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Peptidomics analysis reveals changes in small urinary peptides in patients with interstitial cystitis/bladder pain syndrome

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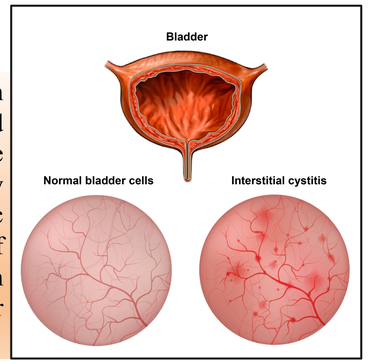
Overview

What is interstitial cystitis

Interstitial cystitis or bladder pain syndrome (IC/BPS) is a chronic and debilitating pain disorder of the bladder and urinary tract with poorly understood etiology. Symptomatic criteria to aid in the diagnosis of IC/BPS includes bladder pain, an increase in urinary urgency or Hunner's ulcers on the bladder wall.



Challenges

High overlap of IC/BPS symptoms with other urological conditions, the diagnosis of IC/BPS remains a significant challenge.

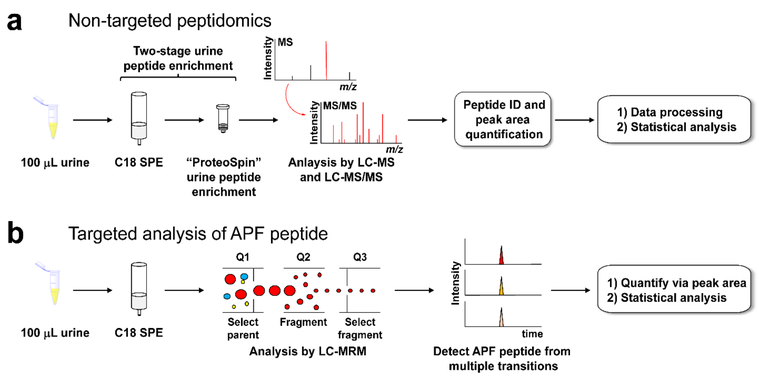
The methods traditionally used in the diagnosis of IC/BPS are highly invasive, including hydrodistension, bladder biopsy, or cystoscopy.

Objectives

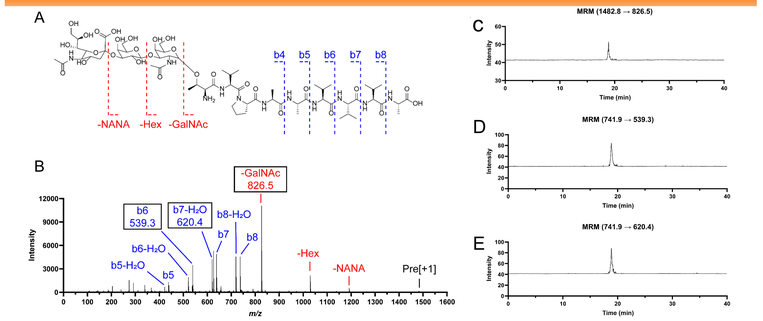
Developing and applying a targeted LC-multiple reaction monitoring (MRM) method to compare the relative quantities of the APF peptide present in urine from both IC/BPS patients and asymptomatic controls.

Exploring differences in the profile of small urinary peptides in IC/BPS from urine collected from IC/BPS patients and asymptomatic controls.

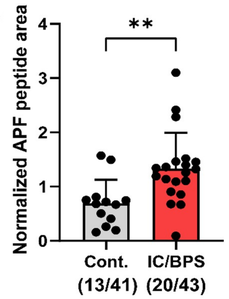
Approaches



Results

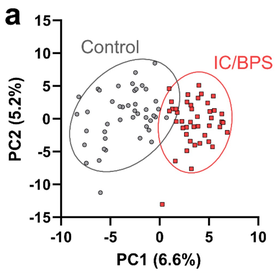


(a) Chemical structure of APF peptide. (b) Annotated MS/MS spectrum (Q-TOF, CID) of APF. (C-E) Representative LC-MRM chromatograms of synthetic APF.



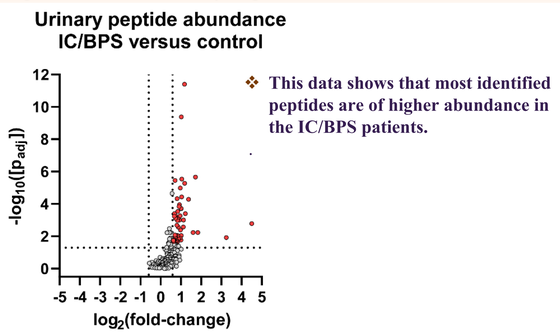
- **Bars show the mean and standard deviation of the normalized peak areas. **p < 0.01 (unpaired t-test).**
- **All data were normalized by dividing the peak area for the APF peptide by the peak area for the internal standard peptide ($FMRGF\text{-}NH_2$).**

Relative abundance of the APF peptide in the urine samples



- **PCA and PLS-DA scores plot shows separation between IC/BPS patients and healthy controls based on peptide profiles.**

(a) PCA and (b) PLS-DA scores plots of non-targeted LC-MS and LC-MS/MS data after preprocessing and normalization by EigenMS.



- **This data shows that most identified peptides are of higher abundance in the IC/BPS patients.**

Volcano plot of detected peptides. Red data points indicate the significantly changing peptides with a >1.5-fold higher abundance in the urine samples from the IC/BPS patients.

Results

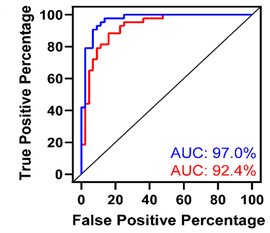
Protein name	Uniprot ID	Gene name	Number of peptides
Osteopontin*	OSTP	SPP1	9
Uromodulin*	UROM	UMOD	6
Polymeric immunoglobulin receptor	PIGR	PIGR	5
CD99 antigen	CD99	CD99	3
Inter-alpha-trypsin inhibitor heavy chain H4*	ITIH4	ITIH4	3
Secreted and transmembrane protein 1	SCTM1	SECTM1	3
Protein AMBP	AMBP	AMBP	2
Complement C1r subcomponent-like protein	C1RL	C1RL	2
Collagen alpha-1(III) chain	CO3A1	COL3A1	2
Endothelial protein C receptor	EPCR	PROCR	2
Hemoglobin subunit beta	HBB	HBB	2
Insulin	INS	INS	2
Kininogen-1*	KNG1	KNG1	2
Basement membrane-specific heparan sulfate proteoglycan core protein*	PGBM	HSPG2	2
Roundabout homolog 4	ROBO4	ROBO4	2
Alpha-1-acid glycoprotein 1/2*	A1AG1/A1AG2	ORM1/ORM2	1
Alpha-1-antitrypsin	A1AT	SERPINA1	1
Actin, cytoplasmic 1/2	ACTB/ACTG	ACTB/ACTG1	1
Albumin*	ALBU	ALB	1
Collagen alpha-1(I) chain	CO1A1	COL1A1	1
Collagen alpha-1(X) chain	COAA1	COL10A1	1
Collagen alpha-1(XVIII) chain	COIA1	COL18A1	1
Collagen alpha-1(XXII) chain	COMA1	COL22A1	1
Cystatin-A	CYTA	CSTA	1
Fibrinogen beta chain	FIBB	FGB	1
Gelsolin	GELS	GSN	1
Histone H1.2	H12	H1-2	1
Histone H1.4	H14	H1-4	1
Insulin-like growth factor-binding protein 7	IBP7	IGFBP7	1
Insulin-like growth factor II	IGF2	IGF2	1
Immunoglobulin heavy constant gamma 1/2	IGHG1/IGHG2	IGHG1/IGHG2	1
Kallikrein-1	KLK1	KLK1	1
Vesicular integral-membrane protein VIP36	LMAN2	LMAN2	1
Nidogen-1*	NID1	NID1	1
Neuropeptide W precursor	NPW	NPW	1
Phosphoinositide-3-kinase-interacting protein 1	PI3IP1	PIK3IP1	1
Extracellular superoxide dismutase [Cu-Zn]	SODE	SOD3	1
Neurosecretory protein VGF	VGF	VGF	1
Xylosyltransferase 1	XYLT1	XYLT1	1

List of proteins that generated the 71 significant urinary peptides identified in this study, along with their Uniprot IDs, gene names, and the number of peptides detected from each protein. Proteins names marked with a * were previously identified as changing in IC/BPS by prior proteomic studies .

a

Peptide sequence	Protein ID (Uniprot)	VIP
Peptide-1	CD99	2.59
Peptide-2	CD99	2.23
Peptide-3	CD99	2.13
Peptide-4	KNG1	2.01
Peptide-5	XYLT1	2.01

b



(a) List of five peptides with VIP scores > 2.0 from PLS-DA. These five peptides were used for AUROC analysis. (b) The ROC curves from the logistic regression (blue) or random forest (red) model using the five peptides listed in panel (a).

Conclusion

Our study revealed differences in the profiles of small urinary peptides for IC/BPS patients compared to age-matched controls which is consistent with increased protease activity in IC/BPS. Our study also enabled the direct measurement of APF peptide abundance in IC/BPS and control urine. Our results indicate that the full-length APF peptide was not consistently found in the urine of IC/BPS patients at levels sufficient to reliably differentiate IC/BPS patients from healthy individuals.

References

1. Kim, H. J. *Int. Neurourol. J.* **(2016)**. 20, 13-17.
2. You, S. et al. *Int. Neurourol. J.* **(2012)**. 16, 159-168.
3. Chen, Z. et al. *BMC Urol.* **(2016)**.16, 11.
4. Keay, S. K. et al. *Proc. Natl. Acad. Sci. U. S. A* **(2004)**. 101, 11803-11808.
5. Urologyspecialist.com/interstitial-cystitis.
6. Regnexx.com/blog/interstitial-cystitis.

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