

CAIRIBU O'BRIEN CENTERS

SUMMER STUDENT RESEARCH JAMBOREE PROGRAM

July 2024 - 4th Annual

Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

The CAIRIBU Interactions Core is led by PI, Kristina Penniston, PhD, RDN and funded by the NIDDK (U24-DK-127726)

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FATTY ACID SUPPLEMENTATION IN BENIGN PROSTATIC HYPERPLASIA

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

METHODS: Human prostate stromal (BHPPrS1) cells were treated with vehicle, a complex I inhibitor (25nM rotenone), and rotenone in combination with the fatty acid of interest. A dose-response curve for palmitoleic acid (PA), and linolenic acid (ALA) was generated by measuring the oxygen consumption rate (OCR) on Resipher. The most effective doses of PA and ALA were further tested on BHPPrS1 cells in the presence of rotenone. An essential mitochondrial protein marker and extracellular collagen were measured using immunocytochemistry (ICC).

RESULTS: Complex I inhibition in BHPPrS1 cells resulted in a decreased OCR compared to the vehicle control. Treatment with 60nM PA and 25nM ALA was most effective in rescuing the respiration rate. Both 60nM PA treatment and 25nM ALA treatments surpassed the respiration rates of BHPPrS1 cells treated with 100nM OA, the effective positive control. ICC for mitochondria showed a decrease in protein expression with complex I inhibition, which was rescued most effectively by ALA. ICC for extracellular collagen I suggests ALA and PA may also be effective in reducing extracellular collagen, like OA.

CONCLUSIONS: These preliminary results show promising trends, but further experiments must be performed to determine the biological and statistical significance of these findings. The enhanced respiration in BHPPrS1 cells treated with PA and ALA indicates a potential protective role of these fatty acids against rotenone induced mitochondrial dysfunction. This is further supported by the rescue of mitochondrial protein expression on ICC. Additionally, the potential of long chain fatty acids as anti-fibrotic intervention requires further investigation. This promising preliminary study indicates a class of treatments with unexplored potential.

Androgen-dependent epididymal development requires active WNT/ β -catenin signaling

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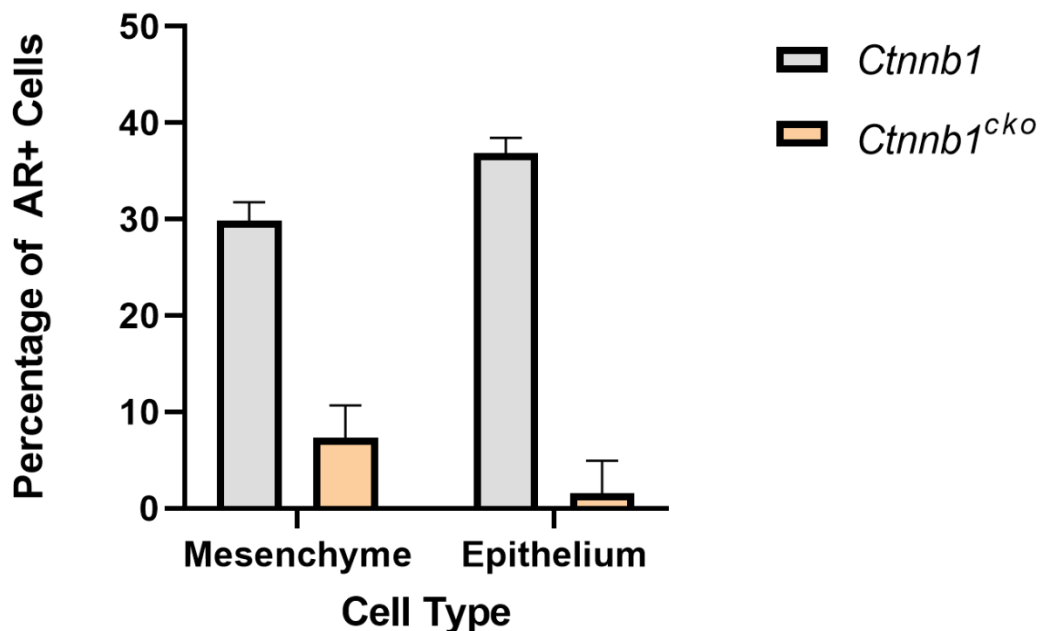
INTRODUCTION AND OBJECTIVE: Male infertility affects approximately 7% of the male population. One of the major causes of male infertility is congenital defects of the genitourinary system, including the epididymis, which is a crucial organ for post-testicular sperm maturation, transport, and storage. During development, the precursor of male reproductive tract organs, the Wolffian duct, is stabilized and then undergoes morphogenesis and differentiation, forming an extensively coiled epididymis. The epididymal coiling has been predominantly promoted by testis-derived androgens, which act on the androgen receptor (AR) in the mesenchyme. Recently, we made an unexpected discovery that WNT/ β -catenin signaling is another critical mesenchymal signaling in transforming the Wolffian duct into a coiled tube. In the absence of mesenchymal β -catenin, the epididymis failed to coil and became severely cystic. β -catenin can act as a transcriptional coactivator in regulating developmental gene expression. Therefore, this summer project aims to determine whether and how the deletion of mesenchymal β -catenin affects AR expression in Wolffian ducts.

METHODS: We performed immunohistochemical staining of AR in control and β -catenin knockout Wolffian ducts at E16.5. The percentage of AR-positive cells in the epithelial and mesenchymal compartments were quantitatively analyzed.

RESULTS: I examined two mice in each group and found that the average percentage of AR+ cells in the epithelium and mesenchyme were both decreased in the absence of mesenchymal β -catenin. Specifically, the percentage of AR+ cells in the epithelium was 35% in the control and 10% in the β -catenin knockout group; the percentage of AR+ cells in the mesenchyme was 30% in the control and 7% in the β -catenin knockout group.

CONCLUSIONS: The deletion of mesenchymal β -catenin potentially affects AR expression pattern during epididymal morphogenesis. It is worth noting that I have examined only two knockout samples, and additional samples are needed to cement this conclusion. Understanding the role of WNT/ β -catenin signaling in epididymal development could shed light on male infertility and lead to potential therapeutic targets for congenital defects.

AR+ Cell Staining in E16.5 *Ctnnb1* Male Mice



UDS 2.0: Digitizing Retrospective UDS and Augmented Analysis

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Introduction and Objective: Urodynamic studies (UDS) are the gold standard for assessing lower urinary tract function. UDS measure detrusor pressure and urinary flow rate during filling and voiding (Fig1). This approach yields a wealth of complex data, yet most clinically used indices of bladder function reduce UDS data to single instantaneous metrics (such as detrusor pressure at maximum flow). Pressure-volume (PV) analysis is used to analyze cardiac function and can be reproduced in the bladder using digitized UDS. The aim of this study is to characterize bladder energetics using PV analysis with semi-automated analysis of digitized UDS tracings.

Methods: 140 patients who underwent UDS were a part of this study. All UDS tracings (pressure and flow curves over time) were digitized and recorded in patient specific spreadsheets. The data for a subject was imported into python using a custom code for analysis. Volume measurements were calculated from the bladder filling rate and the flow rate for the filling and voiding phases. PV curves were created, and bladder work was calculated.

Results: Analysis is ongoing and representative results from a single subject are shown in Figure 1. This demonstrates good agreement between digitized and original UDS tracings which is possibly less sensitive to artifact. Qmax is within 13% of what is shown in the UDS report. Bladder volume and PV curves are shown in Figure 2 and permits calculation of bladder work during filling and voiding as a novel metric of bladder function.

Conclusion: Digitized UDS data is representative of traditional UDS readouts and enables both quantitative analysis of UDS tracings and deeper analysis using PV curves to quantify bladder work and efficiency. Going forward, we will use this approach to study and describe bladder function and correlate with clinical characteristics to identify new ways to predict and measure responses to therapeutic interventions.

Fig1:

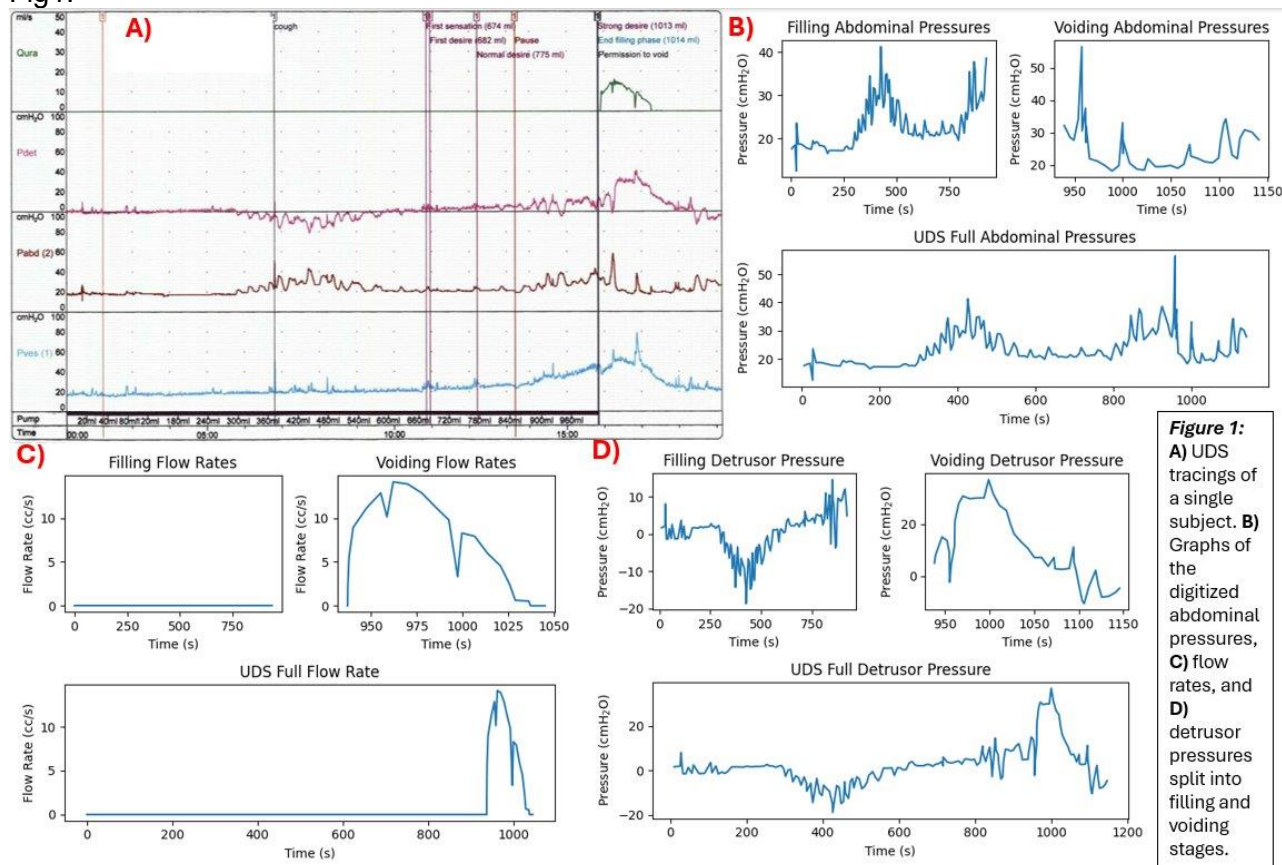
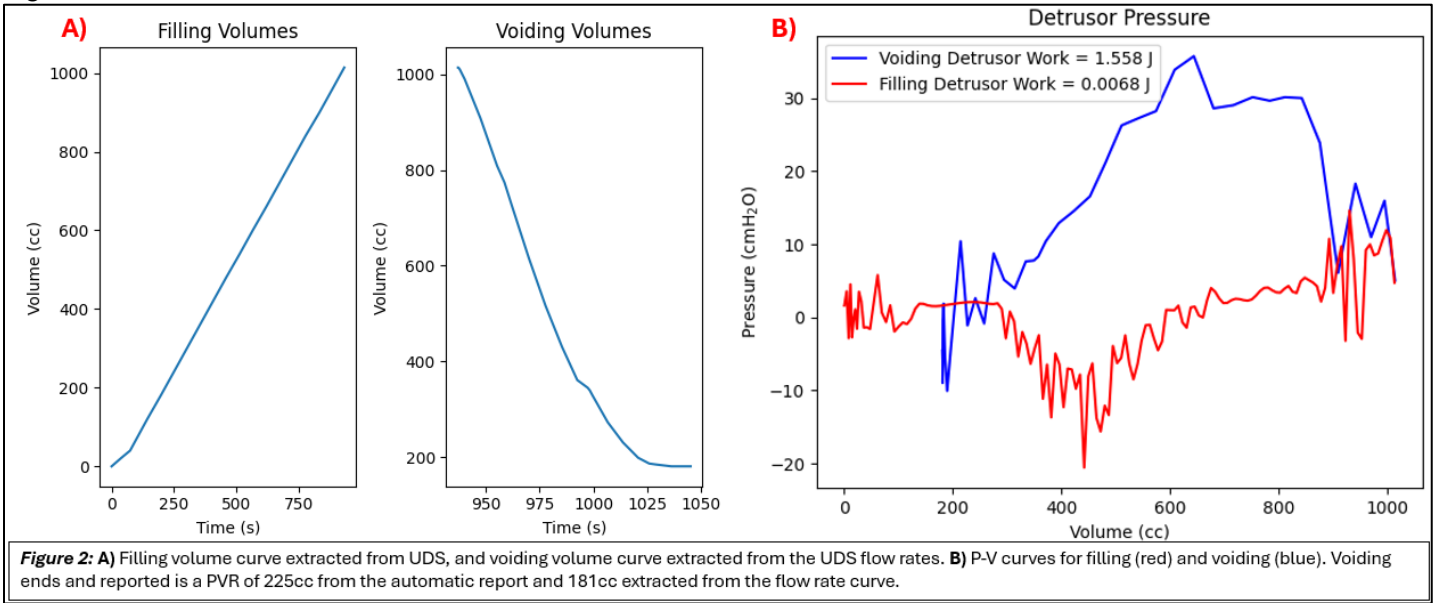


Figure 1:

A) UDS tracings of a single subject. B) Graphs of the digitized abdominal pressures, C) flow rates, and D) detrusor pressures split into filling and voiding stages.

Fig2:



Hematuria suppresses UTI-induced urothelial damage and inflammation

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INTRODUCTION AND OBJECTIVE: In UTIs, hosts shed their superficial urothelial cells in response to bacterial invasion in the urinary tract to reduce infection by elimination and urination. In addition to shedding, patients also frequently experience hematuria, but little is known about this symptom. It is often assumed to be harmful to the urothelium for two reasons: (1) heme's oxidative potential and (2) heme stimulates bacterial growth. In this project, we test this hypothesis of whether hematuria is a damaging symptom to the urothelium.

METHODS: We iron loaded the urinary system with ferric iron or with heme by induced hemolysis using phenylhydrazine (PHZ). UTI was induced by titration of UTI-89 inoculation in male and female mice (20uL of 107 CFU), and monitoring tissue damage by urothelial shedding from the luminal surface of the bladder (CK20+cells) compared to preserved basal cells (CK5+cells).

RESULTS: UTI induced detachment and shedding of superficial cells (between 6-12hrs post infection). Ferric iron loading worsened, and ferric iron deficiency suppressed bacterial growth and urothelial shedding. In contrast, hematuria neither induced bacterial growth nor enhanced cell shedding. In fact, hematuria prevented cell shedding (n=75 mice). Retention of urothelial superficial cells and suppression of erythema was grossly visible even when invasive bacterial intracellular colonies were present.

To find a mechanism of the heme effect, we investigated the pyroptotic pathways. We first confirmed that GsdmD KO mice suppressed urothelial shedding. Next, we found that hematuria suppresses GsdmD RNA expression and upstream regulators of GsdmD, such as Nlrp3 and cleaved casp1 and 11 proteins were depressed by hematuria in superficial and intermediate cells. Pyroptosis associated cytokines, chemokines, neutrophil chemoattractants, interleukins and inflammatory cell death regulators were also downregulated. Similarly, neutrophil invasion into the interstitium of the bladder was suppressed.

Lastly, we find hematuria's inhibitory cell death effect is not due to iron metabolic changes as Hmox1 and Hrg1 KO recapitulate the hematuria rescue, rather we unexpectedly find it to be dependent on TLR pattern recognition receptors.

CONCLUSIONS: Hematuria, a frightening sign of urological disease, is surprisingly anti-inflammatory and cell protective in UTI-cystitis.

Investigating Two-Component System Signaling in UTI Pathogen *Klebsiella pneumoniae*

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INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP) pose significant challenges in medical contexts due to the limited treatment options available. CRKP harbors two-component systems (TCS) that potentially play a critical role in its pathogenicity. Our overall goal is to elucidate the sensory inputs and functions of the CrrAB TCS in CRKP. Specifically, my goal is to develop functional epitope-tagged constructs of CrrB and a putative interaction partner to investigate the signal transduction mechanism of the CrrB TCS *in vivo*.

METHODS: Tagged constructs were made using cloning techniques to express CrrB and its putative interaction partner 2305 from pUC19 and pACYC plasmids in *K. pneumoniae* strains. The 2305 gene is part of many genes of unknown function surrounding the CrrAB TCS that are highly upregulated in a hyperactive clinical mutant of CrrB (CrrB*) according to an RNA-seq done by Genevieve. Therefore, to test the functionality of our FLAG-tagged 2305 construct, I introduced the expression vectors I cloned into the *crrB** Δ 2305 background and measured changes in gene expression of the CrrAB locus and surrounding genes (*crrA*, 2301, and 2304 genes) by qPCR. To ensure proteins were produced I performed a western blot. Protein lysates were run on an SDS gel and visualized using anti-flag antibodies. However, these initial fusions of 2305 did not complement. Subsequently, we developed an alternative Venus-tagged construct. Pending experiments include a pull-down assay that will assess a His-tagged CrrB, that has been tested for complementation by its resistance profile, and the Venus-tagged 2305 for protein-protein interaction *in vivo*.

RESULTS: qPCR analysis of the *crrA*, 2301, and 2304 genes revealed that the FLAG-tagged constructs did not complement. Western blot results indicated that these constructs did not express the protein. Subsequently, a C-terminal Venus-tagged 2305 construct was made and demonstrated to interact with CrrB by two-hybrid screening. Future experiments are expected to validate functionality and production, allowing a subsequent pull-down assay for 2305.

CONCLUSIONS: Although FLAG-tag constructs proved unusable, we are confident that Venus-tagged constructs will be more successful due to the two-hybrid screening. These results will provide a mechanistic understanding of the role of the CrrAB TCS in antibiotic resistance and high virulence. It will facilitate future development of novel therapeutics targeting CRKP infections.

Identification of Variable Number Tandem Repeats in the MUC1 Gene Associated with Chronic Kidney Disease Using Exome Sequencing

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Introduction and Objective: Despite advances in genomic medicine, not all types of disease-causing variants can be easily identified, even with genome or exome sequencing. One of the genetic variation that can be difficult to uncover, but is known to be associated with genetic diseases are Variable Number Tandem Repeats (VNTRs). One type of genetic variation that is difficult to uncover but is associated with genetic diseases is Variable Number Tandem Repeats (VNTRs). VNTRs are usually detected by fragment analysis or PCR analysis, and only recently have new bioinformatic tools been able to uncover VNTRs in exome sequencing data. VNTRs are highly polymorphic DNA sequences repeated a variable number of times in the genome, ranging from a few to hundreds of base pairs (bp)¹. The objective of this project was to implement the tool published by Mukamel et al. in Science 2021 to identify a known VNTR in the *MUC1* gene.

Methods: To implement Mukamel's tool in a cohort of 3300 individuals with kidney disease and exome sequencing data, there was a need to dockerize it. Dockerization is the process of packaging an application and its dependencies into a Docker container. This allows the application to run consistently across different computing environments. Samtools, a collection of tools designed for managing and analyzing high-throughput sequencing data, was used to sort and filter the exome data specific to the *MUC1* gene. One of the difficulties in identifying VNTRs in exome sequencing data is its uneven coverage. Mosdepth was used to calculate the depth coverage of the *MUC1* gene. Since substantial computational power is needed to run Mukamel's tool on a large number of samples, the script was moved to the cloud and written in Common Workflow Language (CWL) after dockerization to efficiently process all 3300 BAM files, which are currently stored there.

Results: To dockerize the script it was first cleaned and run on a small set of 150 samples downloaded from the National Institute of Standards and Technology (NIST). A Docker image was then created to ensure the script's compatibility across various machines, including virtual cloud environments. Presently, a Common Workflow Language (CWL) script is being developed to process all 3300 files in the cloud efficiently.

Conclusions: Dockerizing Mukamel's tool and providing it as an open-source tool to identify VNTRs is expected to allow other researchers to uncover novel gene-disease associations.

¹ Di Maio, S., Zöschner, P., Weissensteiner, H. *et al.* Resolving intra-repeat variation in medically relevant VNTRs from short-read sequencing data using the cardiovascular risk gene *LPA* as a model. *Genome Biol* **25**, 167 (2024). <https://doi.org/10.1186/s13059-024-03316-5>

VALIDATION OF A COLORIMETRIC ASSAY FOR URINARY ANALYSIS OF PHOSPHORUS

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INTRODUCTION AND OBJECTIVE: In swine, phosphorus (P) is needed at an optimal concentration. To prevent deficiencies, a common practice is to over supplement P concentrations, which creates environmental concerns. Through previous studies, attempts have been made to accurately define Ca/P requirements. A new method recently proposed, provides a better estimate of Ca/P requirements based on measures of urinary Ca/P ratios. If applied, this method could allow for more rapid adjustment of dietary P. Currently, P analysis of urine is measured through a nitric perchloric acid digest (NPAD). This method is an expensive and time-consuming process. Due to these factors, our study aims to update the conventional analysis of urine through a simple colorimetric assay.

METHODS: Five samples from five different sows were collected for P assays. The NPAD breaks down the proteins that bind P, while the simple assay uses trichloroacetic acid (TCA) to precipitate the proteins and release P. A colored complex is formed and its absorbance (abs) is then read on a spectrophotometer. To analyze the released P, a standard curve is created with known P concentrations. By creating a line of best fit from these known concentrations, the abs values for the unknown samples are plugged back into the linear equation to obtain the mg P/ dl urine.

RESULTS: Our lab is amid aligning the colorimetric assay to fit the wide scope of the concentrations of phosphorus in a urine sample. Currently, one urine sample had P concentrations below the limit for spectrophotometer detection. This is being corrected by adjusting the volume of the sample used. Once completed, the five samples will all have their concentrations calculated. Next, we plan to perform a NPAD. This step will allow for us to analytically compare the two experiments and decide if there is a significance between the two. We would expect for the mg P/dl of urine analyzed with the simple colorimetric assay to be within a 10% range of the mg P/dl of urine analyzed with the NPAD.

CONCLUSION: These future results will provide a definitive answer on whether the simple colorimetric assay can replace the NPAD with little adjustments to the procedure. If the values are within a ten percent range of each other, then we can conclude that the TCA is able to denature any proteins in urine and effectively release all available P for the colorimetric assay. If the values are not within the 10% range, we can theorize that there is something interacting at the same wavelength, whether that is along with some, all, or zero P released. While this updated methodology is only a portion of this overarching project, the potential for expanded use in commercial settings would be an attractive solution.

WHAT CAN WE LEARN THROUGH SOCIAL MEDIA MINING ABOUT THE BURDEN OF URINARY INCONTINENCE FACED BY AFFECTED INDIVIDUALS AND THEIR CAREGIVERS?

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INTRODUCTION AND OBJECTIVE: Urinary incontinence (UI) is a debilitating dysfunction of the lower urinary tract that has no cure. The burden of UI requires research advances for more effective and tangible treatment options. Those affected by UI as well as those that support and care for them are faced with the challenges that UI presents. With this in mind, we aimed to explore social media to identify salient issues of bother or concern of those living with UI and how those issues may impact their support systems.

METHODS: We searched for UI-related posts on the social media website reddit. The process is described (Figure). We included only posts that directly addressed a UI problem. Posts with pictures were kept so long as the picture was directly related to UI and comments addressed UI as well. We thematically evaluated posts and calculated sentiment scores for all (Figure).

RESULTS: We assembled and analyzed 137 reddit posts and comments. We were able to calculate sentiment scores for 96% of original posts and 100% of top three comments. Posts were from those afflicted with UI (46%), caregivers (45%), and unknown (9%). Posts and comments with negative sentiment were generally those articulating UI challenges, frustrations, and problems, including topics of bedwetting, estrogens creams, odor, laundry, diapers, relationships, and solutions. Posts and comments with positive sentiment were supportive (e.g., “hang in there,” etc.) and/or posts that suggested practical solutions to problems.

CONCLUSIONS: Results demonstrated an active online community of both UI sufferers and their caregivers voicing challenges and problems as well as empathy. Social media thus appears to be a lucrative source of patients’ and caregivers’ experiences with UI. As UI is not definitively treatable for most, more effective and accessible strategies to cope with and mitigate symptoms are needed. Social media may be a rich source of patient- and caregiver-centered perspectives about UI that could be used by providers to discuss and develop coping strategies. Future research should examine other social media platforms, use more sophisticated methods, and consider mechanisms for using social media to communicate evidence-based information and support to patients and their caregivers.

Create Reddit Application Processing Interface (API) & Download the Python Reddit API Wrapper 'praw' package



Identified subreddits to use: had keyword as subreddit name OR a popular subreddit thought to contain desired information (popularity determined with subreddit stats which gives subreddit follower info.) Criteria was at least 1000 followers to be 'popular'

Keywords:
urinary
urgency,
incontinence,
urine,
overactive
bladder,
interstitial

Categories: advice, frustration/venting, seeking advice, caregiver ethics, humor, empathy.
Topic: bedwetting, odor, diapers, non-invasive treatments, laundrying, how to

Post Organization: Original posts were placed into categories. Original Posts as well as comments were assessed for topic being discussed.



Scrape Reddit: For subreddits directly containing keywords as name, python pulled top 10 posts. For subreddits not containing keyword as name python randomly pulled 10 posts with useage of keyword quieres. (e.g. r/nursing with keyword 'incontinence' python returned 10 random posts). We manually collected the top three comments from each post as well as link, poster, date posted, score, and # of comments.

Sentiment Analysis: Original posts and comments were analyzed for setniment via TextBlob which calculates a sentiment score and polarity score.



DOES FRUIT AND VEGETABLE CONSUMPTION JEOPARDIZE METHENAMINE TREATMENT FOR URINARY TRACT INFECTION?

Ali Mustafa,¹ Kristina L. Penniston²

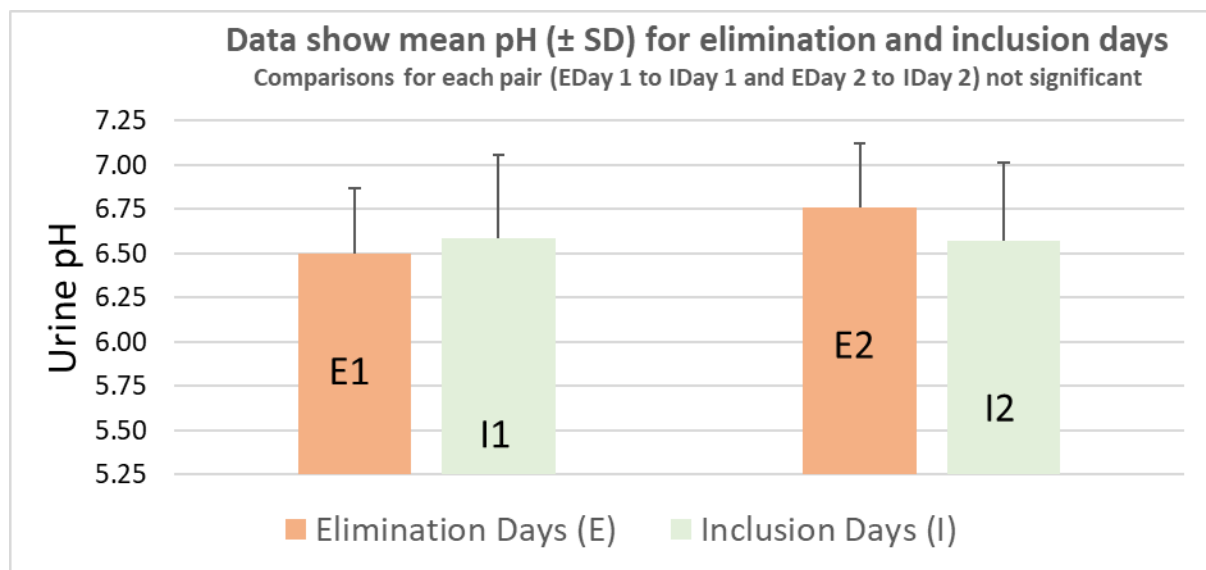
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INTRODUCTION AND OBJECTIVE: Methenamine is used to treat urinary tract infections (UTIs) and works optimally when urine pH is <5.5 . For this reason, it is often administered in a formulation containing hippuric acid, a pH-lowering agent. Because fruits and vegetables (FV) provide a dietary alkaline load due to their organic acids, which undergo hepatic conversion to bicarbonate, patients are often advised to limit FV consumption during methenamine therapy to maintain desired urine acidity. However, the efficacy of this practice is poorly supported by evidence. Moreover, elimination of FV reduces the nutritional quality of the diet in multiple ways. We evaluated the impact of FV on urine pH. We hypothesized that consumption of FV would not have a significant effect on urine pH.

METHODS: With IRB approval, healthy participants agreed to measure their urine pH over 4 days, 2 days of which were to be devoid of FV and the other 2 days to include ≥ 5 servings. Participants used dipsticks to measure and document their urine pH during midstream voids at 3 timepoints each day: (1) upon waking, (2) midday (separated when possible from a meal by 3-4 hours), and (3) before bed. To monitor dietary compliance, participants were asked to record the number of FV servings consumed daily. Group means $\pm$ SD were calculated and compared for significance with Student's t-tests.

RESULTS: Participants ($n=10$, 44% female, average age 21 years) consumed an average of 0.4 ± 0.9 FV servings on elimination days and 6.2 ± 1.5 on inclusion days ($P < 0.001$ for difference). Urine pH did not differ on elimination vs. inclusion days (Figure), $P > 0.30$ for all time comparisons. When we examined men vs. women, there were no differences in FV consumption nor in urine pH on elimination or inclusion days. When controlling for those who ate >6 vs. ≤ 6 servings FV on inclusion days, there were no differences in urine pH compared to elimination days.

CONCLUSIONS: Our results question the necessity of dietary restrictions during methenamine. If the temporary elimination of FV does not significantly alter urine pH, there is little concern that their inclusion will jeopardize the therapeutic effect of methenamine, particularly if given as methenamine hippurate. FV are healthy foods, responsible for maintaining a robust gut microbiome and reducing inflammation. Therefore, assuming the durability of our results in patients with UTI, providers should stop recommending FV elimination during UTI therapy.



Spns1 is an iron transporter essential for megalin-dependent endocytosis.

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Introduction and Objective: The kidney's ability to filter and reabsorb essential nutrients is vital for preventing diseases such as proteinuria, where proteins are lost in urine. This study explores the function of Spns1, an endolysosomal membrane protein, in mediating iron transport and its impact on endocytosis within the kidney.

Methods: A novel Cre-LoxP system targeting megalin-expressing cells was used to conditionally knockout Spns1. Additionally, iron homeostasis was manipulated through diet and genetic engineering, by knocking out the DMT1 gene, to assess the functional reversibility of the observed phenotypes. Quantitative PCR confirmed the efficiency of the knockout and its downstream effects on other iron-regulating genes. Western blotting provided insights into alterations in key proteins involved in endocytosis and iron metabolism. Confocal microscopy was used to observe structural and morphological changes in the kidney tissues.

Results: Knockout of Spns1 significantly impaired the function of the megalin-cubilin complex, essential for protein reabsorption, leading to proteinuria and altered cellular iron levels. Iron loading experiments in wild-type mice mimicked the phenotypic disruptions observed in Spns1 knockout mice, which confirms the gene's role in iron-induced endocytosis inhibition.

Conclusions: Spns1 is critical for maintaining efficient iron transport and endocytic functions in kidney proximal tubules. The loss of Spns1 in the proximal tubules causes cytoplasmic iron overload, which in turn impairs the proximal tubules' ability to endocytose proteins effectively. This results in proteinuria. The study underlines a complex feedback mechanism in which iron levels regulate apical endocytosis and cellular processes within the kidney. Additionally, the findings suggest that Spns1's function might not be compensated by other family members like Spns2 and Spns3 due to its unique role in iron homeostasis.

Local and Remote Tissue-Specific Macrophage Responses during Urinary Tract Infections

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INTRODUCTION AND OBJECTIVE: In the steady state, the myeloid cell compartment of tissues is highly heterogeneous, consisting of macrophages, dendritic cells, and monocytes. Each of these cells plays an essential role in tissue homeostasis, repair, and immunity. Although infections may reside at a single location, the impact of the infection may be systemic due to the release of cytokines, chemokines, and products from pathogens, such as lipopolysaccharide in uropathogenic *Escherichia coli* (UPEC) urinary tract infections (UTI). We hypothesize that UTIs impact the remote tissue (kidney, lung, heart, liver, peritoneal cavity, and spleen) macrophage populations.

METHODS: Bladders of female C57BL/6J mice were infected with UPEC strain UTI89 (n = 10) via transurethral catheterization and the bladders of control female C57BL/6J mice PBS was used (n = 5). Cells were isolated from the bladder, kidney, liver, peritoneal cavity, heart, lung, and spleen and stained with a flow cytometry panel to identify macrophage populations. Cells were analyzed by spectral flow cytometry and FlowJo software. Serum was collected and analyzed for cytokines in the circulation.

RESULTS: Following the bladder inoculation with UPEC, a phenotypic change of the macrophage populations in the bladder and remote tissues was observed compared to the controls. In the bladder, an increase in the monocyte-derived macrophage populations and the recruitment of monocytes and neutrophils was observed following a UTI. A significant increase in the number of macrophages was observed in the bladder ($P < 0.05$), heart ($P < 0.05$), peritoneal cavity ($P < 0.05$), liver ($P < 0.01$), and lung ($P < 0.01$) during a UTI compared to the controls. No significant differences in macrophage numbers between the UTI and the controls were observed in the kidney and lung. There was a significant increase in the inflammatory cytokines IL-1 β ($P < 0.05$), TNF- α ($P < 0.01$), and MCP-1 ($P < 0.01$) in the circulation of mice with UTI compared to the controls.

CONCLUSIONS: The generated data indicates a dynamic change in the composition of the bladder macrophage populations and the remote tissue macrophage populations during UTI. However, it is unknown if the alterations in the macrophage populations are maintained over time or if these populations revert to the steady state following the resolution of infection, which may impact the subsequent local infections of the different tissues. Future studies will elucidate the functional properties of the macrophage populations specific to the steady state and following UPEC infection in the bladder and remote tissues.

Immunohistochemical detection of serotonin producing neuroendocrine cells and serotonin responding interstitial cells of Cajal in the adult male mouse and human urethra

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

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

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

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

IMPACT OF GNRH AGONIST LEUPROLIDE ON THE GENITOURINARY STRUCTURE AND BEHAVIOR OF MALE SPRAGUE DAWLEY RATS

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INTRODUCTION AND OBJECTIVE: Leuprolide is a medication that disrupts the body's hormonal balance. It is used to treat cancers and delay puberty. However, the long-term consequences of this disruption on urogenital development and behavior remain unclear. We investigated how Leuprolide impacts the development of male rat gonads, assessed its reversibility, and potential influences on behavior.

METHODS: 12 Sprague Dawley rats were obtained from Charles River Laboratories. Under specific conditions, their housing was under a 12-hour light/dark cycle with 3 rats per cage. Standard rodent chow was provided ad libitum with Nylabone chew toys for enrichment by animal care staff. Leuprolide was administered subcutaneously as an extended-release depot. The dosage regimen mimicked clinical human dosages, although specific details were not provided. Two different dosages were used on two groups: 0-2.0 mg/rat with an injection volume of 0.8 mL and .15 mg/rat with an injection volume of 0.04 mL (less concentrated). These values are considerably doubled in 2 weeks as the growth rate of these rats is 300% in a month from a post-natal standpoint. The study employed a behavioral test called Barnes Maze to assess the effects of Leuprolide on the rats' cognitive function and behavior; focused on spatial learning and memory. After a 2-minute habituation period, rats underwent 6 acquisition trials lasting 5 minutes each (totaling 30 minutes). This was followed by a reverse learning phase with another 6 trials of 5 minutes each (totaling 30 minutes) and a final 4-minute probe trial. Notably, the behavioral testing was conducted over 4 weeks. As hormonal blockage is present, spatial focus and memory were a key influence in assessing the effect on the gonad morphology and other organ development in the rats at two time points: starting at postnatal day 23 (the first injection) and ending at postnatal day 46.

RESULTS: Initial results suggest spatial learning and memory increase based on the Barnes Maze test. The rats learned and re-learned the maze quicker over time, and continued exploring during a separate trial, potentially indicating cognitive development. Evaluation of gonad morphology is ongoing, with early observations showing weight changes in the gonads caused by the hormonal drug. More specifically, a volume reduction due to Leuprolide's known effects stimulates the pituitary gland to release two hormones: Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). These findings will guide the design of a future study focusing on a more detailed timeline of how Leuprolide impacts urogenital development.

CONCLUSIONS: Based on this pilot study, Leuprolide administration in male rats shows promise for improving spatial learning and memory, while also affecting urogenital development. These initial findings warrant further investigation in a larger study to confirm and explore the detailed timeline of Leuprolide's impact on cognitive function and gonad morphology. The complete study could hold significant implications for understanding Leuprolide's mechanisms of action and potential medical applications.

Dysregulation of the Plasma Metabolome Profile in Kidney Stone Formers

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INTRODUCTION AND OBJECTIVE: Kidney stones (KS) have been linked to cardiometabolic disorders, but limited research has evaluated the effect of metabolic disorders including the metabolic syndrome on KS risk. Thus, we investigated this association in a large dataset using an available panel of serum metabolites and genotyping data.

METHODS: Initially, participants in the United Kingdom Biobank (UKBB) with available metabolomics data (n=186,362) were used to examine the relationship between KS prevalence and blood metabolomics in univariate analysis. Subsequent linear regression models were performed that accounted for comorbidities, eGFR, sex, age, smoking activity, physical activity, alcohol use, and approximate lipid intake. Genome wide association studies (GWAS) were conducted on the phenotypes of KS, BMI, serum HDL, TG, and AIP (log₁₀(TG/HDL)), with or without BMI adjustment. Mendelian randomization (MR) analysis was then utilized to search for potential causal relationships. Linkage disequilibrium score regression (LDSC) was performed in advanced analyses to examine the genetic correlation between phenotypes.

RESULTS: A total of 63 metabolites in very large, large, and medium high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL) were identified as significant lipoprotein subclasses as related to KS. Robust multivariate analysis produced 31 significantly associated metabolites including HDL cholesterol ($\beta=-0.04$, 95% CI: [-0.06, -0.03], $p<0.001$) and VLDL triglycerides (TG) ($\beta=0.07$, 95% CI: [0.03, 0.11], $p=0.02$). GWAS identified several key SNP locations associated with KS, BMI, and HDL. Subsequent MR analysis indicated a strong causal relationship between BMI and kidney stones (OR=1.15, 95% CI: [1.04, 2.29], $p=0.008$), and BMI and HDL ($\beta=-0.02$, 95% CI: [-0.04, -0.01], $p<0.001$). MR analysis between KS and HDL after adjusting for BMI was not significant. Genetic correlation results revealed a significant correlation between HDL and KS ($r_g = -0.13$, $p = 0.003$), which was no longer significant when adjusted for BMI ($r_g = -0.05$, $p = 0.26$). AIP ratio and BMI-adjusted KS were also significantly associated ($r_g = 0.13$, $p = 0.001$).

CONCLUSIONS: This study suggests that subjects with KS manifest lipid dysregulation, with higher large VLDL-TG and lower HDL components. These results highlight the role of obesity-related genetic changes as a potential mediator of the comorbid relationship between KS and the metabolic syndrome.

RECURRENT NEURAL NETWORK-BASED SENTIMENT ANALYSIS OF NEPHROLOGY AND UROLOGY PHARMACEUTICAL CLINICAL TRIALS FOR FEMALE ANATOMICAL COMPATIBILITY

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INTRODUCTION AND OBJECTIVE: It was not until 1993 that Congress mandated that women be included in clinical research under the NIH Revitalization Act (Public Law 103-43).¹ However, this has not prevented pharmaceutical companies from including women in token amounts, or approving products initially intended for males that were eventually also used by females.² In particular, a cross sectional study examining >20,000 clinical trials from 2000 to 2020 found severe female underrepresentation in “oncology, neurology, immunology, and nephrology”, some of the most important processes to human health.³ Unfortunately, as the burden of literature review of pharmaceuticals continues to increase for physicians, it becomes impractical for providers to keep up with the nuances of individual drugs. Accordingly, my project is to create a tool for nephrology and urology healthcare providers to rapidly determine the compatibility of a given pharmaceutical with female anatomy. This will be achieved by employing a recurrent neural network or RNN to classify pharmaceuticals as either recommended or not recommended for female patients with nephrology or urology-based conditions. This algorithm will be implemented in a publicly-available website for providers and patients alike to view.

METHODS: Publically available results from clinicaltrials.gov are employed for the RNN's training and testing datasets. Prior to RNN training, a tokenizer is used to convert the textual data into integers. The RNN itself utilizes Keras Tensorflow and supervised learning, after which a sigmoid function categorizes the final result⁴. The website was coded with Shiny R adapted for Python.

RESULTS: Accuracy will be determined by the percentage of the testing dataset the RNN correctly classifies. The skeleton of the website is near completion, currently displaying relevant instructions and information with an input option.

CONCLUSIONS: When finished, this project will be clinically relevant to providers who may use the RNN-integrated website as a second opinion when prescribing new pharmaceutical treatments to patients with female anatomy. The project presents a time-efficient method for determining pharmaceutical compatibility with female patients.

¹ [*NIH revitalization Act of 1993 public law 103-43 - Women and health research*](#). NCBI Bookshelf.

² Steinberg JR *et al* (2021). Analysis of female enrollment and participant sex by burden of disease in US clinical trials between 2000 and 2020. *JAMA Network Open* 4: e2113749.

³ Young L (2016, November 2). [*Drugs aren't tested on women like they are on men, and it could have deadly consequences*](#). Global News.

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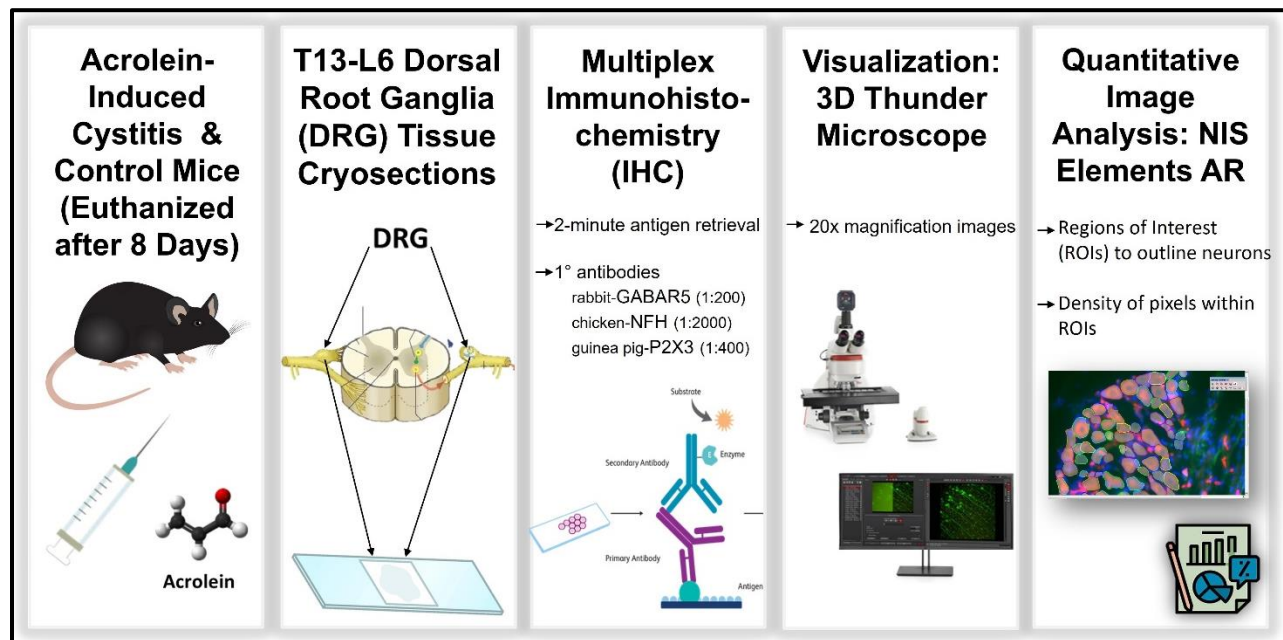
EXAMINING LONG-TERM MOLECULAR CHANGES IN DORSAL ROOT GANGLIA OF A MOUSE MODEL OF ACUTE CYSTITIS

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INTRODUCTION AND OBJECTIVE: Urologic chronic pelvic pain syndrome (UCPPS) encompasses interstitial cystitis/bladder pain syndrome and chronic prostatitis/chronic pelvic pain syndrome, and is characterized by chronic pain in the lower pelvic region and genitalia, along with urinary frequency and urgency. Some patients report experiencing long-lasting secondary effects such as hypersensitivity of the skin, but the underlying pathophysiological mechanisms are unknown and there are no effective treatments. Previous research has revealed that biomarkers of interest such as alpha5 subunit of GABA-A receptor (GABAR5), Neurofilament Heavy (NFH), and P2X3 receptor are upregulated in Dorsal Root Ganglia (DRG) short-term after pain caused by nerve injury, but it is unknown what changes occur after cystitis. Accordingly, after the apparent resolution of voiding dysfunction, long-lasting phenotypes such as paw hypersensitivity may be observed 7-8 days after acute cystitis in mouse models. We hypothesized that after 8 days, GABAR5, NFH, and P2X3 will be upregulated in DRG of acrolein-induced cystitis mice compared to control mice.

METHODS:



RESULTS: Pilot studies determined optimal concentrations of antibodies using a 2-minute antigen retrieval step, including rabbit-GABAR5 1:200; chicken-NFH 1:2000, guinea pig-P2X3 1:400. Using these conditions, staining for NFH and P2X3 were satisfactory, though GABAR5 staining was more difficult to assess. We prioritized L5-L6 DRG, since those contain bladder and paw afferents. Quantitative image analysis is ongoing. We anticipate that all three markers of interest will show increased pixel density within DRG neurons in cystitis mice compared to control mice.

CONCLUSIONS: If our hypothesis is supported, the results would suggest that acute interstitial cystitis causes a neuropathy that contributes to hypersensitivity and other long-lasting phenotypes, even after urinary symptoms have resolved. These findings could shed light on poorly understood neuropathies experienced as a result of UCPPS and other urological diseases. Based on our findings, antagonists of GABAR5, or other markers, might prove useful for treating hypersensitivity after acute cystitis. Understanding molecular changes that underlie hypersensitivity after cystitis will propel novel therapies to treat or prevent UCPPS.

Optimization of locoregionally delivered mesenchymal stem cell-derived extracellular vesicles as an acute kidney injury therapeutic

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INTRODUCTION AND OBJECTIVE:

Acute kidney injury (AKI) often leads to chronic kidney disease (CKD), an urgent public health emergency for which existing therapies are mostly palliative. Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) carry anti-inflammatory and pro-regenerative molecules with the potential to induce localized tissue regeneration in AKI patients. Here, we intend to optimize the dose of locoregionally delivered MSC-derived EVs for treating cisplatin-induced AKI in C57BL/6 mice models.

METHODS:

Umbilical cord-derived mesenchymal stem cells (UC-MSCs) were cultured in 5% CO₂ at 37 °C in low glucose DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. For MSC-EV isolation, FBS-free medium was used. AKI was induced in male C57BL/6 mice (6 weeks old) by intraperitoneal injection of cisplatin at a dose of 20 mg/kg body weight on day 0. Following EV isolation using an exosome isolation kit and EV characterization, varying doses (400-800 µg/kg) of MSC-EVs were administered locoregionally into the renal artery on day 3. On day 5, serum and tissue samples were collected to assess the therapeutic effect of MSC-EVs in ameliorating AKI.

RESULTS:

We expect a high EV dose of 800 µg/kg body weight to be optimal for locoregional delivery in C57BL/6 mice. Consequently, we anticipate observing a significant decrease in serum BUN and creatinine levels, along with reduced tissue fibrosis, tubular damage, and inflammatory responses on day 5 in MSC-EV-treated mice compared to untreated AKI controls.

CONCLUSION:

In optimizing the dosage of locoregionally delivered MSC-EVs, we aim to further leverage their regenerative properties and develop a more effective treatment for cisplatin-induced AKI. This research could pave the way for future studies further optimizing cell-free tissue regeneration methodologies: novel solutions to an increasingly urgent public health challenge.

Using Gene Expression Analysis to Identify Genetic Drivers of Hypercalciuric Urolithiasis

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INTRODUCTION AND OBJECTIVES: 1 in 10 people will experience kidney stones in their lifetime. Genetic predispositions of stone disease, including hypercalciuria, increases the risk of development. Multiple studies have identified genes associated with this disorder, however there is no evidence of *how* these genes interact to create stones in patients. To address this, we sought to identify the alterations in gene regulatory networks between cases and controls that may explain the genetic drivers of kidney stones using RNA sequencing.

METHODS: IRB approval was obtained for this study (2022-0363). Peripheral blood samples were collected from hypercalciuric patients (n=24) and non-stone formers (n=25). RNA was extracted, sequenced, and aligned to the hg38 reference genome at the University of Wisconsin Gene Expression Centre. Principal Components Analysis (PCA) was used to identify sources of variation in the data. Differential expression analysis was performed using the Wald Test within DESeq2 R package v.1.40.2. DEGs with a p-value (adjusted for multiple tests) of <0.05 and a log fold change of >2 was considered significant.

RESULTS: Average age of participants within case and control groups was 54.0. The proportion of women in the case group and control group was 54% and 64% respectively. PCA identified batch and sex effects in the data, and these were included in the final model for differential expression analysis. When all patients were analysed together, no differentially expressed genes (DEGs) were identified. However, after results were stratified by sex, a DEG was discovered in females, along with 2 other genes that were near significance (P<0.01), (Table 1).

Table 1: Top genes identified after differential expression analysis on RNA separated by sex.

Women				
Gene ID	BaseMean	Log Fold Change	Stat	P _{adj}
TALAM1	1.04E+02	-2.32	-5.20E+00	0.003
ANO5	7.00E+01	0.63	4.45E+00	0.06
UBASH3A	2.98E+02	-0.51	-4.29E+00	0.09
Men				
Gene ID	BaseMean	Log Fold Change	Stat	P _{adj}
XRRA1	2.93E+02	1.36	4.32E+00	0.25
GATD3	1.16E+02	3.84	4.10E+00	0.33
ENSG00000278879	2.87E+01	1.42	3.93E+00	0.41

CONCLUSION: Results showed the top gene among females was the *TALAM1* long-noncoding RNA which has been implicated in cancer aggressiveness and malignancy. Further evaluations are planned to identify interactions among genes in the dataset. This research will contribute to knowledge about the underlying mechanisms of stone disease, which may inform novel treatments in the future.

IDENTIFICATION OF BIOMARKERS OF EARLY STAGE BENIGN PROSTATIC HYPERPLASIA

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INTRODUCTION AND OBJECTIVE:

Benign prostatic hyperplasia (BPH) can be found in almost 90% of men by 85 years of age, yet the pathogenesis of BPH remains unclear. Current treatments for BPH, such as transurethral resection of the prostate or laser prostatectomy, aim to treat the entirety of the prostate rather than focusing exclusively on the BPH-related changes within the gland. This study aims to pinpoint factors that contribute to the development of BPH and identify biomarkers specific to earlier stages of the disease.

METHODS:

The patient cohort for this study consists of men with cases of prostate cancer and BPH. Samples from the archived tissue were annotated for areas of BPH and normal prostate tissue. Sections of prostate were dissected for epithelial and stromal tissue separately. The epithelial and stromal samples were profiled by RNA sequencing using Smart-3-SEQ. Biomarker candidates were identified using DESeq2. Tissue microarrays were constructed for BPH and normal prostate. Immunohistochemistry (IHC) for selected biomarker candidates were performed on the BPH and normal tissues.

RESULTS:

We identified four broad groups of BPH lesions based on histology and RNA profiled 20 samples of each. Group 1 is composed of only stroma but expressed genes that have previously been found to be biomarkers of BPH. This group also displayed stromal progenitor and embryonic features. Group 2-5 lesions all contained both stroma and epithelium and have expression profiles different from that of normal prostate tissue, but similar to each other, suggesting that the five groups may all be related to each other. Ongoing IHC will test biomarkers that are specific for group 1 lesions.

CONCLUSIONS:

In this study, we are using laser capture microdissection combined with RNA profiling and IHC to examine the stromal and epithelial components of BPH lesions. We showed that the group 1 stroma nodules share similar transcriptomic signatures with the BPH lesions that contain both epithelium and stroma, suggesting that BPH starts as a fibroblastic process and later acquires epithelium. The identification of a group 1 specific IHC biomarker will aid in the further characterization of this process.