AWARD NUMBER: W81XWH-21-1-0465

TITLE: Pannexin 1 Channel, a Novel Molecular Mediator and Potential Therapeutic Target for Interstitial Cystitis

PRINCIPAL INVESTIGATOR: Sylvia O. Suadicani, PhD

CONTRACTING ORGANIZATION: Albert Einstein College of Medicine, Bronx, NY

REPORT DATE: July 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2022	Annual	15Jun2021 – 14Jun2022

4. TITLE AND SUBTITLE

Pannexin 1 Channel, a Novel Molecular Mediator and Potential Therapeutic Target for Interstitial Cystitis

5a. CONTRACT NUMBER
W81XWH-21-1-0465

5b. GRANT NUMBER
PR200644

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sylvia O. Suadicani, PhD

E-Mail: sylvia.suadicani@einsteinmed.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY, 10461-1900

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project focus on Interstitial Cystitis (IC) and mechanisms whereby stress and urothelial dysfunction lead to development of IC symptoms. The premise of the studies is that stress disrupts the urothelial barrier function, which results in chronic infiltration of urinary K+ into the bladder wall that generates an environment for abnormal Panx1 channel activation and expression that ultimately lead to IC and its symptoms by augmenting proinflammatory responses, intercellular signaling and stimulation of bladder sensory fibers. In this reporting period of the project, the findings that we obtained from studies conducted with the animal model of 96hrs of continuous illumination stress (CIS) demonstrated that stress not only triggers IC-like symptoms of urinary frequency, but that these symptoms persist long after stress cessation and are accompanied by a sustained increase in urothelial ATP signaling and by changes in expression of Panx1 and other key molecular mediators of the urothelial mechanotransduction system. These findings are in line with our overarching hypothesis that stress-induced disruption of the urothelial barrier triggers IC symptoms by amplifying ATP release and signaling through mechanisms that involve upregulation Panx1 expression and function in the bladder.

15. SUBJECT TERMS

Interstitial Cystitis; stress-induced IC symptoms; urothelial dysfunction; ATP signaling; pannexin 1 (Panx1) channels.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	17	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

This research project focuses on Interstitial Cystitis (IC), a chronic and debilitating bladder condition characterized by symptoms that include urinary urgency, frequency, and pelvic pain. The IC etiology is still unclear, but there is accumulating evidence that an increase in permeability of the bladder urothelium contributes to the onset and perpetuation of IC symptoms. Current treatment approaches focused on repairing the urothelial barrier are not always effective, indicating that the deleterious effects of a "leaky" urothelium may persist long after recovery of the barrier function. The studies proposed in this project aim at better understanding mechanisms triggering IC in the context of urothelial barrier dysfunction and identifying novel molecular targets to advance therapeutic approaches for IC patients.

Studies will test the overarching hypothesis that urothelial Pannexin 1 (Panx1) channels play a key role in events leading to bladder sensitization, micturition dysfunction and pelvic pain in IC by amplifying ATP signaling and activating the bladder inflammasome. Specifically, we propose that chronic diffusion of urinary K^+ through a leaky urothelium generates an environment within the bladder wall that results in abnormally high Panx1 channel activation and expression that augment pro-inflammatory responses, intercellular signaling and stimulation of bladder sensory fibers, which ultimately lead to IC and its characteristic urinary and pelvic pain symptoms. At short term, these studies will significantly impact the field of basic and clinical research in IC by bringing forth new mechanisms and disclosing Panx1 channels as novel molecular mediators and therapeutic targets for IC.

2. KEYWORDS:

Interstitial cystitis (IC); IC/Bladder Pain Syndrome (IC/BPS); Chronic pelvic pain; Stress; Bladder urothelium; Pannexin 1; ATP signaling; Inflammasome.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

This project has two major goals:

1 – Investigate the participation of pannexin 1 (Panx1) channels in mechanisms that lead to bladder sensitization, micturition dysfunction and pelvic pain in a stress-induced IC model with disruption of the urothelial barrier.

- Timeline: months 4 to 40 of the award.
- Percentage of completion: 25%

2 – Determine the potential of Panx1 as a novel therapeutic target for interstitial cystitis associated with urothelial barrier dysfunction.

- Timeline: months 13 to 42 of the award.
- Percentage of completion: not started.

What was accomplished under these goals?

In this first year of the award, we made significant progress towards achieving the goals and completing the tasks set for the studies proposed in Aim 1 of this project.

Specific Aim 1 – *Investigate the participation of pannexin 1 (Panx1) channels in mechanisms that lead to bladder sensitization, micturition dysfunction and pelvic pain in a stress-induced IC model with disruption of the urothelial barrier.*

We specifically focused on conducting the activities planned in Major Task 1 of the project's Statement of Work (SOW), Subtasks 1, 2, 3, 4 and 6.

Major Task 1: *Characterize the time course of changes in Panx1 expression and function in the bladder of mice submitted to CIS and determine the extent to which these changes correlate with the course of development and the persistence of IC-like symptoms in this animal model*. (Timeline: months 4 – 40).

We successfully completed Subtasks 1 and 2, which involved obtaining IACUC and ACURO approval for the proposed *in vivo* studies using an animal model in which stress is the trigger of IC symptoms, a factor that is not only clinically relevant but also of major relevance in modern society and particularly in the lives of military personnel and their families. Among the most well-established stress models, we chose the prolonged constant illumination stress (CIS) model that results in a leaky urothelium due to disruption of tight junctions and enhanced desquamation of superficial urothelial cells, similar to what has been observed in histological examination of bladder biopsies from IC patients.

Subtask 3: Following IACUC/ACURO approval, we initiated the activities in Subtask 3, which focused on generating the CIS model and characterizing the time course of onset and maintenance of the stress-induced IC-like urinary symptoms in the CIS mice.

In the CIS model, the mice are individually housed and maintained for 96 hours under constant illumination (regular housing illumination levels). Animals in the control group are kept under conventional illumination (12hrs day/night cycle). At the end of the 96hrs of CIS, the animals are returned to regular housing conditions (3 mice per cage, 12hrs day/night cycle).

The emergence of IC-like urinary symptoms in CIS mice is then assessed non-invasively using the voided stain on paper (VSOP) method to evaluate changes in voiding behavior.

In the VSOP method, the animals are housed individually in cages with a bottom grid and a filter paper beneath it, and with food and water *ad libitum*. Mice are allowed to acclimate to the VSOP chambers overnight and spontaneous voiding behavior is then assessed continuously along two days. Urine-stained spots are then examined under UV light, drawn on paper, counted, and voiding frequency calculated for both daytime (mice sleep phase) and night-time (mice active phase).

VSOP was conducted at baseline (before CIS initiation), then at the end of CIS (96hrs CIS), and at 7- and 14-days post-CIS.

In Figure 1, we present the data that we have obtained from independent rounds of experiments performed with female mice (each round = 3 CIS and 3 control mice per experimental timepoint).

As shown in Figure 1, exposure to 96hrs of CIS induced a significant increase in the diurnal urinary frequency of CIS-mice. Notably, at 7- and 14-days post-CIS the diurnal urinary frequency of CIS-mice remained at levels higher than those of non-stressed controls.

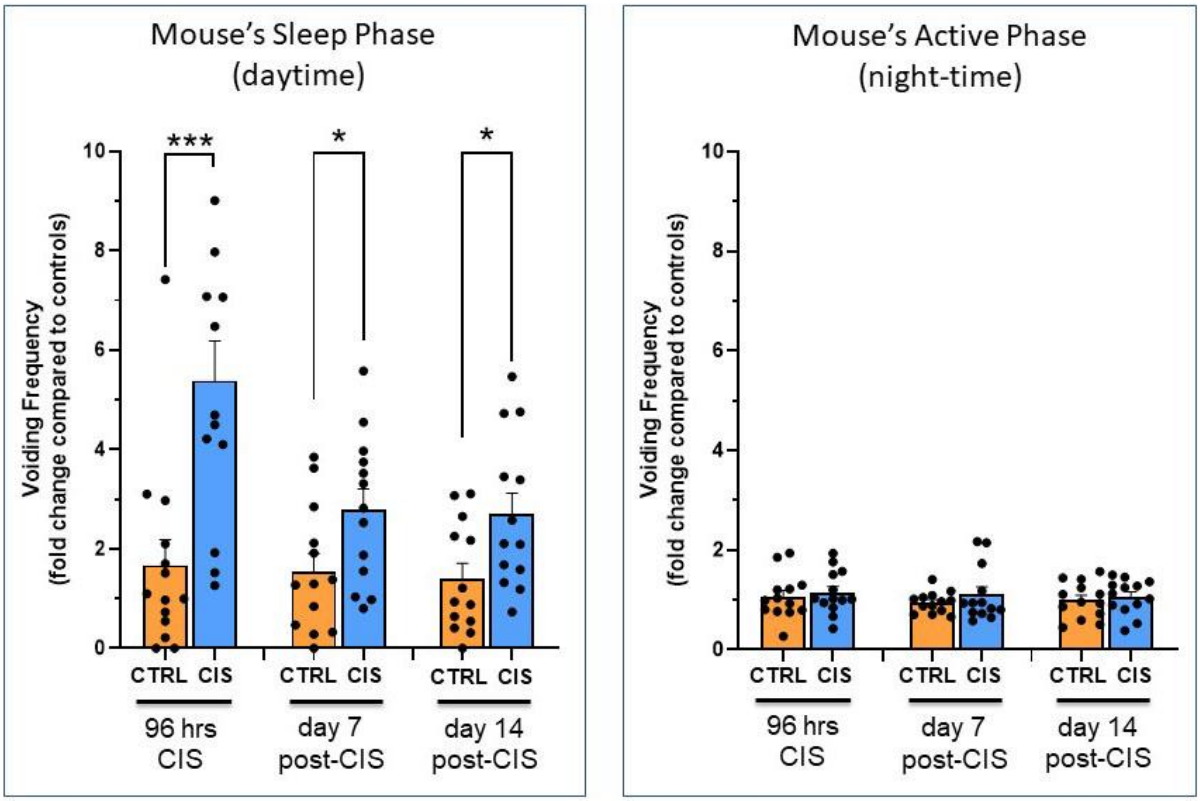


Figure 1 – *Exposure to 96hrs of constant illumination stress (CIS) triggers the development of characteristic IC-like symptoms of urinary frequency in female mice that are evident during the animals sleep phase, indicating the development of nocturia-like symptoms similar to observed in IC patients.* Voiding behavior was assessed at baseline and animals were then divided in CIS and control (CTRL) groups. The CIS mice was then exposed to 96hrs of continuous room illumination and CTRL mice maintained at housing conditions (12hrs light/dark cycle). Changes in voiding behavior were then assessed continuously along 2 days at 96hrs-CIS, and then at 7- and 14-days after stress cessation. Voiding frequency was normalized by each baseline line value and then by control values at each time point. Data is represented as Mean±SEM, n=12-14 mice per group and experimental time point. Mann Whitney test was used to determine statistical differences between CIS and CTRL at each time point (*$p<0.05$; ***$p<0.0001$).

Interestingly, while this persistent effect of stress on voiding function was evident during the animals' sleep phase (daytime), it was not observed during their active phase (night-time) (Figure 1). This finding indicates the development of stress-induced nocturia in the animal model. Nocturia is one of the cardinal symptoms in patients with IC, and urinary urgency and bladder pain are both seeing as factors leading to nocturia in patients. To our knowledge, the observed development of nocturia in the CIS model is first report of stress-induced nocturia in a mouse model, and it is highly encouraging for not only nicely recapitulating the urinary symptoms in IC patients but also further supporting the use of this model in these studies.

Subtask 4: *Molecular analyses of the bladder tissues*. Following the longitudinal evaluation of bladder function at 96hrs of CIS and then at 7- and 14-days post-CIS, the animals were euthanized at 21-day post-CIS and the bladders harvested. The urothelium was separated from the underlying lamina propria and detrusor muscle under a dissecting microscope and processed to quantify expression levels of Panx1 channels and of other molecular targets.

In our preliminary studies, we observed that exposure to 96hrs of CIS induced a significant increase in Panx1 expression in the bladder mucosa at levels that were 2.5-fold higher than in non-stressed controls ($p<0.001$). Such upregulation of Panx1 is consistent with the observed significant increase in voiding frequency in CIS mice (see Fig. 1) and is in line with the proposed role played by this channel in the emergence of the IC-like symptoms in this animal model.

We have now observed that at 21 days after stress cessation, the Panx1 protein expression in the urothelium of CIS mice was downregulated and reduced to levels that were significantly lower than those observed in non-stressed controls (Figure 2). This finding is consistent with the progressive reduction on stress-induced IC-like urinary symptoms observed in the CIS mice after stress cessation. However, such level of Panx1 downregulation is somewhat at odds with the functional data. As shown in Figure 1, at 14 days post-CIS, the voiding frequency of CIS mice was reduced when compared to that observed at 96hrs of CIS but remained higher than that of non-stressed controls ($p<0.05$). One explanation for this apparent discrepancy can be provided by data obtained from quantification of expression levels of urothelial TRPV4 channels and P2X7 receptors. As shown in Figure 2, at 21 days post-CIS, the expression levels of P2X7R are not different but TRPV4 levels are higher in the urothelium of CIS mice when compared to those in non-stressed controls. P2X7R and TRPV4, like Panx1, are components of the urothelial mechanotransduction system and play a key role in urothelial ATP signaling. Most importantly, they have been shown to also activate Panx1 channels. In this regard, it is likely that an upregulated urothelial TRPV4 expression and activity would significantly contribute to enhance Panx1 activation and overall function of these channels, and consequently, the persistence of IC-like symptoms long after stress cessation.

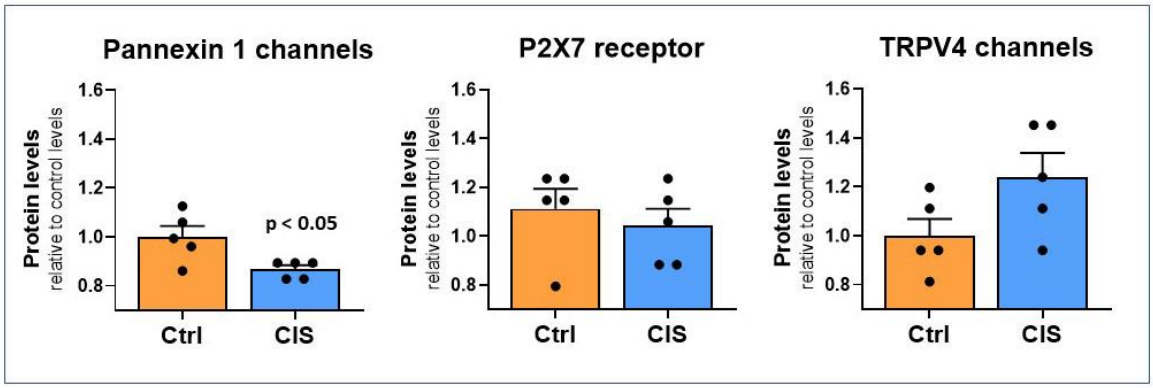


Figure 2 – Protein expression levels of Pannexin 1 channels, P2X7 receptors and TRPV4 channels in the bladder urothelium of control mice (Ctrl) and CIS mice at 21-days after stress cessation. Protein levels normalized to loading control and expressed relative to average control levels. Data is represented as Mean±SEM, n=5 mice per group. Mann Whitney test was used to determine statistical differences.

Subtask 5: *In vivo bladder dye-uptake to assess changes in Panx1 function.* The studies planned as part of the activities under this subtask will provide insights into the potential Panx1-TRPV4 functional interplay and its role in the emergence and persistency of IC-like urinary symptoms in the CIS mouse model. Studies have already been initiated, involving generation of new cohorts of CSI and controls to start assessments at 21-days post-CSI and correlate findings with those already obtained from the molecular analyses (see Subtask 4).

Subtask 6: *Assessment of urothelial ATP signaling.* Activities in this subtask involved immediate collection and freezing of spontaneously voided urine from CIS and control mice at baseline and then at 96hrs of CIS and at 7- and 14-days after stress cessation. ATP levels in the voided urine were then quantified using the luciferin-luciferase chemiluminescence assay. Quantification of

voided ATP levels is routinely used as a non-invasive approach to assess changes in urothelial ATP release and indirectly evaluate changes in bladder sensory function. Urothelial ATP release is increased in animal models of bladder overactivity. ATP levels in the urine of IC patients has also been shown to be higher than those in healthy individuals, in line with the proposed central role that ATP signaling plays in the regulation of bladder sensation and detrusor contractility.

In our preliminary studies, we observed that voided ATP levels were significantly elevated at 96hrs of CIS. We have now confirmed this initial finding and further demonstrated that the effects of stress on urothelial ATP signaling persist long after stress cessation. As shown in Figure 3, at 7- and 14-days post-CIS, ATP levels in the voided urine of CIS mice were 4 to 5-fold higher than those of non-stressed controls. This finding supports our proposal that stress alters the mechanosensory function of the bladder urothelium, and that the dysregulation of urothelial ATP release and signaling is one of the main factors driving the higher activation and sensitization of the bladder sensory afferents, which ultimately lead to the emergence IC-like urinary symptoms in the CIS mouse model and its persistence long after stress cessation.

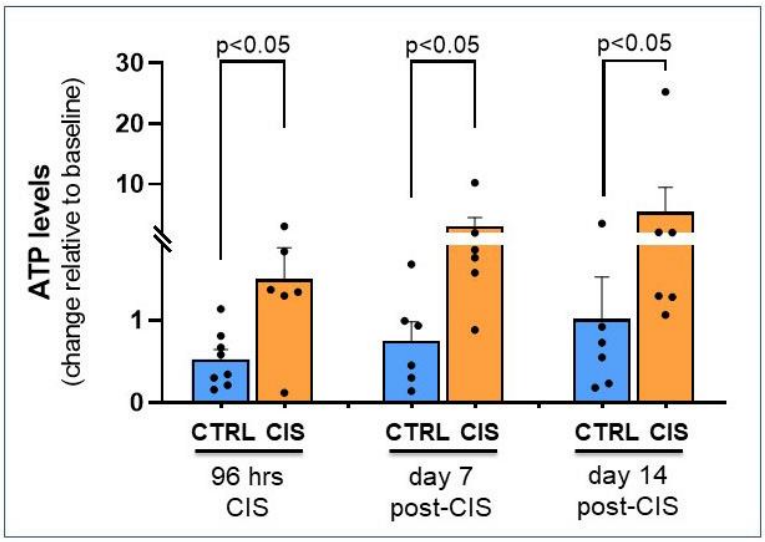


Figure 3 – *Exposure to 96hrs of constant illumination stress (CIS) increases urothelium ATP release, which indicates a stress-induced amplification of urothelial mechanosensory responses that persisted long after stress cessation.* Voided ATP levels were quantified at baseline and animals were then divided in CIS and control (CTRL) groups. The CIS mice was then exposed to 96hrs of continuous room illumination and CTRL mice maintained at regular housing conditions (12hrs light/dark cycle). Voided ATP levels were then quantified at 96hrs-CIS, and at 7- and 14-days after stress cessation and normalized to baseline levels. Data is represented as Mean±SEM, n=8 CTR and n = 6 CIS. Mann Whitney test was used to determine statistical differences between CIS and CTRL at each time point.

Major Task 2: *Determine the role played by exposure of bladder tissues to high K^+ as the trigger of events leading to changes in Panx1 channel activation and expression, and downstream local inflammatory responses involving Panx1-mediated activation of the NLRP3 inflammasome.* (Timeline: months 8 – 14).

Activities planned under this Major Task 2 of the SOW involve use of primary bladder cell cultures prepared from wildtype and Panx1-null mice (Subtask 1), and quantification of mRNA and protein levels of Panx1, inflammasome components and associated cytokines (Subtask 2). We started the experiments as scheduled, but soon after placed them on hold. The reason be that at that time, we came across a study showing that treatment with a Rho Kinase 1 inhibitor (Y-27632) markedly increased the long-term proliferation of human keratinocytes (PMID:20516646). The protocol that we routinely use in our lab to prepare urothelial cell cultures, the same used by others, has limitations. These are mainly due to the characteristic slow

proliferation and fast differentiation of urothelial cells that impact the quality and viability of the cultures past one week of plating. The possibility to circumvent these limitations would be extremely beneficial not only for the studies proposed in this award, but for our other ongoing and future studies and studies by others in the field. We therefore worked towards implementing the use of this Rho Kinase 1 inhibitor in our protocol and were nicely surprised with the outcome. We observed that treatment of freshly isolated urothelial cells with Y-27632 also supported the long-term proliferation of urothelial cells, particularly of basal and intermediate cells, which under regular culture conditions are rapidly lost. We also investigated if prolonged treatment with Y-27632 altered urothelial Panx1 expression, which would preclude its use in our studies. We did not observe significant changes in Panx1 protein levels in urothelial cultures after 1 or 2 weeks in Y-27632 when compared to control 3-day old untreated cultures. We also tested removing Y-27632 one week after plating the cells and did not observe significant changes in cell morphology, viability or Panx1 expression. This is important as will also allow us to conducted studies in which we expected the potential involvement of downstream Rho Kinase 1 signaling. Another beneficial outcome that came from this optimization of our protocol is that we are not only able to keep the primary cells longer in culture but also to passage them without losing their original characteristics. This feature has now allowed us to successfully generate an hTERT-immortalized mouse urothelial cell line (mUC2-hTERT), which is something that we have been trying for years. Use of these cells in parallel with the primary cells will significantly facilitate the studies proposed in this project, as we resume the activities proposed in this Task.

What opportunities for training and professional development has the project provided?

This project was not originally intended to provide training and professional development opportunities. However, with the start of the project, these opportunities came from the role that Dr. Suadicani (PI) plays as the basic science mentor for fellows in the Female Pelvic Medicine and Reconstructive Surgery (FPMRS) fellowship program of the OBGYN Department at Einstein and Montefiore. In this 3-year program, the fellows have protected time to conduct basic and translational research as part of their thesis and requirement for their specialty certification. While discussing ideas for new projects that the fellows could conduct in the lab and talking about ongoing projects, Dr. Whitney Clearwater demonstrated a deep interest for this project, as it directly aligned with her clinical interests. She has since been working closely with Dr. Suadicani in this project, specifically on the activities involving the assessment of changes in voiding function and time course of IC-like urinary symptoms in the CIS model. This opportunity has allowed Dr. Clearwater to look at IC from a basic science perspective, learn the use of pre-clinical models and functional assessments, and to gain a better understanding of IC pathophysiology. Her participation in the project has also been beneficial to the research team by bringing a clinical perspective to our discussions. The participation of Dr. Clearwater has also been recognized by her peers and further her professional development through presentation of our findings in two major scientific meetings, namely the annual meeting of the Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction (SUFU, February 2022) and American Urological Association (AUA, May 2022). Of note, our study was selected for a podium presentation at SUFU, as one of the top 10 basic science abstracts.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

In Year 2 of the award, we will continue to work diligently towards accomplishing the goals of this project. Specifically, for the studies planned in:

Specific Aim 1:

Major Task 1, we will generate new cohorts of wildtype female CIS and non-stressed controls to continue with the functional assessments and characterization of the time course of IC-like symptoms in the CIS mice (Subtask 3), harvest bladder tissues at the various experimental time points for molecular analyses (Subtask 4), *in vivo* assessment of Panx1 function (Subtask 5) and urothelial ATP release (Subtask 6). We will also start these studies with the female Panx1-null mice cohorts.

Major Task 2, we will resume the *in vitro* studies with the primary urothelial cell cultures that will be prepared from wildtype and Panx1-null mice using the optimized protocol that we described in the accomplished goals section of this report. We will also run experiments in parallel with our new hTERT-immortalized mouse urothelial cell line to assess reproducibility of findings from primary cells, which will validate the use of these cells in future studies.

Specific Aim 2:

Major Task 1 – *Pharmacological inhibition of Panx1 channels.* We will conduct the planned scale up/down pilot studies with wildtype female mice submitted to 96hrs of CIS to determine best dose range for intravesical and i.p. treatments with the Panx1 blockers probenecid and BB-FCF (Subtask 1).

Major Task 2 – *Bladder targeted Panx1 knockdown.* We will conduct *in vitro* testing of liposome containing Panx1-siRNA to assess and optimized efficacy in knocking down Panx1 expression (Subtask 1) and will then conduct scale up/down pilot studies with female mice to determine best concentration and exposure time for intravesical delivery and treatment with liposome-containing Panx1-siRNA (Subtask 2).

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Findings obtained from studies conducted with the animal model of constant illumination stress (CIS) reemphasized the importance of stress in the etiology and exacerbation of IC symptoms. They also provided support for the premise that dysregulation of the urothelium mechanosensory function plays a central role in the emergence and persistence of IC symptoms. In addition, by further demonstrating that in the CIS model the animals develop symptoms that closely resemble those of IC patients, this project is now not only validating the use of this model by others in future mechanistic and pre-clinical studies but is also providing the field with a model that is simple and does not require specialized equipment to be generated. The optimization that we made in the protocol that is broadly used in the field to culture primary urothelial cells and the generation of the hTERT-immortalized mouse urothelial cells are also expected to be beneficial to the scientific community as they will significantly facilitate the performance of *in vitro* studies that are essential to gain mechanistic understanding of urothelial function and dysfunction. Overall, this project has already made and will make a notable impact in the field in terms of improving both our basic knowledge of factors and mechanisms in IC etiology and the available experimental tools to study IC.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications

Nothing to Report.

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations. *presentation produced a manuscript.*

Clearwater, W., Wang, Y., Suadicani, S.O. *"Exposure to constant illumination results in persistent changes in voiding behavior of female mice"*. SUFU 2022 Winter Meeting, February 22 – 26, 2022, San Diego, CA. Poster #4, Podium Presentation in the Top 10 Basic Science Abstract Presentations session. Neurourology and Urodynamics, Volume 41, Issue S1, Supplement: SUFU 2022 Abstracts Issue, February 2022, Page S9.

Clearwater, W., Wang, Y., Urban-Maldonado, M., Suadicani, S.O. *"Effects of constant illumination on mouse voiding behavior".* Moderated Poster Session. American Urological Association (AUA) Annual Meeting, May 13-16, 2022, New Orleans, LA. Abstract: MP49-10. *Journal of Urology*, Volume 207, Issue Supplement 5, May 2022, Page e857.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Sylvia Suadicani
Project Role:	Principal Investigator (PI)
Researcher Identifier (ORCID ID):	0000-0001-6811-4029
Nearest person month worked:	5
Contribution to Project:	Dr. Suadicani directed the project, supervised the activities of all the involved investigators, participated in the generation of the animal model and performance of functional assessment of bladder function and tissue harvesting, reviewed the data analyses, headed the discussions and interpretation of findings from all the experiments conducted during this report period.
Name:	Whitney Clearwater
Project Role:	Fellow
Researcher Identifier (ORCID ID):	0000-0002-1578-1853
Nearest person month worked:	3
Contribution to Project:	Dr. Clearwater worked closed with Dr. Suadicani in the generation of the animal model and performance of functional assessment of bladder function, data analysis, interpretation and presentation of findings from the *in vivo* studies conducted during this report period.
Funding Support:	Female Pelvic Medicine and Reconstructive Surgery Fellowship Program (FPMRS) of the OBGYN Department at the Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY.

Name:	Marcia Maldonado
Project Role:	Senior Research Technician
Researcher Identifier (ORCID ID):	0000-0001-8791-0880
Nearest person month worked:	12
Contribution to Project:	Mrs. Maldonado conducted the *in vitro* studies with urothelial cell cultures, prepared the cultures, optimized the culture protocol, generated the hTERT-immortalized mouse urothelial cell line, assisted in tissue harvesting and molecular studies, ordered supplies and animals, conducted the husbandry and maintenance of the mouse colonies and of the animal model.
Name:	Mia M. Thi
Project Role:	co-Investigator
Researcher Identifier (ORCID ID):	0000-0001-6157-5842
Nearest person month worked:	3
Contribution to Project:	Dr. Thi assisted in the supervision, performance, data analysis and interpretation of findings from the urothelial ATP release and signaling studies.
Name:	Kelvin P. Davies
Project Role:	co-Investigator
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Davies assisted in the supervision, data analysis and interpretation of the molecular studies conducted with bladder tissues.
Name:	Yi Wang
Project Role:	Research Associate/Instructor
Researcher Identifier (ORCID ID):	0000-0001-6623-3905
Nearest person month worked:	1
Contribution to Project:	Dr. Wang assisted in the performance of the molecular studies and data analysis, and in tissue harvesting.
Funding Support:	In-kind

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: Attached abstracts presented at scientific meetings.

- Clearwater, W., Wang, Y., Suadicani, S.O. *"Exposure to constant illumination results in persistent changes in voiding behavior of female mice"*. SUFU 2022 Winter Meeting, February 22 – 26, 2022, San Diego, CA. Abstract #4, Podium Presentation in the Top 10 Basic Science Abstract Presentations session.

  Neurourology and Urodynamics, Volume 41, Issue S1, Supplement: SUFU 2022 Abstracts Issue, February 2022, Page S9.

- Clearwater, W., Wang, Y., Urban-Maldonado, M., Suadicani, S.O. *"Effects of constant illumination on mouse voiding behavior".* Moderated Poster Session. American Urological Association (AUA) Annual Meeting, May 13-16, 2022, New Orleans, LA. Abstract: MP49-10.

  Journal of Urology, Volume 207, Issue Supplement 5, May 2022, Page e857.

Neurourology and Urodynamics, Volume 41, Issue S1, Supplement: SUFU 2022 Abstracts Issue, February 2022, Page S9.

Funding: Department of Defense PR161914/PR200027, NIH K08 DK118176-01A1

#4 | EXPOSURE TO CONSTANT ILLUMINATION RESULTS IN PERSISTENT CHANGES IN THE VOIDING BEHAVIOR OF FEMALE MICE

Whitney Clearwater, MD, MPH[1], Yi Wang, PhD[2], Sylvia Suadicani, PhD[2]

[1]Albert Einstein College of Medicine, Montefiore Medical Center, Dept. OB/GYN, Bronx, NY, [2]Albert Einstein College of Medicine, Montefiore Medical Center, Dept. Urology, Bronx, NY

Presented By: Whitney Clearwater, MD, MPH

Introduction: Stress caused by exposure to prolonged constant illumination (CI) has been shown to result in desquamation of superficial urothelial cells and disrupt the urothelial barrier in the mouse bladder. A leaky urothelium is a recognized factor in mechanisms that can drive bladder hypersensitivity and emergence of symptoms of urgency and urinary frequency. However, little is known of the effects of this type of stressor on bladder function. The goal of this study was to determine if exposure to constant illumination stress (CIS) alters the mouse voiding behavior, and if the effects of CIS persist after stress cessation.

Methods: Female C57BL/6CrN mice (20-weeks old) were randomly assigned to control (submitted to conventional 12h/day room illumination) or CIS (96hrs of constant room illumination) groups. Voiding behavior in the animals' active (night-time) and sleep (daytime) phases was characterized using the voided stain of paper (VSOP) method. VSOP was conducted at baseline (pre-CIS), at the day after ending the 96hrs-CIS and at 7 and 14 days after CIS cessation. Spontaneously voided urine was collected at these time points to indirectly evaluate urothelial sensory function by quantifying changes in urine ATP levels using the luciferin-luciferase assay. Data was expressed relative to pre-CIS levels and non-stressed controls, and statistical differences determined at a $p < 0.05$ level using the Mann-Whitney test.

Results: Exposure to 96hrs-CIS induced a 2.5-fold increase in urinary frequency of CIS-mice (pre-CIS: 0.25 ± 0.04 voids/hour vs 96hr-CIS: 0.40 ± 0.06 voids/hour, $n = 5$, $p < 0.03$). A 7- and 14-days post-CIS, the urinary frequency of CIS-mice remained 2.7- and 2.4-fold higher than that of non-stressed controls, respectively. Remarkably, this persistent effect of CIS was observed in the animals' sleep phase (daytime) but not in their active phase (night-time), indicating development of nocturia. Further investigation showed that voided ATP levels were markedly elevated after 96hrs-CIS and remained at 2.5-fold higher levels than controls at 14 days, indicating dysregulation of urothelial mechanosensory responses.

Conclusion: Exposure to 96 hours of constant illumination causes persistent, long-lasting changes in voiding function that may be due to urothelial sensory dysfunction. The CIS model can provide a reliable and simple tool to investigate mechanisms underlying the effects of stress on urinary function.

Funding: DoD-CDMRP W81XWH-21−1-0465

#5 | THE MTOR PATHWAY IS INVOLVED IN A MURINE MODEL OF CHRONIC PROSTATITIS

Praveen Thumbikat[1], Pranav Prasoon[2], Kenny Roman[2]

[1]Northwestern University, [2]University of Pittsburgh

Presented By: Kenny Roman, PhD

Introduction: Patients with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) suffer from chronic pelvic pain. Surveys conducted by the National Institute of Health (NIH) reported a significant impact on the quality of life of CP/CPPS patients with chronic symptoms due to few viable therapeutic targets. Studies showed that various cortices in the brain (e.g., prelimbic, anterior cingulate cortex, etc) are impaired in patients diagnosed with CP/CPPS after several years. Interestingly, a strong link between mTOR signaling in the anterior cingulate cortex and the development of neuropathic pain has emerged in several rodent models. Here, we seek to examine whether the mTOR pathway plays a key role in anterior cingulate cortex sensory processing in mice with chronic pelvic pain in a model of CP/CPPS called experimental autoimmune prostatitis (EAP).

Methods: Our experiments were conducted on male C57BL/6J (B6) mice with or without EAP (6 weeks old; $n = 3$/group). To induce EAP, the experimental group received a subcutaneous injection of rat prostate antigen (1 mg/ml) mixed with an adjuvant (1:1). Behavioral pain testing for pelvic tactile allodynia was conducted on days 0, 7, 14, 20, and 30. On day 30, mice were perfused and the anterior cingulate cortex and spinal cord lumbosacral regions were excised from mice with EAP and respective control cohorts. Next, we processed the anterior cingulate cortex and spinal cord for either immunofluorescence or immunoblots.

MP49-09

OXIDATIVE STRESS DRIVES NOCTURNAL POLYURIA VIA INTRARENAL SPAK-NCC PATHWAY: A THERAPEUTIC POTENTIAL OF A NOVEL ANTIOXIDANT, SILICON COMPONENT AGENTS

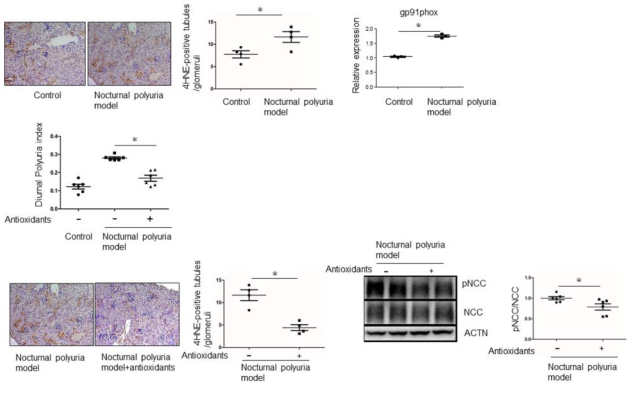
Yosuke Sekii, Hiroshi Kiuchi, Takahiro Imanaka, Sohei Kuribayashi, Koichi Okada, Kentaro Takezawa, Shinichiro Fukuhara, Norio Nonomura, Suita, Japan*

INTRODUCTION AND OBJECTIVE: For decades, the mechanisms that cause nocturnal polyuria in the elderly have been poorly understood. We have recently shown in a novel animal model that it is triggered by inappropriate activation of the SPAK-NCC pathway in the kidney. The pathway is, in part, regulated by oxidative stress, and we hypothesized that oxidative stress may cause nocturnal polyuria. In this study, we aimed to show the impact of oxidative stress on nocturnal polyuria and to evaluate the efficacy of a novel antioxidant, Silicon component agents.

METHODS: 19-week-old C57BL6/J male mice were fed L-NAME (NO synthase inhibitor) and a 1% high-salt diet for 2 weeks to create a nocturnal polyuria model. Oxidative stress in kidney was assessed by immunohistochemical staining for 4-hydroxynonenal (4-HNE) and real-time PCR of NADPH oxidase subunit, which regulates the production of ROS. Silicon component was used as antioxidant stress agents: The silicon component we co-developed is orally ingestible and features sustained hydrogen generation to inhibit oxidative stress production. Urine volume was measured by the aVSOP method after two weeks of treatment with the silicone-containing diet or silicone-free diet. The diurnal polyuria index(inactive urine volume /daily urine volume) was calculated for the index corresponding to nocturnal polyuria in humans. Intrarenal NCC activation was assessed by phosphorylation of NCC using Western blotting.

RESULTS: 4-HNE-positive cells in the distal renal tubules and subunit of NADPH oxidase were significantly increased in nocturnal polyuria group compared with control ($P<0.05$), suggesting the association between oxidative stress and nocturnal polyuria. Oral treatment with silicone component decreased these oxidative stresses. In addition, the component inhibited phosphorylation of NCC, leading to decease of diurnal polyuria index ($P<0.05$).

CONCLUSIONS: Oxidative stress overactivated intrarenal SPAK-NCC pathway and caused nocturnal polyuria, which was improved by Silicon component agents. Oxidative stress plays a crucial role in nocturnal polyuria, and Silicon component agents could be a promising treatment for age-related nocturnal polyuria.



Source of Funding: Japan Society for the Promotion of Science (JSPS) (Grant number: JP19K18582)

MP49-10

EFFECTS OF CONSTANT ILLUMINATION ON MOUSE VOIDING BEHAVIOR

Whitney Clearwater, Yi Wang, Marcia Urban-Maldonado, Sylvia Suadicani, Bronx, NY*

INTRODUCTION AND OBJECTIVE: Stress caused by exposure to prolonged constant illumination (CI) has been shown to result in desquamation of superficial urothelial cells and disrupt the urothelial barrier in the mouse bladder. A leaky urothelium is a recognized factor in mechanisms that can drive bladder hypersensitivity and emergence of symptoms of urgency and urinary frequency. However, little is known of the effects of this type of stressor on bladder function. The goal of this study was to determine if exposure to constant illumination stress (CIS) alters the mouse voiding behavior, and if the effects of CIS persist after stress cessation.

METHODS: Female C57BL/6CrN mice (20-weeks old) were randomly assigned to control (submitted to conventional 12h/day room illumination) or CIS (96hrs of constant room illumination) groups. Voiding behavior in the animals' active (night-time) and sleep (daytime) phases was characterized using the voided stain of paper (VSOP) method. VSOP was conducted at baseline (pre-CIS), at the day after ending the 96hrs-CIS and at 7 and 14 days after CIS cessation. Spontaneously voided urine was collected at these time points to indirectly evaluate urothelial sensory function by quantifying changes in urine ATP levels using the luciferin-luciferase assay. Data was expressed relative to pre-CIS levels and non-stressed controls, and statistical differences determined at a $p<0.05$ level using the Mann-Whitney test.

RESULTS: Exposure to 96hrs-CIS induced a 2.5-fold increase in urinary frequency of CIS-mice (pre-CIS: 0.25 ± 0.04 voids/hour vs 96hr-CIS: 0.40 ± 0.06 voids/hour, $n=5$, $p<0.03$). At 7- and 14-days post-CIS, the urinary frequency of CIS-mice remained 2.7- and 2.4-fold higher than that of non-stressed controls, respectively. Remarkably, this persistent effect of CIS was observed in the animals' sleep phase (daytime) but not in their active phase (night-time), indicating development of nocturia. Further investigation showed that voided ATP levels were markedly elevated after 96hrs-CIS and remained at 2.5-fold higher levels than controls at 14 days, indicating dysregulation of urothelial mechanosensory responses.

CONCLUSIONS: Exposure to 96 hours of constant illumination causes persistent, long-lasting changes in voiding function that may be due to urothelial sensory dysfunction. The CIS model can provide a reliable and simple tool to investigate mechanisms underlying the effects of stress on urinary function.

Source of Funding: DoD-CDMRP W81XWH-21-1-0465 (PI: Suadicani)

MP49-11

FIDGETIN-LIKE 2 (FL2) DEPLETION AT THE SITE AND TIME OF CAVERNOUS NERVE INJURY IMPROVES BLADDER FUNCTIONAL OUTCOMES

Yi Wang, Moses Tar, Marcia Urban-Maldonado, Lisa Baker, David Sharp, Sylvia Suadicani, Kelvin Davies, Bronx, NY*

INTRODUCTION AND OBJECTIVE: Radical prostatectomy (RP) is the most common treatment for localized prostate cancer. Unfortunately, the procedure carries high risk for the development of lower urinary tract symptoms (LUTS). These urogenital complications are highly detrimental to the post-surgical well-being of men, for whom there are presently poor treatment options. We recently demonstrated in a rat model of nerve injury associated with RP, that Fidgetin-Like 2 (FL2) depletion through application of FL2-siRNA at the site and time of cavernous nerve injury (CNI), resulted in significantly enhanced functional nerve recovery, as determined by cavernosometry [1]. In the present studies, we determined if the same approach would also improve bladder function outcomes in the rat model of CNI.

RIGHTSLINK