



Data in Brief

Transcriptome analysis of bladder biopsy from interstitial cystitis/bladder pain syndrome patients



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ABSTRACT

Interstitial cystitis and bladder pain syndrome (IC/BPS) are terms used to describe a heterogeneous chronic pelvic and bladder pain disorder. Despite its significant prevalence, the disease etiology is not well understood and providing diagnosis and treatment can be challenging. In our study, published recently in the *Journal of Urology* (Colaco et al., 2014), we describe the use of microarrays as a tool to characterize IC/BPS and to determine if there are clinical factors that correlate with gene expression. This data-in-brief article describes the methodology for that study, including data analysis, in further detail. Deposited data can be found in the Gene Expression Omnibus (GEO) database: [GSE57560](http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57560).

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Specifications

Organism/cell line/tissue	<i>Homo sapiens</i>
Gender	Female
Strain(s)	Patient bladder biopsy tissue
Sequencer or array type	SurePrint Human Gene Expression v2 microarrays (Agilent)
Data format	Raw data, extracted from scanned images using Agilent Feature Extraction Software
Experimental factors	Low bladder capacity versus normal capacity and control
Consent	All patients gave written informed consent for this study.

Direct link to deposited data

Deposited data can be found in the Gene Expression Omnibus (GEO) database: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57560>. [GSE57560](http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57560) – Correlation of gene expression with bladder capacity in interstitial cystitis/bladder pain syndrome.

Experimental design, materials and methods

Sample procurement

A flow diagram outlining of the experimental design for this study can be found in Fig. 1. Experimental biopsy tissue was collected either

during cystoscopy or at surgery (for patients who were undergoing cystectomy for end stage disease) under general anesthesia from patients diagnosed with interstitial cystitis/bladder pain syndrome (IC/BPS) [1–5]. Cystoscopy patients first underwent hydrodistention at 100 mL of H₂O for a period of 5 min. Study biopsies were taken post hydrodistention from the posterior bladder wall using a cold-cup technique and a portion of each biopsy specimen was sent for normal clinical analysis by the Wake Forest Medical Center pathology department (following standard hospital protocol). The remaining sample was immediately submerged in 200 µL of RNeasy Lysis Buffer and stored at –20 °C until processing. For patients undergoing cystectomy, an amount of tissue similar to that which would be collected at biopsy was harvested from the posterior bladder through a single scalpel incision and submerged in 200 µL of RNeasy Lysis Buffer and stored at –20 °C in a similar fashion. Bladder capacity data and cystoscopic findings for these subjects were retrieved from the patient's last cystoscopy recording in the medical record and included in this analysis (Table 1). For the purposes of this study, low bladder capacity was defined as <400 mL (volume at hydrodistention) [6].

Control tissue was likewise collected during cystoscopy. As these patients did not have any clinical indication for the performance of hydrodistention, this procedure was not done (Table 1).

RNA extraction and QC

Biopsy tissue was homogenized by sonication and total RNA was extracted using RNeasy Minelute Plus columns (includes on-column DNase digestion) and reagents (Qiagen, Valencia, CA) according to the

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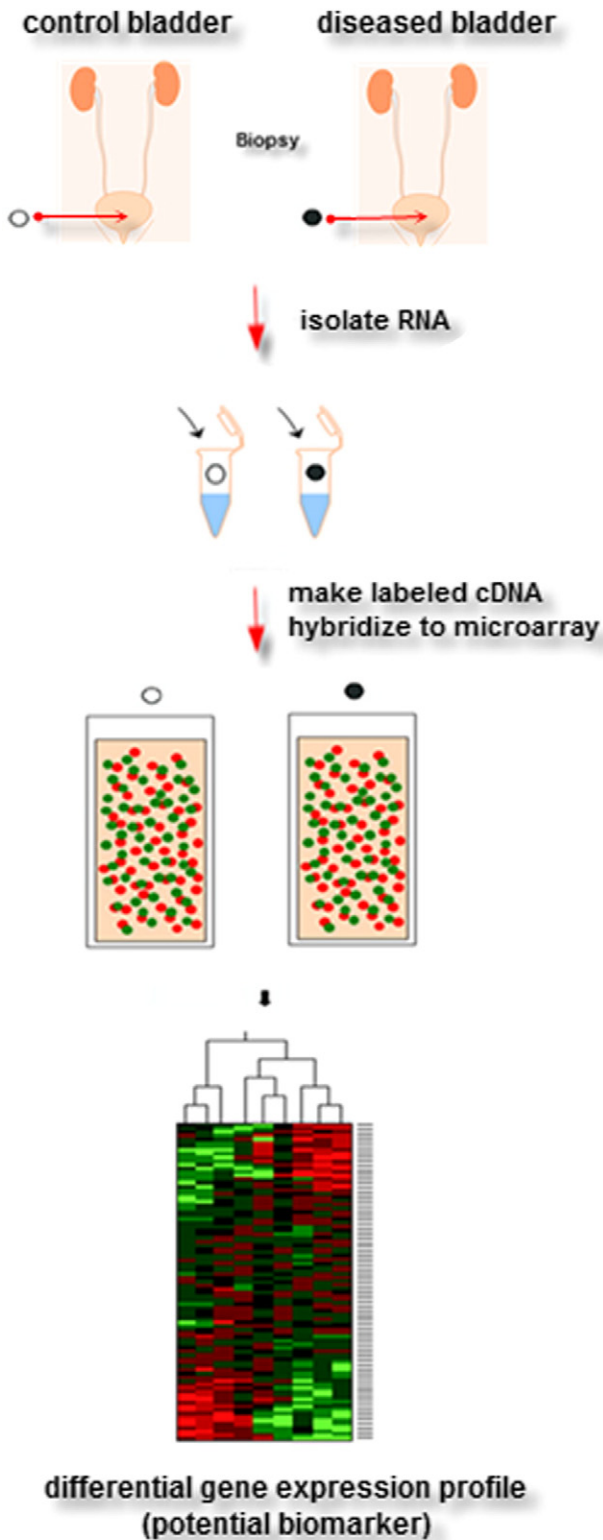


Fig. 1. Flow diagram representing the outline of experimental steps. Briefly, total RNA, isolated from bladder biopsies procured in the clinic, was reverse transcribed, labeled, and hybridized to whole genome microarrays. The arrays were then scanned and the data were analyzed for differential gene expression between disease and control groups.

manufacturer's instructions. RNA was eluted from the columns in nuclease-free water and RNA quantity and purity was determined spectrophotometrically on a Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, DE) by measuring absorbance at 260/280 nm (mean for all samples = 2.09 ± 0.02) and RNA quality was determined using an

Agilent Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA; mean RIN = 9.4 ± 0.24 for all samples). Total RNA was shipped on dry ice to a microarray core facility (City of Hope Functional Genomics Core, Duarte, CA) for processing.

Gene expression analysis by microarray

Cyanine-3 (Cy3) labeled cRNA was prepared from 0.5 μ g total RNA using the One-Color Low RNA Input Linear Amplification PLUS kit (Agilent) according to the manufacturer's instructions, followed by RNeasy column purification (QIAGEN, Valencia, CA). Dye incorporation and cRNA yield were checked with the NanoDrop ND-1000 Spectrophotometer. 1.5 μ g of Cy3-labeled cRNA (specific activity >10.0 pmol Cy3/ μ g cRNA) was fragmented at 60 °C for 30 min in a reaction volume of 250 μ L containing 1 \times Agilent fragmentation buffer and 2 \times Agilent blocking agent following the manufacturer's instructions. On completion of the fragmentation reaction, 250 μ L of 2 \times Agilent hybridization buffer was added to the fragmentation mixture and hybridized to SurePrint Human Gene Expression v2 microarrays (Agilent Technologies) containing 60-mers for 50,599 biological features for 17 h at 65 °C in a rotating Agilent hybridization oven. After hybridization, microarrays were washed 1 min at room temperature with GE Wash Buffer 1 (Agilent) and 1 min with 37 °C GE Wash Buffer 2 (Agilent), then dried immediately by brief centrifugation. Slides were scanned immediately after washing on the Agilent DNA Microarray Scanner (G2505B) using one color scan setting for 1 \times 44 k array slides (Scan Area 61 \times 21.6 mm, Scan resolution 10 μ m, Dye channel is set to Green and Green PMT is set to 100%). The scanned images were analyzed with Feature Extraction Software 9.1 (Agilent) using default parameters (protocol GE1-v1_91 and Grid: 012391_D_20060331) to obtain background subtracted and spatially de-trended Processed Signal intensities. Features flagged in Feature Extraction as Feature Non-uniform outliers were excluded. The anonymized clinical data were deposited in the Gene Expression Omnibus database (GEO <http://www.ncbi.nlm.nih.gov/geo/> under accession number GSE57560).

Data processing for gene expression and gene ontology/pathway analysis

Following log transformation of the Processed Signal intensity data, principle component analysis (PCA) and unsupervised hierarchical clustering were performed (QIAGEN Omics Explorer) to determine the similarity among samples within and between groups (Fig. 2). Further statistical analyses to measure differentially expressed transcripts (DETs) unique to each phenotype were then performed by applying Student's t-test (@fold change ≥ 1.5 ; $p \leq 0.05$) for the pair-wise comparison (GeneSifter© Analysis Edition 4.0) using Benjamini and Hochberg FDR correction [7]. The list of DETs generated from the pair-wise comparison (e.g. *low bladder capacity* versus *normal bladder capacity*) with an adjusted $p \leq 0.05$ was imported into Ingenuity Pathway Analysis software for gene ontology and pathway analysis.

Discussion

Gene expression analysis provides insight into the pathobiology underlying IC/BPS. We demonstrate that low capacity and normal capacity IC/BPS bladders have significantly different molecular characteristics, and this difference may reflect a fundamental difference in disease processes. Meanwhile, IC/BPS patients with normal bladder capacity exhibit similar molecular profiles to control subjects [7]. Given the promising results of this pilot study we are conducting further research into the correlation between molecular and clinical findings and the development of an IC/BPS biomarker.

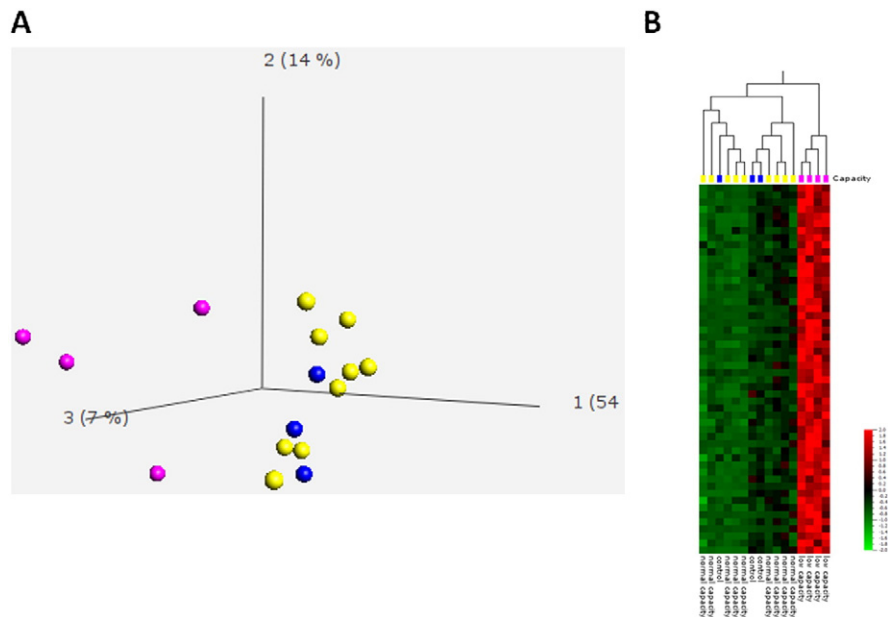


Fig. 2. Gene expression in IC cases and controls. Hierarchical clustering, based on bladder capacity, was performed on whole genome microarray data. For the principle components analysis (PCA; Panel A) the significance threshold was set at $p \leq 0.05$. Each colored circle on the plot represents the cumulative expression of 4363 transcripts for that sample (Panel A). The heatmap displays the top 48 differentially expressed genes based on bladder capacity (Panel B). Panels A & B: violet = low capacity; yellow = normal capacity; blue = controls.

Table 1
Clinical data and demographic information for cases and controls. Demographic and clinical data for each of the 16 subjects. Subject #11 had biopsy performed during cystoscopy then underwent cystectomy at a later date.

Subject	Age	Capacity	O'Leary-Sant	PUF	Glomerulations	Ulceration	Cystectomy
Normal capacity							
1	47	900	30	26	Mild	No	No
2	27	1400	30	25	Mild	No	No
3	57	1350	16	17	Moderate	No	No
4	32	900	26	26	Moderate	No	No
5	37	1000	28	30	Severe	No	No
6	25	900	31	27	Severe	No	No
7	24	1200	19	22	Severe	No	No
8	23	600	33	27	Severe	No	Yes
9	63	400	23	15	Mild	No	No
Low capacity							
10	57	300	32	26	Severe	No	No
11	46	225	35	30	Moderate	No	Yes
12	67	175	35	33	Severe	Yes	Yes
13	65	275	25	17	Mild	Yes	Yes
Control							
14	76	Not reported	Not reported	Not reported	NA	No	No
15	43	1600	14	14	NA	No	No
16	53	850	25	25	NA	No	No

Conflict of interest

The authors declare no competing interests.

Acknowledgments

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