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TITLE: Phenotyping of IC/BPS Using Microbial Community States: Validation of a Novel Noninvasive Biomarker

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CONTRACTING ORGANIZATION: University of California, Los Angeles, CA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Growing research into interstitial cystitis/bladder pain syndrome (IC/BPS), a prevalent, debilitating urologic condition characterized by pain perceived to derive from the bladder, suggests a potential role for the diverse microbial communities of the urinary tract in disease development and progression. To explore the pathophysiology of IC/BPS, we will examine the relation of these urinary bacterial and fungal communities and their impact on the bladder urothelium to patient symptomatology, phenotypes, and treatment outcomes, culminating in an investigation of how these microbial communities could guide diagnosis, classification and treatment decisions for these patients. The novel application of large-scale systems biology approaches to the urinary tract and the integration of these data with detailed clinical phenotyping will facilitate profound advances in the approach to and understanding of this condition and will serve as the foundation for additional studies developing new preventative and therapeutic approaches.

15. SUBJECT TERMS
Interstitial Cystitis, Bladder Pain Syndrome, Microbiome, Urobiome, Fungi, Machine Learning

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TABLE OF CONTENTS

1. **INTRODUCTION:**

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a debilitating, incurable chronic condition whose central feature is intrusive pain attributed to the bladder in the absence of other demonstrable pathology, such as urinary tract infection (UTI). This is a diagnosis of exclusion; thus, patients diagnosed with IC/BPS likely encompass multiple disease phenotypes reflecting different pathologies with overlapping symptomatology. With this kind of heterogeneity, it has been challenging to advance our understanding of IC/BPS disease mechanisms or improve care and management. The similarity of IC/BPS symptoms to UTI suggests a microbial involvement, and modern, sensitive microbial detection methods have further implicated the hypothesis that IC/BPS-associated microbes interact with the bladder to perpetuate a chronic inflammation responsible for the painful symptoms of IC/BPS. However, while alterations in urinary bacterial community diversity are evident in IC/BPS, the differences observed thus far have provided little diagnostic utility or insight into disease mechanisms. Assuming multiple etiologies for the symptoms of IC/BPS patients, we hypothesize that perturbations in microbial communities associated with IC/BPS will only become meaningful in concert with objective clinical phenotyping identifying more homogenous disease phenotypes. We seek to better define these IC/BPS phenotypes utilizing an expanded set of questionnaires focused on the symptomatic domains implicated in our Discovery award. In addition, we will create novel indices to quantitate the salient features of each phenotype for use in clinical care and research assessment. Previously, we generated a simple PCR-based tool to assess urinary microbial biomarkers for their potential as a diagnostic test which demonstrated good clinical potential to identify unique IC/BPS phenotypes. We also will further optimize this PCR-based assay to develop a rapid, scalable tool for the diagnosis and phenotyping of IC/BPS patients. We will also examine the utility of this microbial profiling assay in guiding treatment decisions, which may dramatically improve IC/BPS care. Lastly, we will explore *in vitro* the host-microbe interactions that influence development of bladder-specific pain. We will study the virulence, invasiveness, and inflammatory potential of patient-derived primary microbial isolates and their impact on urothelial activation and gene expression. Together, these goals combine clinical phenotyping, microbial community profiling, and *in vitro* disease modeling to define clinical manifestations, microbial patterns, treatment responses, and molecular etiologies of IC/BPS phenotypes. This approach will allow the development of clinically useful disease measures and testing paradigms and promote a more comprehensive understanding of how IC/BPS pathogenesis is associated with variations in commensal and pathobiont microorganisms.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Interstitial Cystitis, Bladder Pain Syndrome, Microbiome, Fungi, Clinical Microbial Isolates, Host-Pathogen Interactions, Urobiome, Clinical Phenotyping, Biomarkers, Predictive Algorithms, Machine Learning

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - The Major tasks of the project as specified in the SOW are listed below and described in greater detail in the table below. The targeted timeline and current progress are specified for each subheading.

1. *Patient Recruitment and Sample Accrual*
2. *Deep Clinical Phenotyping of IC/BPS Subjects*
3. *Bacterial And Fungal Community Profiling*
4. *Validation Of Microbial Markers as Prognostic Indicators*
5. *Exploration Of Host-Microbe Interactions Related to IC/BPS*
6. *Probe Fungal Antagonism of Lactobacilli in Urothelial Co-Cultures*

Aim 1: **To evaluate the diagnostic accuracy and prognostic utility of a novel microbial biomarker panel to better define IC/BPS phenotypic and clinical characteristics.**	**Target Timeline (Mo.)**	**Actual Progress**
Major Task 1: PATIENT RECRUITMENT AND SAMPLE ACCRUAL		
<u>Subtask 1.</u> Ethical Study Review 1.1. Local IRB Approval 1.2. HRPO/ACURO Approval	1-3	Completed
<u>Subtask 2.</u> Sample Accrual 2.1. Identification of Appropriate Patient Candidates 2.2. Collection of Patient Urine and Clinical Data 2.3. Initial Sample Processing and Aliquoting 2.4. Cataloging of Patient Clinical Information	3-12	In Progress (25% Complete)
<u>Subtask 3.</u> Patient Follow-up/Post-Treatment Assessment 3.1. Assignment of Subjects to Standardized Treatment Pathway 3.2. Collection of Post-treatment Urine, Clinical Data, and Response Outcomes 3.3. Secondary Sample Processing and Aliquoting 3.4. Clinical Database Management and Secure Archiving	6-18	In Progress (10% Complete)
Major Task 2: DEEP CLINICAL PHENOTYPING OF IC/BPS SUBJECTS		
<u>Subtask 1.</u> Definition of IC/BPS Phenotypes Using Machine Learning 1.1. Unsupervised Clustering of Clinical Data to Define Subgroups 1.2. Detailed Description of Salient Features of Each Subgroup 1.3. Determination of Discriminatory Factors as Potential Prognostic Markers	9-15	1.1, 1.2: Completed 1.3: In Progress (50% Complete)
<u>Subtask 2.</u> Development of Novel IC/BPS Phenotypic Indices 2.1. Definition of New Clinical Measures to Quantitate Unique Symptomatic Domains Using Discriminatory Factors in 1.3. 2.2. Determination of Face and Construct Validity 2.3. Validation of Proposed Measures on Independent Population	15-24	In Progress (60% Complete)
Major Task 3: BACTERIAL AND FUNGAL COMMUNITY PROFILING		
<u>Subtask 1.</u> Microbial Profiling by Next-Generation Sequencing 1.1. DNA Extraction from Urine and Quality Control Assessment 1.2. Library Creation and Next Generation Sequencing (ITS1, 16S) 1.3. Bacterial and Fungal Taxonomic Assignment of OTUs	12-20	Not Yet Started (0% Complete)
<u>Subtask 2.</u> Validation of Community Profiling by qPCR 2.1. Primer Design and Validation 2.2. Assessment of qPCR Assay Validity and Reliability 2.3. Determination of Test-Specific *Diagnostic* Thresholds	12-24	In Progress (75% Complete)

<u>Subtask 3.</u> Bioinformatics/Biostatistical Analysis 3.1. Description of Control and IC/BPS Microbial Populations 3.2. Confirmatory Reanalysis of Populations Altered in IC/BPS 3.3. Correlation of Microbial Population Features with Urinary Phenotypes defined in Major Task 2. 3.4. Validation of qPCR correlations with NGS findings	18-26	Not Yet Started (0% Complete)
Major Task 4: VALIDATION OF MICROBIAL MARKERS AS PROGNOSTIC INDICATORS		
<u>Subtask 1.</u> Correlation of qPCR Microbial Biomarker Assay with Symptomatically-Defined Phenotypes 1.1. Statistical Correlation of qPCR Assay with Phenotypic Classifications Defined in Major Task 1 1.2. Iterative optimization of qPCR Assay with additional taxa defined in Major Task 3 as needed 1.3. Determination of Test-Specific *Phenotypic* Thresholds	18-26	Not Yet Started (0% Complete)
<u>Subtask 2.</u> Determination of Potential of qPCR Microbial Biomarker Assay to Predict Patient Responses to Standardized IC/BPS Treatments 2.1. Statistical Correlation of Baseline qPCR Assay with Treatment Responses to IC/BPS Therapies 2.2. Correlation of individual taxa with improvements in specific symptomatic domains 2.3. Assessment of Intraindividual Changes in Microbial Composition Related to Symptomatic Improvements	22-30	Not Yet Started (0% Complete)
***Aim 2:* To examine host-microbe interactions influencing the development of bladder-specific pain.**		
Major Task 5. EXPLORATION OF HOST-MICROBE INTERACTIONS RELATED TO IC/BPS		
<u>Subtask 1.</u> Generation of Primary Clinical Microbial Isolate Library 1.1. Growth of bacterial and fungal clinical isolates using a wide range of media and growth conditions 1.2. Expansion and Purification of Isolates By Serial Propagation 1.3. Identification and Characterization by Sequencing and Microscopic Examination 1.4. Cataloging of Isolates Against Symptoms of Originating Subject	3-12	1.1, 1.2: Completed 1.3, 1.4: In Progress (50% complete)
<u>Subtask 2.</u> Examination of Virulence of Primary Candida Strains 2.1. Examine adhesion and cytotoxicity of Candida clinical isolates in urothelial co-cultures 2.2. Investigate invasive potential of Candida isolates in a genitourinary, epithelial model 2.3. Assessment of Candida Strain filamentation potential 2.4. Statistical Correlation of Virulence with Symptoms in Originating Subject	12-30	2.1, 2.2, 2.4: In Progress (65% Complete) 2.3: Completed
<u>Subtask 3.</u> Analysis of impact of Candida isolates on urothelial cells 3.1. Measure cytokine production by urothelial cells following co-culture with clinical isolates 3.2. Targeted qPCR-based examination of expressional changes in implicated inflammatory, activation, and nociceptive pathways 3.3. Examine global urothelial transcriptional changes following co-culture with clinical isolates 3.4. Statistical Correlation of Inflammatory and Transcriptional Changes with Symptoms in Originating Subject	12-30	In Progress (40% Complete)
Major Task 6. PROBE FUNGAL ANTAGONISM OF LACTOBACILLI IN UROTHELIAL CO-CULTURES		

<u>Subtask 1</u>. Assess Primary Lactobacillus Strain Effect on Candida Virulence 1.1. Exclusion, Displacement, and Competition Experiments to Assess Inhibition of Candida Adhesion by Primary Lactobacilli Strains 1.2. Examine Impact of Primary Lactobacillus Strains on Candida Filamentation 1.3. Assess Inhibition of Candida-mediated Cytotoxicity in Urothelial Co-culture of Unique Primary Lactobacillus Strains	18-22	Not Yet Started
<u>Subtask 2.</u> Test Pathogenicity of Lactobacillus Strains for Urothelial Cells 2.1. Examine Cytotoxicity of Lactobacillus Strains on Urothelial Monolayers 2.2. Measure Cytokine Production by Urothelial Cells following Co-culture with Clinical Lactobacillus Isolates 2.3. Targeted, qPCR-based Examination of Expressional Changes in Implicated Inflammatory, Activation, and Nociceptive Pathways	20-30	Not Yet Started
Major Task 7. DATA SYNTHESIS		
<u>Subtask 1.</u> Compilation of all data and manuscript preparation 3.1. Statistical Modeling and Integration of Multiple Datasets 3.2. Manuscript Writing and Submission	24-36	Not Yet Started

- **What was accomplished under these goals?**

1) *Patient Recruitment and Sample Accrual:* During this award period, we were initially focused on regulatory compliance and have since received both institutional, federal, and DOD approval for this longitudinal study. We have also established a specialized pelvic pain clinic to help facilitate trial recruitment and management, including onboarding a specialized nurse capable of assisting with interventional therapies planned as part of the care pathway for these patients. We have begun recruitment and are on track to complete all patient intake appointments during the next award period.

2) *Deep Clinical Phenotyping of IC/BPS Subjects*: We utilized machine learning with the symptomatic patterns present in our initial panel of IC/BPS subjects to identify unique bladder pain phenotypes, which was published in our attached manuscript (Appendix). While IC/BPS is defined as an unpleasant sensation perceived to be related to the bladder with associated urinary symptoms, due to difficulties discriminating pelvic visceral sensation, IC/BPS likely represents multiple phenotypes with different etiologies that present with overlapping symptomatic manifestations, which complicates clinical management. We hypothesized that unique bladder pain phenotypes or “symptomatic clusters” would be identifiable using machine learning analysis (unsupervised clustering) of validated patient-reported urinary and pain measures. Patients (n = 145) with pelvic pain/discomfort perceived to originate in the bladder and lower urinary tract symptoms answered validated questionnaires [OAB Questionnaire (OAB-q), O’Leary-Sant Indices (ICSI/ICPI), female Genitourinary Pain Index (fGUPI), and Pelvic Floor Disability Index (PFDI)]. In comparison to asymptomatic controls (n = 69), machine learning revealed three bladder pain phenotypes with unique, salient features. The first group chiefly describes urinary frequency and pain with the voiding cycle, in which bladder filling causes pain relieved by bladder emptying. The second group has fluctuating pelvic discomfort and straining to void, urinary frequency and urgency without incontinence, and a sensation of incomplete emptying without urinary retention. Pain in the third group was not associated with voiding, instead being more constant and focused on the urethra and vagina. While not utilized as a feature for clustering, subjects in the second and third groups were significantly younger than subjects in the first group and controls without pain. These phenotypes defined more homogeneous patient subgroups which responded to different approaches to therapy on chart review.

To continue this research, we are currently validating this phenotyping algorithm in an independent population using data from the Multidisciplinary Approach to Pelvic Pain (MAPP) Research Network obtained from the NIH NIDDK repository. We have identified consistent and reproducible discriminatory symptom clusters that are associated with each phenotype. We are using these features to create new Clinical Measures that we can use to both quantitate the severity of the unique symptomatic domains and measure improvements in individual symptom clusters over time, particularly as we take patients down a standardized clinical pathway.

3) *Exploration Of Host-Microbe Interactions Related to IC/BPS:* While we were ramping up recruitment efforts, we have focused on the characterization of the *in vitro* behavior of clinical fungal isolates in co-culture with human urothelial cells. We have expanded our initial library of urinary fungal strains to more than 35 total, including more than 25 *Candida spp*. We have focused our attention on the characterization of *Candida* isolates in independent culture and co-culture conditions. The release of lactate dehydrogenase (LDH) by urothelial cells was used to quantify the urothelial cell killing following *Candida* challenge using a CytoTox 96 nonradioactive cytotoxicity assay. *Candida* strains from patients with pain demonstrate differential killing of benign urothelial cell lines (Fig. 1A), with significantly higher levels of cell damage noted for those pain-associated strains. This effect required live *Candida* cells; no urothelial killing was observed after heat killing of the pathogenic strains. We also measured cytokine and chemokine production by urothelial cells in response to co-cultures with lower multiplicity of infection (MOI) of *Candida* isolates to examine if the different strains promoted different patterns of inflammation. Pain-derived strains stimulated greater production of pro-inflammatory cytokines such as IL-6, IL-8, and IL-1β and reduced levels of immunoregulatory cytokines IL-10 and TNFα (Fig. 1B), mirroring published results from IC/BPS patients. We are currently attempted to dissect the urothelial responses to these Candida isolates. As the effect of Candida appears to require live cells, we have been repeating these assays using fractions of cultured media derived from individual strain cultures to determine if the effect is mediated by direct cell-cell contact, fungal products, fungal components (such as cell wall components) or extracellular vesicles.

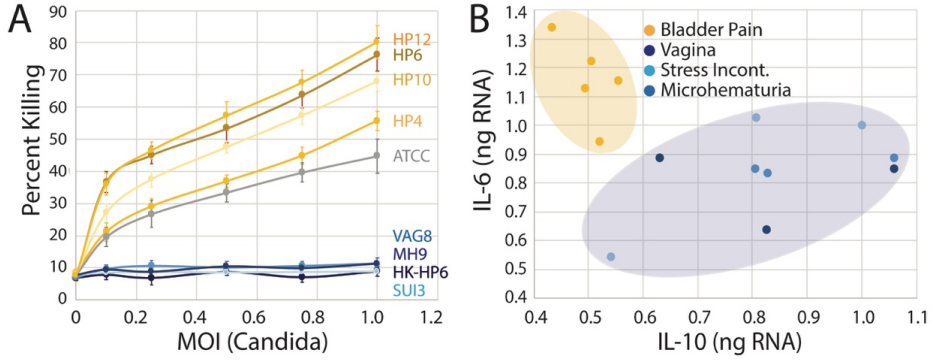

Figure 1. *Urinary fungal isolates from patients with and without bladder pain elicit different urothelial inflammatory responses.* Urothelial cells were co-cultured *C. albicans* strains purchased from ATCC (SC5314) or derived from subjects with or without bladder pain. (A) The *C. albicans* strains from patients with bladder pain (HP4, HP6, HP10, HP12) induced significantly more urothelial cell death than vaginal strains (VAG8) or isolates from asymptomatic patients with microhematuria (MH9), stress incontinence (SUI3). Heat-killed HP6 (HK-HP6) did not induce appreciable killing. This data is representative of thirteen pain-associated and nine asymptomatic unique *C. albicans* strains. (B) Levels of the pro-inflammatory cytokine IL-6 were plotted against the immunoregulatory cytokine IL-10, revealing the distinct differences in cytokine production profiles for strains isolated from patients with (yellow) and without (blue) pain.

Filamentation is widely accepted as a major virulence factor for C. albicans and reflects the adaptation of individual strains to infect specific tissues. To assess filamentation, *Candida* strains grown overnight in YPD broth culture were transferred to hyphal-induction medium (supplemented with fetal bovine serum), and the proportion of unconstricted germ-tubes in each culture assessed at 30-minute intervals by microscopic examination. This measurement of filamentation efficiency of our panel of clinical Candida isolates again revealed a clear division in strain behaviors by symptomatology of the patient of origin (Fig. 12). Pain-associated strains exhibited higher filamentation potential than either strains derived from patients without pain or common laboratory strains (ATCC). These early data lend credence to the concept that altered inflammation may be mediated by microbial virulence factors distinct to IC/BPS-associated fungal strains. We will continue these studies by analyzing fungal biofilm formation using fluorescence microscopy.

We have also completed the genomic sequencing of these isolates and are currently analyzing the results, primarily examining for mutations (small nucleotide polymorphisms) or changes in copy number. We are currently examining genes encoding known virulence factors and regions associated with antifungal resistance. We are hoping to continue to isolate fungal strains from the same patients in longitudinal fashion to allow examination of the *in vivo* evolution of fungal strains with the course of disease, examining for the correlation of symptomatic changes with genetic changes seen over time.

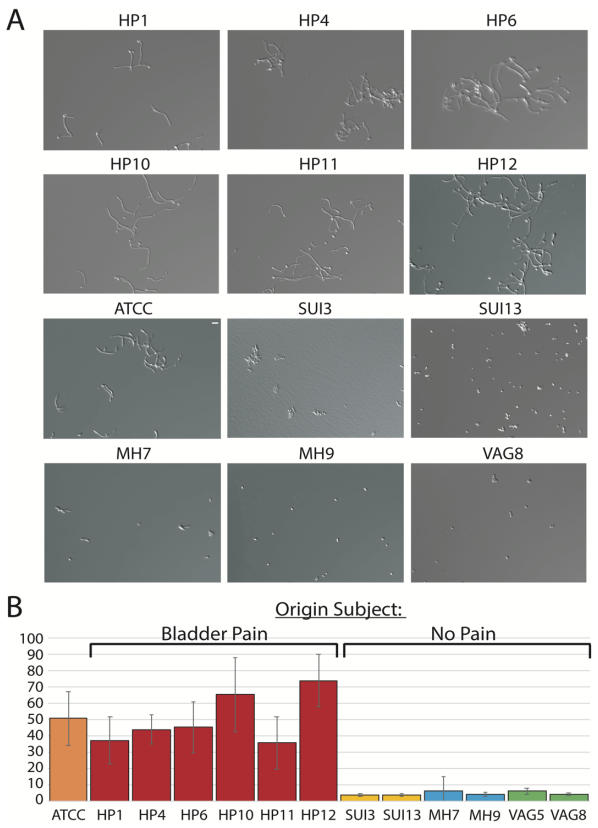

Figure 2. *Urinary C. albicans isolates from patients with IC/BPS exhibit more filamentation than strains from patients without painful GU symptoms.* (A) Phase contrast microscopy of *C. albicans* primary isolates. *C. albicans* strains from bladder pain patients exhibit more filamentation following transfer into fetal bovine serum-containing media than strains isolated from subjects with non-pain urinary complaints. (B) Hyphal length was quantified in at least 10 high power fields. HP: high pain (red); SUI: stress urinary incontinence (yellow); MH: microscopic hematuria (blue); VAG: vaginal isolate (green); ATCC: American Type Culture Collection

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

- *Dr. Ackerman attended the Annual Meetings of* Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction, American Urological Association and American Urogynecologic Society/International Urogynecological Association. In addition, Dr. Ackerman attended the intensive Molecular Mycology course at the Marine Biological Laboratory in Woods Hole, MA, to help advance the detailed characterization of the fungal clinical isolates described above.

- **How were the results disseminated to communities of interest?**

  - The initial results of our machine learning approaches to phenotyping bladder pain were presented as a Webinar during the Interstitial Cystitis Association's

Spring into Summer Self-Care Adventure, entited “Phenotyping Bladder Pain” in May 2022. (https://youtu.be/niHEjvoLmW4)

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - With the gradual ramping up of clinical and surgical volumes, we expect to continue recruitment and sample acquisition to complete subject recruitment in the next study period. We will attempt to accelerate the timeline originally envisioned, which may be more easily facilitated by the creation of our specialized pelvic pain clinic. We will also continue to expand the library of clinical microbial isolates.
  - Once subject recruitment is complete, we will plan on proceeding with the bacterial and fungal community profiling. In the interim, we have been evaluating improvements to our microbial analysis pipeline (previously based on amplicon sequencing) with the incorporation of novel microbial profiling techniques such as adaptive metagenomics, which can provide more comprehensive characterization of microbial communities in biological samples. Such approaches can also provide deeper genomic characterization of individual strains, which may facilitate a breakdown of the microbial genomic characteristics associated with bladder pain without the need to isolate and grow each individual strain.
  - We are also planning on completing the proposed *in vitro* exploration of host-microbe interactions related to IC/BPS. We have characterized markers of virulence, such as filamentation, in yeast strains isolated from patients with bladder pain, as well as the induction of host cell killing and inflammatory mediators (described above). We are currently trying to dissect the mechanism by which these fungi mediate these effects. As live fungi are needed to mediate the pathogenic impact of pain-associated clinical isolates on urothelial cells, we are currently trying to determine if this effect is mediated by direct interaction of the fungi with the host cells or by the impact of soluble mediators produced by fungi. We are also in the process of genomic analysis of these strains with the hopes of identifying common bladder pain-associated genomic alterations. We hope to complete the comprehensive characterization of these strains in the next study period.
  - Much of the next period will focus on deeper analysis of the accumulated data with the more advanced statistical approaches to evaluate for potential interactions between the microbial, inflammatory and genetic data thus far. In addition, we are currently intensely focused on the dissemination of some of these initial results in several manuscripts at various stages of review and preparation.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  - Our clinical phenotyping of patients endorsing pain related to the bladder (Mwesigwa *et al.* 2021) provides an alternative schema for the management of patients with perceived bladder pain. While several different phenotyping approaches exist, this data-driven approach appears to have utility in

predicting treatment responses, which we hope will improve the management of this debilitating condition in the future. We are currently attempting to validate those findings in an independent population in this study. Potentially the most impactful finding from this report, however, is the recognition that isolated myofascial pain and dysfunction can be experienced by patients as bladder pain co-existing with urinary symptoms, such as urgency and frequency. This condition, while manifesting with symptoms that are extremely similar to subjects with bladder-derived pain, has a unique etiology and responds to a different set of therapies. The recognition that musculoskeletal dysfunction can cause these symptoms may help to provide these patients with better therapies aimed at recognizing and correcting the underlying causes (joint dysfunction, gait issues, back injuries, etc.) and addressing the dysfunction (e.g., with physical therapy), avoiding prolonged and unnecessary therapies with pharmacologic agents (e.g., centrally-acting medications such as gabapentin and amitriptyline) or invasive bladder-directed therapies (e.g., cystoscopy or bladder instillations) that are unlikely to impact thri disease course.

- **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**
     - Due to the Covid-19 pandemic, both laboratories and clinics have been gradually resuming normal activity levels throughout the study period. Clinically, restrictions on in-person clinical care have now been removed, but we have such a backlog in care that we are still not back to normal. This has limited clinical access for many patients with chronic conditions. To address this, we have established a specialized clinic solely for patients with bladder pain to facilitate easier access for these patients. This also streamlines our research pathways to help facilitate the pathways for care and testing during the study protocol. This clinic was only recently established, but has already allowed us to focus on study recruitment and management and more quickly add additional patients to our cohort.
   - **Changes that had a significant impact on expenditures**
     - *We are still in the process of hiring the post-doctoral fellow that was anticipated to assist with a large portion of this work. We have this position listed currently and hope to have a candidate identified shortly.*

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Nothing to Report
- **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.**

Mwesigwa P, Jackson NJ, Caron A, Kanji F, Ackerman JE, Webb J, Scott VCS, Eilber KS, Underhill DM, Anger JT, **Ackerman AL**. Unsupervised Machine Learning Approaches Reveal Distinct Phenotypes of Perceived Bladder Pain. Front. Pain Res., 05 November 2021 doi: 10.3389/fpain.2021.757878. PMID: 35036991

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

Interstitial Cystitis/Bladder Pain Syndrome
by: **A. Lenore Ackerman,** H.Henry Lai
in: American Urological Association Core Curriculum
Ed: Larissa Bresler, Matthew Rutman, et al.
Published: January 27, 2022

- **Other publications, conference papers, and presentations.**
  - *Other Publications*:

McGinn-Szlachta AW, **Ackerman AL.** Asymptomatic Bacteriuria, Atypical UTI, and the Urinary Microbiome. AUA Update Series. Volume 40. Lesson 30*. Published: September 1, 2022.*

- *Conference Papers:*

Ackerman AL, Szlachta-McGinn A, Lagree K, Khalique MU, Caron AT, Ackerman JE, Cheng Z, Eilber KS, Anger JT, Underhill DM. Virulent Urinary Fungal Strains in Interstitial Cystitis/Bladder Pain Syndrome Increase Host Inflammatory Response and Induce Urothelial Cell Killing. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

Szlachta-McGinn A, Tipton C, Diaz N, Martin R, Nickel JC, **Ackerman AL**. Characterizing Urogenital Fungi in Women with Bothersome Urogenital Symptoms Compatible with Inflammation

and/or Infection: A Retrospective Cohort Analysis. American Urogynecologic Society/International Urogynecological Association Scientific Meeting, June 2022, Austin, TX.

Szlachta-McGinn A, Ackerman JE, **Ackerman AL**. Quantitative Polymerase Chain Reaction to Identify Common Uropathogens in Urine in a Cohort of Female Urogynecology Patients With and Without Lower Urinary Tract Symptoms. American Urogynecologic Society/International Urogynecological Association Scientific Meeting, June 2022, Austin, TX.

- *Presentations:*

Classifying Patients with Pelvic Pain To Guide Treatment Decisions	47th Annual UCLA State-of-the-Art Urology Conference	March, 2022
Host-Microbe Interactions in Lower Urinary Tract Disorders	Society for Basic Urologic Research 2021 Annual Meeting	November, 2021
Fungal Infections in Urology, Past and Future	Society for Infection and Inflammation in Urology at the AUA	September, 2021

- **Website(s) or other Internet site(s)**
  - Nothing to Report
- **Technologies or techniques**
  - We have developed a diagnostic model for the classification of pelvic pain that correlates in preliminary studies with treatment outcomes. This is described in the accompanying paper (Mwesigwa *et al.* 2021).
- **Inventions, patent applications, and/or licenses**
  - Nothing to Report
- **Other Products**
  - We continue to develop a library of clinical fungal isolates from women with and without bladder pain. This currently has 35 strains of 5 different genera and we continue to add to this collection to facilitate further study.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**
  - Dr. A. Lenore Ackerman, Dr. David Underhill

Name:	*L. Marcella Henao*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*5*

Contribution to Project:	*Ms. Henao has expanded our library of fungal clinical isolates and continues to assist with their clinical characterization.*
Funding Support:	*DOD Award*

Name:	*Crystal Cisneros*
Project Role:	*Clinical Research Coordinator*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Ms. Cisneros is coordinating our dedicated pelvic pain clinic, which oversees the recruitment and continuous care of bladder pain subjects enrolled in this study. She also oversees data management and the regulatory aspects of the project.*
Funding Support:	*DOD Award*

- **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?**
  - Cedars-Sinai Medical Center, where Dr. David Underhill and his lab are located, served as a partner institution supported by this award.
    - **Organization Name:** Cedars-Sinai Medical Center
    - **Location of Organization:** Los Angeles, CA
    - **Partner's contribution to the projec**t *(identify one or more)*
      - **Collaboration:** The research team of Dr. Underhill has helped significantly with research looking at the fungal species isolated from patients with bladder pain, specifically looking at surrogate markers for virulence, including the quantitation of filamentation.
      -

8. **SPECIAL REPORTING REQUIREMENTS**
   - Nothing to Report

9. **APPENDICES: (following)**
    - Updated Curriculum Vitae (Ackerman)
    - Mwesigwa et al. *Front Pain Res*. 2021. PMID: 35036991

A. Lenore Ackerman, M.D. Ph.D.

CURRICULUM VITAE

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Professional Contact Information:
UCLA Medical Center
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Education:

Bachelor of Science	Yale University	1998
Doctor of Philosophy	Yale University	2004
Doctor of Medicine	Yale University	2008
Internship	UCLA-David Geffen	2008-2009
Residency	UCLA-David Geffen	2009-2014
Fellowship	UCLA-Reagan Medical Center	2014-2016
Clinical Scholars Program	Cedars-Sinai Medical Center	2016-2018
UrogynCREST	American Urogynecologic Society, Duke University	2020-2022

Licensure:
Medical Board of California, #A112910
CA Fluoroscopy – Xray Supervisor and Operator, #RHC00202007

Board Certification:

American Board of Urology	2018-
Female Pelvic Medicine and Reconstructive Surgery	2018-

Professional Experience:

Staff Physician	Cedars-Sinai Medical Center Dept. of Surgery, Division of Urology	2016-2020
Assistant Professor	Cedars-Sinai Medical Center Female Pelvic Medicine and Reconstructive Surgery	2016-2020
Adjunct Asst. Professor	UCLA-David Geffen Urology	2016-2020
Assistant Professor, Director of Research	UCLA-David Geffen Urology Women's Pelvic Health Center	2020-

Professional Activities: Professional Society Membership

Western Section – American Urologic Association	2018-
Society for Basic Urologic Research	2016 -
International Urogynecological Association	2016 -
Society for Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction	2015 -
American Urogynecological Society	2015 -
Federation of Clinical Immunology Societies	2015 -
Society for Women in Urology	2014 -

Los Angeles Urological Society	2008 -
American Urological Association	2008 -
American Medical Association	2004 -
American Association of Immunologists	2000 –

Committee/Professional Service

Women's Health Technology (WHT) Coordinated Registry Network (CRN), POP Working Group	2018-2019
Recurrent Urinary Tract Infections Guidelines Committee (American Urological Association)	2018 -
Basic Science Program Committee (Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction)	2018 -
AUA Clinical Problem-Solving (CPS) Module: Recurrent Uncomplicated Urinary Tract Infections in Women	2019
Section Editor, Microbiome and Urologic Infections, Grand Rounds in Urology	2020 -
Committee Member, Terminology on Female Bladder Pain (International Urogynecological Association/American Urogynecologic Society)	2020 -
Chair, Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction Grant Research Committee	2020 -
Guest Editor, Frontiers in Cellular and Infection Microbiology, Urobiome Section	2020 -
Differentiating Asymptomatic Bacteriuria/Chronic Bladder Colonization from Recurrent UTI Clinical Guidance Committee (AUGS)	2021 -
Study Section, NIDDK P20 FORWARD	2022 –
Sub-Committee Lead on Infection and Inflammation, SIU Academy, Société Internationale d'Urologie (SIU)	2022 -

Community Service Activities

Volunteer – Urology Community Outreach, Leo Baeck	2019
Volunteer – Medicine for Humanity	2015
Volunteer – Yale-HAVEN Free Clinic	2005-2008
Coordinator – Medical Reserve Corps	2004-2008
Program Coordinator – Graduate Student Research Symposium, Yale University,	2000
Volunteer – Genetics Education Outreach Program, Yale University	1999-2002
Volunteer – Pediatric Emergency Department, Yale-New Haven Hospital	1998-2000

Editorial Services

Frontiers in Pain Research	2021 -
Frontiers in Cellular and Infection Microbiology	2021 -
European Urology	2018 -

	Neurourology and Urodynamics	2016 -
	Journal of Urology	2015 -
	International Urogynecological Journal	2015 -
	Urology	2015 -
	PLOS One	2015 -

Professional Activities:

CSMC Clinical Competency Committee, Member	2017-2020
CSMG Clinical Care Optimization/Urology Member	2019-2020
UCLA MSTP Admissions Committee	2020 -
UCLA Urology Equity, Diversity, and Inclusion Subcommittee on Education and Training	2021 –

Honors and Special Awards:

Visiting Professor, University of Texas Southwestern Departments of Urology and Geriatrics	2021
Urology Care Foundation Rising Star in Urology	2021
Diokno-Lapides Essay Contest, Second Place	2021
American Urologic Association: Best Poster Infections/Inflammation/Cystic Disease of the Genitourinary Tract: Kidney and Bladder II	2020
Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction- Basic Science Poster Award, Second Place	2019, 2022
American Urologic Association: Research Forum: First Place Early-Career Investigators Showcase	2018
American Urologic Association: Best Poster Basic Research and Pathophysiology II	2017
American Urologic Association: Best Poster Practice Patterns, Quality of Life and Shared Decision Making V	2017
Cedars-Sinai Patient Satisfaction Award	2016-2018
Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction-Best Basic Science Poster	2016
Pfizer/Urology Care Foundation Research Scholar	2015
Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction Resident Travel Award	2012
American Urogynecologic Society Resident Research Scholar	2011

	Yale Minority Science, Technology, and Research Fellow	1996
	Yale University Class of 1954 Fellow	1995-1997
	Yale University Hiram B. Manville Scholar	1995
	Yale Club of Atlanta Community Service Fellow	1995
	Coca-Cola National Scholar	1994

Research Grants and Fellowships Received:

CuresAccelerator/ Cures Within Reach	Repurposing Pelvic Floor Electrical Stimulation for the Treatment of Chronic Pain Repurposing Research /year 2022-2023 Led by a US-Based Racial/Ethnic Minority PI Principal Investigator 10% effort	
Department of Defense Congressionally-Directed Medical Research Program	Phenotyping of IC/BPS Using Microbial Community States: Validation of a Novel Nonivasive Biomarker Peer Reviewed Medical Research Program (PRMRP) Expansion Award Principal Investigator 10% effort	2021-2024
National Institute of Aging (NIH)	The Role of the Vaginal Microbiota in Urgency Urinary Incontinence in Older Women Grants for Early Medical/Surgical Specialists' Transition to Aging Research (R03) Principal Investigator 10% effort	
National Institute of Diabetes and Digestive And Kidney Diseases (NIH)	The Urinary Mycobiota and Host Inflammation in Lower Urinary Tract \ Symptoms Mentored Clinical Scientist Research Career Development Award (K08) Principal Investigator 75% effort	2019-2024
Society for Urodyn. Female Pelvic Med. and Urogenital Reconstruction	The Role of the GU Microbiota in predicting Responses to Sacral Neuromodulation for Overactive Bladder in Older Women Neuromodulation Award Principal Investigator 1% effort	2019-2021
University of California Los Angeles, Clinical and Translational Science Institute	The Microbiome of Interstitial Cystitis Subjects with Hunner's Ulcers Technology Core Vouchers 1% effort Co-Investigator	2019-2020
University of CA Los Angeles, Clinical	The Role of the Vaginal Microbiota in Urgency Urinary Incontinence Iris Cantor-UCLA Executive Advisory	2019-2020

and Translational Science Institute	Board/CTSI Pilot Award Principal Investigator	1% effort	
University of CA Los Angeles, Clinical and Translational Science Institute	The Urinary Microbiota and Host Inflammation in Lower Urinary Tract Symptoms KL2 Mentored Clinical Scientist Training Award Principal Investigator	75% effort	2019-2020
Shaffer Family Foundation	Vaginal Fractionated CO2 Laser Treatment – Effects on the Genitourinary Microbiome Co-Investigator	1% effort	2018-2020
Department of Defense Congressionally-Directed Medical Research Program	The Urinary Fungal Mycobiome and Host Responses in Patients with Interstitial Cystitis PRMRP Discovery Award Principal Investigator	5% effort	2017-2019
Society for Urodyn. Female Pelvic Med. and Urogenital Recon.	The Microbiome as a Predictor of Outcome of Intravesical Botox Chemodenervation Award Principal Investigator	1% effort	2017-2019
University of California Los Angeles, Clinical and Translational Science Institute	Temporal Dynamics of the Female Genitourinary Microbiota Technology Core Vouchers Principal Investigator	1% effort	2016-2017
Urology Care Foundation	Inflammatory Responses and the Microbiome in OAB Urologic Research Training Award Principal Investigator	50% effort	2015-2016
National Institute of Allergy and Infectious Diseases (NIH)	Cellular Mechanisms of Cross-presentation in Dendritic Cells Individual National Research Service Award Minority Fellowship Principal Investigator	100% effort	2000-2004
National Heart, Lung, and Blood Institute (NIH)	Generation of Soluble Antibody/T-cell Receptor Chimeras as Immunosuppressive Adjuncts in Xenotransplantation Under-represented Minority Summer Research Fellowship Principal Investigator	100% effort	1998

Research Projects and Research Focus or Interest:

Clinical Trials	I have been involved in multiple trials of novel therapeutics in the treatment of benign urologic diseases, serving as site coordinator for multiple industry-sponsored trials of oral and local therapies for interstitial cystitis and overactive bladder. Current interests include the

	manipulation of the genitourinary microbiome in the treatment and prevention of lower urinary tract symptoms and infections.
Clinical Research	My primary interest is the application of machine learning to the diagnosis and phenotyping of benign urologic diseases to provide more objective diagnostic schemata and effective screening in the primary care setting as well as better prognostication in treatment decision-making.
Basic Research	I have a particular interest in the relationship between genitourinary microbial communities and host inflammatory responses and how the interactions between them influence lower urinary tract physiology and pathology. My laboratory is focused on identifying the associations of particular microbial communities with benign urologic disease phenotypes and modeling these phenotypes in in-vitro systems and animal models.

Invited Lectures and Presentations:

Molecular Urinary Tract Infection Testing	American Urological Association Annual Meeting	New Orleans, LA	2022
Where the Field of Pelvic Pain Needs to Go	Society for Infection and Inflammation in Urology at the AUA	New Orleans, LA	2022
Recurrent UTI in women: Is there a role for cranberry or other non-antibiotic solutions?	SUFU at the American Urological Association Annual Meeting	New Orleans, LA	2022
Early Investigator Spotlight	Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction	San Diego, CA	2022
Point-Counterpoint: MUS vs. Bulking Agent in a 55-year-old Woman with SUI	Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction	San Diego, CA	2022
The Genitourinary Microbiome and UTI: How a Better Understanding of the Urobiome Influences Understanding of UTI	30th Annual Perspectives in Urology: Point Counterpoint	San Diego, CA	2021
Host-Microbe Interactions in Lower Urinary Tract Disorders	Society for Basic Urologic Research 2021 Annual Meeting	Virtual	2021
Use of the Urinary Microbiome To Guide Clinical Care of Lower Urinary Tract Symptoms	2021 UTSW Visiting Professor in Urology and Geriatrics	Dallas-Ft Worth, TX	2021
The Genitourinary Microbiome In Women: Microbes, Menopause, and Micturition	2021 UTSW Visiting Professor in Urology and Geriatrics	Dallas-Ft Worth, TX	2021
Microbiome Abnormalities in the Lower Urinary Tract SUFU Invited Speaker	International Continence Society 2021 Annual Meeting	Virtual	2021
Fungal Infections in Urology, Past and Future	Society for Infection and Inflammation in Urology at the AUA	Virtual	2021
Panel Discussion: Challenging Cases in Complicated Recurrent UTI	American Urological Association Annual Meeting	Virtual	2021
Clarifying Recent Guidelines and Clinical Studies on	Online Webinar by Solv Wellness	Virtual	2021

Non-antibiotic Management of Recurrent UTIs			
Careers Panel	KUH R25 Summer Undergraduate Research Symposium	Virtual	2021
Pathways to Surgeon-Scientist Panelist	EMPIRE "Hidden Curriculum" AYA NY Section	Virtual	2021
Big Data in Benign Urological Disease	SUFU 2021 Winter Meeting Basic and Translational Science	Virtual	2021
Say Hello to My Little Friends: How We Use the Microbiome In Clinical Urology	SUFU 2021 Winter Meeting General Session	Virtual	2021
A New Perspective on the Definitions and Management of GU Infection	UPMC Magee Women's Hospital Urogynecology Grand Rounds	Virtual	2021
Microbiome: How is it used in Urogynecology?	PFD Week 2020 (American Urogynecologic Society Annual Meeting	Virtual	2020
Urinary Tract Infections	FPMRS Fellows Webinar American Urogynecologic Society	Virtual	2020
Take Home Messages: Infection/Inflammation	American Urological Assoc. Annual Meeting	Virtual	2020
Beyond Bacteria: Fungi In the Urinary Tract	UROBIOME 2020	San Diego, CA	2020
Recurrent Urinary Tract Infections: Imaging and Molecular Diagnostics	Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction	Scottsdale, AZ	2020
Recurrent Urinary Tract Infection: Lessons from the Guidelines	American Urologic Association Annual Meeting	Chicago, IL	2019
Female Pelvic Pain: Why Does It Hurt Down There?	Advanced Practice Provider Program at the AUA	Chicago, IL	2019
Vaginal Laser Therapy in Today's Urology Practice: What the Evidence Tells Us	SUFU at the American Urological Association Annual Meeting	Chicago, IL	2019
The Urinary Mycobiome and Lower Urinary Tract Symptoms	Duke University, Annual Multidisciplinary Benign Urology Research Day	Durham, NC	2019

Expert Panel-Recurrent UTI A Case-based Approach	Western Section, American Urological Association	Maui, HI	2018
The Mycobiome of the Urinary Tract and Its Potential Role in Urologic Conditions	University of California, San Diego	San Diego, CA	2018
The Urinary Microbiota and Host Inflammation in Lower Urinary Tract Symptoms	American Urological Association Annual Meeting Research Forum	San Francisco, CA	2018
Recent Advances in Urinary Microbiota and the Diagnosis of UTI	Mediterranean Incontinence and Pelvic Floor Society Annual Meeting	Rome, Italy	2018
Pathways to Independence in Urologic Research: It Takes a Village	Individualizing Treatment of Urinary Incontinence NIDDK/NIH	Bethesda, MD	2018
Imaging in FPMRS: Ultrasound	Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction	Austin, TX	2018
The Urinary Microbiota: A New Paradigm in the Study of Lower Urinary Tract Symptoms	Yale University, Department of Urology	New Haven, CT	2017
Female Sexual Dysfunction in the Older Patient	Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction	Scottsdale, AZ	2017
Instructor, Female Pelvic Medicine and Reconstructive Surgery, re: AUA/SUFU Guidelines Curso de Actualizacion en Urologia	Sociedad Argentina de Urología/Ameican Urological Association	San Miguel de Tucuman, Argentina	2016
Genitourinary Fistulae: Approaches, Tips and Techniques	Congresso Argnetino de Urologia, Sociedad Argentina de Urología/American Urological Association Joint Symposium	San Miguel de Tucuman, Argentina	2016
Overactive Bladder: A New Perspective on Bladder Hypersensitivity Syndromes	Congresso Argnetino de Urologia, Sociedad Argentina de Urología/American Urological Association Joint Symposium	San Miguel de Tucuman,Argentina	2016

Basic Sciences Symposium. Inflammatory Responses and the Microbiome in OAB.	American Urological Association Annual Meeting	San Diego, CA	2016

Teaching Activities and/or Other off-campus:

UCLA Teaching Activities:

Urology Residents:
- On Call Teaching Rounds (2020 -)
- Yearly Journal Club (2020 -)
- Lectures bimonthly on the Urology Core Curriculum (2020 -)
- Individual teaching on consulting cases, operative cases, clinical supervision (2020 -)
- Participation/Teaching during weekly Grand Rounds, Case Conferences, Morbidity and Mortality Conferences (2020 -)

Female Pelvic Medicine and Reconstructive Surgery Fellows:
- Weekly Didactic, Pre-opperative Conferences (2020 -)
- Individual teaching on consulting cases, operative cases, clinical supervision (2020 -)
- Quarterly FPMRS Los Angeles Area Journal Club (2016 -)
- Academy for Pelvic Surgery Regional Cadaver Lab for FPMRS Fellows (2016 -2019)

Research Fellows:
- Weekly Laboratory Meetings (2020 -)
- Weekly Host-Microbe Journal Club (2020 -)
- Participation/Teaching during monthly Research In Progress Seminars (2020 -)

Research Mentees:
- Ariel Moradzadeh, M.D. (2017-2018)
- Victoria C.S. Scott, M.D. (2018-2019)
- Kai Dallas, M.D. (2019-2021)
- Colby P. Souders, M.D. (2019-2020)
- Ashley Caron, B.S. (2019-2021)
- Julia Guo, B.S. (2019-2020)
- Sarah B. Andebrhan, MPH (2020-2021)
- Patricia L. Mwesigwa, M.D. (2020-2021)
- Michele Torosis, M.D. (2020-)
- Daniele Kaefer. M.D. (2020-2022)
- Rotimi Nettey, M.D. (2020-2022)
- Natalia Garcia Peñaloza, B.S. (2021-)
- Nuha Khalfay, B.S. (2021-)
- Alec Szlachta-McGinn, M.D. (2021-)
- Taylor Sadun, M.D. (2021-)

CME/Educational Events:

Atypical UTI and the Urobiome	AUAUniversity Podcast	2022

Female Pelvic Triangle Microbiomes	Webinar, sponsored by Solv Wellness	2022
Phenotyping Bladder Pain	Webinar, sponsored by the Interstitial Cystitis Association and Desert Harvest	2022
Classifying Patients with Pelvic Pain To Guide Treatment Decisions	47th Annual UCLA State-of-the-Art Urology Conference	2022
Optimizing the Treatment of Complicated UTIs	AUAUniversity Podcast	2022
Defining Properly Collected Urine: Thresholds of Urinalysis for Microscopic Hematuria Evaluation in Women	Grand Rounds in Urology	2022
Value of Genetic Testing and Next Generation Sequencing in Diagnosis Of UTI	46th Annual UCLA State-of-the-Art Urology Conference Live Virtual Conference	2021
Mycoplasma and Ureaplasma Molecular Testing Does Not Correlate with Irritative or Painful Lower Urinary Tract Symptoms	Grand Rounds in Urology Webcast	2021
Prolapse and Sexual Dysfunction after Menopause: Diagnostic Approaches and Treatments	Annual Cedars-Sinai Update In Obstetrics and Gynecology Cedars-Sinai Medical Center	2020
Does My Vagina Need a Makeover? Prolapse, Female Sexual Dysfunction, And Vaginal Rejuvenation	What's New in Urology: A Primer for the Primary Care Physician Cedars-Sinai Medical Center	2019
Obstructive Sleep Apnea and Personal Urological Health	Otolaryngology Symposium Cedars-Sinai Medical Center	2019
Urinary Incontinence and Pelvic Organ Prolapse: Common Complaints	Community Urology CME Event Cedars-Sinai Medical Center	2017
Why Does It Hurt Down There? Primary Care and Pelvic Pain	What's New in Urology: A Primer for the Primary Care Physician Cedars-Sinai Medical Center	2018
Management of Urinary Incontinence	Community Urology CME Event Cedars-Sinai Medical Center	2017
Urinary Complications of Prostate Cancer Treatment	Urologic Cancer Update Cedars-Sinai Medical Center	2017
Urinary Incontinence: Diagnosis And Management	Annual Cedars-Sinai Update In Obstetrics and Gynecology Cedars-Sinai Medical Center	2016

Grand Rounds/Noon Conferences Lectures:

Overactive Bladder and Storage LUTS: Machine Learning and the Microbiome	Internal Medicine Grand Rounds Harbor-UCLA Medical Center	2021
The Urinary Microbiota: A New Perspective On the Definitions and Management of GU Infections	USC FPMRS Rounds USC-Keck Medical Center	2021
Lower Urinary Tract Symptoms in Older Women:Using the Microbiome to Improve Care	UCLA Geriatrics Grand Rounds UCLA Medical Center	2021
Case-based Review of the New AUA Guidelines for the Management of Recurrent Urinary Tract Infections	UCLA FPMRS Grand Rounds UCLA Medical Center	2021
Urinary Microbiota in Benign Lower Urinary Tract Symptoms: New Approaches to Disease Phenotyping	UCLA Urology Grand Rounds UCLA Medical Center	2021
The Urinary Microbiota in LUTS: Microbial Perspectives on Diagnosis and Classification	UCLA FPMRS Grand Rounds, UCLA Medical Center	2020
Female Sexual Dysfunction in Older Patients	Obstetrics and Gynecology Grand Rounds, Cedars-Sinai Medical Center	2020
The Urinary Microbiota in Older Women: Influences of Sex Hormones on LUTS	Center for Research in Women's Health and Sex Differences, CSMC	2020
The Urinary Microbiome in Inflammation of the Urogenital Tract	Microbe Club, Cedars-Sinai Medical Center	2020
Urinary Microbiota and Host Inflammation In Lower Urinary Tract Symptoms	Yale University Department of Urology Grand Rounds, Yale-New Haven Hospital	2020
The Urinary Microbiome in LUTS: A New Paradigm	BIDMC Urology Grand Rounds, Beth Israel-Deaconess Medical Center	2019
Urinary Microbial Profiling in Lower Urinary Tract Symptoms	Urology Grand Rounds, Virginia Commonwealth University	2019
The Urinary Microbiome and Urinary Tract Infection	UCLA FPMRS Grand Rounds, UCLA Medical Center	2019
The Vaginal Microbiome in Health and Disease	Obstetrics and Gynecology Grand Rounds, Cedars-Sinai Medical Center	2019

Recent Advances in our Understanding Of the Diagnosis and Treatment of Urinary Tract Infection	Urgent Care Grand Rounds Cedars-Sinai Medical Center	2018
The Urinary Mycobiome in the Development of Lower Urinary Tract Symptoms	IBIRI Center Group Meeting Cedars-Sinai Medical Center	2017
Host Responses to the Urinary Microbiome in Lower Urinary Tract Symptoms	Immunology Research In Progress Cedars-Sinai Medical Center	2017
A New Perspective on Bladder Hypersensitivity Syndromes: The Urinary Microbiome	Urology Grand Rounds, Cedars-Sinai Medical Center	2017
The Urinary Microbiome and Bladder Hypersensitivity Syndromes	General Surgery Grand Rounds, Cedars-Sinai Medical Center	2017
Interstitial Cystitis: Etiology, Management and Mismanagement	Cedars-Sinai Medical Center Obstetrics and Gynecology Grand Rounds	2016

Undegraduate/Graduate Courses:

Medical Impact of Basic Science	Teaching Assistant, Yale University	2001-2003
Graduate Teaching Center at Yale	Science Teaching Consultant	2000-2002
Biological Mechanisms of Reaction to Injury	Teaching Assistant, Yale University	2000
Biology of AIDS	Adjunct Professor, Albertus Magnus College	2000
Cellular Basis of Behavior	Teaching Assistant, Yale University	1999

PUBLICATION/BIBLIOGRAPHY:

RESEARCH PAPERS

Research Papers (Peer Reviewed)

A. RESEARCH PAPERS – PEER REVIEWED

1. Fleming KG, **Ackerman AL**, Engelman DM. The effect of point mutations on the free energy of transmembrane alpha-helix dimerization. J Mol Biol. 1997 Sep 19;272(2):266-75. doi: 10.1006/jmbi.1997.1236. PMID: 9299353.

2. **Ackerman AL**, Cresswell P. Regulation of MHC class I transport in human dendritic cells and the dendritic-like cell line KG-1. J Immunol. 2003 Apr 15;170(8):4178-88. doi: 10.4049/jimmunol.170.8.4178. PMID: 12682250.

3. **Ackerman AL**, Kyritsis C, Tampé R, Cresswell P. Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens. Proc Natl Acad Sci USA. 2003 Oct 28;100(22):12889-94. doi: 10.1073/pnas.1735556100. PMID: 14561893.

4. **Ackerman AL**, Cresswell P. Cellular mechanisms governing cross-presentation of exogenous antigens. Nat Immunol. 2004 Jul;5(7):678-84. doi: 10.1038/ni1082. PMID: 15224093.

5. **Ackerman AL**, Kyritsis C, Tampé R, Cresswell P. Access of soluble antigens to the endoplasmic reticulum can explain cross-presentation by dendritic cells. Immunol. 2005 Jan;6(1):107-13. doi: 10.1038/ni1147. PMID: 15592474.

6. Shen H, **Ackerman AL***, Cody V, Giodini A, Hinson ER, Cresswell P, Edelson RL, Saltzman WM, Hanlon DJ. (*co-first author) Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. Immunology. 2006 Jan;117(1):78-88. doi: 10.1111/j.1365-2567.2005.02268.x. PMID: 16423043.

7. **Ackerman AL**, Giodini A, Cresswell P. A role for the endoplasmic reticulum protein retrotranslocation machinery during cross presentation by dendritic cells. Immunity. 2006 Oct;25(4):607-17. doi: 10.1016/j.immuni.2006.08.017. PMID: 17027300.

8. Kiyosaki K, **Ackerman AL**, Histed S, Sevilla C, Eilber K, Maliski S, Rogers RG, Anger J. Patients' understanding of pelvic floor disorders: what women want to know. Female Pelvic Med Reconstr Surg. 2012 May-Jun;18(3):137-42. doi: 10.1097/SPV.0b013e318254f09c. PMID: 22543763.

9. Lee U, **Ackerman AL**, Wu A, Zhang R, Leung J, Bradesi S, Mayer E, Rodriguez LV. Chronic Psychological Stress in High Anxiety Rats Induces Sustained Bladder Hyperalgesia. *Physiol Behav*. 2015 Feb; 139:541-548. doi: 10.1016/j.physbeh.2014.11.045. PMID: 25449389.

10. **Ackerman AL**, Lee UJ, Jellison F, Tan N, Patel M, Raman SS, Rodriguez LV. MRI suggests increased tonicity of the levator ani in women with Interstitial Cystitis/Bladder

Pain Syndrome. *Int Urogyn J.* 2016 Jan;27(1):77-83. doi: 10.1007/s00192-015-2794-6. PMID: 26231233.

11. **Ackerman AL**, Jellison FC, Lee UJ, Bradesi S, Rodriguez LV. Glt1 glutamate receptor mediates the establishment and perpetuation of chronic visceral pain in an animal model of stress-induced bladder hyperalgesia. Am J Physiol Renal Physiol. 2016 Apr 1;310(7):F628-F636. doi: 10.1152/ajprenal.00297.2015. PMID: 26697981.

12. Cohen SA, Chaudhry Z, Oliver JL, Kreydin EI, Nguyen MT, Mills SA, **Ackerman AL**, Kim JH, Tarnay CM, Raz S. Comparison of Times to Ureteral Efflux After Administration of Sodium Fluorescein and Phenazopyridine. J Urol. 2017 Feb;197(2):519-523. doi: 10.1016/j.juro.2016.07.099. PMID: 27664579.

13. Ramart P, **Ackerman AL***, Cohen SA, Kim JH, Raz S. (*co-first author)The Frequency of Urinary Incontinence after Suburethral Mesh Removal Requiring Anti-Incontinence Procedure. Urology. 2017 Aug;106:203-209. doi: 10.1016/j.urology.2017.01.060. PMID: 2847668.
14. Clemens JQ, Mullins C, **Ackerman AL**, Bavendam T, van Bokhoven A, Ellingson BM, Harte SE, Kutch JJ, Lai HH, Martucci KT, Moldwin R, Naliboff BD, Pontari MA, Sutcliffe S, Landis JR; MAPP Research Network Study Group. Urologic chronic pelvic pain syndrome: insights from the MAPP Research Network. Nat Rev Urol. 2019 Mar;16(3):187-200. doi: 10.1038/s41585-018-0135-5. PMID: 30560936.
15. Weinberger JM, Houman J, Caron AT, Patel DN, Baskin AS, **Ackerman AL**, Eilber KS, Anger JT. Female Sexual Dysfunction and the Placebo Effect: A Meta-analysis. Obstet Gynecol. 2018 Aug;132(2):453-458. doi: 10.1097/AOG.0000000000002733. PMID: 29995725.
16. Moradzadeh A, Jamnagerwalla J, Eilber KS, Anger JT, **Ackerman AL**. High catastrophizing in subjects with painful mesh complications have worse outcomes. Urology. 2019 Feb;124:83-90. doi: 10.1016/j.urology.2018.05.050. PMID: 30076941.
17. **Ackerman AL**, Lai HH, Parameshwar PS, Eilber KS, Anger JT. Symptomatic Overlap in Overactive Bladder and Interstitial Cystitis/Painful Bladder Syndrome - development of a new algorithm. BJU Int. 2019 Apr;123(4):682-693. doi: 10.1111/bju.14568. PMID: 30253040.
18. Patel DN, Zhao HH, Houman J, **Ackerman AL**, Eilber KS, Anger JT. Comparative effectiveness of one versus two-stage sacral neurostimulation device placement. Neurourol Urodyn. 2019 Feb;38(2):734-739. doi: 10.1002/nau.23908. PMID: 30620133.
19. **Ackerman AL**, Anger JT, Khalique MU, Ackerman JE, Tang J, Kim J, Underhill DM, Freeman MR; NIH Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP). Optimization of DNA extraction from human urinary samples for mycobiome community profiling. PLoS One. 2019 Apr 25;14(4):e0210306. doi: 10.1371/journal.pone.0210306. PMID: 31022216
20. Nickel JC, Stephens A, Landis JR, Mullins C, van Bokhoven A, Anger JT, **Ackerman AL**, Kim J, Sutcliffe S, Krol JE, Sen B, Hammond J, Ehrlich GD; Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. Urinary fungi associated with urinary symptom severity among women with interstitial cystitis/bladder pain syndrome (IC/BPS). World J Urol. 2020 Feb;38(2):433-446. doi: 10.1007/s00345-019-02764-0. PMID: 31028455.
21. Anger J, Lee U, **Ackerman AL,** Chou R, Chughtai B, Clemens JQ, Hickling D, Kapoor A, Kenton KS, Kaufman MR, Rondanina MA, Stapleton A, Stothers L, Chai TC.

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B. RESEARCH PAPERS – PEER REVIEWED (IN PRESS)

1. Szlachta-McGinn A, Douglass KM, Chung UYR, Jackson NJ, Nickel JC, **Ackerman AL.** Molecular Diagnostic Methods Versus Conventional Urine Culture for Diagnosis and Treatment of Urinary Tract Infection: A Systematic Review and Meta-Analysis. *In Press.*

C. RESEARCH PAPERS – PEER REVIEWED (SUBMITTED)

2. Zhao H, Souders CP, Chen A, Masterson J, Naser-Tavakolian A, Daskivich TJ, Ley EJ, **Ackerman AL**. Frequency of Inpatient Specialty Consultations for Patients Hospitalized with SARS-CoV-2. *Submitted.*
3. Rogers A, Zeng L, **Ackerman AL**. Consistent Therapy Delivery with eCoin® for Urgency Urinary Incontinence Demonstrates High Patient Satisfaction. *Submitted.*
4. Torosis M, Jackson NJ, Nitti V, **Ackerman AL**. Overactive Bladder Patients With and Without Urgency Incontinence: A Spectrum of One Condition or Different Phenotypes?" *Under Revision.*

D. RESEARCH PAPERS – NON-PEER REVIEWED

1. **Ackerman AL**, Anger JT. The Role of the Urinary Microbiome in Urological Disease. AUA News, September, 2017.
2. **Ackerman AL**, Raz S. Complete Mesh Removal is Appropriate for Chronic Mesh Related Pain. AUA News, October, 2017.
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4. Souders CP, **Ackerman AL**. Ureaplasma/Mycoplasma Colonization is Negatively Associated with Chronic Urinary Symptoms. AUA News, November, 2020.
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Chapters

1. The role of power morcellation and controversies
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in: Use of Robotic Technology in Female Pelvic Floor Reconstruction
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Published: October 18, 2017

2. Native Tissue Repair After Failed Synthetic Materials
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in: Native Tissue Repair For Incontinence and Prolapse
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3. Bulbocavernosus Muscle and Fat Pad Supplement
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in: Hinman's Atlas of Urologic Surgery, Fourth Edition
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4. Interstitial Cystitis/Bladder Pain Syndrome
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Letters to the Editor None

Reviews

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Editorials

1. **Ackerman AL** and Raz S. Difference of Opinion – Are Synthetic Slings Safe? Opinion: No. Int Braz J Urol. 2016 Jul-Aug;42(4):640-4. doi: 10.1590/S1677-5538.IBJU.2016.04.03. PMID: 27564272.
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Papers in Preparation (Research Completed)

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2. **Ackerman AL**, Khalique MU, Cheng Z, Ackerman JE, Anger JT, Eilber KS, Underhill DM. Microbiological phenotyping of bladder and pelvic pain reveals distinct clinical profiles with implications for prognosis and treatment.
3. Yang J, Scott VCS, Thum LW, Khalique MU, Ackerman JE, Underhill DM, Jacobs J, **Ackerman AL.** Temporal Dynamics of the Female Geintourinary Microbiome.
4. **Ackerman AL,** Liu G, Hu X, Scott VCS, Kreydin E, Shi W, Raz S**.** Bacterial persistence on Transvaginal Slings is Associated with Delayed-Onset Chronic Pain.
5. **Ackerman AL**, Lima BP, Mellano EM, Ramart P, Pizarro-Rojas M, Shi W, Lux R, Raz S. A Pro-Inflammatory Mesh-Associated Microbiota in Transvaginal Slings Explanted from Patients with Delayed-Onset Chronic Pain.

Abstracts

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2. **Ackerman AL** and Cresswell P. Regulated MHC Class I Transport in Human Dendritic Cells Facilitates Proper Immune Activation and Limits Autoimmunity. Keystone Symposium on Dendritic Cells, Keystone, Colorado, March 2003.

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10. **Ackerman AL**, Le N-B, Jellison F, Rogo-Gupta L, Chow D, Hartshorn TG, Rodriguez LV, Raz S. Anatomical, functional, and quality of life outcomes of transvaginal sacrouterine ligament suspension for vaginal vault prolapse. Society of Urodynamics and Female Urology Annual Meeting, New Orleans, LA, February 2012. Recipient of Resident Travel Award.

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13. **Ackerman AL**, Lee UJ, Tan N, Raz S, Raman SS, Rodriguez LV. Alterations in the Pelvic Floor Musculature on Pelvic MRI in Patients with Interstitial Cystits. American Urological Association Annual Meeting, Atlanta, GA, May 2012.

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15. Mellano EM, Lima B, Ramart P, **Ackerman AL**, Lux R, Shi W, Raz S. The Role of Bacterial Biofilms and Chronic Inflammation in the Delayed Development of Pain following Transvaginal Placement of Mesh Slings for Incontinence. AUGS Annual Meeting, October 2015, Seattle, WA.

16. Kang D, Hartshorn T, Pollard M, Choi J, Rodriguez L, Kim JH, **Ackerman AL**, Cohen S, Ramapart P, Raz S. Patient Quality of Life after Removal of Vaginal Mesh. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting,

    Scottsdale, AZ, February 2015.

17. Choi JM, Ramart P, Kang D, **Ackerman AL**, Cohen S, Raz S. Abdominal Sacrocolpopexy Mesh Complications: Presentation and Surgical Removal Techniques. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale, AZ, February 2015.

18. **Ackerman AL**, Lima BP, Ramart P, Mellano E, Lux R, Shi W, Raz S. The Role of Bacterial Biofilms and Chronic Inflammation in the Delayed Development of Systemic Side Effects Following Transvaginal Placement of Mesh Slings for Incontinence. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale, AZ, February 2015.

19. **Ackerman AL**, Lee UJ, Jellison FC, Margolis DC, Tan N, Patel M, Raz S, Raman SS, Rodriguez LV. MRI Imaging Suggests Increased Tonicity of the Levator Ani Muscle Complex in Women with Interstitial Cystitis/Bladder Pain Syndrome. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale, AZ, February 2015.

20. Cohen SA, Mellano EM, Chaudhry Z, **Ackerman AL**, Ramart P, Scott VC, Kim J, Raz S. Cystocele Repair Using Autologous Iliotibial Band. Presentation: Video 15. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale, AZ, February 2015.

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22. Choi JM, Ramart P, Kang DC, Cohen SA, **Ackerman AL**, Raz S. Pubovaginal Sling with Tensor Fascia Lata. American Urological Association Annual Meeting, New Orleans, LA, May 2015.

23. Cohen SA, Viragh KA, Nakamura LY, **Ackerman AL**, Ramart P, Kang DC, Choi JM, Kim J-H, Raman SS, Raz S. Using Translabial Ultrasound as an Effective Tool to Visualize Mesh Erosion into the Urethra and Bladder. American Urological Association Annual Meeting, New Orleans, LA, May 2015.

24. Ramart P**, Ackerman AL**, Cohen SA, Kang DC, Choi JM, Kim J-H, Raz S. Urinary incontinence after suburethral mesh removal requiring anti-incontinence procedure. International Continence Society Annual Meeting, Montreal, Canada, October 2015.

25. **Ackerman AL**, Eilber KS, Caron AT, Pollard ME, Anger JT. Outcomes of Pregnancy Following Surgery for Pelvic Organ Prolapse: A Systematic Review. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, New Orleans, LA, February 2016.

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27. Kreydin EI, Kim MM, Oliver JL, Cohen SA, **Ackerman AL**, Kim J-H, Raz S. Higher Urine Levels of Environmental Toxins Are Associated with Increased Incontinence and Nocturia in Men. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, New Orleans, LA, February 2016.

28. Kreydin EI, Oliver JL, Kim MM, **Ackerman AL**, Cohen SA, Kim J-H, Raz S. 'Til Death Do Us Part: The Relationship Between Urinary Incontinence and Marital Status Among US Women and Men. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, New Orleans, LA, February 2016.

29. Cohen SA, Oliver JL, Kreydin EI, Chaudhry Z, Nguyen MT, Mills SA, **Ackerman AL**, Kim J-H, Tarnay CM, Raz S. Comparison of Times to Ureteral Efflux After Administeration of Sodium Fluorescein and Phenazopyridine. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, New Orleans, LA, February 2016.

30. Ramart P, **Ackerman AL**, Cohen SA, Kim J-H, Raz S. Urinary Incontinence After Suburethral Mesh Removal Requiring Anti-Incontinence Procedures. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, New Orleans, LA, February 2016.

31. **Ackerman AL**, Anger JT, Eilber KS, Funari V, Tang J, Kim J, Freeman MR. Identification of a Diverse Fungal Community ("Mycobiome") in the Normal Female Human Lower Urinary Tract. Society of Urodynamics, Female Pelvic Medicine, and

Urogenital Reconstruction Annual Meeting, New Orleans, LA, February 2016. Recipient of Best Basic Science Poster Award.

32. Kreydin E, Oliver J, Kim M, Cohen S, **Ackerman AL**, Raz S, Lerner L. Prostate Ablation and Enucleation: Comparison of Patient Characteristics and 30-day Surgical Outcomes Using a National Database. American Urological Association Annual Meeting, San Diego, CA, May 2016.

33. Kreydin E, Kim M, Oliver J, Cohen S, **Ackerman AL**, Chaudhry Z, Nguyen MT, Kim JH, Raz S. Til Death Do Us Part: The Relationship between Urinary Incontinence and Marital Status among US Women and Men. American Urological Association Annual Meeting, San Diego, CA, May 2016.

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35. **Ackerman AL**, Eilber KS, Tang J, J. Kim3 , Freeman MR, Anger JT. A Diverse, Viable Fungal Community ("Mycobiome") Exists in the Urine of Healthy Asymptomatic Females. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale, AZ, September/October 2016, V22:5 p. S35. Oral Poster 1.

36. Samimi P, Handler SJ, **Ackerman AL**, Eilber KS, Anger JT. Recurrent UTI in Women: Patient Characteristics, Natural History, and Referral Practice Patterns. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale,AZ, September/October 2016, V22:5 p. S103. Poster 76.

37. Handler SJ, Samimi P, **Ackerman AL**, Anger JT, K. S. Eilber KS. Practice Patterns for the Evaluation of Asymptomatic Micoscopic Hematuria in Women in a Single Healthcare System: Room for Improvement? Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale, AZ, September/October 2016, V22:5 p. S103. Poster 77.

38. Nguyen MT, Cohen SA, Mei JY, **Ackerman AL**, Oliver J, Kreydin KI. Preliminary Report on the Use of the Vastur Lateralis-Fascia Lata Graft for Repair of Anterior Vaginal Wall Prolapse. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, Scottsdale, AZ, September/October 2016, V22:5 p. S149. Poster 180.

39. **AL Ackerman**, KS Eilber, J Tang, J Kim, MR Freeman, JT Anger. A Diverse, Viable Fungal Community ("Mycobiome") Exists in the Urine of Healthy Asymptomatic Females. American Urogynecologic Society Annual Meeting/Pelvic Floor Disorders Week, September 2016, Denver, CO.

40. Van den Broek I, Fu Q, Ackerman AL, Anger JT, Kushon S, Chansky K, Millis K, Percy A, Agreste T, Van Eyk JE. Accuracy of Volumetric Absorptive Microsampling for Quantification of Protein Biomarkers. The Association for Mass Spectrometry: Applications to the Clinical Lab, January 2017, Palm Springs, CA.

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42. Weinberger J, Houman J, Caron A, Baskin A, **Ackerman AL**, Eilber KS, Anger JT. Female Sexual Dysfunction Treatment: A Meta-Analysis of the Placebo Effect Across Randomized Controlled Trials. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2017, Scottsdale, AZ.

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46. Thum DJ, Wood LM, Moradzadeh A, Hannemann A, Li A, **Ackerman AL**, Anger JT, Eilber KS. Concomitant Treatment of Stress Urinary Incontinence and Gynecologic Oncology Surgery: Are We Undertreating? Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2017, Scottsdale, AZ.

47. **Ackerman AL**, Eilber KS, Tang J, Kim J, Underhill DM, Anger JT, Freeman MR. Optimization of DNA Extraction from Human Urinary Samples for Microbial Community Profiling. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2017, Scottsdale, AZ.

48. **Ackerman AL**, Tang J, Eilber KS, Kim J, Anger JT, Underhill DM, Freeman MR. Decreased Urinary Fungal Burden and Diversity in Overactive Bladder. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2017, Scottsdale, AZ.

49. **Ackerman AL**, Anger JT, Tang J, Eilber KS, Kim J, Freeman MR, Underhill DM. Alterations in the Urinary Fungal Mycobiome in Patients with Bladder Pain and Urinary Urgency. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2017, Scottsdale, AZ.

50. **Ackerman AL**, Jie Tang J, Eilber KS, Kim J, Nickel JC, Ehrlich G, Underhill DM, Jennifer Anger JT. Shared Alterations in Urinary Bacterial Communities in Patients

with Interstitial Cystitis and Overactive Bladder. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2017, Scottsdale, AZ.

51. Parameshwar PS, Borok JF, Wood LN, **Ackerman AL**, Eilber KS, Anger JT. Writing in the Margins of Sexual Function Questionnaires: A Qualitative Analysis From Women With Pelvic Floor Disorders. American Urological Association Annual Meeting, May 2017, Boston, MA.

52. **Ackerman AL**, Anger JT, Tang J, Eilber KS, Kim J, Freeman MR, Underhill DM. Alterations in the Urinary Fungal Mycobiome in Patients with Bladder Pain and Urinary Urgency. American Urological Association Annual Meeting, May 2017, Boston, MA.

53. **Ackerman AL**, Lai HH, Eilber KS, Anger JT. Symptomatic Overlap in Overactive Bladder and Interstitial Cystitis/Painful Bladder Syndrome. American Urological Association Annual Meeting, May 2017, Boston, MA.

54. Weinberger J, Houman J, Caron A, Baskin A, **Ackerman AL**, Eilber KS, Anger JT. Female Sexual Dysfunction Treatment: A Meta-Analysis of the Placebo Effect Across Randomized Controlled Trials. American Urological Association Annual Meeting, May 2017, Boston, MA.

55. Juzar Jamnagerwalla J, Anger JT, Eilber KS, **Ackerman AL**. High Catastrophizing in Patients with Self-reported Painful Mesh Complications Have Poorer Outcomes. American Urological Association Annual Meeting, May 2017, Boston, MA.

56. **Ackerman AL**, Tang J, Eilber KS, Kim J, Anger JT, Underhill DM, Freeman MR. Decreased Urinary Fungal Burden and Diversity in Overactive Bladder. American Urological Association Annual Meeting, May 2017, Boston, MA.

57. **Ackerman AL**, Jie Tang J, Eilber KS, Kim J, Nickel JC, Ehrlich G, Underhill DM, Jennifer Anger JT. Shared Alterations in Urinary Bacterial Communities in Patients with Interstitial Cystitis and Overactive Bladder. American Urological Association Annual Meeting, May 2017, Boston, MA.

58. Parameshwar PS, Kayondo M, **Ackerman AL**, Eilber KS, Anger JT, Tarnay CM. Post-Operative Pain Management In Patients Undergoing Perineal And Vaginal Reconstructive Surgery: An Alternative To Narcotics. American Urogynecologic Society Annual Meeting/Pelvic Floor Disorders Week, October 2017, Providence, RI.

59. Kuhlmann P, Chen A, Johnson J, Hubbard L, **Ackerman AL**, Eilber KS, Anger JT. Concomitant Procedures Performed at the Time of Midurethral Sling Affect Post-operative Urinary Retention Rate. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2018, Austin, TX.

60. Moradzadeh A, Jamnagerwalla J, Eilber KS, Anger JT, **Ackerman AL**. High Catastrophizing in Subjects with Self-Reported Painful Mesh Complications Have Poorer Outcomes. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2018, Austin, TX.

61. Scott V, Tang J, Drell T, Simm J, Salumets A, Metsis M, Underhill D, **Ackerman AL**. Evaluation of the Vaginal Mycobiome in Asymptomatic Pre-menopausal Women. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2018, Austin, TX.

62. Scott V, **Ackerman AL**, Liu G, Shi W, Raz S. Immunofluorescence Localization of Bacterial Biofilms on Explanted Transvaginal Mesh Slings Removed for Chronic Pain. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2018, Austin, TX.

63. Anger JT, **Ackerman AL**, Spivia W, van den Broek I, Crear D, Eilber KS, Freeman M, Kim J, Fu Q, Van Eyk J. Differential Protein Expression in Patients with UCPPS: A MAPP Study. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2018, Austin, TX.

64. **Ackerman AL**, Scott V, Liu G, Shi W, Raz S. Characterization of Bacteria Identified on Explanted Mesh Slings Using Next-generation Sequencing Techniques. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2018, Austin, TX.

65. Scott V, **Ackerman AL**, Liu G, Shi W, Raz S. Immunofluorescence Localization of Bacterial Biofilms on Explanted Transvaginal Mesh Slings Removed for Chronic Pain. Annual Meeting. American Urological Association; May 2018; San Francisco, CA, USA.

66. **Ackerman AL**, Scott V, Liu G, Shi W, Raz S. Characterization of Bacteria Identified on Explanted Mesh Slings Using Next-generation Sequencing Techniques. Annual Meeting. American Urological Association; May 2018; San Francisco, CA, USA

67. Scott V, Tang J, Drell T, Simm J, Salumets A, Metsis M, Underhill D, **Ackerman AL**. Evaluation of the Vaginal Mycobiome in Asymptomatic Pre-menopausal Women. Annual Meeting. American Urological Association; May 2018; San Francisco, CA, USA.

68. Kuhlmann PK, Chen A, Johnson J, Hubbard L, **Ackerman AL**, et al. Concomitant Procedures Performed at the Time of Midurethral Sling Affect Post-operative Urinary Retention Rate. Annual Meeting. American Urological Association; May 2018; San Francisco, CA, USA.

69. Anger J, Spivia W, van den Broek I, Crear D, **Ackerman AL**, et al. Differential Protein Expression in Patients with UCPPS: A MAPP Study. American Urological Association Annual Meeting, May 2018, San Francisco, CA, 199:4 e506: J Urol; April, 2018.

70. van den Broek I, Mouapi KN, Mastali M, Holewinski R, Venkataraman V, Fu Q, **Ackerman AL**, Kim J, Freeman M, Anger JT, Millis K, Percy A, Van Eyk J. A stable-isotope labeled protein and peptide mixture for global normalization in targeted and data-independent quantitative bottom-up proteomics. ASMS Conference on Mass Spectrometry and Allied Topics, June 2018, San Diego, CA.

71. Kuhlmann PK, Chen A, **Ackerman AL**, Anger JT, Eilber KS. Higher BMI and Concomitant Pelvic Organ Prolapse Repair are Protective Against Intraoperative Bladder Perforation During Midurethral Sling Placement. American Urogynecologic Society Annual Meeting/Pelvic Floor Disorders Week, October 2018, Chicago, IL.

72. Patel DN, Gonzales-Alabastro C, Houman J, **Ackerman AL**, Eilber KS, Anger JT. Comparative Effectiveness of One- Versus Two-Stage Sacral Neuromodulation Device Placement. American Urogynecologic Society Annual Meeting/Pelvic Floor Disorders Week, October 2018, Chicago, IL

73. **Ackerman AL**, Khalique MU, Ackerman JE, Tang J, Eilber KS, Anger JT, Underhill DM. The Urinary Microbiota and Host Inflammation in Lower Urinary Tract Symptoms. Western Section American Urological Association Annual Meeting, Maui, HI, October 2018.

74. Scott VCS, Thum LW, Ackerman JE, Khalique MU, Eilber KS, Anger JT, Underhill DM, **Ackerman AL**. Temporal Dynamics of the Genitourinary Microbiome. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

75. Burton C, O'Brien R, Eilber KS, **Ackerman AL**, Anger JT. Identifying barriers to urinary incontinence care among primary care providers. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

76. Scott VCS, Lerner LB, Anger JT, Van Kuiken M, **Ackerman AL**. Satisfaction of women urologists with maternity leave, childbirth timining and work-family balanace ten years later. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

77. Souders C, Caron AT, Obi N, Clark K, McClelland L, **Ackerman AL,** Anger JT, Eilber KS. 60 minutes and vaginal mesh: did the media tell the whole story? Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

78. Cohen T, Burton C, Francis S, Patel D, Othman N, Lam P, **Ackerman AL,** Eilber KS, Anger JT. Improving patient experience with sacral neuromodulation: a human factors approach. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

79. Caron AT, Scott VCS, Eilber KS, Anger JT, **Ackerman AL.** Evaluation of recurrent uti-like symptoms should prompt consideration for alternative etiologies. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

80. Stewart C, Maniskas S, **Ackerman AL**, Eilber KS, Freedland S, Anger JT. Evidence linking comestibles to interstitial cystitis and bladder pain syndrome. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

81. Scott VCS, Lerner LB, Anger JT, Van Kuiken M, **Ackerman AL**. Re-evaluation of birth trends and pregnancy complications among female urologists: have we made any progress? Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

82. Burton C, Gonzalez-Alabastro C, Choi E, Parameshwar P, Gonzalez G, Bresee C, Eilber KS, **Ackerman AL,** Anger JT. Overuse of specialty care for women with urinary incontinence. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

83. Souders C, McClelland L, Caron AT, Alazzeh M, Zukotynski B, Ackerman AL, Eilber KS, Anger JT. Transvaginal mesh litigation has significant geographic variation. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

84. Caron AT, **Ackerman AL,** Parameshwar P, Eilber KS, Anger JT. Outcomes of pregnancy following surgery for pelvic organ prolapse: a systematic review. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

85. Stewart CA, Zhao H, Gonzales G, Gonzales-Alabastro C, Zaghiyan K, Moore B, Magner D, **Ackerman AL,** Anger JT, Eilber KS. Outcomes of sacrocolpopexy with concurrent colorectal surgery for multicompartment prolapse repair. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

86. Zhao H, Stewart CA, Gonzalez G, Gonzales-Alabastro C, **Ackerman AL,** Eilber KS, Anger JT. De novo defecatory symptoms and posterior compartment prolapse as a complication of sacrocolpopexy. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting, March 2019, Miami, FL.

87. Stewart CA, Zhao H, Gonzales G, Gonzales-Alabastro C, **Ackerman AL,** Eilber KS, Anger JT. Outcomes of prophylactic sling at the time of robotic assisted sacrocolpopexy. American Urological Association Annual Meeting, May 2019, Chicago, IL.

88. Kuhlmann P, Stewart CA, Souders C, Moradzadeh A, Houman J, **Ackerman AL,** Anger JT, Eilber KS. What is "vaginal rejuvenation"? A survey of medical professionals and lay persons. American Urological Association Annual Meeting, May 2019, Chicago, IL.

89. Cohen T, Burton CS, Francis S, **Ackerman AL**, Eilber KS, Anger JT. Improving patient experience with sacral neuromodulation: a human factors approach. American Urological Association Annual Meeting, May 2019, Chicago, IL.

90. Anger JT, Spivia W, **Ackerman AL,** Eilber KS, Kim J, Fu Q, Michael Freeman MR, Van Eyk J. The Serum Proteome Correlates with Clinical Phenotypes of UCPPS: a MAPP Study. American Urological Association Annual Meeting, May 2019, Chicago, IL.

91. Zhao H, Stewart CA, Souders CP, Gonzalez G, Gonzales-Alabastro C, **Ackerman AL,** Eilber KS, Anger JT. De novo Defecatory Symptoms and Posterior Compartment Prolapse as a complication of Sacrocolpopexy. American Urological Association Annual Meeting, May 2019, Chicago, IL.

92. Dallas K, **Ackerman AL**, Eilber K, Rogo-Gupta L, Scott V, Troung M, Cohen T, Anger J. Validated Surgical Steps of the Robotic Assisted Sacrocolpopexy Operation. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA. c.
93. Burton C, Markowitz M, Kanji F, Stewart C, Scott V, Eilber K, **Ackerman AL**, Anger J. Validation of the Pelvic Floor Awareness and Knowledge Survey (PFAKS). Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA. c.
94. Burton C, Gonzalez G, Bresee C, Scott V, Eilber K, **Ackerman AL**, Wieslander C, Anger J. Urinary Incontinence Referral Patterns in Academic and County Hospitals: The Impact of Econsult. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
95. Kuhlmann P, Ackerman J, Khalique M, Caron A, Kanji F, Anger J, Eilber K, Underhill D, **Ackerman AL**. Urinary Aerococcus Defines a Severe, Treatment-refractory Phenotype of Urgency Urinary Incontinence in Older Women. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
96. Scott V, Souders C, Khalique M, Ackerman J, **Ackerman AL**. Is Ureaplasma Truly a Urinary Tract Pathogen? Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
97. Guo J, Souders C, McClelland L, Anger J, Eilber K, **Ackerman AL**. Safety and Legal Environment for Vaginal Lasers: Uncovering the Evidence Behind the FDA Safety Communication. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
98. Kuhlmann P, Dallas K, Masterson J, Patel D, Casteneda P, Reddy A, Tsai K, **Ackerman AL**. Risk Factors for Intraoperative Bladder Perforation at the Time of Midurethral Sling Placement. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
99. Masterson J, Kuhlmann P, Dallas K, Reddy A, Tsai K, Castaneda P, Eilber K, Anger J, **Ackerman AL**. Overactive Bladder Following Midurethral Sling Placement: De Novo or Persistent? Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
100. Burton C, Lo E, Kanji F, Caron A, Cohen T, Miller D, Wenger N, Scott V, **Ackerman AL**, Eilber K, Anger J. Implementation of a Primary Care Intervention to Improve Care for Women with Urinary Incontinence. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
101. **Ackerman AL**, Caron A, Ackerman J, Anger J, Eilber K, Kaufman M. 'Persistency': A Novel Urinary Symptom Measure Encompassing the Myofascial Component of LUTS. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA

102. Dallas K, Kuhlmann P, Masterson J, Reddy A, Tsai K, Casteneda P, **Ackerman AL**, Eilber K, Anger J. Urinary Tract Infection after Mid-urethral Sling: Rates and Risk Factors. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
103. Kuhlmann P, Masterson J, Dallas K, Tsai K, Reddy A, Casteneda P, **Ackerman AL**, Eilber K, Anger J. Development of Post-operative Urinary Retention after Midurethral Sling Placement: Can We Counsel Patients on Duration?. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
104. Dallas K, Caron A, Anger J, Eilber K, **Ackerman AL**. Application of Machine Learning Algorithms to Classify Storage Lower Urinary Tract Symptoms. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
105. Burton C, Gonzalez G, Kanji F, Caron A, Bresee C, Eilber K, **Ackerman AL**, Anger J. Rates of Tertiary Procedures among Women Referred for Urinary Incontinence. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA. c.
106. Souders C, **Ackerman AL** Underrepresentation of FPMRS in Federal Funding for Benign Urologic Conditions. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
107. **Ackerman AL**, Khalique M, Ackerman J, Cheng Z, Eilber K, Anger J, Underhill D. Microbial Composition Defines Pelvic Pain Phenotypes in Reproductive-Age Women. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
108. Dallas K, Anger J, Caron A, Eilber K, **Ackerman AL**. Validation of the Diagnostic Accuracy of Diagnostic Groupings of Patients with Storage Lower Urinary Tract Symptoms Generated by Machine Learning Algorithms. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
109. Cohen T, Burton C, Cohen K, **Ackerman AL**, Eilber K, Anger J. Using a Human Factors Approach to Develop Interventions Aimed at Improving Patient Experience with Sacral Neuromodulation. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
110. Burton C, Tran J, Gonzalez G, Bresee C, Choi E, Scott V, **Ackerman AL**, Eilber K, Anger J. Urinary Incontinence Care for Older Adults: Difference or Disparity. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2020 February; Scottsdale, AZ, USA.
111. **Ackerman AL**, Caron AS, Ackerman JE, Anger JT, Eilber KS, Kaufman M. 'Persistency': A Novel Urinary Symptom Measure Encompassing the Myofascial Component of LUTS. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
112. **Ackerman AL**, Khalique MU, Ackerman JE, Cheng Z, Eilber KS, Anger JT, Underhill DM. Microbial Composition Defines Pelvic Pain Phenotypes in Reproductive-age Women. American Urological Association Annual Meeting, May 2020, Virtual Meeting.

113. Scott VCS, Souders CP, Khalique MU, Ackerman JE, **Ackerman AL**. Is Ureaplasma a Urinary Tract Pathogen? American Urological Association Annual Meeting, May 2020, Virtual Meeting.
114. Dallas K, Caron AS, Anger JT, Eilber KS, **Ackerman AL**. Application of Machine Learning Algorithms to Classify Storage Lower Urinary Tract Symptoms. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
115. Dallas K, **Ackerman AL**, Eilber KS, Rogo-Gupta L, Scott VCS, Troung M, Cohen T, Anger JT. Validated Surgical Steps of the Robotic Assisted Sacrocolpopexy Operation. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
116. Burton CS, Markowitz MA, Kanji F, Scott VCS, **Ackerman AL**, Eilber KS, Anger JT. Validation of the Pelvic Floor Awareness and Knowledge Survey. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
117. Kanji F, Burton CS, Cohen T, Bresee C, Scott VCS, **Ackerman AL**, Eilber KS, Anger JT. Developing a Urinary Incontinence Screening Tool for Use in Primary Care: Trials and Tribulations. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
118. Burton CS, Tran J, Gonzalez G, Bresee C, Choi E, Scott VCS, **Ackerman AL**, Eilber KS, Anger JT. Urinary Incontinence Care for Older Adults: Difference or Disparity? American Urological Association Annual Meeting, May 2020, Virtual Meeting.
119. Kuhlmann PK, Ackerman JE, Khalique MU, Caron AS, Kanji F, Anger JT, Eilber KS, Underhill DM, **Ackerman AL**. Urinary Aerococcus Defines a Severe, Treatment-refractory Phenotype of Urge Incontinence in Older Women. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
120. Lai HH, Clemens JQ, Newcomb C, Appleby D, **Ackerman AL**, Anger JT, Nickel JC, Gupta P, Rodriguez L, Landis JR. Comparison of Deep Phenotyping Features of UCPPS Patients with and without Hunner Lesion – A MAPP Research Network Study. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
121. Burton CS, Lo EM, Kanji F, Caron A, Cohen T, Miller D, Wenger N, Scott VCS, **Ackerman AL**, Eilber KS, Anger JT. Implementation of a Primary Care Intervention to Improve Care for Women with Urinary Incontinence. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
122. Gupta P, Lai HH, Anger JT, **Ackerman AL**, Gallop R, Landis JR, Clemens JQ. Impact of History of Urinary Tract Infections on Patients with Urologic Chronic Pelvic Pain Syndromes: Findings from Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Study. American Urological Association Annual Meeting, May 2020, Virtual Meeting.
123. Hopp M, **Ackerman AL**, et al. Sexual and Urinary Health in OSA Patients. American Academy of Otolaryngology-Head and Neck Surgery Annual Meeting, September 2020, Virtual Meeting.
124. Dallas K, Anger JT, Elliott CS, **Ackerman AL**. Racial Disparities Exist in Outcomes in a Population Based Cohort of 35,000 Women Undergoing Myomectomy for Fibroid Disease. PFD Week 2020 – American Urogynecologic Society Annual Meeting, October, 2020, Virtual Meeting.

125. Tholemeier LN, Choi E, Bresee CT, Kanji FF, **Ackerman AL**, Anger JT, Eilber KS. IMACTIV: A Pilot Study of the Impact of Unrestricted Activity Following Mid-Urethral Sling Surgery. PFD Week 2020 – American Urogynecologic Society Annual Meeting, October, 2020, Virtual Meeting.

126. Nettey O, Caron AT, Khalique UM, Ackerman JE, Eilber KS, Anger JT, **Ackerman AL**. Natural History of the Urinary Microbiome in Women Across the Menopausal Transition. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; 2021 February; Virtual Meeting.

127. Chen A, Caron AT, Kanji FF, Kuhlmann P, Eilber KS, Anger JT, **Ackerman AL**. The Utility of Catheterization to Improve the Accuracy of Microscopic Hematuria Evaluation in Women. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; 2021 February; Virtual Meeting.

128. Choi E, Anger JT, Ackerman JE, Ramsay JW, Eilber KS, Stothers L, **Ackerman AL**. "Chronic UTI": An Exploration of Patient Perceptions of Refractory Painful Genitourinary Symptoms. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction. Annual Meeting; 2021 February; Virtual Meeting.

129. Kanji FF, Burton C, Cohen T, Bresee C, Scott VCS, **Ackerman AL**, Eilber KS, Anger JT. Developing a Urinary Incontinence Screening Tool for Use in Primary Care: Trials and Tribulations. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; 2021 February; Virtual Meeting.

130. Mwesigwa PL, Caron AT, Kanji FF, Ramsey JW, Scott VCS, Eilber KS, Anger JT, **Ackerman AL**. Unsupervised Machine Learning Approaches Reveal Distinct Pelvic Pain Phenotypes. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; 2021 February; Virtual Meeting.

131. Torosis M, Caron AT, Ramsey JW, Eilber KS, Anger JT, **Ackerman AL**. Urologic Manifestations of High-Tone Pelvic Floor Myofascial Dysfunction. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; 2021 February; Virtual Meeting.

132. Nettey O, Caron AT, Khalique UM, Ackerman JE, Eilber KS, Anger JT, **Ackerman AL**. Natural History of the Urinary Microbiome in Women Across the Menopausal Transition. American Urological Association Annual Meeting; 2021 September; Virtual Meeting.

133. Nickel JC, Stephens A, **Ackerman AL**, Anger JT, Lai HH, Ehrlich GD. The Normal Healthy Urinary Microbiome in the MAPP Network Study: Relation to Gender, Age and Menopausal Status. American Urological Association Annual Meeting; 2021 September; Virtual Meeting.

134. Torosis M, Jackson NJ. **Ackerman AL**. Are Overactive Bladder Patients With and Without Urgency Incontinence the Same Phenotype? PFD Week 2020 – American Urogynecologic Society Annual Meeting, October, 2021, Phoenix, AZ .

135. **Ackerman AL**, Szlachta-McGinn A, Lagree K, Khalique MU, Caron AT, Ackerman JE, Cheng Z, Eilber KS, Anger JT, Underhill DM. Virulent Urinary

Fungal Strains in Interstitial Cystitis/Bladder Pain Syndrome Increase Host Inflammatory Response and Induce Urothelial Cell Killing. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

136. Szlachta-McGinn A, Douglass KM, **Ackerman AL**. Next Generation Sequencing Versus Conventional Urine Culture For Diagnosis and Treatment of Urinary Tract Infection: A Systematic Review and Meta-Analysis. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

137. Shoureshi P, Caskey R, Anger JT, **Ackerman AL,** Dubinskaya A, Eilber KS. Biologic Grafts as a Safe Alternative to Mesh for Sacrocolpopexy. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

138. Volkin D, Kim J-H, Tarnay C, **Ackerman AL**, Nitti V. Fixed-Life Versus Rechargeable Sacral Neuromodulation (SNM): Patient Choice and Device Specific Satisfaction. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

139. Kaefer D, Chiang J, **Ackerman AL.** Rate of Progression to Sepsis in Patients Presenting in the Outpatient Setting for Evaluation of Acute Cystitis**.** Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

140. Sadun TY, Cisneros C, **Ackerman AL.** Duration of Non-Obstructive Lower Urinary Tract Symptoms in End-Stage Renal Disease Patients Undergoing Renal Transplantation: A Systematic Review. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

141. Sadun TY, Jackson NJ, **Ackerman AL.** AUA-Symptom Scale is Not Reliable for Use in Measuring Storage Phase Dysfunction. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

142. Nettey O, Dallas K, **Ackerman AL.** To Hyst or Not: Concomitant Vaginal Hysterectomy at Time of Colpocleisis. Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction Annual Meeting; February 2022; San Diego, CA.

143. Kaefer D, Chiang J, **Ackerman AL**. Rate of progression to sepsis following presentation for acute cystitis. American Urological Association Annual Meeting; May 2022; New Orleans, LA.

144. Sadun TY, Jackson NJ, **Ackerman AL**. AUA-Symptom Scale Is Not Reliable For Use in Measuring Storage Phase Dysfunction. American Urological Association Annual Meeting; May 2022; New Orleans, LA.

145. Weinberger JM, Henao LM, Cisneros C, Ackerman JE, **Ackerman AL**. Urotypes in Young Women with Pelvic Pain: A Pilot Analysis of Clinical Urinary Microbiome Classification. American Urological Association Annual Meeting; May 2022; New Orleans, LA.

146. Nettey O, Dallas K, **Ackerman AL**. To Hyst or Not: Concurrent Vaginal Hysterectomy at Time of Colpocleisis. American Urological Association Annual Meeting; May 2022; New Orleans, LA.

147. Cisneros C, Grisales T, Kim JK, Nitti VW, Rosenman AE, Tarnay CM, **Ackerman AL.** Low Medication Compliance in New Urogynecology Patients. American Urological Association Annual Meeting; May 2022; New Orleans, LA.

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149. Torosis M, Carey E, Christensen K, Kaufman M, Kenton K, Kotarinos R, Lai HH, Lee UJ, Lowder JL, Meister M, Spitznagle T, Wright K, **Ackerman AL**. A clinical consensus treatment algorithm for patients with high tone pelvic floor dysfunction: A Delphi study of national experts. American Urogynecologic Society/International Urogynecological Association Scientific Meeting, June 2022, Austin, TX.

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Unsupervised Machine Learning Approaches Reveal Distinct Phenotypes of Perceived Bladder Pain: A Pilot Study

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Interstitial cystitis/bladder pain syndrome (IC/BPS) is defined as an unpleasant sensation perceived to be related to the bladder with associated urinary symptoms. Due to difficulties discriminating pelvic visceral sensation, IC/BPS likely represents multiple phenotypes with different etiologies that present with overlapping symptomatic manifestations, which complicates clinical management. We hypothesized that unique bladder pain phenotypes or "symptomatic clusters" would be identifiable using machine learning analysis (unsupervised clustering) of validated patient-reported urinary and pain measures. Patients (n = 145) with pelvic pain/discomfort perceived to originate in the bladder and lower urinary tract symptoms answered validated questionnaires [OAB Questionnaire (OAB-q), O'Leary-Sant Indices (ICSI/ICPI), female Genitourinary Pain Index (fGUPI), and Pelvic Floor Disability Index (PFDI)]. In comparison to asymptomatic controls (n = 69), machine learning revealed three bladder pain phenotypes with unique, salient features. The first group chiefly describes urinary frequency and pain with the voiding cycle, in which bladder filling causes pain relieved by bladder emptying. The second group has fluctuating pelvic discomfort and straining to void, urinary frequency and urgency without incontinence, and a sensation of incomplete emptying without urinary retention. Pain in the third group was not associated with voiding, instead being more constant and focused on the urethra and vagina. While not utilized as a feature for clustering, subjects in the second and third groups were significantly younger than subjects in the first group and controls without pain. These phenotypes defined more homogeneous patient subgroups which responded to different therapies on chart review. Current approaches to the management of heterogenous populations of bladder pain patients are often ineffective, discouraging both patients and providers. The granularity of individual phenotypes provided by unsupervised clustering approaches can be exploited to help objectively define more homogeneous patient subgroups. Better differentiation

of unique phenotypes within the larger group of pelvic pain patients is needed to move toward improvements in care and a better understanding of the etiologies of these painful symptoms.

Keywords: interstitial cystitis, bladder pain syndrome, urinary symptoms, phenotypes, pelvic pain/discomfort, lower urinary tract symptoms, unsupervised machine learning

INTRODUCTION

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a frequently debilitating, chronic condition whose central diagnostic feature is pain attributed to the bladder (1). Direct costs of care are high; IC/BPS is chronic, incurable, and frequently treatment-resistant. Estimates fluctuate widely, but community-based surveys suggest that as many as 7% of women in the US may express this symptom (2). However, IC/BPS is a diagnosis of exclusion, defined by the AUA/SUFU guidelines only "in the absence of infection or other identifiable causes" (3, 4). There is no international consensus on this definition or diagnostic criteria, which has made estimates of prevalence, treatment responses, mechanistic data, and long-term outcomes inconsistent and unreliable. As a result, many women with bladder pain symptoms may never receive an accurate diagnosis (2). A lack of diagnostic and prognostic indicators makes it challenging to assign effective care and identify appropriate therapies, leaving patients highly debilitated (5).

While the central feature of IC/BPS is the perception of bladder pain, patients exhibit a diverse range of accompanying genitourinary (GU) symptoms such as urinary urgency, frequency, nocturia, dyspareunia (sexual pain), pelvic pain, and incontinence (6). These types of symptoms overlap considerably with other GU tract pathologies such as overactive bladder (OAB), vaginitis, dysfunctional voiding, and other pelvic pain syndromes (7, 8). Early data from the NIH Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network previously created separate measures of pain and urinary severity (9), observing that the severity of each varied independently. In addition, each symptom domain was associated with different, co-morbid features, such as depression, suggesting that the variations in urinary symptoms and other pain features may reflect fundamentally distinct disease subsets.

Despite this large symptomatic diversity, IC/BPS is typically managed and studied as a single clinical condition. The combination of inconsistent mechanistic data, the large diversity of symptom expression, and the inability of any specific treatment to prove effective in more than a subset of patients suggest that IC/BPS is not a single clinical condition, but instead may reflect several unique pathologies that manifest with similar, overlapping symptoms (10, 11). It is likely this heterogeneity in IC/BPS populations that has made scientific advances difficult, confounding potential insights into disease physiology. That lack of understanding has in turn limited progress in diagnosis, prevention, and treatment. We hypothesize that the current definition of IC/BPS, requiring only the perception of bladder discomfort and co-existing urinary symptoms, encompasses multiple, mechanistically distinct disease phenotypes. Progress in clinical care and management of IC/BPS requires refinement of our diagnostic and prognostic schema by identifying independent biologies or data that can be used to define each phenotype more objectively to correlate with pathogenesis and treatment outcomes. In this study, we define unique IC/BPS phenotypes through deeper clinical profiling and begin to examine the response of these newly defined phenotypes to different treatment modalities.

MATERIALS AND METHODS

Patient Recruitment

After approval from the local Institutional Review Board (IRB# Pro00046154), 521 sequential patients complaining of any element of pelvic pain were evaluated from the Cedars-Sinai Women's Urology Clinic for possible inclusion. For the IC/BPS cohort ($n = 145$), we enrolled premenopausal women (maximum age of 45) suspected of having IC/BPS by their treating provider at initial consultation. Diagnosis was made by one of four board-certified, Female Pelvic Medicine and Reconstructive Surgery specialty urologists from a detailed history and physical exam using the definition established by the American Urological Association (AUA)/Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction (SUFU): IC/BPS is "an unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms of more than 6 weeks duration, in the absence of infection or other identifiable causes" (12). For inclusion, patients could not have any other urologic diagnoses considered to explain their urinary or pain symptoms (such as overactive bladder, recurrent urinary tract infections, urinary retention, or dysfunctional voiding). To ensure the perception of bladder pain was a symptomatic feature in the IC/BPS cohort, we implemented an additional inclusion criterion requiring direct patient endorsement of bladder pain. Previously, in a retrospective comparison of self–reported symptoms for subjects with a range of GU symptoms, we created a novel measure of perceived bladder pain that accurately distinguishes IC/BPS from OAB and asymptomatic controls, known as the bladder pain composite index (BPCI) (7). To ensure a more homogeneous group specifically expressing pain attributable to the bladder, we used the criterion of a BPCI score >4 for inclusion. Asymptomatic, age-matched controls ($n = 69$) were recruited independently from patients seen in the same clinic who had been referred for urologic evaluation for a range of benign, asymptomatic findings, such as renal cyst or microscopic hematuria. All of these subjects had a BPCI score <3 and asymptomatic responses on all additional questionnaires.

Subjects who had previously undergone invasive therapies prior to evaluation for inclusion, such as bladder instillations, prior pelvic surgery, intradetrusor Botox®, or sacral neuromodulation, were excluded, as were patients with active urinary tract infection, pregnancy, diabetes, neurologic or rheumatic disease (e.g., multiple sclerosis), current smoking, or a vaginal pessary. As worsening pain in association with menstrual cycle can be a common feature of IC/BPS, subjects who experienced flares or worsening pain during phases of the menstrual cycle were allowed to participate, but were excluded if they complained only of isolated cyclic pain at menses (dysmenorrhea/menorrhalgia). Baseline demographics and clinical data including age, body mass index (BMI), comorbidities, past surgeries, and medication usage, including hormonal medications, were captured at enrollment.

Symptomatic Assessment Instruments

After signing informed consent, subjects completed the female Genitourinary Pain Index (fGUPI) (13) and Interstitial Cystitis Symptom and Problems Indices (ICSI/ICPI), (14), to quantitate and describe the typical pelvic symptoms associated with bladder pain. The Overactive Bladder Questionnaire Short Form (OABq-SF), (15), and Pelvic Floor Distress Index short form (PFDI-20) (16)) were also administered to measure the nature, severity, and impact of other urinary and pelvic symptoms.

Cluster Analysis

Cluster analysis methodology utilized a two-step approach to generate symptomatic clusters for the IC/BPS population. Controls were not included in the derivation of clusters. First, hierarchical clustering using Ward's method generated a cluster dendrogram (**Figure 2A**). This provided an estimation of the number of likely clusters within the studied population of IC/BPS subjects, of which two or three groups appeared the most appropriate. The elbow method, in which the explained variation in the data is plotted as a function of the number of possible clusters (14), implicated a cluster number of three as the optimal solution for the number of phenotypic groups in our cohort (**Figure 2B**). Second, we applied the *K*-means machine learning algorithm (17) as the principal clustering technique to divide the symptomatic bladder pain cohort into three (the *k* determined in step 1) subgroups using phenotypic variables derived from the symptomatic assessment instruments. All measurements were standardized using z scores for continuous variables and 0 or 1 for categorical variables. Continuous variables were log transformed to approximate a normal distribution where indicated. Patient cluster distribution visualized by principal coordinate analysis with the Bray-Curtis dissimilarity measure.

Self-Organizing Maps

Self-organizing maps (SOM), an unsupervised technique of clustering and dimensionality reduction, consist of an arbitrary number of nodes where each node represents a point in the original, multi-dimensional input space. As new points are added, they are classified by pairwise Euclidean distance with the nearest neighboring node. The grid of nodes is trained such that nearby nodes resemble each other more than nodes that are further away. A final step uses hierarchical clustering to group the nodes into a user-defined number of metaclusters, which is then visualized in two-dimensional space (18). This analysis utilized a Gaussian neighborhood function, in which all nodes are adjusted in n-dimensional space toward the current data point, but closer nodes are displaced more. This contrasts with a circular function, in which only the nodes nearest to the closest node are adjusted and those adjustments are all of equal magnitude. Neighborhood relations (typically rectangular or hexagonal) dictate the topology (interconnectivity) of the SOM. We selected a rectangular topology, which has fewer connections between nodes. SOM size was 6×4 with 1,000,000 iterations at a learning rate of 0.1, which was determined experimentally to provide the greatest convergence (19). This solution resulted in a convergence index of 0.78.

Cluster Stability

To ensure cluster assignment stability, we resampled a large number of replications (10,000) with replacement (bootstrapping) and identified the cluster assignments for each iteration. The Rand (20) and Jaccard (21) indices were used to assess the agreement between each reference clustering and the clustering obtained for the subsampled validation cohort; percentile bootstrap 95% confidence intervals were generated for the Rand and Jaccard indices. The Rand index represents the overall percent observed agreement in cluster assignment, while the Jaccard coefficient represents the percent overlap between cluster assignments. The Jaccard coefficient measures only the times when the same assignment was made as a proportion of the total times the cluster was assigned in either the original or bootstrap sample. As a result, the Jaccard coefficient is a more sensitive measure of cluster similarity by excluding instances where neither the original nor bootstrap sample assign an observation to the cluster. Jaccard coefficient values > 0.7 are generally considered to indicate very good agreement. A challenge with establishing agreement is that *K*-means randomly assigns observations to a new cluster given an arbitrary number designation; thus, an emergent cluster of the same observations may be assigned a different cluster number in the bootstrapped sample. To account for differences in cluster number assignment when determining these metrics, we reassigned cluster numbers based on the highest interrater reliability (kappa) between the original cluster and the bootstrapped clusters.

Thematic Analysis

To determine if there were additional features common to each symptom cluster that could not be captured by the patient-reported outcomes administered in this study, we performed a modified thematic analysis approach. Descriptions of patient complaints and features were captured from the history of present illness and assessment of the specialist's initial consultation within the electronic medical record. Primary patient complaints/bothersome symptoms as well as anticipated symptomatic features (including aggravating and relieving factors, presence/absence and patterns of flares, most bothersome symptom, and terminology used to describe the nature of their bladder/pelvic pain) were cataloged by two reviewers blinded

to both subject cluster assignment and the nature of the characteristic cluster symptomatic features.

Treatment Responses

For the 145 IC/BPS subjects, treatment responses were assessed by chart review for therapies prescribed after inclusion. Response to treatment was noted to be positive if the immediate post-treatment note documented a positive patient perception of improvement with therapy (yes/no). Medical charts were reviewed by one of two researchers who were blinded to both subject cluster assignment and cluster features. While patients were categorized to specific clusters from data obtained prior to their treatments, there was no communication with the patients about this categorization or its hypotheses; patients chose their care plans and reported their responses according to the typical standard of care with their treating provider. Only those patients for whom a determination about responses to treatment could adequately be made were included in the analysis. Only 105 subjects could be included in this analysis, as we did not have adequate information to determine treatment responses for 40 subjects (27%), either because they were unable to complete the course of treatment or to follow-up with their treating provider after treatment due to clinic closures associated with the SARS-CoV2 pandemic. A proportion of the subjects within each phenotype responding to each treatment was determined (within group analysis) as was the proportion of total responders to each therapy for each phenotype (within cohort analysis).

Statistical Analysis

The R Studio integrated development environment was used for the unsupervised clustering analysis with the stats (version 3.6.1), cluster (version 2.1.0), mclust (version 5.4.7) randomForest (version 4.6–14), kohonen (version 3.0.10), and popsom (version 5.2) packages. Differences in patients' demographic and clinical characteristics were compared by using the Wilcoxon signed rank tests for paired data and the Pearson chi square, Fisher exact, or Mann-Whitney *U*-tests for independent data as appropriate (2-tailed). Differences in proportions were compared using the two-sample *z*-test. Results are considered significant at an alpha level <0.05.

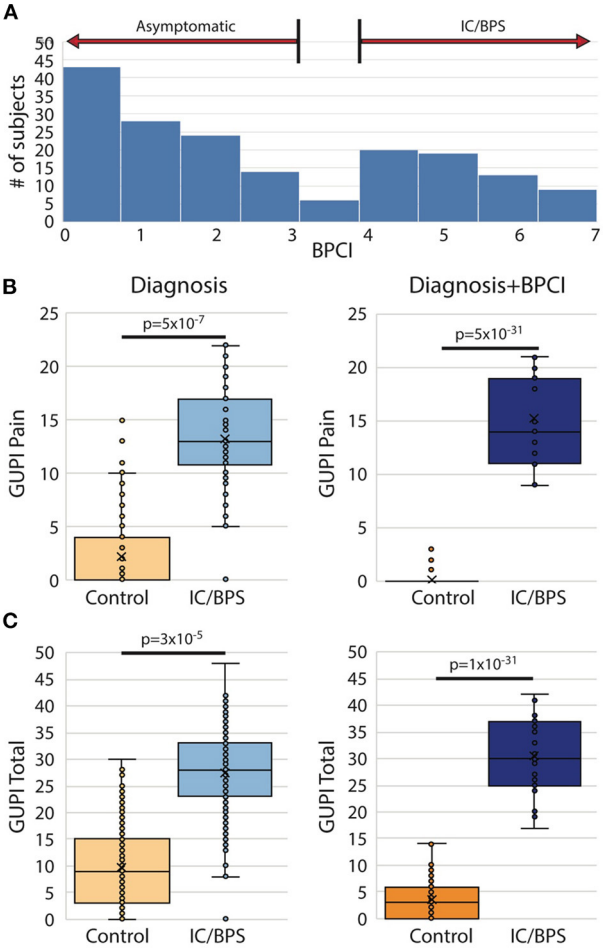


FIGURE 1 | Bladder pain composite index (BPCI) defines more homogeneous IC/BPS and control populations. **(A)** BPCI score distribution for 521 subjects with and without urinary symptoms recruited as possible participants revealed a clear division between patients with and without bladder pain and was used to define our study populations for this proposal. **(B,C)** Use of clinical diagnosis of IC/BPS results in substantial symptomatic overlap in **(B)** pain severity (fGUPI Pain domain) and **(C)** overall urinary symptoms (fGUPI total score) with subjects identified as controls (left), while separation based on a BPCI>4 provides more homogeneous, distinct populations (right).

RESULTS

Objective Symptom Measure in Combination With Physician Diagnosis Provides Better Identification of IC/BPS Subjects Than Clinician Diagnosis Alone

From the original group of 521 potential subjects with genitourinary pain, 183 (35%) were assigned a diagnosis of IC/BPS. Although pain levels in this group were significantly elevated in comparison to control subjects, identification of subject groups by diagnosis was not sufficient to separate IC/BPS patients from controls symptomatically (**Figure 1A**). In addition, approximately 25% of patients included in the control group expressed some bother associated with urinary/pelvic symptoms, as determined by an fGUPI quality of life subscale >4. As such contamination between cases and controls could confound the description of unique pain phenotypes, we selected only the 145 pre-menopausal women of the 183 who bore a clinical diagnosis of IC/BPS or bladder pain *and* also scored 4 or higher on the BPCI, a measure of pain selectively associated with the bladder. Sixty-nine age-matched, asymptomatic subjects with BPCI < 3 were selected from the potential subject pool as controls. As the hormonal changes in the peri- and postmenopausal period add an additional level of complexity and obscure the relation of symptomatology to bladder pain, only pre-menopausal women were included. This selection avoids the complicating comorbidities and co-existing genitourinary symptoms, such as detrusor underactivity and overactive bladder, common in post-menopausal women. The combination of narrow inclusion

TABLE 1 | Patient demographics.

	IC/BPS ($n = 145$)	Controls ($n = 69$)	*P*-values
Age: years (SD)	31.8 (6.9)	34.1 (6.4)	0.08
Average prescription meds: number (SD)	0.8 (1.1)	0.5 (0.8)	0.28
BMI: kg/m^2 (SD)	25.4 (7.1)	26.3 (5.5)	0.63
Hormonal birth control: percent (n)	23.5% (34)	23.2% (16)	0.96
Comorbidities: percent (n)			
Anxiety	13.1% (19)	5.7% (4)	0.06
Depression	6.9% (10)	5.7% (4)	0.75
Endometriosis	2.1% (3)	0% (0)	0.08
Fibromyalgia	2.1% (3)	0% (0)	0.08
GERD	0.7% (1)	4.3% (3)	0.16
Hyperlipidemia	0.7% (1)	4.3% (3)	0.16
IBS	10.3% (15)	2.8% (2)	*0.02
Migraine	4.1% (6)	2.8% (2)	0.63
Nephrolithiasis	1.4% (2)	7.2% (5)	0.07
PCOS	2.1% (3)	1.4% (1)	0.73

GERD, gastroesophageal reflux disease; IBS, irritable bowel syndrome; PCOS, polycystic ovary syndrome.*

criteria using clinician diagnosis in conjunction with objective scoring on the BPCI corrected for confounders within and between study groups to reliably identify IC/BPS subjects *and* separate symptomatic patients from controls (**Figures 1B,C**). The baseline characteristics of the included participants are summarized in **Table 1**.

Unsupervised Clustering of IC/BPS Subjects Into Unique Clusters

While IC/BPS is defined as *bladder pain* in the absence of infection or other organic pathology, difficulties discriminating pelvic visceral sensation and a lack of well-defined language describing pelvic pain complicate IC/BPS diagnosis and subclassification. As a quantitative approach to identifying more homogeneous IC/BPS subpopulations, we used *K*-means clustering, an unsupervised machine learning approach, to recognize distinct phenotypes based on the patient-reported measures examining GU symptomatology from the 145 IC/BPS subjects. *K*-means clustering necessitates defining *k*, the fixed number of clusters you anticipate within the dataset. To define this value, we first applied hierarchical Ward's clustering to the symptomatic assessment instruments alone, revealing that a three-cluster solution best fit the dataset of patients with perceived bladder pain (**Figure 2A**). To validate this choice of cluster groups, we employed the elbow method to identify the number of clusters after which adding more groups provides little improvement in the model. Using this approach (**Figure 2B**), the clinical data also confirmed that clustering into three groups yielded the most meaningful number of cluster profiles. We then used *K*-means clustering analysis (17) to divide subjects into three (*k*) clinical phenotypes based only on the patient responses to symptomatic questionnaires. This cluster solution assigned 56 subjects to group 1, 31 patients to group 2, and 58 patients to group 3. The meaningful separation of these groups by symptomatic assessment measures was visually confirmed by principal coordinate analysis using Bray-Curtis dissimilarity measures (**Figure 2C**).

Baseline Clusters Show Distinct Bladder Pain Phenotypes

To explore the face validity of the unsupervised clustering, we combined a review of the symptomatic questionnaire scores (**Table 2**) with a qualitative chart review of the phenotyped subjects. Regardless of phenotype, almost all patients categorized as IC/BPS displayed elevated pain levels on a visual analog scale (fGUPI4) as well as significant urinary frequency (ICSI2, ICPI1) and discomfort below the waist (fGUPI1d). The first group described a pattern of bladder-specific pain (ICSI4) that was aggravated by bladder filling (fGUPI2c) and relieved by emptying (fGUPI2d) (**Figure 3A**), a constellation of symptoms we dubbed bladder-specific pain symptoms (BPS). Of all the groups, this group most commonly expressed sensitivities of their bladder pain symptoms to dietary triggers, such as acidic foods or caffeine, on chart review, although this domain was not assessed in all subjects.

The second group exhibited persistent, non-cyclic pelvic pain unrelated to bladder filling or emptying, which we designated non-urologic pelvic pain (NUPP). In these patients, the fGUPI revealed higher levels of pain localized to the vagina and urethra (fGUPI1a,b,c), coexisting with both dyspareunia and dysuria (fGUPI2a,b) (**Figure 3A**). NUPP subjects typically had lower scores on questions regarding bladder pain and discomfort (ICSI4 and ICPI4) and denied pain related to the voiding cycle (fGUPI2c,d). On chart review, the NUPP group tended to describe a more diffuse pelvic discomfort, with more specific pain localized to the urethra and distal vagina/introitus. All of the groups exhibited a significant impact on quality of life and dissatisfaction with their current symptoms, but the NUPP group appeared the least impacted of the three groups (fGUPI QOL subscale 7.78 $\pm$ 3.04 for NUPP vs. 9.56 $\pm$ 1.90 BPS and 10.26 $\pm$ 1.77 MFP) (**Figure 3B**).

The third group, designated as myofascial pain (MFP), exhibited a wide range of symptoms that were elevated, such as urinary frequency, bladder discomfort, and pelvic pressure, in comparison to controls. This group was distinguished by significantly more defecatory symptoms (PFDI20 questions 7–14), an increased sensation of incomplete emptying of the bladder (PFDI-20q5, fGUPI5), and small amounts of urine leakage (PFDI-20q18), typically without awareness. While these subjects expressed similarly elevated "pain below the waist" (fGUPI1d) as the other groups, they had the lowest proportions of pain in other pelvic locations specified on questions 1 and 2 on the fGUPI, which specify types of pain in the pelvis through a set of eight yes/no questions (**Figure 3A**). However, on the PFDI-20, a questionnaire designed to assess for symptoms of pelvic organ prolapse (POP), the MFP group consistently demonstrated higher symptomatic bother than

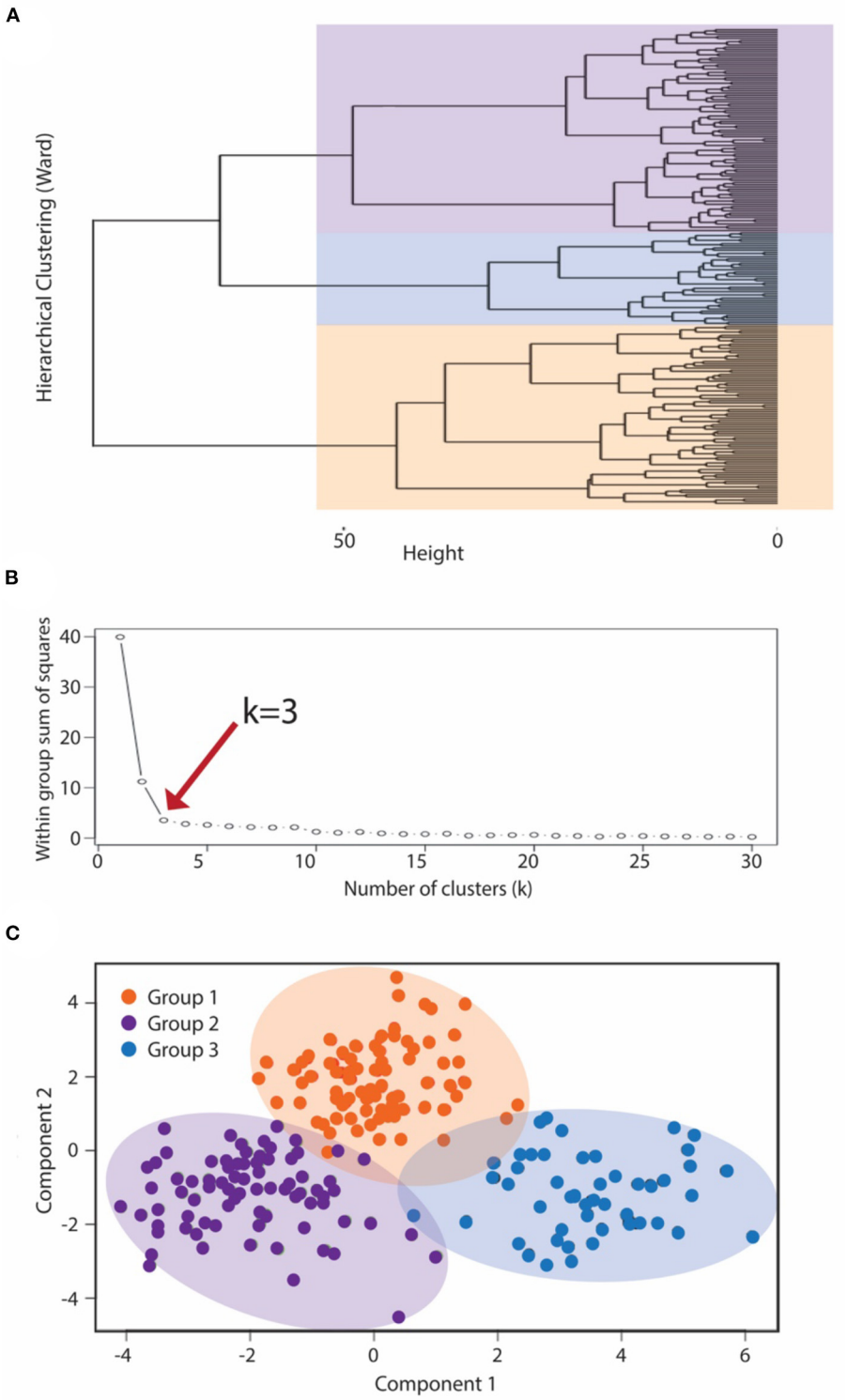


FIGURE 2 | K means clustering to subcategorize pre-menopausal female subjects with IC/BPS. To classify IC/BPS patients into phenotypes, we utilized a k-means algorithm of unsupervised clustering for an independent group of subjects. **(A)** Dendrogram visualizing the order and distances of subjects for merges during hierarchical clustering by Ward's method, with the overlaid colors representing the three-group solution as the optimal *k*. **(B)** The number of phenotypes (clusters), k, was also determined by the minimum number of groups yielding the most meaningful cluster profiles according to the within group sum of squares method ("elbow" method). **(C)** Centroid plot for the three-cluster solution by *K*-means clustering of symptomatic profiles demonstrated clear separation of these clusters into three groups, corresponding to the BPS group (purple), MFP group (orange), and NUPP group (blue).

TABLE 2 | Geometric means for patient scores on individual symptomatic questions.

Question	Symptom feature	MFP (Std Dev)	BPS (Std Dev)	NUPP (Std. Dev)	*P*-values		
					MFP vs. BPS	MFP vs. NUPP	BPS vs. NUPP
ICSI1	Strong need to void with no warning	2.98 (±1.41)	3.16 (±1.48)	1.04 (±1.14)	0.59	<0.001	<0.001
ICSI2	Urinary frequency within 2 h	4.19 (±1.04)	4.29 (±0.96)	2.16 (±1.37)	0.65	<0.001	<0.001
ICSI3	Nighttime urination	2.95 (±1.36)	1.97 (±1.15)	1.10 (±1.11)	<0.001	<0.001	<0.001
ICSI4	Pain or burning in the bladder	2.13 (±1.67)	2.61 (±1.48)	1.16 (±1.23)	0.16	<0.001	<0.001
ICSI Total	**ICSI 1–4**	12.45 (±3.76)	11.95 (±2.90)	5.45 (±3.04)	0.49	<0.001	<0.001
ICPI1	Frequent daytime urination	3.12 (±1.02)	3.33 (±0.62)	1.46 (±1.14)	0.26	<0.001	<0.001
ICPI2	Getting up at night to urinate	3.10 (±1.08)	2.53 (±1.19)	0.92 (±1.14)	0.03	<0.001	<0.001
ICPI3	Need to urinate with little warning	2.84 (±0.90)	2.40 (±1.20)	0.88 (±1.11)	0.08	<0.001	<0.001
ICPI4	Bladder burning, pain, discomfort, or pressure	3.10 (±1.30)	3.05 (±1.38)	1.75 (±1.46)	0.88	<0.001	<0.001
ICPI Total	**ICPI 1–4**	12.16 (±2.55)	11.31 (±2.47)	5.05 (±3.02)	0.12	<0.001	<0.001
OLS	**ICSI + ICPI**	24.61 (±5.62)	23.26 (±4.92)	10.51 (±5.67)	0.24	<0.001	<0.001
OABq2	Uncomfortable urge to urinate	4.77 (±1.38)	4.76 (±1.11)	2.33 (±1.22)	0.95	<0.001	<0.001
OABq3	Sudden urge to urinate with no warning	4.19 (±1.70)	3.74 (±1.62)	1.92 (±1.17)	0.22	<0.001	<0.001
OABq4	Accidental loss of small amounts of urine	3.97 (±1.87)	2.05 (±1.42)	1.89 (±1.26)	<0.001	<0.001	<0.001
OABq5	Nighttime urination	4.45 (±1.69)	3.67 (±1.66)	2.02 (±1.05)	0.04	<0.001	<0.001
OABq6	Waking at night to urinate	4.55 (±1.46)	3.93 (±1.53)	2.20 (±1.17)	0.07	<0.001	<0.001
OABq8	Urine loss associated with strong urgency	3.87 (±1.89)	2.59 (±1.85)	1.41 (±0.97)	0.002	<0.001	<0.001
OABq SF	**OABq 2–6, 8**	25.81 (±7.34)	20.74 (±5.80)	11.79 (±4.18)	<0.001	<0.001	<0.001
fGUPI1A	Discomfort at the entrance to the vagina	0.48	0.38	0.58	0.27	0.38	0.07
fGUPI1B	Discomfort in the vagina	0.48	0.43	0.61	0.59	0.25	0.10
fGUPI1C	Discomfort in the urethra	0.43	0.47	0.55	0.69	0.29	0.46
fGUPI1D	Discomfort below the waist	0.74	0.76	0.59	0.86	0.16	0.05
fGUPI2A	Pain or burning during urination	0.41	0.53	0.71	0.19	0.11	0.18
fGUPI2B	Pain or discomfort with sexual intercourse	0.54	0.63	0.64	0.44	0.39	0.97
fGUPI2C	Pain or discomfort as your bladder fills	0.58	0.79	0.25	0.03	0.002	<0.001
fGUPI2D	Pain or discomfort relieved by voiding	0.71	0.63	0.22	0.50	<0.001	<0.001
fGUPI3	How often was your pain	3.90 (±1.10)	3.67 (±1.13)	2.98 (±1.15)	0.36	<0.001	0.002
fGUPI4	Average pain or discomfort	6.39 (±1.76)	6.13 (±1.52)	2.98 (±1.61)	0.49	0.01	0.02
fGUPI Pain	**Total fGUPI 1–4**	14.66 (±4.24)	14.45 (±3.67)	10.11 (±3.42)	0.26	<0.001	<0.001
fGUPI5	Sensation of not emptying your bladder	3.37 (±1.37)	2.65 (±1.67)	1.67 (±1.64)	0.04	<0.00	0.002
fGUPI6	Urinate again within 2 h	3.60 (±1.16)	4.05 (±1.02)	2.01 (±1.42)	0.06	<0.001	<0.001
fGUPI Urinary	**Total fGUPI 5–6**	6.96 (±2.30)	6.71 (±2.08)	3.70 (±2.62)	0.59	<0.001	<0.001
fGUPI7	Impact on activities	2.29 (±0.64)	1.89 (±1.04)	1.27 (±1.14)	0.06	<0.001	0.002
fGUPI8	Distraction by symptoms	2.65 (±0.75)	2.66 (±0.61)	2.18 (±0.92)	0.94	0.02	0.001
fGUPI9	Satisfaction with current symptoms	5.32 (±0.83)	5.01 (±0.97)	4.33 (±1.62)	0.13	0.002	0.007
fGUPI Bother	**Total fGUPI 7–9**	10.26 (±1.77)	9.56 (±1.90)	7.78 (±3.04)	0.09	<0.001	<0.001
fGUPI Total	**Total fGUPI 1–9**	31.87 (±6.67)	30.72 (±5.22)	21.59 (±6.21)	0.13	<0.001	<0.001
PFDI20-1	Pressure in the lower abdomen	2.81 (±1.78)	1.91 (±1.67)	1.64 (±1.52)	0.01	<0.001	0.36
PFDI20-2	Heaviness or dullness in the abdomen	2.83 (±1.60)	1.76 (±1.60)	1.32 (±1.44)	0.001	<0.001	0.13
PFDI20-3	Vaginal bulge	0.76 (±1.40)	0.34 (±0.87)	0.54 (±1.16)	0.06	0.39	0.32
PFDI20-4	Splint to defecate	1.58 (±1.13)	0.40 (±0.92)	0.57 (±0.99)	<0.001	<0.001	0.33
PFDI20-5	Feeling of incomplete emptying	3.39 (±1.22)	1.62 (±1.57)	1.07 (±1.25)	<0.001	<0.001	0.04
PFDI20-6	Splinting to void	0.87 (±0.75)	0.12 (±0.59)	0.02 (±0.13)	0.001	<0.001	0.21
POPDI-6	**Total PFDI 1–6**	51.0 (±18.7)	25.7 (±15.0)	21.5 (±18.9)	<0.001	<0.001	0.19
PFDI20-7	Straining to have a bowel movement	2.26 (±1.18)	0.76 (±1.13)	1.07 (±1.21)	<0.001	<0.001	0.16
PFDI20-8	Tenesmus	2.32 (±1.56)	0.74 (±1.09)	1.18 (±1.31)	<0.001	<0.001	0.05
PFDI20-9	Loss of formed stool	1.03 (1.35)	0.02 (±0.13)	0.02 (±0.13)	<0.001	<0.001	0.98

(Continued)

TABLE 2 | Continued

Question	Symptom feature	MFP (Std Dev)	BPS (Std Dev)	NUPP (Std. Dev)	*P*-values		
					MFP vs. BPS	MFP vs. NUPP	BPS vs. NUPP
PFDI20-10	Loss of liquid stool	1.56 (±1.62)	0.23 (±0.66)	0.16 (±0.56)	<0.001	<0.001	0.50
PFDI20-11	Flatal incontinence	2.03 (±1.43)	0.21 (±0.69)	0.39 (±0.98)	<0.001	<0.001	0.25
PFDI20-12	Pain with bowel movements	1.29 (±1.51)	0.14 (±0.43)	0.43 (±0.81)	<0.001	<0.001	0.02
PFDI20-13	Urgency to have a bowel movement	2.75 (±1.03)	0.70 (±1.08)	0.59 (±1.01)	<0.001	<0.001	0.57
PFDI20-14	Rectal prolapse	1.37 (±1.52)	0.05 (±0.30)	0.07 (±1.03)	<0.001	<0.001	0.79
CRADI-8	**Total PFDI 7–14**	45.7 (±16.6)	8.9 (±4.8)	12.2 (±12.0)	<0.001	<0.001	0.03
PFDI20-15	Frequent urination	3.05 (±1.39)	2.83 (±1.22)	1.32 (±1.33)	0.44	<0.001	<0.001
PFDI20-16	Urine leakage with urgency	2.6 (±1.63)	1.24 (±1.48)	0.41 (±0.85)	<0.001	<0.001	<0.001
PFDI20-17	Urine leakage related to cough, laugh, sneeze	1.98 (±1.47)	1.05 (±1.33)	1.00 (±1.21)	0.003	0.001	0.82
PFDI20-18	Small amounts of urine loss	2.46 (±1.47)	0.86 (±1.22)	0.55 (±1.06)	<0.001	<0.001	0.15
PFDI20-19	Difficulty emptying your bladder	2.02 (±1.65)	1.28 (±1.46)	0.73 (±1.17)	0.03	<0.001	0.03
PFDI20-20	Pain or discomfort in lower abdomen	3.05 (±1.04)	2.18 (±1.59)	1.66 (±1.61)	0.007	<0.001	0.08
UDI-6	**Total PFDI 15–20**	63.2 (±4.72)	39.3 (±17.6)	23.6 (±16.7)	<0.001	<0.001	<0.001
Age	Years	31.34 (±19.7)	31.53 (±7.18)	37.8 (±6.3)	0.13	<0.001	0.001

fGUPI 1A-2D are yes/no questions; the responses are shown as proportions. All other questions are Likert scales, for which the means and standard deviations are shown. ICSI, Interstitial Cystitis Symptom Index; ICPI, Interstitial Cystitis Problem Index; OLS, O'Leary-Sant Indices total score; OABqSF, Overactive Bladder Questionnaire Short Form; fGUPI, Genitourinary Pain Index; PFDI-20, Pelvic Floor Distress Inventory Short Form; POPDI-6, Pelvic Organ Prolapse Distress Inventory 6; CRADI-8, Colorectal-Anal Distress Inventory 8; UDI-6, Urinary Distress Inventory Short Form. Significant p-values are indicated in red.

the other groups, except in their endorsement of a vaginal bulge (PFDI-20q3). This group had higher scores than those historically described for patients with POP (16), but without the discriminatory feature of a vaginal bulge or evidence of prolapse on physical exam (16). Instead, chart review showed obvious findings of myofascial pain on physical exam, manifest as either tenderness to palpation of or distinct trigger points in the levator muscles and hip flexors (primarily obturator internus) (22).

Subjects in this group had nearly identical pain levels, urinary symptoms and severity, and quality of life impact as the BPS group. The major difference in their symptoms was the associated defecatory symptoms. The symptomatic questionnaires, however, include multiple questions describing the nature of the pain (**Figure 3C**). ICPI4 asks about "burning, pain, discomfort or pressure" in the bladder, while ICSI4 specifies "pain or burning" only. MFP subjects tended to score higher on ICSI4 than on ICPI4. The MFP group has significantly lower proportions of subjects who endorsed pain with bladder filling (fGUPI2c) than the BPS group but endorsed similar pain to the BPS group "below the waist" (fGUPI1d). Questions 1 and 2 on the PFDI-20, respectively, describe "pressure" and "heaviness or dullness"; the MFP subjects scored significantly higher on these two questions than either the BPS or NUPP groups. This pattern of scores suggests that the MFP group experiences pelvic discomfort more accurately described as a pressure or discomfort, while the BPS group describes more frank pain, particularly related to bladder filling.

Cluster Stability

To ensure the stability of our cluster assignment, we resampled (500) with replacement (bootstrapping) a large number of replications (10,000) and identified the cluster assignments for each iteration. From these we computed the percent observed agreement (Rand Index) and percent overlap (Jaccard Coefficient), for which values over 0.7 indicate good cluster stability. The Rand indices were 0.86 (95%CI: 0.85–0.87) for BPS, 0.85 (95%CI: 0.084–0.86) for NUPP and 0.75 (95%CI 0.74–0.76) for MFP, demonstrating high percent observed agreement of the bootstrapped samples. The clusters had Jaccard coefficients of 0.68 (95%CI: 0.67–0.69) for BPS, 0.55 (95%CI: 0.53–0.57) for NUPP and 0.49 (95% CI: 0.47–0.52) for MFP. These measures of agreement suggest that for the NUPP and MFP clusters, the high observed percent agreement was driven by the absence of observations being assigned to these clusters. However, the BPS cluster showed stability based on both percent agreement and percent overlap. Overall, the cluster stability measures indicate that only the BPS cluster would be likely to be detected using a similar definition in a different sample.

Measures Examining Each Symptom Complex Can Discriminate Bladder Pain Phenotypes

We next utilized a random forest model (23) to identify important features used to classify subjects in the *K*-means algorithm (**Figure 4A**). From the 20 questions with the largest impact on phenotypic classification, we then selected the

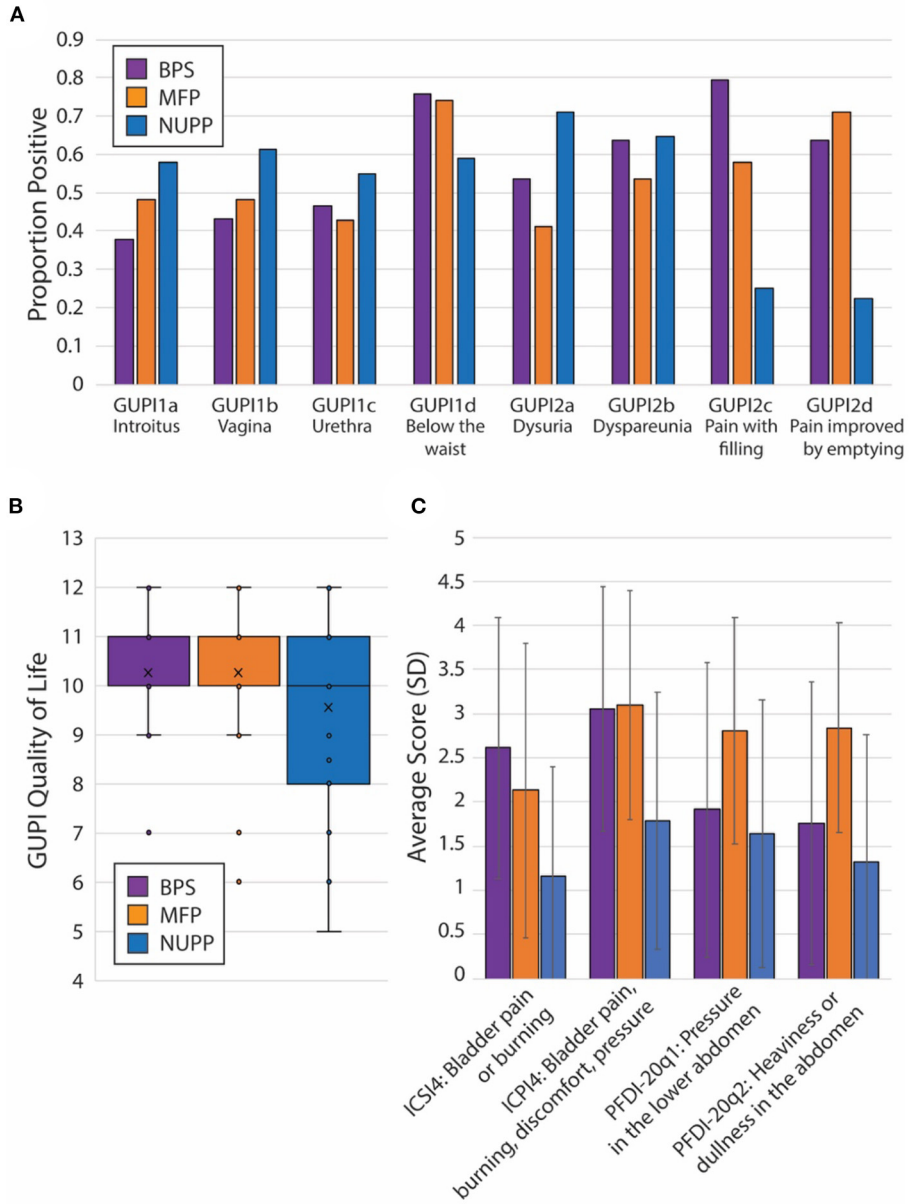
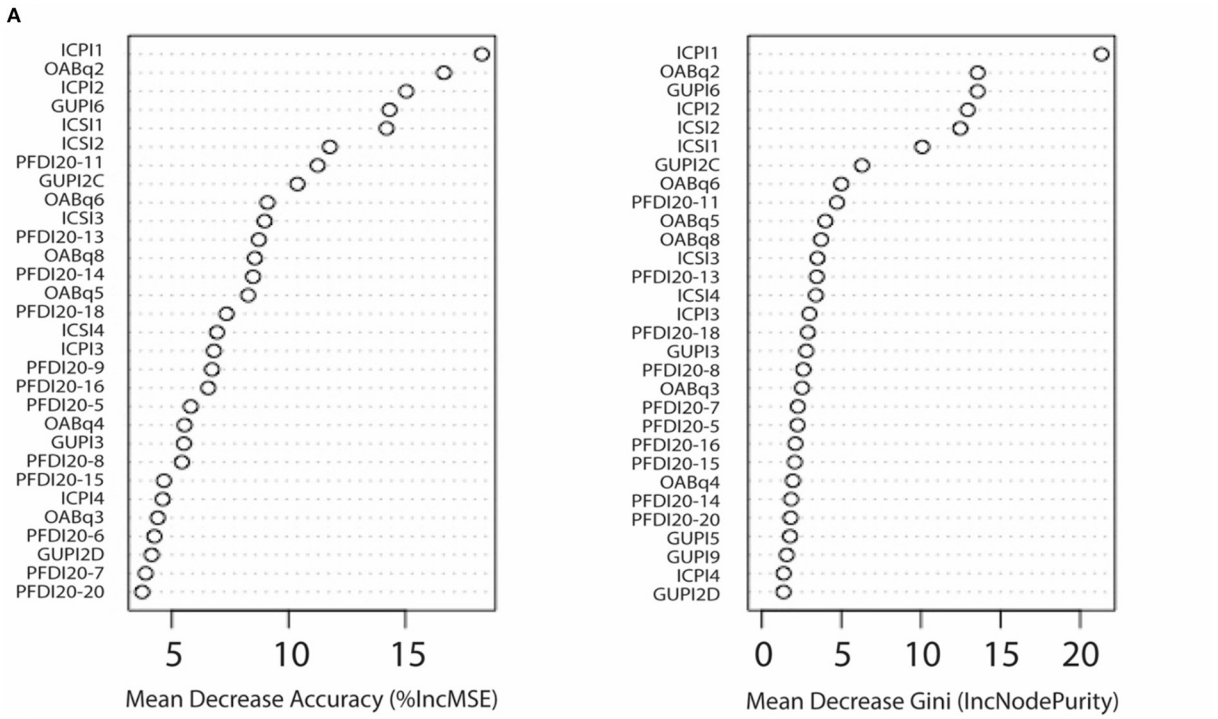


FIGURE 3 | Unique features of each IC/BPS phentoypic cluster. **(A)** Individual proportions of IC/BPS subjects endorsing pain symptoms by location (fGUPI1) and activity (fGUPI2) by phenotype. **(B)** Box and whisker plot displaying the composite quality of life score on the fGUPI for each phenotype in the inset legend. **(C)** Mean scores for four independent measures of bladder-related pain, pressure or discomfort are shown by phenotypic group. Error bars display the standard deviations. Significances for all pairwise comparisons are listed in **Table 2**.

questions with largest association with specific phenotypic groups, as expressed in a heat map of the scaled values (**Figure 4B**). These discriminatory questions were combined to express the relative severity of these symptom domains for each phenotypic group, plotted in box and whisker plots for the three IC/BPS phenotypes in comparison to controls (**Figure 5**). While all IC/BPS subjects exhibited highly elevated symptomatic bother (**Figure 5E**), the severity of each symptom complex differed greatly. For the BPS group, a bladder pain measure was generated from the weighted composite of ICSI4 (bladder pain or burning), fGUPI2c (pain with bladder filling), and fGUPI6 (urinary frequency). These specific features were highly restricted to the BPS group (**Figure 5A**). For the NUPP group, a measure of non-urologic pelvic pain was generated from the sum of fGUPI1a, b, and c (pain in the introitus, vagina, and urethra, respectively) and fGUPI2a and b (dysuria and dyspareunia, respectively). Using this measure, the NUPP group was easily distinguished from the other pain groups and controls (**Figure 5B**). To represent the symptoms of the MFP group, we incorporated the scaled scores for



B

Group	ICSI1	ICSI2	ICSI3	ICSI4	ICPI1	ICPI2	ICPI3	ICPI4	OABq2	OABq3
BPS	0.3774883	0.4649473	0.1663942	0.3187467	0.4704907	0.3809281	0.2399216	0.269421	0.4347571	0.2875459
NUPP	-0.7927792	-0.8501239	-0.6270561	-0.4952718	-0.8592459	-0.8698645	-0.7738525	-0.5232004	-0.8713232	-0.7327045
MFP	0.499213	0.4043212	0.6827239	0.1312936	0.4074524	0.6201973	0.7672583	0.2852615	0.5054011	0.5949631

Group	OABq4	OABq5	OABq6	OABq8	GUPI1A	GUPI1B	GUPI1C	GUPI1D	GUPI2A	GUPI2B
BPS	-0.0982779	0.2910722	0.3269804	0.1239677	-0.1307397	-0.094744	0.00846087	0.12655446	0.06252391	0.04451401
NUPP	-0.4038545	-0.729151	-0.7692068	-0.6212424	0.14011745	0.18272733	0.08642231	-0.2006624	0.2330721	0.05610156
MFP	0.88711822	0.5813611	0.5699206	0.7654298	0.08676268	0.01416239	-0.1624087	0.05882984	-0.2215412	-0.1000145

Group	GUPI2C	GUPI2D	GUPI3	GUPI4	GUPI5	GUPI6	GUPI7	GUPI8	GUPI9	PFDI20_1
BPS	0.375736	0.258684	0.1470574	0.1039663	0.1984834	0.5301172	0.01075017	0.1363838	0.1272467	0.04801009
NUPP	-0.5669556	-0.5196213	-0.3706936	-0.2545444	-0.5581863	-0.8310409	-0.3795954	-0.2947967	-0.3126019	-0.2400143
MFP	0.1266564	0.3026792	0.2975643	0.1978253	0.4980579	0.2305907	0.60924745	0.1943142	0.2438881	0.29546843

Group	PFDI20_2	PFDI20_3	PFDI20_4	PFDI20_5	PFDI20_6	PFDI20_7	PFDI20_8	PFDI20_9	PFDI20_10	PFDI20_11
BPS	0.06457626	-0.0756674	-0.1963683	-0.0492963	-0.0886352	-0.3120485	-0.2845143	-0.2986393	-0.2185263	-0.4004539
NUPP	-0.3377174	-0.0605223	-0.1094688	-0.5016251	-0.2570875	-0.1051088	-0.0447314	-0.278911	-0.255552	-0.1812358
MFP	0.42222961	0.26565734	0.6100945	0.9433983	0.621507	0.8547537	0.69416125	1.1152218	0.9018217	1.1741591

Group	PFDI20_12	PFDI20_13	PFDI20_14	PFDI20_15	PFDI20_16	PFDI20_17	PFDI20_18	PFDI20_19	PFDI20_20
BPS	-0.256411	-0.2894806	-0.3105189	0.2709751	0.08367501	-0.0799789	-0.140802	0.09512008	0.08420715
NUPP	-0.0551604	-0.2916436	-0.266016	-0.5992287	-0.5705926	-0.2371303	-0.399201	-0.3843791	-0.3915318
MFP	0.65034014	1.1164971	1.1196012	0.408591	0.76876212	0.56939337	0.9719705	0.43348139	0.46916856

FIGURE 4 | Determination of important clinical features defining the unique IC/BPS phenotypes. **(A)** Variable importance plot demonstrating how important each variable is in classifying the data. Mean decrease Accuracy expresses how much accuracy the model loses by excluding individual variables. The mean decrease in Gini coefficient measures how much each variable contributes to generating homogeneous nodes, with higher values indicating greater importance in the model. **(B)** Mean scores for each phenotype are expressed as Z scores to provide a normalized distribution of scores relative to the mean of the overall population regardless of individual item scale. These were then expressed in heat maps for each question to provide a visual representation of scores uniquely associated with a single phenotype, with the highest scoring questions shown in the darkest red.

fGUPI5 and PFDI-20q5 (sensation of incomplete emptying), PFDI-20q1 (abdominal pressure), and PFDI-20q7 (straining to defecate) into a single measure. While this constellation of symptoms, bladder pressure and incomplete elimination symptoms, was most elevated in the MFP group, varying degrees of these symptoms were present in all three groups over the baseline seen in asymptomatic controls (**Figure 5C**). The subjects only infrequently complained of symptoms not

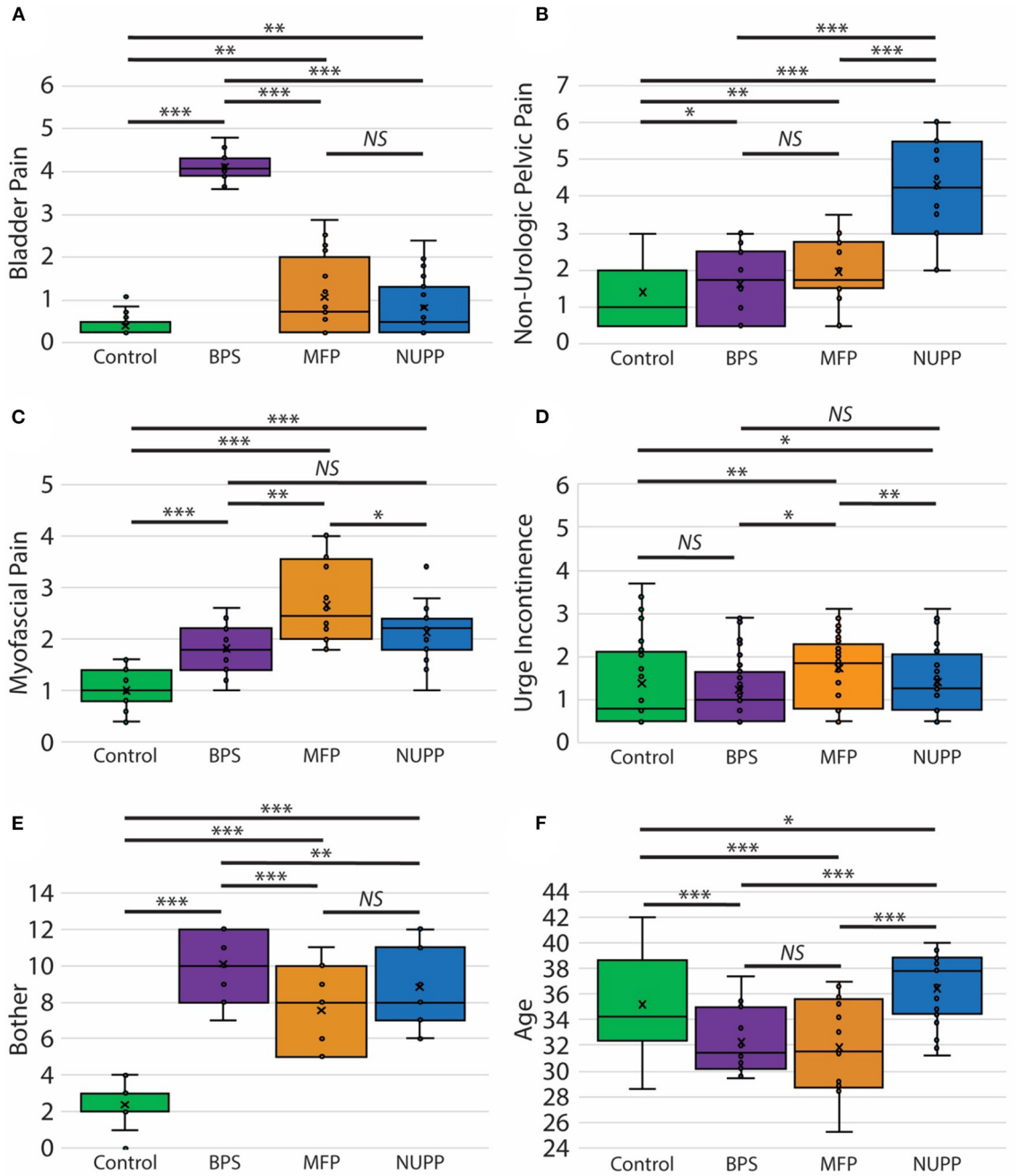


FIGURE 5 | Clinical features of pelvic pain patients identified by unsupervised clustering. Measures of the phenotypic characteristics of the presumptive IC/BPS clusters are plotted as box and whisker plots. Subjects exhibited distinct associated pain symptoms. The BPS group (purple) was homogenously high for bladder pain related to bladder filling **(A)**, while the NUPP group (blue) demonstrated pelvic pain focused on the urethra and vagina unrelated to urination **(B)**. The MFP group (orange) had features of myofascial pain **(C)**. None of these groups exhibited significant urgency incontinence **(D)** and were all significantly bothered by their symptoms in comparison to controls **(E)**. The BPS and MFP groups were significantly younger than controls or NUPP patients **(F)**. ***$p < 0.0001$, **$p < 0.005$, and *$p < 0.05$.

classically associated with IC/BPS, such as urgency incontinence measured by the urge incontinence composite index (UICI) (7). While incontinence symptoms were highest in the MFP group, the scores for all groups remained low overall (**Figure 5D**). Interestingly, the BPS and MFP groups were significantly younger than NUPP subjects and asymptomatic controls (**Figure 5F**), suggesting an association of these phenotypes with younger age.

Overlap Between Phenotypes Within the IC/BPS Population

While most IC/BPS subjects displayed only one predominant symptom cluster, some individuals exhibited features of more than one group. These overlapping symptoms raised the question of whether these phenotypes could manifest independently. We utilized a Kohonen SOM (24), a type of neural network trained using unsupervised learning, to explore the relative expression of the clinical features defining each phenotype across the cohort, using the symptomatic measures defined above. Graphically, patients are clustered into an arbitrary number of "bins," each represented in the figure by a circle, and the relative expression of each symptom domain (bladder pain, myofascial pain and non-urologic pelvic pain as defined in the measures above) for that bin is represented by the size of each pie slice within the circle (**Figure 6A**). Given the low number of subjects, the number of bins was kept small ($n = 24$ in a 6×4 grid) to allow for meaningful groups of similar patients to emerge. Only a portion of the total subjects ($n = 81/214$; 38%) including the pain groups and controls fell into a "pure" phenotype category (asymptomatic controls, BPS, NUPP, and MFP). These "pure" phenotypes, in which a single symptomatic feature (or "pie slice") was clearly dominant, are designated by a colored ring surrounding the bin. The red heat map in **Figure 6B** represents the number of patients in each bin corresponding in space to those depicted in **Figure 6A**. The remaining 79 IC/BPS patients fell into bins expressing combinations of two or more of these symptom clusters (cyan), including a 'global pain' phenotype (magenta) in which subjects demonstrated high levels of all three types of pain symptoms. Interestingly, almost half of the control subjects expressed symptoms placing them in bins with other symptomatic patients, although they did not complain of significant bother associated with these symptoms. The similarity of each of the symptom patterns (each bin shading from green to white) to the neighboring bin's pattern is also expressed as a heat map (in green) in **Figure 6C**. The global pain phenotype (upper right in grid) exhibited the largest symptomatic difference from the other groups. The pure MFP (ringed in orange) and MFP-dominant phenotypes were more dissimilar to the surrounding symptom combinations than the BPS and NUPP phenotypes were to each other, mirroring the hierarchical clustering in **Figure 2**.

Unique IC/BPS Phenotypes Demonstrate Different Responses to IC/BPS Therapies

After observing segregation of the original IC/BPS population into phenotypes with divergent clinical features, we hypothesized each would respond differently to IC/BPS therapies. We performed retrospective chart review of subjects for each group to determine patient-perceived responder rates for four common IC/BPS therapies: oral bladder analgesics, intravesical instillations, pelvic floor physical therapy (PFPT), and oral amitriptyline (**Table 3**, **Figure 7**). Of patients who had attempted each therapy, the BPS group responded best to bladder-directed therapies, such as bladder analgesics and intravesical instillations. The response to intravesical instillations was significantly better in the BPS phenotype than in either the NUPP or MFP groups ($p < 0.001$ for both). We observed that the NUPP group consistently demonstrated low response rates to all therapies. In contrast, MFP subjects responded well to PFPT, with almost 80% of those who could be assessed responding positively to this therapy (79 vs. 12% for NUPP and 9% for BPS; $p < 0.0001$ for both). Unfortunately, the number of patients in this group that were not seen in follow-up after PFPT was enriched due to clinic closures associated with the pandemic. Regardless, these findings are suggestive that IC/BPS phenotypes can be identified that require different therapeutic strategies to achieve symptomatic control.

DISCUSSION

Deeper clinical characterization of female premenopausal patients with the subjective sensation of bladder pain reveals three distinct phenotypes which suggest divergent sources of perceived pain. Each of the phenotypes had recognizably different clinical features as well as different responses to the various treatments frequently employed for IC/BPS patients, data which support differing pathophysiologies underlying these phenotypes.

For many chronic pain conditions, such as IC/BPS, the condition is defined by a process of exclusion of other organic pathologies. The exclusion of other conditions does not, however, mean that the remaining affected individuals are homogeneous. Depending on the inclusion criteria, patients endorsing the sensation of bladder pain or discomfort may include multiple symptomatologies: true pain deriving from aberrant sensation in the bladder, other forms of pelvic pain referred to the bladder, centralized or systemic allodynia including features of pain ascribed to the bladder, or other localized pelvic pain misattributed to, but unrelated to the bladder. These different symptom origins may be described similarly by patients but are unlikely to represent a single clinical condition or respond to identical treatment approaches. While there is growing consensus that the recognition of Hunner's lesions in IC/BPS should prompt different considerations for medical and surgical management (25), phenotyping of the remaining majority of IC/BPS patients who lack obvious bladder pathology has been challenging.

In addition, subjects with IC/BPS are at high risk for experiencing other forms of pelvic pain or dysfunction, such as irritable bowel syndrome, which has been attributed to neuronal cross-talk at the level of the spinal cord (26–30). IC/BPS also commonly co-exists with a range of functional somatic syndromes, such as fibromyalgia and myalgic encephalomyelitis, as well as psychiatric comorbidities, such as anxiety and depression. Given the complexity of presentation for many of these patients, phenotyping of patients into more homogeneous groups has proven difficult; no classification system has yet achieved wide-spread use (31–33).

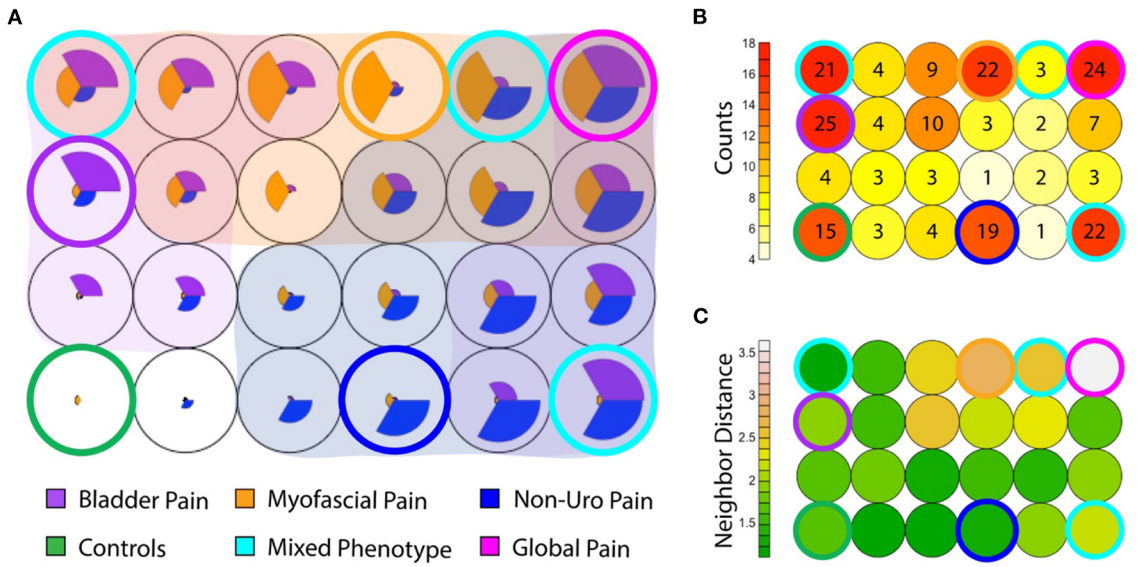


FIGURE 6 | Coexisting symptom domains determined by Kohonen self-organizing map (SOM). **(A)** The SOM groups subjects (including IC/BPS subjects and asymptomatic controls) into 24 (6 × 4) bins. Within each, the size of each pie slice expresses the average measure score for that subgroup after scaling to normalize values. Asymptomatic controls are at the inferior left corner (green ring), in which no pie slices are seen. The canonical phenotypes, expressing only a single symptom cluster are ringed in blue for NUPP, orange for MFP, and purple for BPS. Other combinations of phenotypes are shaded by an overlay of the respective group color when that symptom profile was expressed within that group. The most common mixed phenotypes are ringed in cyan. The global pain phenotype, in which all three symptom types were highly expressed, is ringed in magenta. **(B)** The numbers of patients within each of the groups in A are expressed in a heat map, with the relative position of each bin corresponding to the layout seen in **(A)**. The number indicates the absolute number of subjects in each bin. Most subjects reside in the control and the ringed phenotypic groups, with a substantial minority present in subgroups expressing more than one phenotype. **(C)** The similarity of each of the symptom patterns to the neighboring bin in Euclidean distance is expressed as a heat map (each bin shading from green to white). For example, the MFP phenotype ringed in orange is highly dissimilar to the surrounding symptom combinations, but the global pain phenotype ringed in magenta is the most dissimilar from all other groups, demonstrating the largest Euclidean distance to its neighbors.

TABLE 3 | Responders to typical IC/BPS therapies by phenotypic cluster.

Phenotype	Oral bladder analgesics†	Intravesical instillations‡	Pelvic floor physiotherapy	Amitriptyline	Total patients with follow-up*
BPS	9 ($n = 12$)	18 ($n = 20$)	3 ($n = 4$)	5 ($n = 8$)	40 (44 therapies attempted)
MFP	1 ($n = 6$)	2 ($n = 4$)	23 ($n = 27$)	1 ($n = 2$)	38 (39 therapies attempted)
NUPP	4 ($n = 13$)	1 ($n = 10$)	5 ($n = 21$)	5 ($n = 15$)	27 (59 therapies attempted)
Responders	14 ($n = 31$)	21 ($n = 34$)	31 ($n = 52$)	11 ($n = 25$)	105

Patients with a positive response are indicated for each therapeutic approach by phenotype. The total numbers of patients attempting each therapy are denoted in parentheses.
†Oral analgesics included phenazopyridine, Urogesic-Blue™ (hyoscyamine, methenamine, methylene blue, and sodium biphosphate), and Uribel™ (hyoscyamine, methenamine, methylene blue, phenyl salicylate, sodium phosphate) or other formulations with similar composition.
‡Standard bladder instillation included lidocaine, sodium bicarbonate, heparin, and triamcinolone.
**The number of attempted therapies for each group are greater than the total number of patients with follow up as multiple patients per group attempted more than one therapy.*

Several previous classification systems, such as UPOINT (34, 35), have focused on optimizing care of patient pain by comprehensively addressing all aspects of their symptomatology. While this can be highly effective at helping to manage individual patient symptoms and improve overall quality of life, the use of such categorization systems requires a significant expertise and familiarity with urologic pain manifestations. More importantly, these systems do not aim to identify the more homogenous groups of patients needed to move forward in mechanistic studies. Thus, the opportunity for cure or prevention of the condition remains elusive for now due to a fundamental lack of understanding of the underlying pathophysiology of bladder pain.

In fact, even the necessity of pain for a diagnosis of IC/BPS is not universally accepted. European Association of Urology (EAU) guidelines consider pain attributed to the bladder a

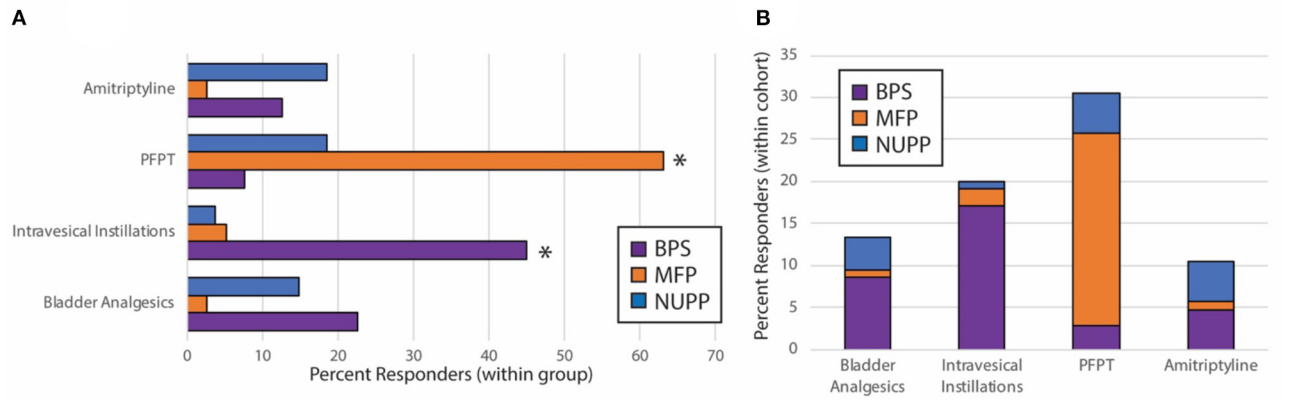


FIGURE 7 | Subjective treatment responses vary by phenotype. Chart review of subjects in the BPS, MFP, and NUPP groups was used to assess binary responsivity (responded yes/no) to several typical therapies prescribed for IC/BPS patients. **(A)** Responder rates for patients were expressed as a percentage of each individual phenotype. The starred treatment groups were statistically different from both of the other groups (*$p < 0.001$ for pairwise comparisons with both other phenotypes). **(B)** The proportion of responders within the total population was expressed for each therapy, demonstrating that the majority of responders to the bladder-directed therapies (bladder analgesics and intravesical instillations) were BPS patients, while the majority of responders to PFPT were MFP subjects. Responses were only assessed for those subjects who attempted the designated therapies.

key diagnostic feature (36), while the American Urological Association (AUA) guidelines expand the concept of pain to also include "sensations of pressure or discomfort" (12). The International Association for the Study of Pain (IASP) recently established a new definition for chronic primary bladder pain syndrome which is "chronic pelvic pain perceived in the region of the urinary bladder that is also associated with at least one other symptom," such as worsening of the pain upon bladder filling and urinary frequency during day time and/or night time. This definition, which is intended to be used in place of IC/BPS, does not require the presence of associated urinary symptoms (37). The guidelines published by the Japanese Urological Association do not require pain for inclusion, stating many patients do not describe pain, instead using the term "hypersensitive" bladder to describe the uncomfortable frequency and nocturia experienced by some individuals without Hunner's lesions.

The differing definitions of IC/BPS have significant consequences in the study of this condition. Our phenotypes reveal that a subset of patients may define their symptoms more as pressure or discomfort than frank pain. This discomfort was more constant and associated with myofascial dysfunction, which was distinct from the perception of pain in the bladder related to voiding cycle. Given the subjective nature of the definitions of IC/BPS, there may be very different populations included if the definition requires a description of "pain" instead of "pain, pressure or discomfort." As no objective markers of IC/BPS exist, attempting to discover an underlying pathologic mechanism in a pooled, inclusive population of subjects with any form of pressure, pain, or uncomfortable frequency may prevent any potential discoveries.

We focused on identifying the subtle differences in bladder-related symptoms in a population of premenopausal women with a narrowly-defined perception of bladder pain to characterize possible phenotypes that could represent different pathologies. Only by defining more homogeneous populations will we be able to improve therapeutic assignment and outcomes. This has even more significance for future research attempting to discover the underlying molecular mechanisms related to bladder or pelvic pain in each population, a requirement for identifying new approaches to improving patient care. A lack of such homogeneous groups may well be the primary confounding factor affecting studies to date. Only with such knowledge will we be able to define the pathways we can target for better treatment and prevention. While it will be necessary to validate the classification system and optimize the associated phenotypic measures in larger populations, this study is a first step toward those goals.

The clear differences seen in these populations in terms of their responses to treatment support the concept that these phenotypic groups represent distinct, independent disease categories or etiologies. The mechanisms of each of these therapies suggests possible origins of the pain seen in each group, which we hope to evaluate further in additional studies. Most responders to bladder-directed therapies, such as bladder analgesics and intravesical instillations were those with bladder-centric pain related to the voiding cycle categorized to the BPS group. This finding implicates a bladder-derived pathology in these patients, as could be mediated by local inflammation (38, 39) or urothelial dysfunction (40), two mechanisms previously proposed for IC/BPS. The great majority of responders to PFPT were MFP subjects, implicating a myofascial involvement for these individuals (22). The NUPP group did poorly with the common approaches attempted in this population, suggesting that the standard IC/BPS algorithm may be inappropriate for these patients. Given the pattern of pain, this group may represent a vestibulodynia subset with pain referred to the bladder, but for whom treatment of the bladder itself provides little relief.

The myofascial pain group is challenging to recognize without more accurate metrics, because it is common for patients with bladder pain to have some myofascial pain that accompanies their bladder-centric symptoms and frequently will respond to PFPT (41). This is reinforced in the SOM analysis, in which nearly half of the patients with dominant bladder-centric pain also manifest some degree of myofascial pain, albeit less severe than the MFP group. In direct comparison, the two groups exhibited highly similar urinary symptoms, but the BPS group could be clearly distinguished by voiding-cycle related pain, exacerbated by bladder filling. In contrast, the largest factors discriminating the MFP population from BPS were inconsistent levels of defecatory dysfunction, a sensation of incomplete emptying, and small volume incontinence. This mirrors well what has been seen in prior phenotyping studies (31). Each of these individual symptoms, however, were only present in a subset of the overall MFP population. While the two groups were easily recognizable at the population level, it may be difficult to classify patients to one of the two groups on an individual basis using the metrics proposed in this study.

This may be the explanation underlying why the Jaccard coefficient (JC) of the MFP group (0.51) is significantly lower than the BPS group (0.69). A "stable" or "excellent" cluster, in which subjects can be consistently assigned to this group in multiple sub-samplings of the population, will have a Jaccard coefficient of more than 0.70. While the symptoms of these patients were highly similar, the presence of pain with bladder filling was highly specific for the BPS group, which likely aided in the stability of group assignment. In contrast, no single clear symptom was consistently found in all MFP subjects. Regardless of this finding, the difference in treatment responses suggests that the differentiation of the MFP subjects from BPS-dominant groups is clinically relevant and pathologically meaningful. Additional studies with more extensive phenotyping may be helpful in identifying more discriminatory symptomatic features, but we propose that MFP be suspected in IC/BPS subjects without clear pain with bladder filling who complain of POP-like symptoms without objective signs of visceral or pelvic floor descent on exam.

The NUPP group exhibited milder urinary symptoms than the other two groups but was still highly bothered by the pain. The pain was prominent in the distal vagina/urethra and was associated not with bladder filling but with voiding. It is easy to see how these painful symptoms could be attributed to the bladder, despite the distinct pattern in their manifestation. These patients do not respond to the bladder-directed therapies of the BPS group, or indeed any of the other typical IC/BPS therapies documented in this study. A large limitation to understanding the nature of this group was the limited exploration of vaginal and urethral symptoms present in the fGUPI. The questions distinguishing the NUPP group were yes/no questions merely describing pain location or aggravating factors, without a more subtle assessment of these pain manifestations or their severity. These symptoms may have been missed or even overemphasized in some. A more detailed exploration of uncomfortable vaginal and pelvic symptoms in combination with a detailed exam is needed to understand the range of symptoms and the relationship of this phenotype to other pain conditions, such as vestibulodynia.

Interestingly, we did observe the expression of features characteristic of multiple phenotypes by smaller numbers of subjects. This may mean these phenotypes are not sufficiently defined. It has been noted elsewhere that patients often find the language of urology unfamiliar at best and have difficulty describing their symptoms accurately (42), which may limit the utility of patient-reported measures in symptomatic phenotyping. Further, the reticence of subjects to describe their experience fully for a variety of reasons is an additional limiting factor (43). On the other hand, the manifestation of multiple phenotypes within a single patient may simply indicate that these phenotypes accurately describe the different patterns of pain, which are distinct from each other but can co-exist. If occurring independently, individual subjects might express more than one phenotype concurrently.

It was common for the BPS subjects to manifest at least some MFP symptoms. Pelvic floor hypertonicity and tenderness is hypothesized to be a consequence of local pelvic pain and is seen in a variety of pelvic pain conditions (22, 41). These data support this. Interestingly, the converse is not similarly true (e.g., high levels of myofascial pain do not frequent co-occur with mild bladder-centric pain). These data reinforce the concept that myofascial pain is an independent condition, more experienced as "pressure or discomfort" that can be expressed or experienced as the perception of bladder pain in the absence of pain related to voiding cycle.

The group with high levels of all types of pain comprises a small, but not insignificant portion of the overall population. Patients with more domains of pain typically have more severe symptoms (34, 44), suggesting that severe pain in one domain could augment other types of pelvic pain in a positive feedback loop. It is also possible that this group may represent patients with all three phenotypes of pain occurring independently or a more centralized pain, as seen in central sensitization syndrome, who may express more global allodynia. The distance of this phenotype from other patient clusters in the SOM suggests additional or different mechanisms at play in these patients, which may make them more unlikely to respond to locally-directed therapies. Prospective evaluation of this population is critical in understanding whether these subjects represent a separate etiology or more of an end-stage of the other "pure" phenotypes if not effectively treated.

The details of the phenotypic classification determined in this study highlight the challenges in attempting to identify more homogeneous phenotypes of perceived bladder pain, even when narrowly defined by multiple inclusion criteria. It is possible that a larger sample size would identify additional phenotypes that were not recognized here and that are less common or inadequately assessed by the information collected in these questionnaires.

The responder analysis was not anticipated in the original study; thus, the utility of this data is limited by its retrospective acquisition, the absence of more detailed, quantitative outcome measures, and lack of standardized care pathways. In addition, lack of follow up for a subset of the population secondary

to reduced clinical access as a result of the SARS-CoV2 pandemic also limited these data. Therefore, while treatment response data are suggestive that the groups respond differently, more detailed prospective studies are needed to validate these preliminary findings.

As this study only included pre-menopausal women, it is not known whether this approach will be generalizable to other age groups or genders. Postmenopausal women can commonly experience a range of genitourinary symptoms associated with that state of relative hormone deficiency, including pain, burning, and dysuria which may complicate this phenotypic classification.

Finally, the IC/BPS subjects included in this analysis did not routinely undergo cystoscopic evaluation as part of their initial treatment course, making it difficult to determine whether a subset of these subjects may have had Hunner's lesions. As Hunner's ulcers are present only in a minority of IC/BPS cases and tend to occur predominantly in older patients, we anticipate that this is a minor portion of this population (45–47). We cannot, however, make any determinations about the influence of Hunner's ulcers on our phenotyping system; the association of individual phenotypes with cystoscopic findings of bladder-specific inflammation or frank ulceration of the urothelium needs to be assessed in future studies. Regardless, as the phenotyping described in this study entirely uses information obtained from patient-reported outcomes at the initial consultation before treatment or examination, such a classification system may still be highly useful, particularly for the non-specialty provider without access to cystoscopic assessment.

Going forward, it will be necessary to validate these phenotypes in larger, multicenter populations. It will be especially important to confirm prospectively that these phenotypes are reliable and accurate predictors of response to treatment. Finally, we must determine if these phenotypes are applicable to other populations such as older women and men. We expect both additional and different symptoms in these groups. Additional studies must determine if new phenotypes are required and how well the model described here can be adapted.

As with all chronic pain conditions, multimodal treatment remains the most effective approach. It is important to address the accompanying extra-pelvic symptoms and recognize the psychosocial and systemic manifestations of this chronic pain condition to improve overall health-related quality of life. We believe, however, that a simple IC/BPS phenotyping into the categories defined here could help focus both initial treatment assignment as well as better facilitate mechanistic studies that could provide insight into disease etiology and promotion. Progress in the care and management of this highly impactful condition is sorely needed; refining our diagnostic categories is a necessary next step in moving toward that goal.

In conclusion, expanded clinical phenotyping of patients reporting perceived bladder pain can identify distinct phenotypes. The different patterns of associated symptoms suggest that these phenotypes may reflect unique pathophysiologies. Further, the varying response of each phenotype to traditional therapies for IC/BPS suggests the utility of such phenotyping and, if validated in prospective studies, may provide a useful approach to understanding the underlying pathophysiologies and improving the diagnosis, treatment, and prevention of bladder pain.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Pro00046154. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PM assisted in the development of the study, conduct of the research, data analysis, and drafting the manuscript. FK and AC conduct of the research and editing of the manuscript. NJ development of the study, data analysis, and editing of the manuscript. JW, VS, KE, and JTA development of the study, conduct of the research, and editing of the manuscript. DU development of the study, direction of the research, and editing of the manuscript. JEA data management and editing of the manuscript. ALA development of the study, conduct of the research, data analysis, and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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