Original Article

Neuroinflammatory gene expression in chronic prostatitis/chronic pelvic pain syndrome patients: insights into etiology and phenotype biology

Daniel A. Shoskes, Karen S. Keslar, Paige Gotwald, Ryan Berglund, Sarah Vij

Glickman Urological and Kidney Institute, The Cleveland Clinic, Cleveland, OH, USA

Contributions: (I) Conception and design: DA Shoskes, KS Keslar; (II) Administrative support: P Gotwald; (III) Provision of study materials or patients: DA Shoskes, R Berglund, S Vij; (IV) Collection and assembly of data: DA Shoskes, P Gotwald, KS Keslar; (V) Data analysis and interpretation: DA Shoskes, KS Keslar; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Daniel A. Shoskes. Cleveland Clinic, 9500 Euclid Ave, Desk Q10-1, Cleveland OH, 44195, USA. Email: dshoskes@gmail.com.

Background: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) has diverse clinical phenotypes and its etiology is multifactorial. Studies to date of gene expression in humans have been limited to small numbers of target genes. NanoString can simultaneously measure hundreds of genes. We wished to study gene expression in blood and urine of CP/CPPS patients compared to controls for neuroinflammatory genes and characterize the results by patient phenotype.

Methods: Blood and urine were collected from 10 men with CP/CPPS and 7 asymptomatic controls. RNA was isolated from urine pellets using Qiagen RNeasy kits. Whole blood was collected and RNA isolated. 100 ng of RNA was used for gene expression analysis with the 770-gene NanoString Human Neuroinflammation gene panel. Data was imported into Rosalind (OnRamp Bioinformatics) for normalization, calculation of fold-changes and P values, and identification of enriched pathways. Gene expression was considered significantly different if there was a greater than 1.5× change compared to controls and corrected P was <0.05.

Results: Mean patient age was 42.2 years, median symptom duration was 15.5 months, median UPOINT domains was 3 and mean total National Institute of Health-Chronic Prostatitis Symptom Index Score was 28.8. In blood, there were 5 genes with significantly different expression to controls, the largest differences found in FOS1 (neuropathic pain control), PROS1 (blood clotting) and DDX58 (antiviral innate immunity). Gene set analysis showed differences in inflammation, angiogenesis and cytokine signaling. In urine there were 48 genes with significantly different expression including SLAMF8 (lymphocyte activation) and LAIR1 (inhibits B and T cell function). Gene set analysis showed differences in carbohydrate metabolism, neurons and neurotransmission, adaptive immunity and inflammatory signaling. Subgroup analysis by UPOINT domain showed unique gene expression in the Organ Specific and Neurologic/Systemic domains in both blood and urine for neurogenic pain and cytokine signaling associated genes

Conclusions: Men with CP/CPPS have a diverse set of neuroinflammatory genes with differential expression compared to controls. Clinical phenotypes have distinct patterns of gene expression. These findings could lead to novel biomarker development, emphasize the importance of multimodal therapy targeting diverse pathways and further validate the biologic basic of clinical phenotyping.

Keywords: Prostatitis; chronic pelvic pain; inflammation

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Introduction

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a common condition with diverse clinical phenotypes (1). A specific etiology for all patients is elusive, with prior evidence for infective, inflammatory, and neuromuscular pathways (2). Prior attempts to find biomarkers for these etiologic pathways have focused on expression of several inflammatory genes in urine and blood (3), genetic polymorphisms (4) and the mediators of neuropathic pain such as nerve growth factor (NGF) (5). Such attempts have been limited by the techniques employed, which can only look at several genes at a time in one sample.

The NanoString nCounter is a novel technology that allows direct measurement of mRNA expression with a small sample for a large number of genes without the need for conversion to cDNA or polymerase chain reaction (6). A premade cassette has almost 800 genes related to inflammation and neuropathic pain. We therefore wished to study the gene expression in blood and urine for this large number of neuroinflammatory genes in men with CP/CPPS compared to asymptomatic controls. This approach allows the simultaneous measurement of related genes whose key role in pathophysiology may lie more in their orchestrated expression rather than the levels of any one gene at a time. We hypothesize that men with CP/CPPS will have unique RNA expression signatures and that men with different clinical phenotypes may have different pathways active.

Methods

The study was approved by the Cleveland Clinic IRB (protocol 19-1515) and appropriate written consent was obtained on all subjects. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). Blood and midstream urine were collected from 10 men presenting to a specialty prostatitis clinic with CP/CPPS using the National Institute of Health (NIH) definition of category III (7) as well as from 7 asymptomatic controls presenting for a vasectomy between January and December 2020. Symptom severity was assessed with the NIH-Chronic Prostatitis Symptoms Index (8) and clinical phenotype documented with UPOINT (9).

RNA was isolated from urine pellets using Qiagen RNeasy kits. Whole blood was collected and RNA isolated using the Tempus Spin RNA isolation kit. 100 ng of RNA was used for gene expression analysis with the 770-gene NanoString Human Neuroinflammation gene panel.

Statistical analysis

Data was imported into Rosalind (OnRamp Bioinformatics) for normalization, calculation of fold-changes and p values, and identification of enriched pathways. MultiDimensional Scaling (MDS) graphs were generated for individual samples. Gene expression was considered significantly different if there was a greater than 1.5x change compared to controls and P was <0.05 corrected for multiple comparisons.

Results

Of the 10 men with CPPS, the mean age was 42.2 years (range 22–59 years) and median symptom duration 15.5 months (range 3 months to 35 years). Using clinical phenotyping there were a median of 3 UPOINT domains positive (range 2–4) however no patients had evidence for infection ("I" domain) and only 1 patient didn't have bothersome urinary symptoms ("U" domain). The mean total NIH-CPSI score was 28.8 (range, 14–39). These values are all typical for CPPS patients we have studied and reported on in the past (10). The control asymptomatic men had a mean age of 42.6 years (range, 30–55 years).

Comparing gene expression between CP/CPPS and control in blood showed moderate differences. The multidimensional scaling (MDS) plot showed broad overlap in all samples (data not shown). There were only 4 genes with expression >1.5× different in CP/CPPS *vs.* controls and a P value <0.05 (*Figure 1*), with 1 being lower in CP/CPPS and 3 being higher (see *Table 1* for gene names and function). The only marginally significant gene set analysis difference between groups was for inflammatory signaling and NF-kB [significance score (SS) 1.11]. By contrast there were more differences seen for the UPOINT phenotypes that had sufficient heterogeneity in the sample. In our patient cohort nobody had the I domain (infection), only 1 didn't have the U domain (urinary) and only 1 didn't have the T domain (tenderness). As seen in *Figure 2*, the MDS plots for yes or no for Organ Specific, Psychosocial, and Neurogenic/Systemic all showed evidence for grouping. For Organ Specific (yes *vs.* no) there were 16 genes with significantly different expression led by BCL2A1 (–2.1× change, P=0.03), CST7 (–1.9× change, P=0.03) and IL1B (–1.9×, P=0.04). By gene set analysis grouping

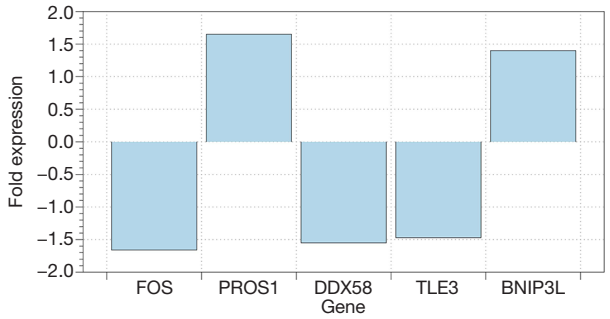

Figure 1 Mean differential gene expression between chronic prostatitis/chronic pelvic pain syndrome patients and controls in blood. Genes listed had at least 1.5× differential expression with P<0.05. For full gene function please see *Table 1*. FOS FBJ murine osteosarcoma viral oncogene homolog, PROS1 Protein S (alpha), DDX58 Dead Box polypeptide 58 (also called RIG-1), TLE3 Transducer like enhancer of split 3, BNIP3L BCL2/adenovirus E1B interacting protein.

there were differences in carbohydrate metabolism (SS 1.4), lipid metabolism (SS 1.3) and NF-kB expression (SS 1.3). Neurologic/Systemic had no genes with differential expression and Psychogenic had only 1 and gene set analysis showed no clear pathway clusters.

By contrast, gene expression between CP/CPPS and control urine samples showed more differences in extent and degree. MDS plots showed clear segregation between CP/CPPS and vasectomy (*Figure 3*). There were 48 genes with expression >1.5× different in CP/CPPS *vs.* controls and a P value <0.05 with greatest differences seen for SLAMF8 (22.3× higher, P=0.0004), LAIR1 (18.8× higher, P=0.004) and C1QC (16.5× higher, P=0.01) (*Figure 4*). Gene set analysis was different for a number of pathways including carbohydrate metabolism (SS 1.8), Neurons and Neurotransmission (SS 1.5), Adaptive Immunity (SS 1.4) and Inflammatory Signaling (SS 1.3). More impressive differences were seen between phenotypes. For Organ Specific (yes *vs.* no) there were 58 genes with significantly different expression led by S100A12 (−298× change, P=0.03), ILIRN (−65.3× change, P=0.008) and OSMR (−49.4×, P=0.004). By gene set analysis grouping there were differences in Astrocyte function (SS 1.6), Cytokine signaling (SS 1.5), Innate Immune Response (SS 1.5) and Growth factor signaling (SS 1.4). Neurologic/Systemic had 4 genes with differential expression including S100A12 (−121× change, P=0.009) and STEAP4 (−26.9× change, P=0.01). Gene set analyses did not show distinct groupings.

Discussion

CP/CPPS is a syndrome characterized by pelvic and genital pain often accompanied by urinary symptoms and sexual dysfunction in the absence of acute or recurrent urinary tract infection (7). No single common etiology appears to explain clinical findings and risk factors (11), with evidence for infection, inflammation, muscle spasm, neuropathic activation and central neurologic changes found depending on the patient population. Lack of uniformity in presentation and response to therapy led to the development of the UPOINT clinical phenotyping system which classifies patients according to 6 clinical domains and guides multimodal therapy based on those findings (10). The domains are clinically diagnosed and have not been externally validated by other biomarkers.

Prior attempts to differentiate CP/CPPS patients from controls by findings or biomarkers have been met with variable success. There is no difference in rates of infection or inflammation (as measured by presence of white cells) in urine or prostatic fluid (12), however there are changes in microbial ecology both in the urine (13) and bowel (14). Several studies have shown differences in specific cytokines between CP/CPPS patients and controls both for expression (3) and genetic polymorphisms (4). Such work has been limited by the number of genes that could be practically analyzed simultaneously. Newer technologies such as NanoString allow the assessment of a large number of genes on relatively small samples. This is especially important since, while a single preselected gene might have a relatively modest increased expression, such modest expression of multiple genes within the same pathway may have a powerful biologic effect. Given the importance of inflammation and neuropathic pain in the proposed etiology of CP/CPPS we chose the premade NanoString neuroinflammation panel for this study.

The first striking finding for gene analysis in our samples was that there were more differences seen between CP/CPPS patients with different phenotypes than there were between patients and controls. This finding strongly supports the hypothesis that CP/CPPS is not a single disease with a common underlying biology for all or most patients but rather a syndrome with heterogeneous phenotypes that have a different biologic basis (15). The second surprising finding was that the majority of differences in gene expression and biologic pathways was for inflammation

Table 1 List of genes discussed in the paper with full names and function

Gene	Full name	Function	Disease association	Significant change in CPPS
BCL2A1	BCL2 related protein A1	Inflammatory mediated apoptosis		Blood Organ Specific domain
BNIP3L	BCL2/adenovirus E1B interacting protein	Inhibits apoptosis from viral infection		Blood CPPS *vs.* Control
C1QC	Complement component 1	Activates complement	Low in Lupus	Urine CPPS *vs.* Control
CD68	Cluster of Differentiation 68	Macrophage marker	Gaucher's disease	Urine CPPS *vs.* Control
CST7	Cystitin F	Immune Regulation		Blood Organ Specific domain
DDX58	Dead Box polypeptide 58 (also called RIG-1)	Antiviral innate immunity		Blood CPPS *vs.* Control
FOS	FBJ murine osteosarcoma viral oncogene homolog	Cell growth, marker of neuronal activity	Neuropathic pain	Blood CPPS *vs.* Control
IL1B	Interleukin-1	Inflammatory cytokine	Autoimmune diseases	Blood Organ Specific domain
ILIRN	Interleukin-1 receptor antagonist	Inhibition of IL-1		Urine Organ Specific domain
LAIR1	Leukocyte associated immunoglobulin-like receptor 1	Inhibition of T and B cells		Urine CPPS *vs.* Control
LILRB4	Leukocyte Ig like receptor, subfamily B member 4 (CD 85K)	Monocyte receptor, inhibits immune response		Urine CPPS *vs.* Control
OSMR	Oncostatin M receptor	Cytokine receptor	Primary cutaneous amyloidosis	Urine Organ Specific domain
PROS1	Protein S (alpha)	Vitamin K dependent anticoagulation		Blood CPPS *vs.* Control
S100A12	S100 calcium binding protein A12	Monocyte antibacterial function	Lupus, inflammatory bowel disease	Urine CPPS *vs.* Control and Organ Specific, Neurologic domains
SLAMF8	Slam Family Member 8	Lymphocyte activation		Urine CPPS *vs.* Control
SLC02B1	Solute carrier organic anion transporter family 2B1	Membrane transport protein		Urine CPPS *vs.* Control
STEAP4	STEAP family member 4	Adipocyte development	Prostate cancer	Blood CPPS *vs.* Control and Urine Neurologic domain
TLE3	Transducer like enhancer of split 3	Transcriptional co-repressor	Prognosis marker for some cancers	Blood CPPS *vs.* Control

CPPS, chronic pelvic pain syndrome.

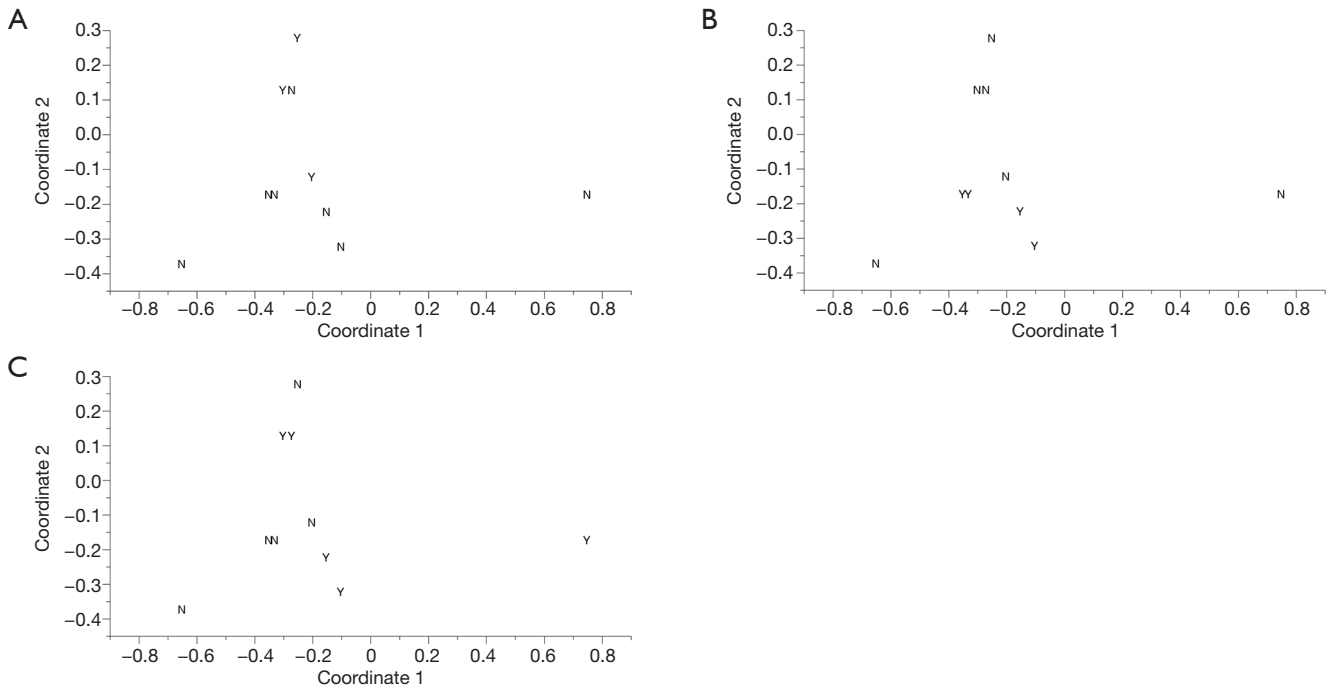

Figure 2 Multidimensional Scaling (MDS) Plots of gene expression for individual chronic prostatitis/chronic pelvic pain syndrome patients in blood by presence of UPOINT domain. Each patient represented by a Y (yes, domain present) or N (no, domain not present). For exactly overlapping data points the 2 symbols were placed side by side. Axis are arbitrary units and overall differences in gene expression are represented by the distance between any two points. (A) Organ Specific Domain. Each patient represented by a Y (yes, Organ specific domain present) or N (no, Organ specific domain not present). (B) Psychosocial Domain Each patient represented by a Y (yes, Psychosocial Domain domain present) or N (no, Psychosocial Domain domain not present). (C) Neurogenic/Systemic Domain Each patient represented by a Y (yes, Neurogenic/Systemic domain present) or N (no, Neurogenic/Systemic domain not present).

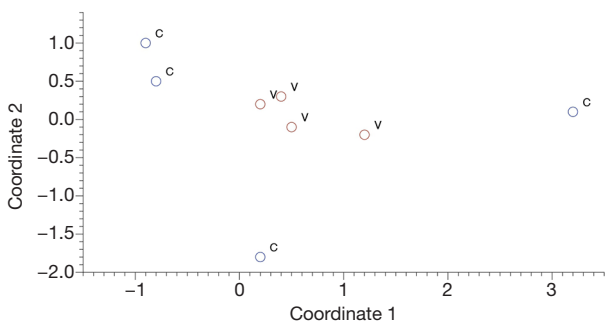

Figure 3 Multidimensional Scaling Plots for chronic prostatitis/chronic pelvic pain syndrome patients (C) and vasectomy controls (V) in urine. Axis are arbitrary units and overall differences in gene expression are represented by the distance between any two points.

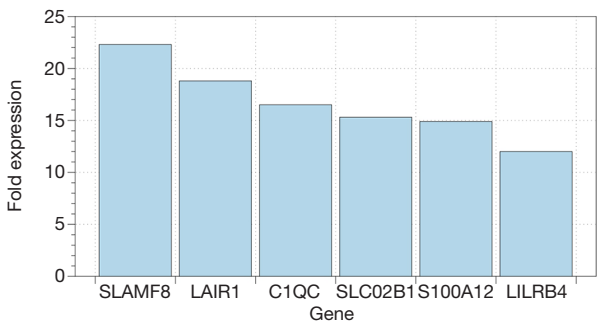

Figure 4 Mean differential gene expression between chronic prostatitis/chronic pelvic pain syndrome patients and controls in urine. Genes listed had at least 10× differential expression with P<0.05. For full gene name and function please see *Table 1*.

rather than neuropathic pain and none of the neuropathic genes were different for patients with the Neurologic/Systemic or Psychogenic domains. Of note, there were patients in the Neurologic/Systemic group with profound systemic symptoms including debilitating fibromyalgia. Even genes strongly associated with neuropathic pain and

previously found in prostate fluid of CP/CPPS patients such as nerve growth factor (NGF) (5) did not have differential expression in urine or blood. This is counter-intuitive to our current thinking of CP/CPPS patients with systemic symptoms having more in common with other centrally mediated neuropathic pain syndromes (16). Indeed, such changes may be present in these patients but not only in organ sites not samples by blood or urine. Furthermore, the presence or transit time of these cells in the blood may make sampling more difficult than cells recovered from the urine.

While the findings in blood did not demonstrate major differences, we did find differential inflammatory and neuropathic gene expression in urine. The greatest changes were seen in genes such as SLAMF8 (17) and LAIR1 (18) which regulate monocytes, T and B cells. Gene set differences included both neurotransmission and inflammation. It has been a central paradox in CP/CPPS that even though "prostatitis" implies inflammation, only a small minority of these patients have inflammation visible on prostate biopsy specimens (19). Nevertheless, inflammatory mediators have been found in the prostate fluid (20) and many patients do respond to anti-inflammatory based therapies (21). Even with a shift in thinking of CP/CPPS as more of a neuromuscular or neuropathic condition, there is persistent evidence of immune cell involvement even if biopsy evidence of inflammation or presence of white cells in expressed prostatic secretions fail to correlate with specific symptoms (22). This can explain in part symptomatic improvement from antibiotics such as quinolones that have immunomodulatory effects (23) and nutraceuticals with anti-oxidant properties such as quercetin (21) and cernilton (24). Such immunomodulation may be insufficient however without multimodal therapy that addresses both muscular spasm (25) and neuropathic pain (26).

Strengths of this study include the broad range of genes tested, inclusion of a well-matched control group and complete clinical phenotyping of the CP/CPPS patients, a feature lacking in all prior genetic studies. Limitations include the relatively small numbers which did not allow a sufficient heterogeneity in clinical phenotypes to adequately examine the impact of the Urinary symptoms or Tenderness of Muscle domains on genetic expression. We also did not have long term follow up to determine whether specific treatments resulted in success based on the gene expression.

In conclusion, men with CP/CPPS have a diverse set of neuroinflammatory genes with differential expression compared to controls. Furthermore, clinical phenotypes have distinct patterns of gene expression and differences between phenotypes were more pronounced than between patients and controls. Surprisingly, those with systemic symptoms of neuropathic conditions did not show differences in neuropathic genes, at least in the blood. This approach could lead to novel biomarker development and further validates the biologic basic of clinical phenotyping.

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Footnote

Data Sharing Statement: Available at https://dx.doi.org/10.21037/tau-21-387

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tau-21-387). *DAS* serves as an unpaid editorial board member of *Translational Andrology and Urology* from Aug 2020 to Jul 2022. DAS consults for Utility Pharmaceuticals and Urogen and has investment interest in Triurol. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work (if applied, including full data access, integrity of the data and the accuracy of the data analysis) in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Cleveland Clinic IRB (protocol 19-1515) and appropriate written consent was obtained on all subjects. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

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