Original Article

Neuroinflammatory gene expression analysis reveals potential novel mediators and treatment targets in interstitial cystitis with Hunner lesions

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Background: We sought to study differential neuroinflammatory gene expression in men with interstitial cystitis (IC) with Hunner lesions compared with asymptomatic controls using NanoString, which uses barcoded probes to measure hundreds of genes. IC is a heterogenous condition lacking reliable biomarkers, and a subset of patients exhibits Hunner lesions, implicating the bladder as an inflammatory pain generator.

Methods: Blood, urine, and bladder biopsies were collected from 6 men with IC and Hunner lesions. 7 asymptomatic controls had blood and urine collected and 2 benign bladder biopsies were obtained from our tissue bank. RNA was isolated and analyzed with NanoString Human Neuroinflammation panel. Gene expression was considered significant if there was a >1.5-fold change and adjusted P value <0.05 compared with controls.

Results: Mean patient age was 61.5 years with 8 years median symptom duration. In bladder tissue, while many cytokine and chemokine genes had higher expression as expected (e.g., *TNF* , *CXCL10*), other significant genes included *TRPA1* (1098-fold increased, expressed in pain sensing neurons) and *TNFRSF17* (735-fold, B-cell related). In urine, there was 114-fold increase in *S1PR4*, which mediates pain via TRP-dependent pathways. A patient on cyclosporine had lower inflammatory gene expression levels relative to other IC patients, but no difference in *TRPA1*.

Conclusions: Men with IC and Hunner lesions have a diverse set of neuroinflammatory genes with differential expression compared to controls. We identified genes linked to neuropathic pain through the TRP pathway and this expression was not reduced by cyclosporine. These findings open a new direction for biomarker and therapeutic discovery.

Keywords: Interstitial cystitis (IC); lower urinary tract symptoms (LUTS); bladder pain syndrome; biomarkers; Hunner lesions

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Introduction

Interstitial cystitis (IC) is characterized by pelvic pain, pressure, or discomfort related to the urinary bladder, often accompanied by urinary urgency or frequency, and relieved by voiding (1). IC is often debilitating, its etiology remains enigmatic, and it affects 2.7–6.5% of the U.S. population (2,3). Patients with IC incur about $7,100 in annual healthcare costs, nearly double the annual healthcare costs than patients without the condition (4). IC is a heterogenous condition, with no one-size-fits-all treatment, but rather treatment tailored to the individual. Commonly, patients are treated with a multimodal regimen of progressively escalated therapies, before they reach acceptable symptomatic control (5). Men are subject to misdiagnoses including prostatitis, benign prostatic enlargement, and epididymitis, and thus provide unique challenges practitioners (6). Due to this, along with higher prevalence of IC in women, few studies have focused on the condition in men. As yet there are no reliable and validated biomarkers to guide both diagnosis and treatment in this population.

There has been recent investigation of IC biomarkers with antiproliferative factor, epidermal growth factor, heparin-binding epidermal growth factor, glycosaminoglycans, and bladder nitric oxide as some of recent focus (7). However, due to difficulty or lack of practicality of assays, such as the need to culture bladder urothelial cells for detection of antiproliferative factor activity, diagnosis remains based on symptomatology and cystoscopic findings. An important subtype of IC is those patients with Hunner lesions found on cystoscopy. These patients are often older, have more severe symptoms and are more likely to respond to immunotherapy such as cyclosporine (2,8). Nevertheless, diagnosis of Hunner lesions is inconsistent and prevalence can vary widely depending on the Urologist's experience identifying them. Therefore, identification of novel objective biomarkers would be helpful to guide effective therapy.

The NanoString nCounter is a novel rapid and automated platform allowing for direct mRNA measurement for up to 800 genes, using a small sample without the need for cDNA or polymerase chain reaction (9). We sought to compare RNA profiles of patients with IC with Hunner lesions relative to healthy controls. We utilized a pre-designed cassette with nearly 800 genes associated with inflammation and/or neuropathic pain, and set out to study gene expression in blood, urine, and bladder tissue for this set of neuroinflammatory genes in men with IC compared to asymptomatic controls. Our approach allows for simultaneous measurement of gene interaction networks, as the orchestrated expression of interrelated genes, rather than any single gene, may play a key role in IC pathophysiology. We hypothesized that men with IC would have unique gene expression signatures associated with neuroinflammatory pathways, relative to asymptomatic controls. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/tau-21-657).

Methods

The study was approved by the institutional IRB (#19-1515) and appropriate written consent was obtained for all subjects. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Blood, urine, and bladder biopsies were collected from 6 men with the clinical diagnosis of symptomatic IC found to have Hunner lesions (10). IC patients were excluded if they were not currently symptomatic with pain and lower urinary tract symptoms (LUTS), or if they had active infection (newly found or being treated with an antibiotic). IC patients were also excluded from our investigation if they were receiving immune-modulating treatments (e.g., corticosteroids), with the exception of cyclosporine for the secondary analysis of comparison of IC patients not on cyclosporine to the patient on cyclosporine. A single bladder biopsy was taken by an attending urologist with recognized expertise in the diagnosis and management of IC, from a visible Hunner lesion. The biopsy was obtained during cystoscopy under general anesthesia using cold cup biopsy forceps prior to lesion fulguration. At least 20 mL urine and approximately 3 mL blood were obtained either during a clinic visit or on the day of surgery prior to starting antibiotics or anesthesia. Urine and blood were similarly collected from 7 asymptomatic men, serving as controls, without signs or symptoms of UTI or LUTSs, presenting for a vasectomy. Benign biopsies were analyzed from our tissue bank, from 2 male patients with no documented history of LUTS or IC. These men underwent biopsy for suspicion of bladder cancer, and biopsies were taken from areas remote to any target lesion. Men were excluded from this group for evidence of UTI on preoperative urine culture, or other diagnosis of LUTSs or pain.

All samples were processed and frozen within 6 hours

of collection. Samples were stored at the appropriate temperature (–20 ℃ for tempus tubes, –80 ℃ for urine pellets, wherein RNA is stable indefinitely) for up to 9 months before being used for analysis. Three mL of blood was collected in Tempus Blood RNA tubes (Applied Biosystems; ThermoFisher Scientific), which contain a stabilizing reagent that immediately lyses blood cells and inactivates RNases. RNA isolation and analysis were performed as previously described (11). The RNA is stable indefinitely when stored at –20 ℃. RNA was isolated from the blood samples using the Tempus Spin RNA Isolation Kit.

Urine samples were centrifuged at 3,000 g for 30 minutes at 4 ℃ in 50 mL conical tubes. The pellets were resuspended in 1 mL PBS, transferred to 2 mL microcentrifuge tubes and centrifuged at 10,000 rpm for 4 minutes at room temperature. The PBS was removed and the urine pellets were then immediately stored at –80 ℃. RNA was isolated from the urine pellets using the Qiagen Rneasy Mini Kit. RNA from blood samples was quantified using a Nanodrop ND100 spectrophotometer. The mean RNA yield was 8.53 µg (range, 3.5–13.6 µg) and the OD260/280 ratios were all >2.1. RNA from urine samples was measured both with a Nanodrop ND100 spectrophotometer to determine the OD260/280 ratios, and the Qubit4 for RNA concentration. The mean RNA yield was 291 ng (range, 0.1 to 1,600 ng) and the mean OD260/280 ratio was 1.8. A total of 100 ng RNA was used for gene expression analysis with the 770-gene NanoString Human Neuroinflammation gene panel.

Statistical analysis

Data were imported into ROSALIND® (https://rosalind.onramp.bio/) for quality control, normalization, analysis of fold-changes and P values, and identification of enriched pathways and gene sets. Differential gene expression was considered biologically and statistically significant if there was a greater than 1.5-fold change compared to controls (the asymptomatic control group for the blood and urine samples, and the tissue bank group for the tissue sample), and Benjamini-Hochberg adjusted P value was $P<0.05$. Patients were clinically phenotyped with the Urinary, Psychosocial, Organ-Specific, Infection, Neurologic/Systemic and Tenderness (UPOINT) system (12). The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Results

In the IC group, mean patient age was 61.5 years (range, 44–69 years) and median symptom duration was 8 years (range, 2–17 years). Number of positive UPOINT domains ranged from 2–4 with a median of 2.5. In the control group, the mean patient age was 42.6 years (range, 30–55 years) and the patients had no reported history of LUTSs. The bladder biopsy patients were 48 and 65 years old, respectively.

Bladder

Bladder biopsy of the subjects with IC as compared to controls showed differential expression in 141 genes. The highest Nanostring gene set global significance scores were for apoptosis, adaptive immune response, and oligodendrocyte function (*Table 1*). The genes with the greatest significant positive fold-change were transient receptor potential cation channel, subfamily A, member 1 (*TRPA1*), tumor necrosis factor receptor superfamily, member 17 (*TNFRSF17*), lymphocyte antigen 9 (*LY9*) interferon regulatory factor 4 (*IRF4*), and chemokine (C-X-C motif) ligand 10 (*CXCL10*) (*Table 2*). The genes with the greatest significant negative fold-change were RNA binding protein, fox-1 homolog (C. elegans) 3 (*RBFOX3*), microtubule-associated protein tau (*MAPT*) (*Table 2*).

Urine

Urine of the subjects with IC as compared to controls showed differential expression in 123 genes. The highest gene set analysis scores were for Wnt (neuropathic pain), adaptive immune response, and angiogenesis (*Table 1*). The genes with the greatest significant positive fold-change were sphingosine-1-phosphate receptor 4 (*S1PR4*), suppressor of cytokine signaling 3 (*SOCS3*), colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage) (*CSF2RB*), neutrophil cytosolic factor 1 (*NCF1*), and ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2) (*RAC2*) (*Table 3*).

Blood

Blood of the subjects with IC as compared to controls showed differential expression in 41 genes. The highest gene set analysis scores were for oligodendrocyte function, carbohydrate metabolism, and matrix remodeling (*Table 1*).

Table 1 Gene set analysis significance scores in bladder biopsy tissue, urine, and blood of IC patients versus healthy controls via NanoString Annotations

Term	Significance score
Bladder tissue	
Apoptosis	4.9464
Adaptive immune response	4.9051
Oligodendrocyte function	4.8428
Innate immune response	4.6728
Cytokine signaling	4.6176
NF-kB	4.5874
Inflammatory signaling	4.5428
Lipid metabolism	4.3747
Wnt (neuropathic pain)	4.3695
Insulin signaling	4.1935
Urine	
Wnt (neuropathic pain)	3.0979
Adaptive immune response	2.9616
Angiogenesis	2.95
Insulin signaling	2.9336
Inflammatory signaling	2.8375
Carbohydrate metabolism	2.8349
Oligodendrocyte function	2.7838
Autophagy	2.7356
Growth factor signaling	2.6501
Innate immune response	2.6483
Blood	
Oligodendrocyte function	1.8446
Carbohydrate metabolism	1.6355
Matrix remodeling	1.605
Neurons and Neurotransmission	1.5946
Epigenetic regulation	1.4982
Autophagy	1.4736
Cellular stress	1.4732
Astrocyte function	1.471
Inflammatory signaling	1.4521
Innate immune response	1.448

The genes with the greatest significant positive fold-change were lipocalin 2 (*LCN2*), CD68 molecule (*CD68*), granulin (*GRN*), interleukin 1 receptor, type II (*IL1R2*), and pleckstrin homology domain containing, family M (with RUN domain) member 1 (*PLEKHM1*) (*Table 4*). The genes with the greatest significant negative fold-change were radical S-adenosyl methionine domain containing 2 (*RSAD2*) and membrane-spanning 4-domains, subfamily A, member 1 (*MS4A1*) (*Table 4*).

Cyclosporine: bladder, urine, and blood

One of the IC patients was on cyclosporine, and thus we performed separate analyses comparing with the other IC patients. Bladder biopsy of the subject on cyclosporine as compared to those not on cyclosporine showed differential expression in 156 genes. *Table 5* shows the genes with the largest difference between the patients not on cyclosporine versus the patient on cyclosporine in bladder, urine, and blood. As expected, key inflammatory genes including *CD14* (501-fold in urine, 3-fold in blood), *CXCL9* (248-fold in urine) and *CXCL10* (ten-fold in bladder tissue) were downregulated in the patient on cyclosporine. Notably, there was no significant difference in *TRPA1* expression between the two groups.

Discussion

Progress in the treatment of IC has been slow, and hampered by a lack of predictive biomarkers and limited consensus on pathophysiology. Patients with Hunner lesions are a distinct subset of IC patients with documented inflammatory lesions in the bladder that may be responsive to fulguration, direct steroid injection and cyclosporine (2,8). Our study used a novel technology to simultaneously measure the expression of neuroinflammatory genes in blood, urine and tissue between men with IC versus asymptomatic controls. While commonly known inflammatory markers were identified as expected, we found novel genes whose function could lead to new therapeutic interventions for IC. In bladder biopsies, we identified significantly different expression of 141 genes. As expected, many cytokine and chemokine genes had higher expression (e.g., *CXCL10* 92-fold higher, *TNF* 28-fold higher). Other significantly differently expressed genes included *TRPA1*

Table 2 Genes with greatest significant differential expression (positive and negative change greater than ten-fold) in bladder tissue between IC patients and healthy controls

Name	Description	Fold change	P value
TRPA1	Transient receptor potential cation channel, subfamily A, member 1	1098.4	0.00004
TNFRSF17	Tumor necrosis factor receptor superfamily, member 17	734.5	0.0009
LY9	Lymphocyte antigen 9	113.7	0.0001
IRF4	Interferon regulatory factor 4	98.6	0.0010
CXCL10	Chemokine (C-X-C motif) ligand 10	92.7	0.0006
CD3D	CD3d molecule, delta (CD3-TCR complex)	90.5	0.00004
LTB	Lymphotoxin beta (TNF superfamily, member 3)	74.6	0.0002
GZMB	Granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	60.9	0.0007
CCR2	Chemokine (C-C motif) receptor 2	57.9	0.0002
FASLG	Fas ligand (TNF superfamily, member 6)	52.2	0.0017
MAPT	Microtubule-associated protein tau	-22.2	0.0007
RBFOX3	RNA binding protein, fox-1 homolog (C. Elegans) 3	-26.4	0.0009

Table 3 Genes with greatest significant differential expression (positive and negative change greater than ten-fold) in urine between IC patients and healthy controls

Name	Description	Fold change	P value
S1PR4	Sphingosine-1-phosphate receptor 4	113.7	0.000003
SOCS3	Suppressor of cytokine signaling 3	45.5	0.0002
CSF2RB	Colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	37.0	0.0002
NCF1	Neutrophil cytosolic factor 1	32.0	0.00008
RAC2	Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	27.1	0.0002
WAS	Wiskott-Aldrich syndrome	25.3	0.0002
SELL	Selectin L	24.8	0.00009
LTB	Lymphotoxin beta (TNF superfamily, member 3)	24.2	0.00007
ZBP1	Z-DNA binding protein 1	23.8	0.0006
IL1R2	Interleukin 1 receptor, type II	21.8	0.0025

(1,098-fold higher) and *TNFRS17* (735-fold higher). Apoptosis, adaptive immune response, and oligodendrocyte pathways had higher significance scores in bladder tissue in patients with IC relative to controls. It is thought that neurogenic inflammation of the mucosa of the bladder can cause central and peripheral nervous system changes, which may be reflected in oligodendrocyte function, and can in turn contribute to IC (13).

TRPA1 was differentially expressed at 1000-fold greater levels in IC patients relative to asymptomatic controls. *TRPA1* is an ion channel that has been shown to be an important component in pain pathways (14,15). It has been implicated in inflammatory pain such as in osteoarthritis, neuropathic pain such as in diabetic neuropathy, migraine pain, and cancer pain. In addition, *TRPA1* has also been shown to play an important role in "dysfunctional pain", a category of pain with an ill-defined cause, where unexplained pain is the main presenting symptom (15).

Table 4 Genes with greatest significant differential expression (positive and negative fold-change) in blood between IC patients and healthy controls

Name	Description	Fold change	P value
LCN2	Lipocalin 2	9.4	0.0001
CD68	CD68 molecule	2.9	0.0005
GRN	Granulin	2.4	0.0004
IL1R2	Interleukin 1 receptor, type II	2.4	0.0204
PLEKHM1	Pleckstrin homology domain containing, family M (with RUN domain) member 1	2.3	0.0052
CD14	CD14 molecule	2.3	0.0028
FCER1G	Fc fragment of ige, high affinity I, receptor for; gamma polypeptide	2.2	0.0011
CD24	CD24 molecule	2.2	0.0231
NRGN	Neurogranin (protein kinase C substrate, RC3)	2.1	0.0147
PTMS	Parathymosin	2.1	0.0040
MS4A1	Membrane-spanning 4-domains, subfamily A, member 1	–1.5	0.0396
RSAD2	Radical S-adenosyl methionine domain containing 2	–2.8	0.0225

Dysfunctional pain conditions include IC, fibromyalgia, temporomandibular disorder, and irritable bowel syndrome.

TRPA1 has been shown to be associated with inflammation and LUTS in the urinary bladder (16). A gain of function mutation in *TRPA1* is responsible for familial episodic pain syndrome, and interestingly, antagonists have been shown to inhibit the gain-of-function mutant channel (17). Thus, *TRPA1* may be an important drug target for pain syndromes. It has been shown that *TRPA1* localized to unmyelinated nerve fibers in the urothelium, suburothelium, and muscle layers of the rat urinary bladder, and that activators of *TRPA1* result in increased voiding frequency, decreased voided volume, and detrusor overactivity (18). It has also been shown to localize to the urothelial and interstitial cells of the urethra, and that *TRPA1* agonists can modify the tone of human urethral preparations, suggesting a role in signaling in the human urinary outflow region (19). In a rat model of IC, inhibition of the *TRPA1* decreased bladder hyperactivity and pain (20). It has also been shown to be differentially expressed in bladder tissue of those with IC (21). Our results build on these findings, and importantly, have demonstrated the presence of a significant upregulation in IC patients with Hunner lesions.

S1PR4, a G protein-coupled receptor never previously demonstrated in the urine, was detected at 114-fold higher expression in the urine of IC patients than healthy controls. It is a member of the endothelial differentiation, G-protein-coupled (EDG) receptor family. It is mainly expressed in the hematopoietic system. A recent study utilizing *S1PR4* knockout mice demonstrated that the main defect was in dendritic cell differentiation and cytokine secretion. In this study, dendritic cells were unable to switch T cells to Th17, and thus the immune response was shifted to a Th2 response (22). Of note, in IC, the Th17 cell-differentiation pathway has been shown to be significantly enriched in patients with IC with Hunner's lesions (23). Patients with IC are 100 times more likely to have inflammatory bowel disease than healthy individuals (24). Interestingly, severity of colitis was significantly reduced in a *S1PR4* knockout inflammatory bowel disease mouse model (22). The molecule FTY720, which has a beneficial effect in treatment of colitis was postulated to act through the synergistic inactivation of both S1PR1 and S1PR4 (25). In a Phase II randomized controlled clinical trial, Etrasimod (APD334), an S1P1, S1P4, and S1P5 receptor modulator, was more effective than placebo in terms of clinical and endoscopic improvements in patients with moderate to severe ulcerative colitis (26).

Kittaka *et al.* have demonstrated a potential link between TRPA1 and S1PR (27). S1P-induced responses in the dorsal root ganglia neurons in mice were partially inhibited by TRPA1 antagonists. The group used a selective TRPA1 antagonist (HC-030031), and showed that it reduced the

Table 5 Genes with greatest significant differential expression (positive fold-change) in bladder tissue, urine, and blood, between patients with IC not on cyclosporine versus the patient on cyclosporine

Name	Description	Fold change	P value
Bladder tissue			
LCN2	Lipocalin 2	980.402	0.005404
MS4A1	Membrane-spanning 4-domains, subfamily A, member 1	410.199	0.007753
CD19	CD19 molecule	245	0.006156
PNOC	Prepronociceptin	178.4	0.00498
S100A12	S100 calcium binding protein A12	177.4	0.008513
SPP1	Secreted phosphoprotein 1	154.133	0.003085
SPIB	Spi-B transcription factor (Spi-1/PU.1 related)	151	0.014443
FPR1	Formyl peptide receptor 1	149	0.013784
MMP12	Matrix metallopeptidase 12	145.746	0.009168
TNFRSF17	Tumor necrosis factor receptor superfamily, member 17	86.0802	0.008041
Urine			
SOCS3	Suppressor of cytokine signaling 3	2,225.79	8.17E–06
CSF3R	Colony stimulating factor 3 receptor (granulocyte)	1,485.36	1.39E–05
CSF2RB	Colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	1,476.84	0.000134
TREM1	Triggering receptor expressed on myeloid cells 1	1,368.23	0.000295
SRGN	Serglycin	1,146.72	7.14E–06
IL1R2	Interleukin 1 receptor, type II	943.802	0.0003
IL1B	Interleukin 1, beta	837.648	1.60E–05
HCAR2	Hydroxycarboxylic acid receptor 2	807.059	6.73E–05
WAS	Wiskott-Aldrich syndrome	767.201	0.000178
TNFRSF1B	Tumor necrosis factor receptor superfamily, member 1B	697.8	0.000242
Blood			
LCN2	Lipocalin 2	17.7917	0.004986
IL1R2	Interleukin 1 receptor, type II	7.53815	0.004441
FPR1	Formyl peptide receptor 1	5.17999	0.001785
CLEC7A	C-type lectin domain family 7, member A	5.07934	0.002594
IRAK3	Interleukin-1 receptor-associated kinase 3	4.3436	0.00594
SOCS3	Suppressor of cytokine signaling 3	4.04615	0.045124
FKBP5	FK506 binding protein 5	3.57747	0.028315
CEACAM3	Carcinoembryonic antigen-related cell adhesion molecule 3	3.09848	0.020186
C5AR1	Complement component 5a receptor 1	3.07902	0.027357
B3GNT5	UDP-glcnac:betagal beta-1,3-N-acetylglucosaminyltransferase 5	2.87869	0.014248

S1P-induced increases of intracellular calcium influx. Their results suggested that calcium influx caused by S1P is mediated by TRPA1. Additional studies are needed to further understand these molecular underpinnings.

Our results, in the context of the above studies, elucidate a link between TRPA1 and S1PR in IC. *TRPA1* has a 1,098-fold expression increase in bladder tissue and *S1PR4* a 114-fold expression increase in urine in those with IC relative to asymptomatic controls. We propose that this combination may lead to an intracellular calcium influx and subsequent ATP release. Indeed, multiple studies have shown that ATP release in urothelial cells is controlled by a rise in intracellular calcium concentration (28,29). It is known that ATP is released from the urothelium in the context of bladder stretch. In a feline model for IC, it has been shown that swelling-evoked ATP release in IC cells was elevated relative to cells of normal cats (30). Further, bladder urothelial cells in patients with IC had augmented extracellular ATP release relative to those of healthy controls (31).

TNFRS17, also known as B-cell maturation antigen (*BCMA*), is preferentially expressed in mature B lymphocytes, and has been implicated in cell development and autoimmune response. Until now, it has not been detected in the bladder. *TNFRS17* has been implicated in lymphomas, leukemias, and multiple myeloma, and autoimmune diseases including SLE. *TNFRS17* has been shown to have inflammatory effects on myeloid cells, and to be expressed in macrophages (32). *TNFRS17* has also been identified as a suitable candidate for CAR-T therapy in multiple myeloma (33).

One of our patients was on cyclosporine therapy. Although inflammatory gene expression levels were significantly lower, as expected, in blood, urine, and tissue, *TRPA1* was not significantly decreased in the cyclosporine patient's bladder tissue. This finding, in the context of our other data and the literature, supports the importance of the identification of novel inhibitors of TRPA1 for the treatment of IC. Given that TRPA1 antagonists have been developed for a variety of pain conditions, there is the possibility that therapy delivered systemically or intravesically could improve symptoms in IC, sparing the long-term toxicities of cyclosporine.

Strengths of this study include the broad range of genes tested and inclusion of a healthy control group. Limitations include the relatively small sample size, a split between control samples for fluids and tissue, an age difference between groups, and lack of histological or proteomic analysis. The small sample size reflects the rarity of IC, particularly in the male population with Hunner lesions. Indeed, the female to male ratio is 5:1, the minority of these patients harbors Hunner lesions, and there has been minimal investigation in this population (34-36). Further, cyclosporine use in the population is exceedingly rare, as it is currently recommended as a fifth line treatment for IC by the American Urological Association (37). Our study population was confined to men, because of their unique challenges and misdiagnoses, the paucity of literature on men with IC, and because they are the focus of the clinical practice. The study calls for further investigations to generalize these findings to women and other patients with IC. Future work should also address the differential genetic signatures of men with IC as compared to those with chronic prostatitis, or benign prostatic hyperplasia, as there is overlap in these conditions and their differentiation in practice can be challenging. Further, the study was not designed to assess for outcomes of IC interventions based on genetic signatures, but this will be a future focus.

In conclusion, men with IC and Hunner lesions have a diverse set of neuroinflammatory genes with differential expression compared to controls. In addition to the expected inflammatory genes, we identified genes linked to neuropathic pain through the TRP pathway and this expression was not reduced by cyclosporine. These findings open a new direction for biomarker and therapeutic discovery.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.org/10.21037/tau-21-657

Data Sharing Statement: Available at https://dx.doi.org/10.21037/tau-21-657

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional IRB (#19-1515) and informed consent was taken from all the patients.

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