Correlation of Gene Expression with Bladder Capacity in Interstitial Cystitis/Bladder Pain Syndrome

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Purpose: Interstitial cystitis and bladder pain syndrome are terms used to describe a heterogeneous chronic pelvic and bladder pain disorder. Despite its significant prevalence, our understanding of disease etiology is poor. We molecularly characterized interstitial cystitis/bladder pain syndrome and determined whether there are clinical factors that correlate with gene expression.

Materials and Methods: Bladder biopsies from female subjects with interstitial cystitis/bladder pain syndrome and female controls without signs of the disease were collected and divided into those with normal and low anesthetized bladder capacity, respectively. Samples then underwent RNA extraction and microarray assay. Data generated by these assays were analyzed using Omics Explorer (Qlucore, Lund, Sweden), GeneSifter® Analysis Edition 4.0 and Ingenuity® Pathway Analysis to determine similarity among samples within and between groups, and measure differentially expressed transcripts unique to each phenotype.

Results: A total of 16 subjects were included in study. Principal component analysis and unsupervised hierarchical clustering showed clear separation between gene expression in tissues from subjects with low compared to normal bladder capacity. Gene expression in tissue from patients with interstitial cystitis/bladder pain syndrome who had normal bladder capacity did not significantly differ from that in controls without interstitial cystitis/bladder pain syndrome. Pairwise analysis revealed that pathways related to inflammatory and immune response were most involved.

Conclusions: Microarray analysis provides insight into the potential pathological condition underlying interstitial cystitis/bladder pain syndrome. This pilot study shows that patients with this disorder who have low compared to normal bladder capacity have significantly different molecular characteristics, which may reflect a difference in disease pathophysiology.

Key Words: urinary bladder; cystitis, interstitial; gene expression; pain; microarray analysis

Abbreviations and Acronyms

BPS = bladder pain syndrome

DET = differentially expressed transcript

FDR = false discovery rate

IC = interstitial cystitis

PCA = principal component analysis

PUF = Pelvic Pain and Urinary Frequency patient symptom scale

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PAINFUL bladder syndrome, ICS and BPS are terms used to describe a chronic disease of uncertain etiology that primarily affects women. Patients experience vague pelvic pain that can be exacerbated by bladder filling and is often associated with urinary frequency and urgency.[1] IC/BPS is a relatively common entity projected to affect approximately

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3 to 8 million women and 1 to 4 million men in the United States.[2,3] It incurs as much as $750 million in medical costs per year.[1] However, despite this burden the etiology of IC/BPS is still poorly understood.[4] Theories include urothelial dysfunction,[5–7] mast cell overactivity,[8,9] neurogenic inflammation[10,11] and an allergic or autoimmune response[12] but evidence is sparse. The presentation is heterogeneous. While the classic presentation involves Hunner ulcers or glomerulations, neither is a prerequisite for diagnosis. Moreover, such lesions develop in few patients who present without ulcers. As such it is believed that ulcerative and nonulcerative IC may be separate subtypes of the same disorder or entirely distinct entities with similar symptoms.[13]

Currently there is no absolute histological or pathological marker for IC/BPS, which is a clinical diagnosis of exclusion. While evaluation requires only confirmed negative urinalysis and urine culture as well as a thorough history and physical examination, patients often undergo prolonged evaluations with invasive testing such as cystoscopy, urodynamics and biopsy to rule out confounding processes before a presumed diagnosis of IC/BPS is made.

In this pilot study we examined gene expression in bladder tissue from female patients who had IC/BPS with varying clinical presentations. We evaluated several clinically relevant parameters as delineators of molecular variation, including bladder capacity during anesthesia measured at 100 ml H_2O pressure, symptomology using validated questionnaires, mastocytosis, presence or absence of glomerulations, symptom history and presence or absence of ulcerations. Although others attempted to molecularly characterize ulcerative[14] and nonulcerative disease,[15] to our knowledge these findings have never previously been correlated with the clinical presentation.

MATERIALS AND METHODS

Subjects

Permission to perform this study was obtained from the Wake Forest University Health Sciences institutional review board. Experimental subjects were prospectively enrolled from the population of female patients between ages 18 and 80 years who presented for evaluation of IC/BPS with no history of another bladder pathology. Controls were drawn from the population of female patients who presented to the same urology clinic for urological evaluation requiring biopsy unrelated to IC/BPS.

Clinical Evaluation and Subject Grouping

Before cystoscopy all subjects underwent assessment, including history and physical examination. The clinical diagnosis of IC/BPS was based on the most current American Urological Association guidelines definition, "An unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms of more than six weeks duration, in the absence of infection or other identifiable causes."[16] Patients were also asked to complete ICSI (Interstitial Cystitis Symptom Index), ICPI (Interstitial Cystitis Problem Index) and PUF preoperatively. These instruments are valid measurements of IC/BPS symptomology and provide a baseline to track the response to therapy.[17,18]

Tissue Procurement

Experimental tissue was collected with the patient under general anesthesia at cystoscopy or surgery, the latter in those undergoing cystectomy for end stage disease. For cystoscopy patients first underwent hydrodistention at 100 ml H_2O for 5 minutes. After hydrodistention the bladder was emptied and the degree of glomerulations was assessed based on the ICDB (Interstitial Cystitis Database) criteria of none, mild, moderate or severe.[19] All cystoscopies and glomerulations were graded by a single physician. After hydrodistention study biopsies were taken from the posterior bladder wall using a cold cup technique. According to standard hospital protocol a portion of each biopsy specimen was sent to the Wake Forest Medical Center pathology department for normal clinical analysis. The remaining sample was immediately submerged in 200 μl RNAlater® and stored at −20C until use.

For patients who underwent cystectomy an amount of tissue similar to what would be collected at biopsy was harvested from the posterior bladder through a single scalpel incision, submerged in 200 μl RNAlater and stored at −20C in similar fashion. Bladder capacity data and cystoscopic findings were retrieved from the patient last cystoscopy recording in the medical record and included in this analysis.

Control tissue was likewise collected during cystoscopy. Since these patients had no clinical indication for hydrodistention, this procedure was not done.

Patient Groups

After evaluating cystoscopy results the patients were divided for molecular analysis into group 1—normal capacity (normal anesthetized bladder capacity, defined as 400 ml or greater during hydrodistention), group 2—low capacity (low anesthetized bladder capacity, defined as less than 400 ml during hydrodistention) and group 3—control (no history of IC/BPS).

Low bladder capacity was defined as less than 400 ml because in the past patients with a bladder capacity of less than 400 ml had significantly better results after surgical treatment of IC/BPS (cystectomy), suggesting that this may represent an alternate pathology than normal capacity disease or a more advanced disease state.[20] Secondary analysis was done using patient subjective symptom scores, degree of glomerulations and tissue mast cell counts as delineating factors.

Sample Processing

Biopsy tissue was homogenized by sonication. Total RNA was extracted using RNeasy® MinElute™ Plus columns,