage contribution from cavitation by altering the collapse the LL-generated bubble away from the stone. Stone damage was quantified using optical

coherence tomography (OCT, OQ Labscope, Lumedica) following treatment with varying number of laser pulses (15 – 1000).

RESULTS: Stones treated in water under non-contact (SD = 0.5 mm) resulted in significantly larger and wider craters compared to those treated with the fiber in contact (SD = 0 mm) with the stone (**Fig. 1b-d** blue and purple lines). Stones treated in air resulted in negligible craters compared to their counterparts in water regardless of SD (Fig. 1b-d warm vs. cool colored lines). The addition of the counter-plate dampened the bubble collapse and significantly reduced the stone damage in water at SD = 0.5 mm (**Fig. 1b-d** black line).

Figure 1.
(a) Counter-plate setup to alter the collapse of the LL bubble and OCT quantification of the damage crater produced by dusting mode (0.2 J and 20 Hz), (b) volume, (c) depth, and (d) surface area following treatment with varying number of pulses and treatment conditions.
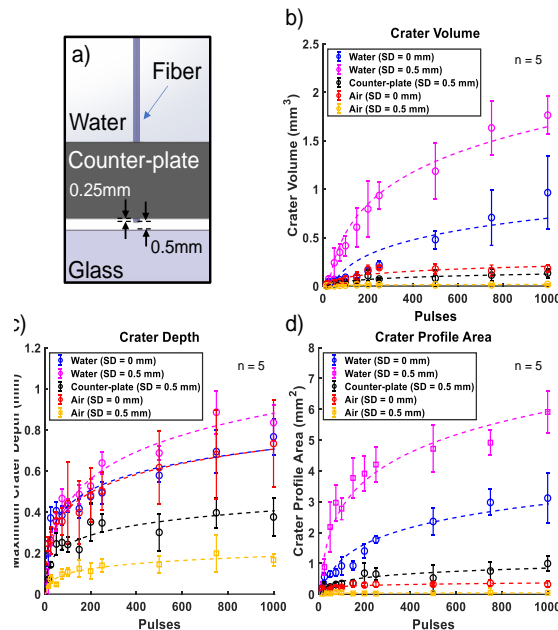
CONCLUSIONS: This study demonstrates that repeated bombardments from the microjet impact of the bubble collapse towards the stone surface is a major contributor of stone damage during dusting mode LL. This has considerable implications on treatment optimization when dusting stones. The standard notion for optimizing LL treatment is to minimize the fiber distance from the stone to deliver the most amount of laser energy to the stone. However, our study demonstrates that dusting in non-contact mode to increase cavitation induced damage at the cost of decreased laser energy absorbed by the stone may significantly improve the damage efficiency of LL dusting.

SACRAL NEUROMODULATION IN RATS: PARAMETERS AND PATHWAYS

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INTRODUCTION AND OBJECTIVE: The neural pathways mediating the effects of sacral neuromodulation remain unclear. We conducted acute cystometry and mapping studies in female rats to determine the neural pathways mediating stimulation outcomes and quantify the effects of stimulation parameters.

METHODS: Experiments were conducted in female CD rats anesthetized with urethane (1.2 g/kg SC) and paralyzed with gallamine. Mapping experiments (n=5) were conducted with stimulation electrodes placed on L6 and S1 nerves (at the sacroiliac junction). Recording electrodes were placed on the motor pudendal, sensory pudendal, and sciatic nerves. Input-output curves of evoked compound action potentials were generated by L6 or S1 stimulation at 3-10 times motor threshold (T). In separate experiments single fill saline cystometry was conducted with L6 or S1 stimulation (1-1.5T, 10 Hz, n=6) and sensory pudendal stimulation (1-1.5T, 10 Hz). Subsequently, additional cystometric experiments were conducted with L6-S1 trunk stimulation (1-6T, 10 Hz, n=4).

RESULTS: Sensory pudendal activity was always evoked by L6 stimulation, but only evoked by S1 stimulation in 4/11 experiments. In 4 mapping experiments using tripolar cuffs stimulation electrodes to minimize current spread, sciatic activation was minimal (n=1) or absent (n=3) by L6 stimulation and absent by S1 stimulation. L6 and S1 stimulation at 1-1.5T had negligible effects on bladder capacity or voiding efficiency, whereas sensory pudendal stimulation increased bladder capacity (179 ± 6%, p < 0.001) and decreased voiding efficiency (-42± 16%, p=0.044). L6-S1 trunk stimulation increased bladder capacity at amplitudes ≥2T in 1 experiment, ≥6T in 2 experiments, and not at any amplitude in another experiment. No parameters of L6-S1 trunk stimulation generated consistent increases in voiding efficiency.

CONCLUSIONS: S1 appears to contribute minimally to sensory pudendal activation. Neither L6 nor S1 stimulation at 1-1.5T, 10 Hz reliably changed bladder capacity or voiding efficiency whereas sensory pudendal stimulation reliably increased bladder capacity. Recent work in humans suggests that threshold amplitude sacral neuromodulation may not immediately impact urinary function in women with incontinence1, consistent with our observations, and suggesting that low-amplitude sacral neuromodulation acts via a plasticity-dependent pathway.

[Reference available from author]

PRELIMINARY RESULTS OF BASELINE CORTICAL NEURAL ACTIVITY IN MEN WITH BENIGN PROSTATIC HYPERPLASIA AND BLADDER OUTLET OBSTRUCTION

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INTRODUCTION: Benign prostatic hyperplasia (BPH) affects the entire micturition cycle including filling and storage phases, creating bothersome lower urinary tract symptoms (LUTS).1 Persistent bladder outlet obstruction (BOO) remodels the entire lower urinary tract including the detrusor, connective tissue and local neural network; however, the extent to which it alters the central nervous system (CNS) in BPH is unknown.2,6 This study explores brain activation patterns on fMRI in men with BPH and BOO.

METHODS: Men ≥ 45 years old who failed conservative BPH therapy planning to undergo BOO procedures were recruited. Eligible men underwent concurrent fMRI-urodynamics (UDS) examination, during which the bladder was filled with sterile water until subjects signaled a strong desire to void.3,4 Subjects were instructed to hold for 30 seconds, after which they were given permission to void. UDS was performed concurrently to monitor the filling/voiding cycle. After voiding/attempt to void, the bladder was drained and the cycle was repeated up to four times. fMRI images were obtained via T1W 3 Tesla MRI images. Significant activated voxels (p<0.05) were identified at strong urge to void and voiding initiation/attempt to void.

Figure 1. Brain activation of seven BPH men prior to BOO procedure, at a) Full urge, and b) Voiding initiation. Blue markings represent areas of deactivation, while red marks indicate areas of activation. Green crosshairs are used to indicate brain regions associated with activation and deactivation. Table 1, patient characteristics.
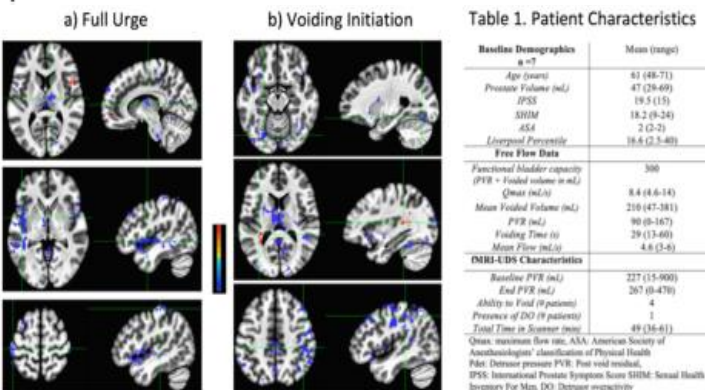
RESULTS: Seven men with baseline demographics are represented in **table 1**. At strong urge to void, there was activation in the right inferior frontal gyrus (IFG) (p<0.05), **fig 1a**. Deactivation was seen bilaterally in thalamus, middle frontal gyrus, insula and parahippocampal gyrus, and additionally in left middle/superior temporal gyrus (p<0.05). At voiding initiation activation was seen in the left angular gyrus/superior temporal gyrus, and the IFG, **fig 1b**. Only four of seven patients were able to void supine during fMRI scan.

CONCLUSION: This is the first study evaluating cortical activity in men with BPH and BOO. At full urge there was significant activation of the IFG, consistent with the meta-analysis data from Harvie et al showing its significance during the voiding phase. However, there were important deactivations including bilateral thalamus, insula, and IFG during initiation and voiding attempts previously shown to be important regions in normal micturition in healthy adults.5,7,8,9 Further studies should evaluate whether deactivation in these regions reflect the inability to void of multiple subjects and/or represent neuroplastic changes of supraspinal micturition control in response to chronic outlet obstruction in men with BPH.

[References for this abstract may be requested from the authors]

BACTERIAL METABOLIC RE-WIRING FUELS TRANSIENT RESISTANCE TO POSITIVELY CHARGED ANTIBIOTICS

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INTRODUCTION AND OBJECTIVE: Antibiotic resistance among bacterial pathogens has become a global health threat, fueling the efforts to develop new antimicrobial treatments. Bacteria can mount resistance to antibiotics through acquisition of mobile genetic elements as well as through chromosomally encoded systems. We have recently reported the presence of an intrinsic, transient resistance mechanism in uropathogenic Escherichia coli (UPEC) that confers resistance to positively charged antibiotics, such as the polymyxins and aminoglycosides. We demonstrated that ferric iron stimulation of the polymyxin resistance (Pmr)B sensor histidine kinase led to transcriptional activation of two regulators: the cognate response regulator PmrA and the non-cognate QseB. These two response regulators are transcriptional regulators. Together, after stimulation of the histidine kinase PmrB, they mediate the transcription of genes that modify the lipopolysaccharide (LPS) of the bacteria. These modifications alter the LPS charge, effectively making it less negatively charged which in turn impairs the attraction of positively charged antibiotics. In this work, we interrogate the regulon of PmrA and QseB in response to ferric iron stimulation. We also demonstrate a novel role for QseB in controlling the metabolic burden of modifying LPS.

METHODS: A well described cystitis UPEC isolate, UTI89, was utilized for these experiments. The transcriptomes of UTI89 and isogenic mutants ΔpmrA, ΔqseB and ΔqseBΔpmrA were analyzed by RNAseq prior to and post ferric iron stimulation. Extracted RNA was sequenced using the Illumina platform. To determine direct transcriptional targets of QseB, a ChiP on chip experiment was performed. To determine survival against antibiotics, survival assays were used. The aminoglycosides amikacin and gentamicin were utilized. The polymyxin, polymyxin B, was also tested. Briefly, cells were subcultured from an overnight culture and allowed to reach the mid-logarithmic growth phase. Cells were then normalized and subjected to a challenge of antibiotic at 5 times the minimum inhibitory concentration (MIC). Samples were taken before the addition of antibiotic and at 15, 60, and 180 minutes post exposure. Percent survival was calculated by comparing strains at each time point to the pre-challenge sample. A challenge with nitrofurantoin, an antibiotic with a neutral charge, was included as a control and due to the kinetics of killing samples were taken at 15, 60, 180, 300, 420, and 540 minutes post challenge. In a similar set up of polymyxin B challenge, samples were taken and assessed for the metabolites: coenzyme A, aspartate, and glutamate. The concentrations of these metabolites were measured using colorimetric kits.

RESULTS: Activation of the PmrAB and QseBC systems lead to LPS modifications that confer resistance to positively charged antibiotics such as the aminoglycosides and polymyxins. We found many genes upregulated in response to ferric iron in wild-type UTI89. Of these genes, 16 encode LPS modifying enzymes, including pmrC and yibD. Analysis of the ΔpmrA, ΔqseB and ΔqseBΔpmrA mutants showed that some of the LPS modifying genes are under the control of both PmrA and QseB, consistent with the abrogation of polymyxin B resistance in the and ΔqseBΔpmrA mutant. We also found that several metabolism genes were upregulated. Many of these were confirmed to also be targets of QseB by the ChiP-on-chip experiment. This revealed that a key LPS modifying reaction mediated by ArnB was under QseB control. This reaction converts an LPS intermediate to a modified version and consumes an oxoglutarate molecule converting it to glutamate in the process. Glutamate is then utilized by the cell and converted back to oxoglutarate through the pantothenate, arginine, and GABA synthesis pathways. Several genes in these pathways are either upregulated after stimulation or controlled by QseB.

CONCLUSION: Combined, these results demonstrate a novel role for QseB as a mediator of metabolism during LPS modification. These results also show a mechanism for resistance to positively charged antibiotics mediated by PmrAB and QseBC.

EFFECTS OF INTRAVESICAL INSTILLATION OF LIPOSOME-CONJUGATED ANTISENSE OLIGONUCLEOTIDE TARGETING NERVE GROWTH FACTOR ON BLADDER OVERACTIVITY IN A RAT MODEL OF NON-BACTERIAL PROSTATIC INFLAMMATION

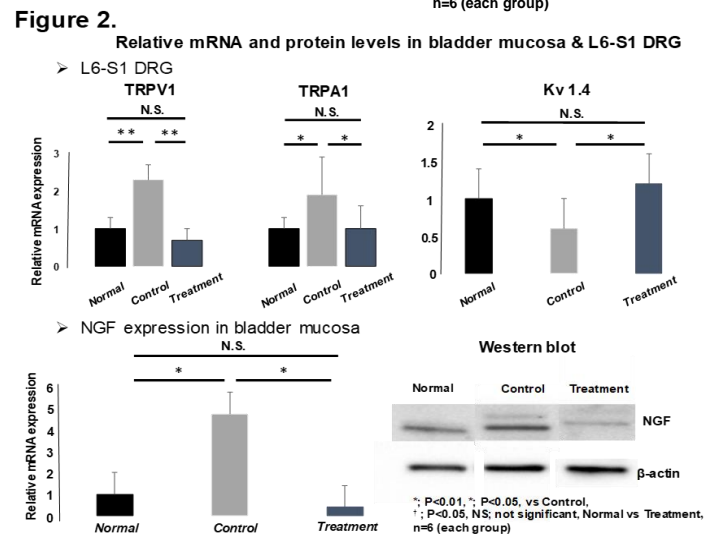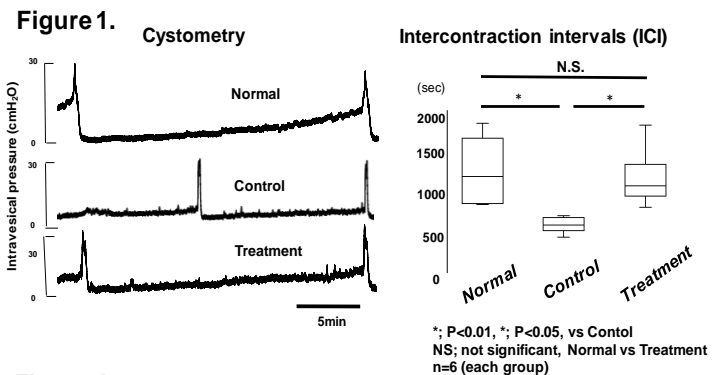(34) Taro Igarashi, Pradeep Tyagi, Tetsuichi Saito, Zhou Wang and Naoki Yoshimura

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INTRODUCTION AND OBJECTIVE: Local upregulation of nerve growth factor (NGF) in the bladder has been implicated in lower urinary tract dysfunction such as bladder overactivity due to afferent sensitization. Also, prostatic inflammation (PI) associated with benign prostate hyperplasia (BPH) has been recognized as a contributing factor to BPH symptoms. Thus, the present study aimed to examine the effect of instillation of liposomes-conjugated NGF antisense oligonucleotide (OND) into the bladder on local overexpression of NGF and bladder overactivity in a rat model of PI.

METHODS: Male SD rats were divided into three groups: (1) Normal group without PI, (2) Control group; with PI induced by 5% formalin injection into the ventral lobes and intravesical instillation of normal saline (NS), (3) Treatment group; with PI and intravesical instillation of liposome-conjugated OND on day 14. Then, on day 28, we evaluated awake cystometry (CMG) and harvested tissues to analyze protein and mRNA levels of NGF in the bladder mucosa, and mRNA levels of C-fiber afferent markers and a potassium channel subunit in L6-S1 dorsal root ganglia (DRG).

RESULTS: IN CMG, Treatment group had significantly longer intercontraction intervals than Control group while there were no significant differences between Treatment and Normal groups (**Fig. 1**). mRNA levels of TRPV1 and TRPA1 in DRG of Treatment group were significantly lower and Kv 1.4 subunit levels were higher than Control group while there were no significant differences between Treatment and Normal groups (**Fig. 2**). Both mRNA and protein levels of NGF in the bladder mucosa in Treatment group were significantly reduced compared to Control group while there were

no significant differences between Treatment and Normal groups (Fig. 2).

Figure 1.

Cystometry

Intercontraction intervals (ICI)

*; P<0.01, *; P<0.05, vs Contol
NS; not significant, Normal vs Treatment
n=6 (each group)

Figure 2.

Relative mRNA and protein levels in bladder mucosa & L6-S1 DRG

➢ L6-S1 DRG

TRPV1   TRPA1   Kv 1.4

➢ NGF expression in bladder mucosa

Western blot

*; P<0.01, *; P<0.05, vs Control,
†; P<0.05, NS; not significant, Normal vs Treatment,
n=6 (each group)

CONCLUSIONS: These results indicate that intravesical treatment with OND suppressed NGF overexpression in the bladder mucosa and improved bladder overactivity and hypersensitivity induced by prostatic inflammation. Therefore, local NGF-targeting therapy could be useful for the treatment of storage symptoms in BPH patients with prostatic inflammation. [Funding: U54 DK112079]

IMMUNOHISTOCHEMICAL ANALYSIS OF EPITHELIAL CELL COMPOSITION IN THE MOUSE PROSTATIC URETHRA

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INTRODUCTION AND OBJECTIVE: Benign Prostatic Hyperplasia (BPH) is the non-malignant enlargement of the prostate that affects 80% of men aged 80 and older. Although an estimated $4 billion is spent annually on the medical management of BPH, treatment is not always effective, warranting further investigation into BPH pathology. BPH is driven by epithelial cell proliferation, which constricts the urethra and causes lower urinary tract dysfunction

(LUTD). Current therapies target luminal epithelial proliferation, however, approximately 8-10% of BPH specimens encompass basal cell proliferation and which remains untargeted. The purpose of this study is to identify whether the proportion of luminal and basal cells change in aging and steroid hormone-induced mouse models of LUTD. We are specifically interested in pathological changes in the mouse prostatic urethra, the region identified in our laboratory to most closely resemble the human prostate.

METHODS: Tissue sections were taken at the midpoint of the prostatic urethra from 3-month old untreated (UNT) (n=6), 24-month old UNT (aged mice, n=4), and 3-month old mice treated with testosterone and estradiol pellets for 1 month (T+E2, n=7). Immunohistochemistry was performed to label p63-positive basal cells and counterstained with hematoxylin to label nuclei. Epithelial cells negative for p63 were counted as luminal cells. The percentage of basal and luminal cells in prostate glands surrounding the urethra and in the urothelium was calculated and averaged for each experimental group.

RESULTS: In urothelium, there was a significantly higher percentage of luminal cells in aged (51.43%) versus young (31.84%, p=0.0125) mice. There was a trend for increase in the percentage of luminal cells in T+E2 (37.75%) versus UNT (31.84%, p=0.1115) urothelium. In the periurethral prostate glands, there was a near-significant increase in the percentage of luminal cells in T+E2 (62.84%) versus UNT (55.40%, p=0.0554) mice.

CONCLUSIONS: These results suggest that there is an increase in prostate luminal cells in the urothelium in LUTD mice, which could be an indication of luminal cell hyperplasia. Furthermore, basal cells were present in mouse prostate glands, suggesting that glands in the prostatic urethra have similar histology to mouse prostate lobes. In contrast, we did not identify basal cell hyperplasia in either LUTD models indicating the need for the development of new strategies to study this pathological phenotype.

UTILITY ESTIMATION FOR NEUROGENIC BOWEL DYSFUNCTION IN THE GENERAL POPULATION

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INTRODUCTION: Neurogenic bowel dysfunction (NBD) affects over 80% of individuals with spina bifida causing bowel incontinence and/or constipation. NBD is also associated with decreased quality of life, depression, anxiety, and decreased employment/ educational attainment. Because NBD is a life-altering condition without a cure, understanding the utility of different health states related to NBD would aid clinicians as they try to counsel families regarding management options and to better understand the quality of life associated with disease management. The aim of this study was to elicit utility scores for NBD using an online community sample.

METHODS: A cross-sectional anonymous survey was completed by 1534 voluntary participants via an online platform (Amazon Mechanical Turk (MTurk, www.mturk.com)), representing an 87% response rate. The survey presented hypothetical scenarios that asked respondents to imagine themselves as an individual living with NBD or as the caretaker of a child with NBD. The time trade-off (TTO) method was used to estimate a utility score, and outcomes for each scenario were calculated using median and IQR. Univariate comparisons of distributions of TTO for demographic data were made using Kruskal-Wallis tests.

RESULTS: The median utility score for NBD was 0.84 [0.70-0.92]. Participants reported that they would give up a median of 5 years of their own life, to prevent NBD in themselves of their child. Utility values for child scenarios were significantly different when stratified by age, gender, race, parental status, marital status, and income. Stratification by current health status did not yield significantly different utility values.

CONCLUSION: NBD is perceived by the community as having a substantial impact to health-related quality of life for children with spina bifida, representing a 16% reduction from perfect health. In general, health state utilities have been increasingly used in healthcare systems to understand how burdensome a population perceives a disease is and to evaluate whether interventions improve quality of life years.

NOVEL APPROACH IN UROLOGICAL RESEARCH REVEALS DIFFERENTIAL ION CHANNEL SUBCELLULAR LOCALIZATION IN HUMAN URINARY BLADDER SMOOTH MUSCLE

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INTRODUCTION AND BACKGROUND: Contraction and relaxation of detrusor smooth muscle (DSM) control micturition. DSM cell excitability and contractility depend on synchronized activity of multiple ion channel types. Our group, in collaboration with urologists, has the unique advantage to study the expression, function, and regulation of human DSM ion channels. Here, we have focused on two key DSM channel families: the TRPM (activation causes membrane depolarization and contraction) and voltage-gated Kv7 (activation causes hyperpolarization and relaxation). To exert their regulatory role, these channels would be expected to be localized to the DSM plasma membrane but so far investigations confirming this are lacking. To test this hypothesis, we selected the three most important family member representatives, the TRPM4, Kv7.4 and Kv7.5 channels in order to investigate their cellular localization.

METHODS: We have employed a novel technique called 'surface biotinylation' in conjunction with immunocytochemistry to examine the overall plasma membrane versus intracellular localization of these three ion channels. Human DSM tissue strips were incubated with non-cell permeable biotin tagged reagents that specifically bind to cysteine and lysine protein residues. Biotinylated surface proteins were then separated using avidin beads, eluted and Western blotting performed to determine the overall surface (plasma membrane) to intracellular localization of these channel proteins. Immuocytochemistry analyses for Kv7.4 and Kv7.5 channels were also performed on freshly isolated human DSM cells.

RESULTS: Surface biotinylation revealed that >85% of total TRPM4 protein was localized to the surface of human DSM cells,

with only ~15% appearing in the intracellular fraction. Similarly, >82% of total KV7.4 and ~66% of total Kv7.5 proteins were also localized on the surface of DSM cells. Interestingly, the Kv7.5 distribution surface/intracellular (~66%/34%) ratio was the lowest among the three channels studied. Immunocytochemistry data analyses revealed that Kv7.4 channels were predominantly surface localized while Kv7.5 displayed a uniform distribution.

CONCLUSION: By employing surface biotinylation, a novel approach in urological research, along with immunocytochemistry, we revealed differential expression of ion channel subunits in human DSM. These exciting new data offer vital clues to the relative importance of the TRPM4 and Kv7 channels in regulating human bladder function.

MITOCHONDRIAL DYSFUNCTION AND CELLULAR SENESCENCE DUE TO AGING CONTRIBUTES TO PROSTATIC FIBROSIS

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INTRODUCTION AND OBJECTIVE: Aging is the single largest risk factor for many common diseases that burden public health. This is especially true in the prostate; as men age, the prostate undergoes a prototypical aging change, fibrosis. The aged fibrotic prostate causes urinary symptoms which will afflict nearly every man if they live long enough. However, the molecular mechanisms responsible for the aging-dependent promotion of fibrosis are largely unknown and understudied. In this study, we sought to reveal the contribution of mitochondrial dysfunction to fibrosis in the aging prostate, ultimately leading to prostate dysfunction, urinary symptoms, and overall poor health.

METHODS: Immunohistochemistry was performed on formalin-fixed, paraffin-embedded human and rodent tissue samples using Ndufs3 and PINK1 as markers for altered mitochondrial homeostasis. Additionally, tissues were assessed for fibrosis using picrosirius red (PSR) staining. BHPrS1 cells, a human prostate stromal cell line, were treated with 25nM rotenone to inhibit mitochondrial electron transport chain complex I. qPCR was performed to assess gene expression of collagen genes.

RESULTS: The demise of mitochondrial function is well established in other aging-associated diseases but has not been investigated in the prostate. Our analysis revealed an increase in cellular senescence and mitochondrial dysfunction in BPH patient tissue compared to normal prostates. Furthermore, selective inhibition of mitochondrial complex I by rotenone in cultured human prostate fibroblasts led to myofibroblast phenoconversion as characterized by increased expression of Col1a1, Col3a1, and ACTA2. To determine the contribution of mitochondrial dysfunction on fibrosis and lower urinary tract dysfunction (LUTD), we examined the prostate lobes in both aging and steroid-induced LUTD in mice. These models have been extensively characterized and exhibit an age-mediated increase in LUTD and fibrosis. We observed a decrease in mitochondrial function and an increase in cellular senescence corresponding to an increase in fibrosis and LUTD.

CONCLUSIONS: This suggests that normal aging-dependent reductions in mitochondrial complex I function in the prostate may promote fibrosis and contribute to urinary dysfunction.

IMPACT OF BISPHENOL-A ON UROGENITAL TRACT MORPHOLOGY IN DEVELOPING MICE

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INTRODUCTION AND OBJECTIVE: Found in everyday consumer plastics, Bisphenol-A (BPA) is a xenoestrogen that has shown positive correlations with the development of chronic urogenital-related illnesses in aging males. The large-scale exposure of BPA is especially concerning due the lacking research being done on the effect of BPA exposure on the urogenital tracts of infants and developing children. In this study, we sought to visualize the effects of early BPA exposure on the urogenital tract morphology and anatomy of developing mice utilizing 3D reconstruction software.

METHODS: BioVis 3D reconstruction was performed on four experimental treatment groups: control, 5 μg BPA/kg, 50 μg BPA/kg, and 500 μg BPA/kg. For each 3D reconstruction, eight morphological structures of the urogenital tract were reconstructed: Seminal Vesicles, Vas Deferens, Urethra, Utricle, Anterior Pituitary Ducts, Ventral Pituitary Ducts, Lateral Pituitary Ducts, and Dorsal Pituitary Ducts.

RESULTS: The pre-disposition for chronic urogenital-related illnesses in aging males exposed to BPA has been well researched but has not been investigated for infants and developing children. Our analysis revealed the morphological structures of the developing urogenital tracts from BPA treated mice, which can be utilized in the future analysis of urogenital tract development and the associated health risks that arise from early BPA exposure in humans. This research also reveals the effects of BPA on lobe development and the potential adverse impacts or changes BPA has on the early development of the urogenital tract.

CONCLUSION: Findings from this study will help the benign urological community in the future by providing the early morphological effects of BPA on the urogenital tract under normal and exposed conditions, which will give us better tools that aid in understanding the developmental origins of disease.

AGE-DEPENDENT EXPRESSION OF TRPM4 CHANNEL IN GUINEA PIG DETRUSOR SMOOTH MUSCLE IS ASSOCIATED WITH ALTERED CONTRACTILITY

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INTRODUCTION AND OBJECTIVE: During development, maturation, or aging, the expression and function of detrusor smooth muscle (DSM) ion channels can change, thus affecting micturition. Increasing evidence supports a novel role of transient receptor potential melastatin type 4 (TRPM4) channels in DSM physiology. However, it remains unknown whether the properties of TRPM4 channels in DSM fluctuate over different life stages. Here, we examined TRPM4 channel protein expression and the effects of TRPM4 channel inhibitors, 9-phenanthrol (9-Phen) and

glibenclamide (Glib), on phasic and tonic contractions of DSM isolated strips from juvenile and adult guinea pigs.

METHODS: Male Hartley-Albino guinea pigs, juveniles (5-9 weeks old; N=23) and adults (6-18 months old; N=23), were used in this study. All studies were on DSM without mucosa. Western blot was employed for the determination of total TRPM4 expression as well as for the detection of intracellular and plasma membrane fractions using cell surface biotinylation labelling. Isometric tension recordings of DSM isolated strips were performed using increasing cumulative concentrations of 9-Phen or Glib. $P<0.05$ (two-tailed) was considered statistically significant for Student's t-test or two-way ANOVA followed by the post hoc Sidak multiple comparison test.

RESULTS: Compared to juveniles, adults displayed a 50-70% reduction ($P<0.05$) in total DSM TRPM4 protein expression, while the surface-to-intracellular expression ratio remained the same for the two age groups. In both adult and juvenile DSM, cell surface TRPM4 protein expression (~80%) predominated over its intracellular fraction (~20%), revealing optimized channel trafficking mechanisms toward the cell membrane. 9-Phen showed lower potencies and/or maximum efficacies in adults than juveniles for inhibiting amplitude ($P<0.05$) and muscle force ($P<0.05$) of spontaneous and 20 mM KCl-induced phasic DSM contractions. Compared to 9-Phen, Glib also attenuated both spontaneous and KCl-induced DSM contractions, but with less pronounced effects in adults.

CONCLUSIONS: We reveal that total, surface, and intracellular TRPM4 channel protein fractions were decreased in adult compared to juvenile DSM while channel trafficking remained intact. Further, 9-Phen exerted a reduced inhibitory effect on adults, both spontaneous and 20 mM KCl-induced phasic DSM contractions, where the expression of the total TRPM4 protein was reduced compared to juveniles. Collectively, our data show age-dependent expression of TRPM4 channel protein and altered function in DSM.

THE EFFECTIVENESS OF PIRFENIDONE AND HALOFUGINONE IN THE REVERSAL OF PROSTATIC FIBROSIS AND BPH IN MICE

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INTRODUCTION: Benign prostatic hyperplasia (BPH) is a very prominent disease among aging men and is characterized by the enlargement of the prostate, resulting in an obstructed urethra and lower urinary tract dysfunction (LUTD). BPH affects a significant portion of the male population and treatment costs billions of dollars in the U.S. annually. Recent studies have suggested the enlargement of the prostate seen in BPH could be the result of prostatic fibrosis. In response to these claims, we decided to test the effectiveness of two antifibrotic drugs, pirfenidone and halofuginone, in treating BPH and resolving the obstruction of the urethra.

METHODS: Using a repeated measures study design, we modeled the lower urinary tract dysfunction associated with human BPH in aging mice (18-24 months). We then measured urinary function using void spot assays (VSAs) for each mouse. We treated the mice with either halofuginone or pirfenidone for a period of six weeks with continued weekly VSAs to assess urinary dysfunction.

At the end of the study, we examined prostatic changes and corresponding fibrosis with Picrosirius red (PSR) staining.

RESULTS: While the halofuginone seemed to have little effect on void spot counts initially, there was a significant decrease in void spots after the third week of treatment ($p<0.05$). The pirfenidone, however, appeared to be more effective as there was an immediate and significant decrease ($p<0.01$) in void spot count. By the end of the study, both halofuginone and pirfenidone decreased void counts, suggesting a resolution of voiding dysfunction. PSR analysis showed an alteration of collagen deposition in both treatment groups.

CONCLUSIONS: Our results indicate that pirfenidone may be a viable candidate for treating LUTD as a result of BPH. It seems halofuginone is less effective than pirfenidone in reducing LUTD from BPH, and further investigation is required to determine the feasibility of clinical trials for halofuginone in particular. An effective and comprehensive treatment for this condition would not only save billions of dollars but also improve the quality of life for millions.

PROFILING DIFFERENCES IN SECRETED AND INTRACELLULAR PROTEOMIC EXPRESSION IN A CELLULAR MODEL OF BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION/OBJECTIVES: Benign prostatic hyperplasia (BPH) and prostate cancer are two distinct conditions that influence prostatic proteome composition. While these conditions develop as men age, pathogenesis is markedly different; thus, a deeper understanding of the molecular mechanisms driving progression is crucial. Recently, a BPH1-derived cancer progression (BCaP) model was created to map the various molecular changes that occur across stages of prostate cancer.1 The use of BPH1 cells as the precursor allows for a clean comparison of genetic and molecular differences between the two disease states, furthering our understanding and treatment of such conditions. Here, we set out to comparatively examine cellular models of prostate protein expression — in the intracellular proteome and the secretome at various stages of prostate disease.

METHODS: BPH1 and four BCaP model lines (NT1, T1, T10 and M1) were maintained in DMEM/F-12 medium supplemented with 5% FBS and 1X penicillin/streptomycin. Once sub-confluent, cells were subjected to two 24-hour treatments in serum-free media. At 48 hours, both conditioned media and cells were harvested and stored at -80oC for subsequent sample preparation. Cell lysis was achieved via sonication and proteins extracted using urea buffer. The extracted proteins were reduced and alkylated via DTT and IAA, respectively. Secreted proteins were concentrated via overnight dialysis or centrifugation and reduced/alkylated as described above. Five hundred μg of protein were subjected to overnight tryptic digestion and the resultant tryptic peptides were purified via C18 SPE cartridges. Samples were analyzed using LC-MS/MS on a nanoAcquity UPLC coupled to a Q Exactive quadrupole orbitrap mass spectrometer. Peptides were fragmented using higher-energy collision dissociation (HCD) then identified and quantified using PEAKS software.

RESULTS: Protein expression profiles are markedly different comparing the tumorigenic lines to those of non-tumorigenic phenotype. In particular, we anticipate a significant difference between the parental BPH1 cells compared to the non-tumorigenic prostatic NT1 cells.

CONCLUSIONS: The changes expected between intracellular and secreted proteomic profiles may offer insights into the different mechanisms of pathogenesis between disease states. Such differences can be exploited in the development of therapeutics that target each disease state and offer clinicians a more effective mode of treatment.

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OXALATE IMPACTS MACROPHAGE METABOLISM AND IMMUNE RESPONSE TO UROPATHOGENIC E.COLI INFECTION

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INTRODUCTION AND OBJECTIVE: Kidney stones have been suggested to be associated with urinary tract infections (UTIs). Escherichia coli (E. coli) is the most common causative pathogen of UTIs and has been isolated from the urine of stone formers as well as calcium oxalate stones. Macrophages play an important role in crystal and pathogen clearance. The objective of this study was to determine the impact of oxalate on macrophage metabolism and immune response to uropathogenic Escherichia coli (UPEC) infection.

METHODS: Differentiated macrophages were exposed to oxalate (50 μM) for 48 hours followed by infection with UPEC (1:2/1:5) for 2 hours. Macrophage viability, mitochondrial membrane potential, and oxidative stress were evaluated using biochemical and fluorescence based assays. Macrophage cellular energetics was assessed using the Seahorse XF Analyzer. The anti-bacterial response was determined by assessing the bacterial load.

RESULTS: Oxalate exposure did not affect macrophage viability. However, oxalate significantly increased mitochondrial membrane potential and oxidative stress in macrophages. In addition, oxalate significantly reduced macrophage cellular energetics and anti-bacterial response.

CONCLUSIONS: These data suggest oxalate impacts macrophage immunometabolism, induces oxidative stress and compromises anti-bacterial response, and this may contribute to the development of kidney stones and associated UTIs.

DEVELOPING A COMPUTATIONAL PIPELINE FOR CHARACTERIZATION OF UROPATHOGENIC ESCHERICHIA COLI

(25) **Grace Morales**[1], Benjamin Abelson[2], Gerald Van Horn[1], Douglass Clayton[2], Maria Hadjifrangiskou[1,2,3], Jonathan Schmitz[1,2,3]

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INTRODUCTION AND BACKGROUND: Urinary tract infections are among the most common bacterial infections. Uropathogenic Escherichia coli (UPEC) are responsible for up to 80% of these infections (1). Discerning symptom-causing from other non-pathogenic E. coli strains can be challenging as they are not able to be identified using traditional typing methods, such as checking for the presence of a toxin (2). The goal of this project is to build a computational pipeline that identifies key features of uropathogenic E. coli.

METHODS: To build the computational pipeline, we will leverage bacterial strains collected via the Vanderbilt Urologic Infection Repository (VUIR). The isolates are stored and linked to patients' deidentified medical records. The goals are to facilitate: genome assembly; genome annotation; single nucleotide polymorphism (SNP)-calling, and eventually; genome wide association studies (GWAS) to statistically cross correlated with clinical outcomes (**Figure 1, Table 1**).
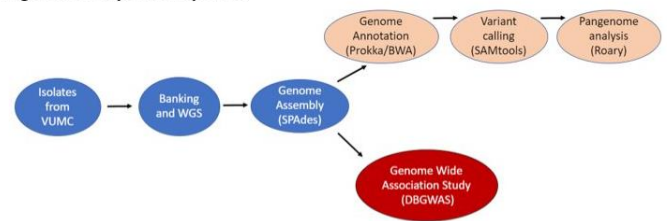

Figure 1: Proposed Pipeline

Table 1:

Feature	Percent of cohort
Sex:	
Females	87.8%
Males	12.2%
Age:	
18-35	10.2%
36-50	24.5%
51-60	26.5%
61-70	20.4%
71-80	12.2%
81+	6.1%
Other:	
Asymptomatic Bacteriuria	24.5%

RESULTS: To begin building the pipeline, an initial cohort of 49 urinary isolates was subjected to Illumina paired-end whole genome sequencing. Each genome was de novo assembled using the SPAdes software through the Geneious platform (3,4). For annotation, the contig files from the assembly were trimmed and put through the Prokka annotation software to assign known genes and functions (5). For pangenome analysis, the Roary pangenome methodology was employed from the Prokka files (6). All SNP-calling was performed using SAMtools (7). As an additional analysis, multi-locus sequence typing (MLST) was performed on all genomes using EnteroBase (8). All programs were utilized using default parameters according to their documentation. With the pilot cohort of 49 E. coli urinary isolates (Table 1), we have thus far tested how genomes will be assembled de novo, annotated, sequence-typed, began initial pangenome analysis (**Figure 2**), and have validated this work against what has previously been reported (9). Genome sizes ranged from 4.9Mb to 5.3Mb. The core genome (genes present in >95% of genomes sampled), is 3010 genes, which corroborates previous studies (9).

CONCLUSIONS: This pipeline will lay foundation for creating a system in which clinical isolates are easily sequenced and analyzed, in order to cross correlate back to patient phenotypes for translational discovery.

[References may be requested from the authors]

Figure 2: Pangenome analysis

13-95% of genomes 3420

95-98% of genomes 1101

>98% of genomes 1929

<13% of genomes 12253
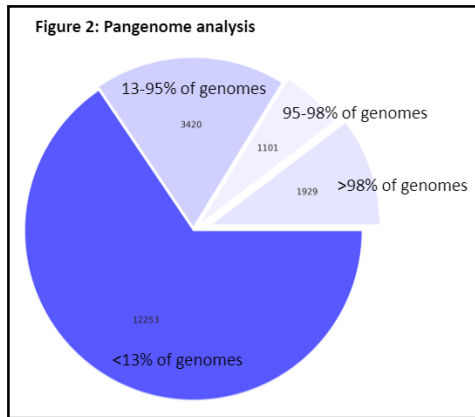
THE CO-LOCALIZATION OF COX-1 AND COX-2 IN AGED MOUSE PROSTATE AND HUMAN PROSTATE

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BACKGROUND: Benign prostatic hyperplasia (BPH) is characterized by the enlargement of the prostate leading to lower urinary tract symptoms (LUTS) with the potential to have a severe negative impact on the quality of life in aging men. Prostatic inflammation may contribute to the pathogenesis of BPH, however the underlying mechanisms for this contribution have yet to be fully elucidated. Cyclooxygenase-2 (COX-2) is an inflammatory mediator that has been found to be selectively overexpressed in diverse cell types within benign and malignant disease, including the prostate. Cyclooxygenase-1 (COX-1) is constitutively expressed, and while primarily involved in maintaining homeostasis in various organs, it has been implicated in inflammation. Despite the association between prostatic inflammation and BPH/LUTS, treatment of symptomatic BPH with a combined therapy of a nonsteroidal anti-inflammatory agent (NSAID), which inhibits Cox-2, and a 5 alpha-reductase inhibitor (5ARI) only showed transient resolution of LUTS in clinical trials. In this study, we compare the changes in expression of both COX-1 and COX-2 in both normal and diseased human prostate. Additionally, our lab has previously shown that lower urinary tract dysfunction spontaneously develops in aged mice. To also investigate a potential COX-1/COX-2 compensatory mechanism in an aging prostate, we also compared the protein expression of both enzymes in the lobes of young and old mice.

METHODS: Formalin fixed paraffin embedded human normal adjacent and BPH tissues from a tissue micro-array as well as mouse prostate (AP, VP, and DLP) from 2 and 24 month old mice, were stained for COX-1 and COX-2 using multispectral quantitative multiplex IHC. Fluorophores were spectrally unmixed and the optical densities for both proteins were quantified using InForm® software. Tissue and cell segmentation were performed for protein localization.

RESULTS: In mouse tissues, co-localization of Cox-1 and Cox-2 is decreased significantly in the stroma of the prostatic lobes in the aged mice compared to the young mice. Additionally, COX-1 is significantly downregulated in the stroma of aged VP and DLP lobes and aged epithelial VP. There were no significant differences in Cox-2 expression between normal and BPH tissues, or in young and old mouse prostate tissues.

CONCLUSIONS: Multiplex IHC revealed the presence of both the constitutive and inducible forms of cyclooxygenase, COX-1 and COX-2, respectively, within individual cells of prostate tissue. Clinical use of COX-2 specific NSAIDs may have unpredictable effects on the production of arachidonic acid metabolites, products of cyclooxygenase activity, that play an important role in the promotion and/or resolution of inflammation. Distinct alterations in the balance between COX-1 and COX-2 in aged mice or in human BPH provide the impetus for future studies to investigate the role of specific arachidonic acid metabolites in BPH/LUTS progression, particularly in the context or prostatic inflammation. Furthermore, age as a factor in the human tissue was not examined in this study. The potential impacts of the changes in COX-1/COX-2 co-localization and alterations to the expression of COX-1 in the aged stroma of the human prostate will require further exploration.

NLRP3-DEPENDENT MECHANISMS DOWNREGULATE GENES CONTROLLING UROTHELIAL BARRIER FUNCTION IN DIABETIC MICE

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INTRODUCTION: Diabetic bladder dysfunction (DBD) is a progressive deterioration of urinary function commonly occurring in patients with diabetes. In addition to high glucose levels, diabetes is associated with metabolic derangement and the production of numerous potentially harmful metabolites which accumulate in the circulation as well as the urine. Bladder urothelia normally maintain an impenetrable barrier to protect underlying smooth muscle but this barrier is known to breakdown during diabetes. We have previously shown in the Akita diabetic mouse model that the NLRP3 inflammasome, a multimeric structure which activates inflammatory cascades, is responsible for diabetic bladder inflammation and dysfunction. In this study we hypothesize NLRP3 activation decreases urothelial barrier function, thus exposing the underlying tissue to high levels of harmful diabetic metabolites. This increases inflammation and bladder dysfunction. Additionally, the loss of barrier function may be a major factor in the increased susceptibility to urinary tract infections. As an initial investigation into this hypothesis, we examined changes in expression of barrier genes in diabetic mice with either intact NLRP3 or in which it has been genetically deleted.

METHODS: Four groups of 15 week old female mice were used: non-diabetic control (non-diabetic, n=9), type 1 diabetic Akita mice (diabetic, n=7), non-diabetic control mice with NLRP3 knocked out (non-diabetic KO, n=12), and diabetic NLRP3 knock-out mice (diabetic KO, n=12). Previously we have shown DBD symptoms in the Akita mice at this time point. Urothelia was harvested from each mouse and used for qPCR studies. The following gene expression markers of barrier function were assessed: zona occludin 1 (ZO1), zona occludin 2 (ZO2), Claudin 4 (CL4), beta catenin (BCT), uroplakin 1b (UP1), and uroplakin 2 (UP2).

RESULTS: Urothelia from diabetic mice exhibit significant decreases in expression of ZO1, ZO2, CL4 and UP1. No significant

changes in BCT or UP2 gene expression are noted. Importantly, these changes in barrier gene expression did not occur in the diabetic KO mice.
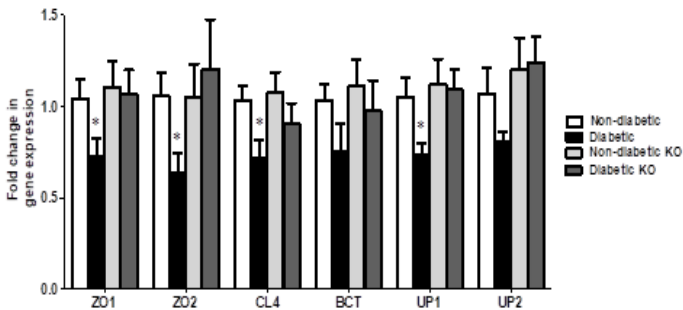
CONCLUSIONS: Diabetes reduces expression of genes regulating urothelial barrier function in a NLRP3-dependent manner. This finding provides insight into how DBD develops, and may identify a much needed therapeutic target for patients living with DBD.

E-CADHERIN IS DOWN-REGULATED IN THE AGING PROSTATE AND ASSOCIATED WITH INCREASED INFLAMMATION IN BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION AND OBJECTIVE: E-cadherin is an adherens junction that is critical for the development and maintenance of the prostate epithelial barrier. Previous studies have shown that E-cadherin is down-regulated in benign prostatic hyperplasia (BPH) and may contribute to increased epithelial permeability and subsequent prostatic inflammation. This study explored the potential associations between loss of E-cadherin and the presence of inflammatory mediators in prostate tissue specimens from young healthy donors and aged BPH patients.

METHODS: Serial prostate sections from a cohort of BPH patients and healthy young donors were immunostained with E-cadherin, Cox-2, CD4, CD8, CD20 and CD68. E-cadherin and Cox-2 H-Scores and the number of inflammatory cells were calculated for the same area in donor, normal adjacent prostate to BPH (NAP) and BPH specimens. Quantification and statistical correlation analyses were performed for comparisons between groups.

RESULTS: E-cadherin was decreased in aged NAP tissues and in BPH compared to donor tissue. E-cadherin down-regulation was associated with increased age and inflammation in NAP compared to young healthy donor prostate.

CONCLUSIONS: These findings suggest that aging is associated with down-regulation of E-cadherin. E-cadherin immunostaining was inversely associated with age and inflammation in the prostate. E-cadherin down-regulation in the aging prostate could contribute to increased prostatic inflammation, subsequently contributing to BPH pathogenesis.

PROSTATE-SPECIFIC DELETION OF CDH1 INDUCES MURINE PROSTATIC INFLAMMATION AND BLADDER OVERACTIVITY

(36) <u>Laura E. Pascal</u>[1], Shinsuke Mizoguchi[1], Wei Chen[1], Lora H. Rigatti[2], Taro Igarashi[1], Rajiv Dhir[3], Pradeep Tyagi[1], Zeyu Wu[1], Zhenyu Yang[1], William C. de Groat[4], Donald B. DeFranco[4], Naoki Yoshimura[1] and Zhou Wang[1,4,5*]

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INTRODUCTION AND OBJECTIVES: Benign Prostatic Hyperplasia (BPH) is an age-related debilitating prostatic disease that is frequently associated with prostatic inflammation and bothersome lower urinary tract symptoms (LUTS). Animal models have shown that formalin- and bacterial-induced prostatic inflammation can induce bladder dysfunction; however, the underlying mechanisms contributing to prostatic inflammation in BPH and bladder dysfunction are not clear. We previously reported that E-cadherin expression in BPH is down-regulated in hyperplastic nodules compared to expression in adjacent normal tissues. Here, we explored the potential consequences of prostatic E-cadherin down-regulation on the prostate and bladder in vivo using an inducible murine model of prostate luminal epithelial-specific deletion of Cdh1.

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. Deletion of E-cadherin was induced by transient administration of tamoxifen when mice reached sexual maturity (7 weeks of age). At 21-23 weeks of age, the prostate, bladder, and prostatic urethra were examined histologically, and bladder function was assessed using Void Spot Assays and cystometry.

RESULTS: Mice with Cdh1 deletion had increased prostatic epithelial hyperplasia, inflammation, and stromal changes at 21-23 weeks of age, as well as changes in bladder voiding function compared to age-matched controls.

CONCLUSIONS: Thus, loss of E-cadherin in the murine prostate could result in prostatic defects that are characteristic of BPH and lower urinary tract symptoms, suggesting that E-cadherin down-regulation could be a driving force in human BPH development and progression.

LOSS OF OSTEOPONTIN FUNCTION PREVENTS THE DEVELOPMENT OF URINARY DYSFUNCTION IN E. COLI-INDUCED PROSTATIC INFLAMMATION

(11) <u>Petra Popovics</u>[1,2,3], Asha Jain[1,2], Francesca Van[1,2], Hannah Ruetten[1,2,4], Mark Cadena[1,2,4], Kristen S. Uchtmann[1,2], Chad M. Vezina[1,2,4], William A. Ricke[1,2]

[1]Department of Urology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI; [2]George M. O'Brien Center of Research Excellence, University of Wisconsin, School of Medicine and Public Health, Madison, WI; [3]K12 Kure, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI; [4]Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI

INTRODUCTION AND OBJECTIVE: Approximately 30% of men with lower urinary tract symptoms (LUTS) are resistant to medical therapies and progress to surgical intervention. Fibrogenic and inflammatory processes in the prostate have been recently implicated in LUTS. Identification of new molecular pathways that provoke inflammatory responses and fibrosis, which are not targeted by current therapies, are vital to improve treatments of LUTS. We previously established that prostatic osteopontin (OPN), a pro-fibrotic cytokine, has increased levels in LUTS. We also revealed that OPN secretion is stimulated by inflammatory cytokines in prostate stromal cells and it further promotes inflammation by increasing the gene expression of IL6, CXCL8, CXCL1 and CXCL2. The current study aims to investigate whether the absence of OPN improves inflammation-induced urinary dysfunction in E. coli-induced prostatic inflammation and whether fibrosis-associated genes are induced by OPN in prostate stromal cells.

METHODS: Uropathogenic Escherichia coli (UTI89) or saline (controls) was instilled via a transurethral catheter (OD 0.8, 100 ul) two times, 3 days apart in C57BL/6J (B6) or B6.129S6(Cg)-Spp1tm1Blh/J (OPN-KO) mice. Urinary function was assessed by weekly void spot assays (VSA) where mice were isolated for 4 hours in cages with filter papers to collect urine. Filter papers were imaged with UV transillumination and analyzed with Void Whizzard Imagej plugin. BHPrS-1 immortalized prostate stromal cells were treated with 500 ng/ml human recombinant OPN for 2 hours. RNA from cells were isolated and mRNA abundance of 790 genes were identified with the Nanostring nCounter Human Fibrosis V2 Panel.

RESULTS: The void spot count was significantly elevated 33 days after instillation in B6 (2.05-fold, p=0.0097) but not in OPN-KO mice compared to saline-instilled controls. Void spot counts were not significantly different between groups at other time-points. Nanostring analysis revealed that recombinant human OPN did not directly induce the expression of genes encoding extracellular matrix elements or processing enzymes stimulating their extracellular assembly. Instead, OPN stimulated the expression of cytokines, including a new pro-fibrotic stromal target gene, IL11.

CONCLUSIONS: These results indicate that manipulation of OPN expression may counteract the development of inflammation-induced urinary dysfunction. OPN in the prostate primarily targets inflammatory pathways and potentially promote fibrosis by exacerbating inflammatory processes. Our future studies will investigate whether prostatic fibrosis is also reduced in mouse prostatic inflammation models in the absence of OPN.

TRENDS AND PATIENT CHARACTERISTICS OF URINE INFECTIONS CAUSED BY EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ESCHERICHIA COLI IN 3 CLINICAL SETTINGS OF A SAN FRANCISCO HEALTHCARE SYSTEM, 2014-2020

(15) Eva Raphael[1], Henry F. Chambers[2]

[1]Department of Family and Community Medicine, University of California, San Francisco, [2]Department of Medicine, University of California, San Francisco

INTRODUCTION AND OBJECTIVE: Prevalence of urinary tract infections (UTI) caused by antimicrobial resistant (AMR) Escherichia coli (E. coli), in particular those producing extended-spectrum beta-lactamases (ESBL-E. coli), is increasing worldwide. A prior study showed differing prevalence of ESBL-E. coli infections by healthcare institution type in San Francisco (Microbial Drug Resistance, 2020), but did not differentiate them as occurring in community vs healthcare settings. A 2019 Center for Disease Control and Prevention report highlighted the increasing prevalence of community-onset AMR infections. We compared trends of community-onset vs healthcare-associated UTIs caused by ESBL-E. coli from 2014 to 2020 in patients from three public healthcare institutions in San Francisco.

METHODS: We collected electronic health record data on all patients diagnosed with E. coli UTI from urine cultures performed at Zuckerberg San Francisco General Hospital (SFGH) microbiology laboratory from January 2014 to June 2020. We obtained demographic and clinical information and E. coli antimicrobial susceptibility results from SFGH (inpatient), San Francisco Health Network clinics (outpatient), and Laguna Honda Hospital (skilled nursing facility [SNF]). We conducted univariate and multivariate logistic regression analyses, with cluster bootstrapping to adjust for patients with multiple UTIs.

RESULTS: Between 2014 and 2020, 8,629 E. coli urine cultures from 1183 inpatients, 4844 from outpatients, and 264 from SNF patients, were performed at SFGH clinical microbiology laboratory. ESBL-E. coli prevalence increased in all clinical settings (inpatient: OR 1.14, 95% confidence interval [CI] 1.09, 1.20; outpatient: OR 1.08, 95% CI 1.00, 1.17; skilled nursing facility: OR 1.20, 95% CI 1.06, 1.35), while resistance to trimethoprim-sulfamethoxazole increased significantly from 33% to 35% among outpatients only (OR 1.03, 95% CI 1.00, 1.05). In multivariate analyses, male gender and hospitalization in the 3 months prior to the UTI episode was associated with ESBL-E. coli infection. Among outpatients, older age (>65) and Latinx race/ethnicity were associated with ESBL-E. coli infection.

CONCLUSION: UTIs caused by ESBL-E. coli increased in 3 different clinical settings over the study period. Male gender and recent hospitalization were associated with ESBL-E. coli UTI. Latinx patients were at higher risk of outpatient ESBL-E. coli. These observations suggest distinct risk factors for ESBL-E. coli UTIs in community vs healthcare settings that need further exploration to address why the prevalence of ESBL E. coli infections continues to increase.

IDENTIFYING CAUSATIVE VARIANTS IN PATIENTS WITH MONOGENIC STONE DISEASE

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INTRODUCTION AND OBJECTIVES: Primary hyperoxaluria (PH) and Dent disease are rare monogenic diseases resulting in kidney stones and renal insufficiency. PH is caused by biallelic mutations to AGXT, GRHPR, or HOGA1 while Dent disease (X-linked) is

caused by single mutations to CLCN5 or OCRL. Previously, Sanger screening resolved 53% of PH and 45% of Dent diagnosed families, and unresolved patients were screened with a next generation sequencing (NGS) panel of ~102 known or candidate stone-forming genes that solved a further 10% of these PH/Dent diagnosed families. The focus of the study was to identify patients carrying single heterozygous missense or atypical splicing variants in genes with autosomal dominant inheritance that could account for the stone phenotype.

METHODS: The pathogenicity of detected variants was evaluated with in-silico tools, including literature review (PubMed, Human Gene Mutation Database), multisequence protein alignments, web-based prediction programs (SIFT, PolyPhen-2, MutationTaster, Human Splice Finder), tertiary/quaternary modeling (SWISSMODEL, PDB, PyMol), and population data (gnomAD).

RESULTS: For the PH unresolved cases, 3 of 225 families were found to have monoallelic mutations to the genes SLC4A1, SLC34A1, and SLC34A3. In a further 51 families, a single potentially pathogenic variant was detected, but proof of causality was not reached. Of the 53 unresolved Dent families, 2 families were likely resolved by monoallelic mutations to the gene HNF4A. Additionally, the etiology of 5 families was likely solved with previously mis-scored biallelic variants in the genes AGXT, SLC34A1 (X2), SLC34A3, or SLC12A1.

CONCLUSIONS: This study shows the value of NGS and careful use of in silico methods for identifying pathogenic variants in our stone forming cohort, enhancing diagnostics and treatment options of monogenic kidney stone formers.

URINARY FLOW DYNAMICS – MRI-BASED COMPUTATIONAL MODELING

**(12)** Ryan Pewowaruk, David Rutkowski, Diego Hernando, Wade Bushman and **Alejandro Roldán-Alzate**

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INTRODUCTION: Benign prostate hyperplasia (BPH) affects a majority of men over the age of 60 in the United States. Along with BPH, lower urinary track symptoms (LUTS) and bladder dimension changes are a common development in this population. The complex biomechanics of the full male urogenital system and their effects on BPH symptom development are still not fully understood. Furthermore, non-invasive analysis and diagnostic methods for prostate and bladder pathologies have previously been limited. Magnetic resonance imaging (MRI) has the potential to analyze many of these factors in a single imaging session. Furthermore, MRI based computational fluid dynamics (CFD) models could provide valuable information about the urinary flow dynamics. In this study we describe a magnetic resonance imaging (MRI) urodynamics protocol and use this information to perform a patient specific computational fluid dynamics (CFD) simulation of bladder voiding.

METHODS: In this IRB-approved and HIPAA–compliant study 3 men with BPH/LUTS and 3 control subjects were recruited. MRI was performed on a clinical 3T scanner using a high-density flexible surface coil array. An MRI urodynamics protocol was implemented which involved voiding during MRI. 3D 'Fast-spin echo' (FSE) T2-weighted acquisitions were performed immediately before and after voiding. A sagittal plane 2D spoiled gradient echo (SGRE) dynamic real-time imaging (RTI) acquisition was performed during voiding. The bladder and urethra were segmented from pre and post voiding 3D images, while bladder cross-sectional area change during voiding was calculated from the 2D RTI. Measurements from both the 3D and 2D images were incorporated in a patient specific simulation of bladder voiding. For the CFD simulation. Bladder wall motion was estimated as described above and imposed to virtually drive voiding. The urethra was assumed to be rigid and its outlet was set to atmospheric pressure.

RESULTS: The urodynamics MRI protocol was successfully completed in all subjects. The estimated displacement maps show that greatest displacement occurs at the dome of the bladder (**Figure 1**). Overall the bladder walls of men with BPH/LUTS moved only 25%-50% as much as the control subjects. The control subjects had little left-right asymmetry (4%-14%) while the BPH patients had large left-right asymmetry (40%-160%). Urodynamics results from CFD are shown in **Figure 2** for a sagittal plane near the center of the bladder.
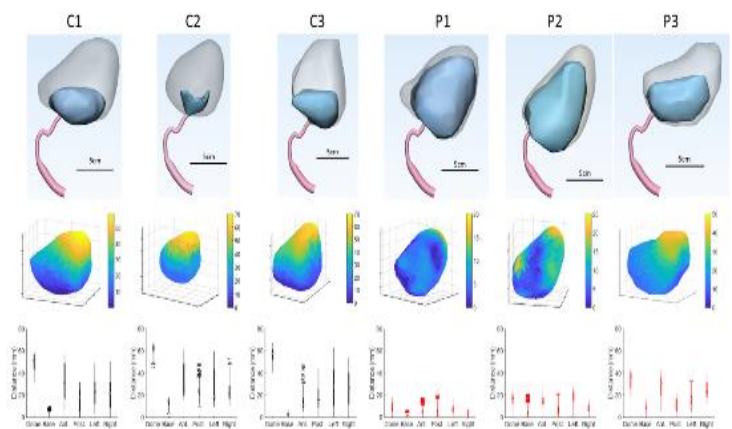
Figure 1: Top row: pre- and post-voiding bladder anatomies for each subject. Middle row: Bladder wall displacement maps (in mm) for each subject. Note that the legend scale is much smaller for the men with BPH/LUTS. Bottom row: Box plots showing regional displacement behavior for each subject (C: Control; P: Patient).
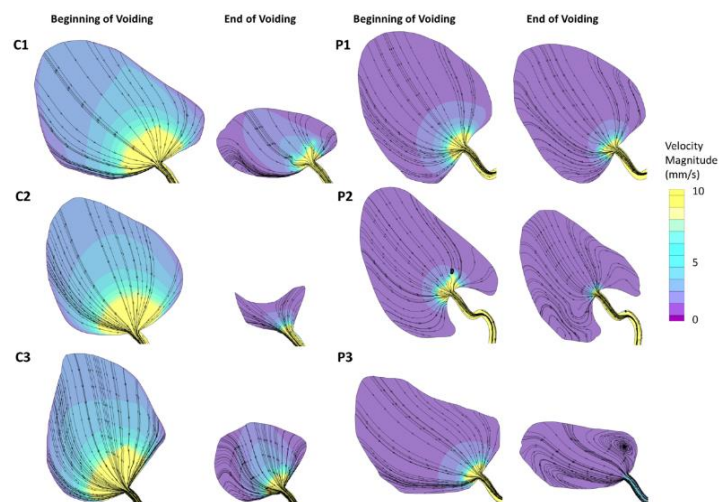
Figure 2: CFD results showing velocity contours and streamlines on a sagittal plane at the center of the bladder for each subject. Time points are displayed both near the initiation and termination voiding.

CONCLUSION: This study demonstrates the feasibility of MRI voiding studies to be performed, to provide new insight into lower urinary tract function in health and disease, and to generate patient-specific simulations of voiding that can be used to guide treatment.

A PHENOME-BASED APPROACH FOR CHARACTERIZING MOUSE URINARY PATHOPHYSIOLOGIES

(47) **Hannah Ruetten**[1], Gervaise Henry[2], Douglas Strand[2], Chad Vezina[1]

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INTRODUCTION AND OBJECTIVES: Lower urinary tract symptoms (LUTS) are a series of urine voiding and storage symptoms that are often attributed to prostatic disease in aging men. LUTS in men are often diagnosed using standardized patient surveys asking about urinary symptoms, however, many research labs use prostatic enlargement as a sole endpoint for both creating animal models and determining potential etiologic mechanisms and therapeutics. This limited focus does not capture the true range of etiologies and symptoms of urinary tract dysfunction in men. Several assays exist to measure changes in urinary function in mice, however it is currently unknown which changes are just changes and which are truly pathologic. One assay used to quantify urinary function changes in mice is the spontaneous void spot assay (VSA). This assay is inexpensive, noninvasive, and protocols are freely available to researchers. Herein we test the ability of the VSA to distinguish mouse models from one another. We also determine common features of diabetic diuresis, irritative urinary dysfunction, and obstructive urinary dysfunction in mice using the BTBR ob/ob (BTBR), E. coli UTI89 infection (UTI89) and Testosterone and Estradiol Supplementation (T+E2) mouse models respectively.

METHODS: We obtained the Void Whizzard output (VSA analysis software) from three mouse models that develop urinary dysfunction. All mice in each group were age and strain matched and compared to their respective controls. We also normalized treated mice to their respective controls and then compared treatments to each other.

RESULTS: We detected distinct features of diabetic diuresis, irritative dysfunction, and obstructive dysfunction using only VSA

Figure 1: Multiple models of urinary dysfunction can be isolated using Void Spot Assay endpoints.

endpoints obtained from the Void Whizzard output. We also were able separate the three models using Void Whizzard outputs and principle components analysis (**Figure 1**).

CONCLUSIONS: These findings support use of VSA to identify urinary dysfunction in mice so that we can create better animal models and improve etiologic and therapeutic testing.

EFFECT OF EPIDURAL KILOHERTZ FREQUENCY SPINAL CORD STIMULATION ON LOWER URINARY TRACT FUNCTION IN A RAT SPINAL CORD TRANSECTION MODEL

(6) **Casey J Steadman**[1]; Chris L Langdale[1]; Warren M Grill[1,2,3,4]

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INTRODUCTION AND OBJECTIVE: Spinal cord injury (SCI) results in bladder dysfunction, including neurogenic detrusor overactivity (NDO), detrusor-sphincter dyssynergia (DSD), and impaired bladder emptying. We recently established the effectiveness of epidural kilohertz frequency spinal cord stimulation (KHF SCS) to modulate lower urinary tract function in intact rats, as well as following the instillation of acetic acid into the bladder to induce hypersensitivity, mimicking the effects seen after SCI. The purpose of this study is to determine the effectiveness of KHF SCS to suppress 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 in rats following complete spinal transection.

METHODS: Male (n=7) and female (n=7) Sprague-Dawley rats (200-300 g) underwent a T10 spinal transection. Four weeks after transection (SCIT), we performed continuous-fill saline cystometry 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. Using a randomized block design, 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)}, as well as trials 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 also stimulated the sensory pudendal nerve at 10 Hz at 80% and 2x MT.

RESULTS: BC increased to 6.8 ± 2.6 mL (mean ± s.d.; n=6) in SCIT rats compared to 0.55 ± 0.32 mL in spinally-intact rats (n=14). KHF SCS generated an apparent decrease in the mean bladder capacity in SCIT animals when normalized to pre-stimulation control trials across all stimulation parameters, with the most notable decreases occurring with 1 kHz 80% MT, 5 kHz 20% MT, and 10 kHz 80% MT. Further, several stimulation parameters produced an increase in VE, with the most notable increases occurring with the stimulation parameters of 1 kHz 40% MT, 1 kHz 80% MT, 5 kHz 20% MT, and 10 kHz 40% MT. While EUS EMG activity and NVC data have yet to be analyzed, we hypothesize that reductions in both EUS EMG activity and the number and area-under-the-curve of NVCs will occur with epidural KHF SCS.

CONCLUSIONS: Our preliminary results suggest that epidural KHF SCS may be a viable approach to modulate bladder function after SCI.
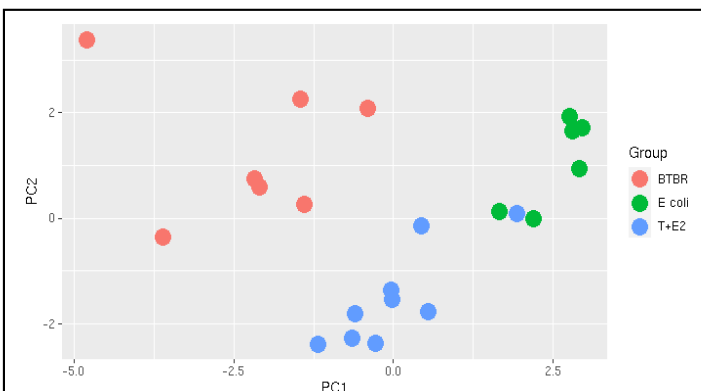
ELUCIDATING THE ORIGIN AND DEVELOPMENT OF BLADDER RESIDENT MACROPHAGES. COLUMBIA UNIVERSITY OPPORTUNITY POOL PROJECT

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Macrophages are part of the phagocyte mononuclear system constantly replaced by the circulating blood monocytes. 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, 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 population.

To identify the unique bladder macrophage populations, we used a multiparameter flow cytometry approach. Bladder macrophages were isolated from 3 and 8-week old mice in the steady-state.

There were four distinct macrophage populations, derived from a resident macrophage population and a recruited monocyte derived macrophage population in the bladders of 3-week-old mice. The resident bladder macrophage populations had low expression of MHC class II on the surface, while recruited macrophages had high surface expression of MHC class II. Bladder macrophages derived from 8-week-old female mice revealed over 90% of the macrophage population had high MHC class II surface expression.

This would suggest either the resident bladder macrophages are in an activated state by MHC class II surface expression, or the resident macrophages in the bladder are replenished by the monocyte-derived recruited macrophage population. The data indicates there is a dynamic change in the bladder macrophage population leading to an activated macrophage phenotype with age.

INTERACTIONS OF COMMENSAL BLADDER LACTOBACILLI WITH URINARY PATHOGENS

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INTRODUCTION AND OBJECTIVE: Lactobacilli are long known to humankind as beneficial bacteria that are extensively used in food preparation and preservation due to their ability to inhibit growth of other bacteria. Lactobacilli are found in human oral, intestinal and vaginal microbiomes and are often applied as probiotic for various health conditions. Now it has also become evident that diverse lactobacilli species inhabit the lower urinary tract. The identity and composition of the lactobacilli in this niche appear to correlate with several urological conditions, including urinary tract infections (UTIs). We set to test whether lactobacilli found in commensal bladder microbiome can directly affect growth of major uropathogens.

METHODS: We used a previously collected library of bladder bacteria containing seven diverse species of lactobacilli among others. Genomes of two lactobacilli strains were sequenced, assembled, and analyzed in silico for ability to produce bacteriocins and other toxins. To assess the interactions between the uropathogens and lactobacilli, we used well-diffusion inhibition assay on MRS agar plates in aerobic and anaerobic conditions. As the major uropathogen we used classic uropathogenic Escherichia coli strains DS17, UTI89, and CFT073. To model other two prominent uropathogens Klebsiella pneumoniae and Enterococcus faecalis, we used respective isolates from the bladder bacteria library. Several clinical isolates of the extended-spectrum beta-lactamase producing (ESBL) E. coli were used in the same inhibition assay to test whether the observed interactions are affected by the present resistance markers.

RESULTS: Assembled genomes of two lactobacilli species Lactobacillus gasseri S1 and Lactobacillus delbrueckii S9 were predicted in silico to produce 2 bacteriocin homologues. These two lactobacilli strains inhibited growth of the three major uropathogens. Screening 17 other lactobacilli strains from the bladder bacteria library established that most of the lactobacilli are capable of inhibiting uropathogenic E. coli CFT073 to different extent. Inhibitory action depended on the growth stage of active lactobacilli and presence of oxygen. S1 and S9 strains also successfully inhibited growth of multidrug resistant ESBL E. coli.

CONCLUSIONS: We tested lactobacilli, isolated from lower urinary tract, for their interactions with major urinary pathogens and found that these lactobacilli can efficiently inhibit pathogen growth in vitro, including growth of multidrug resistance strains. These results open a new avenue for systematic analysis of interbacterial interactions in bladder microbiome using microbiological and clinical methods.

GENETIC TOOLS THAT TARGET MECHANORECEPTORS CAN BE USED TO LABEL BLADDER AFFERENTS

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INTRODUCTION AND OBJECTIVE: There is evidence that mechanosensitive visceral afferents can contribute to visceral pain. However, studying these subpopulations is challenging as they cannot be readily identified with immunohistochemistry (IHC). Most of what is known about sensory innervation of the bladder comes from IHC studies that traditionally focus on nociceptive nerve terminals. Transgenic mouse lines have been used to characterize mechanosensitive afferents in the skin. This study aimed to test these genetic tools to determine whether they can enhance our understanding of bladder innervation.

METHODS: Cre-dependent tdTomato expression was evaluated in mice from two mouse lines: TrkB CreER (induced at 3-4 weeks of age) labeling Adelta mechanoreceptors, and Ret CreER (induced at 8 weeks of age) labeling a combination of Abeta mechanoreceptors, nociceptors, and other neurons. Whole-mount multiplex IHC of bladders from these mice used neuronal markers calcitonin gene-regulated peptide (CGRP) to label peptidergic nociceptors, and neurofilament heavy chain (NFH) to label myelinated Adelta and Abeta neuron fibers, including a variety of mechanoreceptors and Adelta nociceptors.

RESULTS: Axon terminal labeling in the bladder was successful and images revealed identifiable spatial distribution patterns. Across all markers innervation was densest in the neck compared to the body. Nerve fibers in the body trace along the length of the lateral walls diverging in various patterns, some resembling branches separating from a major nerve fiber while others were more complex. There was partial overlap of TrkB and NFH, where larger nerve bundles show co-localization but smaller terminals expressed only individual markers. Ret labeling was more extensive than TrkB and partially overlapped with NFH labeling and with CGRP labeling. CGRP and NFH also partially co-localized suggesting that CGRP nerve terminals do not consist solely of C-fibers, but also include moderately myelinated Adelta fibers. Ongoing analysis will further characterize nerve terminal labeling and discern truly co-localized from intertwining fibers.

CONCLUSIONS: TrkB CreER and Ret CreER mouse lines may serve as valuable genetic tools to evaluate mechanosensitive bladder afferents and their role in pathologic conditions such as bladder pain. Interestingly, these data also suggest a large proportion of Adelta bladder afferents are peptidergic, contradicting pervading assumptions in the field of bladder innervation. This work will contribute to efforts to elucidate bladder pathophysiology and characterize the cellular and molecular underpinnings of bladder pain.

PRELIMINARY RESULTS OF NOVEL NONINVASIVE CORTICAL MODULATION USING TRANSCRANIAL ROTATING PERMANENT MAGNET STIMULATOR IN IMPROVING VOIDING DYSFUNCTION IN FEMALE MULTIPLE SCLEROSIS PATIENTS

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INTRODUCTION AND OBJECTIVES: Voiding dysfunction (VD) is a common neurogenic lower urinary tract dysfunction (NLUTD) in multiple sclerosis (MS) patients. Currently, the only effective management for VD and retention in neurogenic patients is catheterization, prompting us to look for novel therapies. Transcranial rotating permanent magnet stimulator (TRPMS) is a noninvasive, multifocal neuromodulator that simultaneously modulates multiple cortical regions, enhancing their functional connections. In this clinical trial (ClinicalTrials.gov NCT03574610), we investigated the efficacy of TRPMS in modulating brain regions during voiding initiation and its therapeutic effects in mitigating VD in MS women.

METHODS: Six ambulatory MS women with NLUTD and VD [defined as having %post-void residual/bladder capacity (%PCR/BC) ≥ 40% or being in the lower 10th percentile of the Liverpool nomogram] underwent concurrent urodynamic and fMRI evaluation with three cycles of bladder filling/emptying, at baseline and post-treatment. Predetermined regions of interest and their blood-oxygen-level-dependent (BOLD) activation at voiding initiation were identified on baseline functional scans, corresponding to the microstimulators placement on the TRPMS treatment cap to either stimulate or inhibit these regions. **Figure 1a** details the regions to modulate and their corresponding tasks. Patients received ten 40-minute treatment sessions (**Figure 1b**).

Uroflow and validated questionnaires were collected at baseline and post-treatment.

RESULTS: After treatment, patients showed significantly increased activation in regions known to be involved at voiding initiation in healthy subjects, detailed in **Figure 1c**. Additionally, %PVR/BC significantly decreased (p=0.028), indicating clinical improvement in patients' VD. No treatment-related adverse effect was reported.

Regions of interest	Task	Stimulated/Inhibited?
Right inferior frontal gyrus	Voiding initiation	Stimulated
Left dorsolateral prefrontal cortex	Depression mitigation	Stimulated
Bilateral supplementary motor area	Pelvic floor contraction	Inhibited
Right middle frontal gyrus	Pelvic floor contraction	Inhibited
Right dorsolateral prefrontal cortex	Anxiety	Inhibited
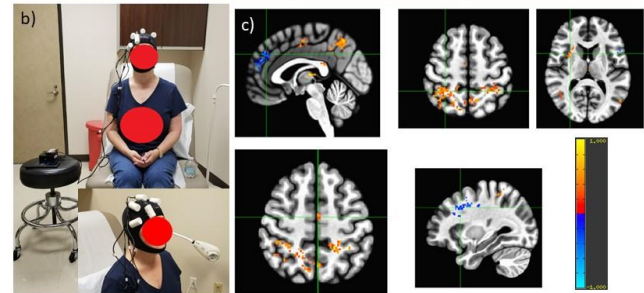
Figure 1. a) Cortical regions to modulate and their corresponding tasks. b) An MS patient receiving TRPMS treatment in our clinical trial. c) Change in fMRI activation post-treatment, averaged over six MS patients (p<0.05). Significantly increased activation was observed in the left cingulate body and posterior cingulate, left precuneus, right insula, thalamus, and supplementary motor areas.

CONCLUSIONS: For the first time we report our preliminary results showing significant improvements in both neuroimaging and clinical data, suggesting that TRPMS is able to effectively and safely modulate cortical and even deeper brain regions involved during voiding initiation, leading to clinical improvements in MS patients with VD. Although only cortical regions were modulated, simultaneous modulation of these regions helped increase the strength of the voiding network, resulting in significant changes in deeper brain regions involved in micturition cycle.

TESTOSTERONE AND ESTRADIOL MEDIATE MALE VOIDING DYSFUNCTION BY REDUCING PROSTATIC SMOOTH MUSCLE PPP112B ABUNDANCE AND IMPAIRING MUSCLE RELAXATION

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INTRODUCTION AND OBJECTIVE: Lower urinary tract symptoms derived from bladder outflow obstruction are nearly ubiquitous in men of advancing age. Decades of research focused on excessive adrenoreceptor-mediated muscle contraction as the likely mechanism. Here, we report a novel mechanism leading to impaired smooth muscle relaxation.

METHODS: We delivered sustained-release testosterone (T) and estradiol (E2) implants to male mice, mimicking changes in

circulating hormone concentrations in aging men. We evaluated urine velocity through the prostatic urethra by contrast enhanced ultrasound. We used genetically enhanced calcium sensors (GCaMP) bred into prostate smooth muscle and tissue bath to evaluate the impact of T+E2 on smooth muscle relaxation in vitro. We evaluated the expression of myosin phosphatase subunits using RT-PCR and western blot. Lastly, we evaluated the urinary and smooth muscle physiology of a mouse with one subunit of the myosin phosphatase knocked out (Ppp1r12b).

RESULTS: A single bolus of phenylephrine (IV), a smooth muscle contractile agonist, increased urine velocity in T+E2 treated but not control mice. Using phenylephrine to increase muscle tension and drive intracellular calcium release in mouse prostate preparations, we discovered that T+E2 treated tissues return more slowly to baseline than controls, indicating impaired smooth muscle relaxation. T+E2 treatment decreased prostatic mRNA and protein abundance of protein phosphatase 1 regulatory subunit 12B (PPP1R12B), a component of the myosin phosphatase complex. Genetic deletion of Ppp1r12b partially phenocopied the physiological responses to T+E2 treatment by slowing prostate smooth muscle relaxation.

CONCLUSIONS: Collectively, these results support the hypothesis that testosterone and estradiol reduce PPP1R12B abundance, inhibiting normal prostatic or urethral smooth muscle dilation in response to adrenergic stimuli, and priming bladder outflow obstruction.

SINGLE CELL TRANSCRIPTOME PROFILING TO DEFINE CELL TYPES IN BRAIN NUCLEI CONTROLLING BLADDER FUNCTION

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The pontine micturition center (PMC) is a nucleus in the rostral pons of the brainstem involved in the supraspinal regulation of micturition. Activity in PMC neurons correlates with detrusor contraction and voiding, and selective ablation of glutamatergic PMC neurons eliminates voiding. Thus, for micturition to occur, an excitatory signal from PMC has to reach preganglionic bladder motoneurons. The glutamatergic PMC neuron population includes subgroups that express additional specific marker genes, and some of these subpopulations may provide descending control of detrusor and sphincter function. Here we identify all neuron subtypes in PMC. Moreover, we map the functional connections of specific bladder controlling neurons using anterograde and retrograde tracing.

We performed RNA sequencing as an unbiased method to define the types of neurons in specific brain regions in mice. Furthermore, we combined neural mapping methods with chemogenetic activation to characterize the functional contribution of those neurons that directly innervate bladder motoneurons in the spinal cord; i.e. whether the newly identified subpopulations of PMC cells play important roles in regulating bladder function.

Our 'PMC-general' atlas of molecularly distinct neuronal groups contains ten clusters of glutamatergic putative-PMC neurons. First, using in situ hybridization we determined which of the gene markers has expression restricted to PMC. Second, in existing

transgenic mouse lines with Cre-expression driven by specific gene promoters we showed that Cre-dependent AAVs colocalize with the Cre-reporting neurons in PMC and that these neurons send axonal projections to the lumbosacral level of the spinal cord. Third, through chemogenetic stimulation we find that several of the subpopulation-marking neurons play important functional roles in regulating detrusor and EUS activity.

The cellular PMC is highly heterogeneous, using single nuclei Drop-Seq (DroNc) we have identified multiple transcriptionally distinct populations. In addition, we have demonstrated anatomical projections to the sacral spinal cord and we determined particular functional roles of neurons of subpopulations within the PMC with respect to bladder function. Ongoing and future studies are focusing on retrograde labeling as a consequence of AAVs injected in PMC for the identification of functional neuron populations in afferent brain sites that project axons specifically to PMC. Our results taken together will generate a deeper understanding of how brain and spinal inputs converge to control bladder filling and voiding, and hence the neurologic mechanisms of LUTS in mice and humans.

MACROPHAGE-DERIVED TUMOR NECROSIS FACTOR CONTRIBUTES TO BENIGN PROSTATIC HYPERPLASIA THROUGH INCREASED FIBROBLAST PROLIFERATION

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INTRODUCTION AND OBJECTIVE: Benign prostatic hyperplasia (BPH) is characterized by enlargement of the prostate, and commonly involves inflammation. High-grade inflammation has been demonstrated to limit the success of current therapies. However, the mechanisms by which immune cells promote expansion of the prostate and whether these can be controlled therapeutically are not well elucidated. Previous evaluation of patient medical records indicated that men with an autoimmune disease (AID) diagnosis were significantly more likely to also be diagnosed with BPH, while systemic therapy of AID using tumor necrosis factor α (TNF)-antagonists reduced BPH diagnoses back to a baseline incidence rate. Here, we investigated the inflammatory component of human BPH tissues and assess whether TNF regulates proliferation of epithelial or stromal cells from BPH tissues.

METHODS: These studies utilized histological evaluation of human BPH tissues from our prostate tissue repository as well as in vitro cell proliferation assays using benign prostate epithelial (BHPrE-1 and NHPrE-1) or stromal (BHPrS-1) cell lines, monocyte-like THP-1 cells, and primary fibroblast cultures isolated from BPH tissues following simple prostatectomy. We also isolated transition zone tissue from small (<60 grams) versus large (>70 grams) human prostates and sorted viable, CD45+EpCAM-CD200- immune cells for single-cell mRNA-sequencing (scRNA-seq) analysis using the 10x Genomics Chromium System. CellRanger and Seurat were

used for data analysis, evaluation of cell clusters, and differential pathway analysis.

RESULTS: All inflammatory cell types accumulate in large BPH tissues and single-cell mRNA-sequencing (scRNA-seq) analyses of CD45+ immune cells indicate significantly altered pathways related to autoimmune inflammatory (AI) conditions in macrophages from large versus small tissues. TNF is primarily expressed by macrophages within the immune compartment and in vitro studies demonstrate that TNF stimulates proliferation in fibroblasts but not epithelial cells. Furthermore, primary fibroblast proliferation is stimulated by conditioned medium from M1-polarized THP-1 cells. Finally, histological evaluation of prostate specimens from seven patients taking TNF-antagonists at the time of procedure indicate these tissues have decreased inflammation and reduced proliferation compared to matched controls.

CONCLUSIONS: These studies implicate macrophage-derived TNF as a driver of BPH. Studies to determine whether TNF targeting may yield therapeutic benefit for BPH patients are ongoing.