
Voiding Pattern Analysis as a Surrogate for Cystometric Evaluation in Uroplakin II Knockout Mice

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Purpose: Previous study has shown that the absence of uroplakin II can cause urinary tract dysfunction, including vesicoureteral reflux and renal abnormalities, as well as micturition pattern changes. We developed a simple surrogate measure of bladder function using ultraviolet visualization of urinary voiding patterns in a uroplakin II knockout mouse animal model.

Materials and Methods: Three male and 3 female WT mice, and 3 male and 3 female uroplakin II knockout mice were evaluated by cystometric analysis and voiding pattern markings. Voiding pattern markings were graded by independent observers on a scale of 1 to 5 according to the degree of dispersion of voided urine. Statistical analysis was then used to correlate voiding dispersion grades with cystometric parameters in the same mice.

Results: The degree of dispersion of voiding pattern markings correlated with several measures of bladder function. Specifically the Pearson correlation coefficients for the observed voiding patterns highly correlated with baseline pressure, threshold pressure and intermicturition pressure measurements made during conscious cystometry in these mice ($p < 0.05$).

Conclusions: Ultraviolet visualization of urinary voiding patterns of mice correlated well with certain measures of standard cystometric evaluations. As such, this method provides a simple, noninvasive method of evaluating mouse bladder function. Implementation of this methodology, which can potentially be automated for high throughput analysis, can accelerate the development of novel therapy for certain important aspects of bladder disease/dysfunction.

Key Words: mice, bladder, urination, uroplakin II

An estimated 35 million Americans have disorders and diseases of the bladder, affecting all ages, races and ethnic groups.¹ For example, OAB syndrome, defined by the International Continence Society as urgency with or without urge incontinence, usually with frequency and nocturia, and occurring in the absence of infection or another obvious pathological condition,² has been reported to occur in 12% to 17% of the Western population.³⁻⁵ Although effects on the quality of life of affected individuals are profound, many individuals do not seek professional help. As recently pointed out, the prevalence of OAB is similar to or higher than the rates of many other chronic diseases, such as asthma, coronary artery disease and peptic ulcer disease.⁶ The direct and indirect costs of urinary incontinence are substantial with the total annual costs in the United

States alone estimated to be \$19.5 billion in 2000, which increased to \$21 billion in 2004.⁷ In fact, urinary incontinence is the most costly urological condition.⁷

Despite the prevalence, the magnitude of the unmet medical need is further highlighted by the fact that pharmacological treatment for OAB/DO is still based on antimuscarinic agents, an approach that has predominated without robust clinical success for 2 decades.^{8,9} The major problem with this approach is that, even when these drugs have documented efficacy, treatment is still limited by side effects with fewer than 20% of patients adhering to treatment after 1 year.⁹ Undoubtedly new drugs with improved efficacy and decreased adverse effects are urgently needed.

In our continuing effort to use animal models and develop novel methods for the improved understanding, diagnosis and treatment of lower urinary tract disease we have turned our attention to the UP KO mouse model. UPs are integral membrane proteins that are synthesized as major urothelial differentiation products forming 16 nm protein particles packed into 2-dimensional crystals (urothelial plaques) covering almost the entire apical urothelial surface.^{10,11} Ablation of the UPIII and II genes led to the partial or complete loss of urothelial plaques and a compromised urothelial barrier function. These alterations in turn were coupled to vesicoureteral reflux and associated renal abnormalities as well as altered micturition patterns detected by a filter paper assay.¹¹⁻¹³ In short, the absence of UPII and III was

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Cystometric parameters and corresponding voiding grades in individual mice

Animal	Bladder Capacity (ml)	Micturition Vol (ml)	Residual Vol (ml)	BP (cm H ₂ O)	TP (cm H ₂ O)	Micturition Pressure (cm H ₂ O)	IMP (cm H ₂ O)	SA	Bladder Compliance	No. Voids/Hr	Voiding Grade
Female:											
UPII KO	0.25	0.13	0.12	4.65	9.13	30.58	6.80	2.15	0.06	6.00	1.70
UPII KO	0.38	0.26	0.12	11.78	24.17	49.72	14.63	2.86	0.03	4.00	2.45
UPII KO	0.38	0.47	0.00	9.84	13.67	34.69	11.01	1.17	0.10	4.00	2.60
WT	0.30	0.23	0.07	3.10	8.70	14.90	5.61	2.51	0.05	5.00	1.84
WT	0.09	0.05	0.04	7.69	14.86	24.81	12.38	4.69	0.13	17.00	1.48
WT	0.17	0.04	0.13	4.00	9.46	29.67	8.46	4.46	0.16	9.00	1.50
Male:											
UPII KO	0.12	0.02	0.10	14.63	16.89	29.10	17.17	2.54	0.05	14.00	4.10
UPII KO	0.25	0.11	0.14	17.57	26.58	53.82	21.57	4.00	0.03	6.00	4.00
UPII KO	0.50	0.31	0.19	9.14	21.14	44.59	14.13	5.00	0.04	3.00	3.98
WT	0.17	0.12	0.05	4.95	14.16	34.11	7.68	2.73	0.02	9.00	1.13
WT	0.30	0.12	0.18	1.63	5.82	19.61	3.72	2.09	0.07	5.00	1.89
WT	0.30	0.17	0.13	4.55	13.70	29.40	7.44	2.89	0.03	5.00	1.22
Parameter vs voiding grade	0.30	0.15	0.30	0.81	0.66	0.55	0.80	0.18	-0.28	-0.09	1.00
p Value				<0.001	<0.02		<0.002				

associated with lower urinary tract dysfunction,^{11,14} including the presence of DO.¹⁰

The specific goal of the present study was to demonstrate a simple method for identifying and evaluating altered bladder function in mice that could facilitate a noninvasive and higher throughput evaluation of the causes of bladder storage conditions, thus, facilitating the identification of novel therapies. To this end we investigated bladder function in male and female WT¹⁰ and UPII KO mice using a filter paper assay to compare ultraviolet visualization of voiding patterns with cystometric parameter estimates in these mice. The data suggest that noninvasive measurement of voiding patterns provides a simple surrogate measure of bladder function in these rodents.

MATERIALS AND METHODS

The UPII KO mice used were produced as previously described.^{11,14} All animals weighed approximately 25 gm. The Animal Care and Use Committees of the Wake Forest University School of Medicine and New York University School of Medicine approved all experimental protocols. Animals were housed in a barrier facility with a 12-hour light/dark cycle and free access to food and water.

Animal Surgery

Surgical procedures in the mice were performed as previously described in rodents.^{15,16} Briefly, control and KO mice were anesthetized via intraperitoneal injections of xylazine (7 to 14 mg/kg) and ketamine (37.5 to 75 mg/kg) formulated for animal use. The ventral abdominal wall, perineum and upper back were shaved with an electrical shaver and cleansed with povidone-iodine. A low midline abdominal incision was made and the bladder was identified. A small incision was made in the bladder dome and a PE-10 polyethylene catheter (Clay Adams, Parsippany, New Jersey) with a cuff was inserted. A 7-inch silk suture was placed around the catheter to anchor it and close the bladder incision. Saline was injected through the catheter to ensure no bladder leakage. The catheter was then tunneled through the subcutaneous space, exited through an orifice created in the back of the animal and secured with a suture. The abdominal incision was closed using a 5-inch silk suture and the free end of the catheter was thermally sealed.

Cystometric Analysis

Cystometric analysis was performed in a manner similar to that previously described.^{15–17} Three days after bladder catheter implantation conscious mice were placed in a mouse metabolic cage and the bladder catheter was connected to a 2-way valve connected to a pressure transducer and infusion pump. The pressure transducer was connected via an ETH 400 transducer amplifier (CB Sciences, Dover, New Hampshire) to a MacLab/8e data acquisition board (Analog Digital Instruments, Castle Hill, New South Wales, Australia). The pressure transducers and analog-to-digital board were calibrated in cm H₂O before each experiment.

Room temperature saline was infused into each bladder at a rate of 1.5 ml per hour. Micturition volume was measured with a silicone coated collecting funnel that directed urine into a collecting tube connected to a force displacement transducer. Intravesical pressure and micturition volume were continuously recorded. Analysis began after the voiding pattern of the mice stabilized. Only mice with BP less than 20 cm H₂O and 30 to 60 minutes of reproducible micturition cycles were analyzed.

The table lists all cystometric parameters measured. Bladder function was evaluated by urodynamic parameters, including 1) bladder capacity—the volume of infused saline at micturition, 2) BP—the lowest BP recorded during cystometry, 3) TP—BP immediately before micturition, 4) micturition pressure—peak BP during micturition, 5) micturition volume—volume of urine discharged during micturition, 6) residual volume—volume of infused saline minus micturition volume,¹⁸ 7) IMP—mean pressure between micturitions, 8) SA—an approximate index of spontaneous bladder contractions between micturitions, ie DO, 9) bladder compliance—the change in pressure between voiding contractions and 10) micturition frequency. SA was calculated by subtracting BP from IMP. Note that this parameter is identical to that previously described as mean intermicturition oscillatory pressure, that is mean intermicturition oscillatory pressure = intermicturition pressure – BP.

Because of differences between human and murine cystometric methods, there is no strict clinical correlate for all parameter estimates obtained. However, micturition pressure may correspond to voiding pressure, bladder capacity may correspond to post-void residual volume plus the vol-

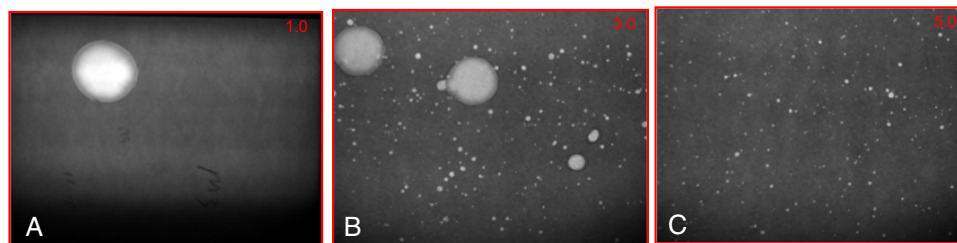


FIG. 1. Representative images of spectrum voiding patterns in individual mice. A, normal, large volume voiding. B, intermediate bladder dysfunction with some normal large voiding patterns and some small frequent nonvoiding contraction patterns. C, virtual dribbling incontinence. Relative grading score (upper right) served as basis for table measurements.

ume of infused saline since the last void, micturition volume may correspond to voided volume and residual volume may correspond to post-void residual volume, while IMP and SA are measures of pressure during the intermicturition interval. As such, the latter parameter estimates would correspond approximately to DO, which is thought to be the clinical correlate associated with urgency.

Determination of the Micturition Pattern

The urination pattern was determined using a filter paper assay, as previously described.^{11–13} Six WT¹⁰ and 6 UPII KO mice (3 males and 3 females each) were housed in a 12/12-hour day/night cycle, germ-free animal facility. The mice were placed singly in a special cage fitted with a fine mesh bottom, thus, allowing urine to land on a piece of filter paper, which could be changed periodically. Calibration studies established that the surface areas covered by mouse urine were strongly fluorescent. Thus, they could be easily visualized under ultraviolet light to provide an accurate measurement of urine volume with a linear range of up to approximately 500 μl .

Analysis of Micturition Pattern

Filter paper voiding patterns were evaluated by 4 independent and blinded observers. Each voiding pattern was given a grade of 1 to 5 with a score of 1 representing only a few large micturition spots and 5 representing many small scattered spots. Unweighted and weighted kappa statistics were calculated to assess the degree of chance corrected agreement between pairs of readers.¹⁷ Pearson product moment correlation coefficients were used to quantitate degrees of association between the average grade given to each animal in voiding studies and the cystometric parameters of that mouse from urodynamic studies.

RESULTS

Voiding Studies

Voiding patterns for all mice were studied using previously described methods.^{11,15} Figure 1 shows representative examples of typically observed voiding patterns. These 3 examples show the spectrum of voiding possibilities, ranging from normal, large volume voiding to intermediate bladder dysfunction with some normal large voiding patterns and some small frequent nonvoiding contraction patterns to virtual dribbling incontinence (fig. 1). A relative dispersion scale was used for independent observer rating/ranking of the detected voiding patterns in individual mice, as de-

scribed (fig. 1). Voiding scores were 1.1 to 4.1 in this animal cohort (see table).

Independent grading of voiding pattern markings by 4 observers was found to be in fair to good agreement. Specifically the average unweighted kappa statistic across all pairs of readers was 0.47 (range 0.28 to 0.63), indicative of fair agreement among readers.¹⁷ Weighted kappa statistics give weights proportional to the difference in scores, eg scores that differ by 0 are given a greater weight than those that differ by 1, which are given a greater weight than those that differ by 2, etc. The average weighted kappa statistic across all pairs of readers was 0.67 (range 0.49 to 0.82), which again indicated good weighted agreement among readers.¹⁷ Having confirmed the reliability of observer scores we then determined their relationship to well established, quantitative measures of bladder function in the same mice, as assessed by cystometry.

Cystometric Studies

Cystometric studies done in all mice established a spectrum of micturition activity that correlated well with the observed voiding patterns. Figures 2 to 4 show representative tracings of cystometric records from WT and UPII KO mice. Observed recordings ranged from relatively normal micturition patterns in WT mice to evidence of clear detrusor overactivity in UPII KO mice (figs. 2 to 4). In addition to voiding patterns, figures 2 to 4 show voiding scores, and BP, TP and IMP in the same animals. The table lists cystometric parameter estimates from all WT and UPII KO mice studies with the corresponding score of voiding pattern markings (voiding grade) shown adjacent to urodynamic results. Not surprisingly Pearson correlation coefficients demonstrated that BP, TP and IMP correlated well with the voiding pattern analysis (each $p < 0.05$, see table). Figure 5 shows linear regression relationships for BP and IMP.

DISCUSSION

Although cystometry/urodynamics performed in conscious, freely moving rodents is technically cumbersome, it will likely continue to have a place in the evaluation of bladder function/disease. Nonetheless, the development of a simple noninvasive method to evaluate bladder physiology/dysfunction would also clearly have an important role in animal models of bladder disease. In fact, in previous studies we used a filter paper assay as a simple measure of the volume and frequency of urine output in UP KO mice. As the next logical step toward the development of more noninvasive methodologies, we report the use of the UPII KO mouse to

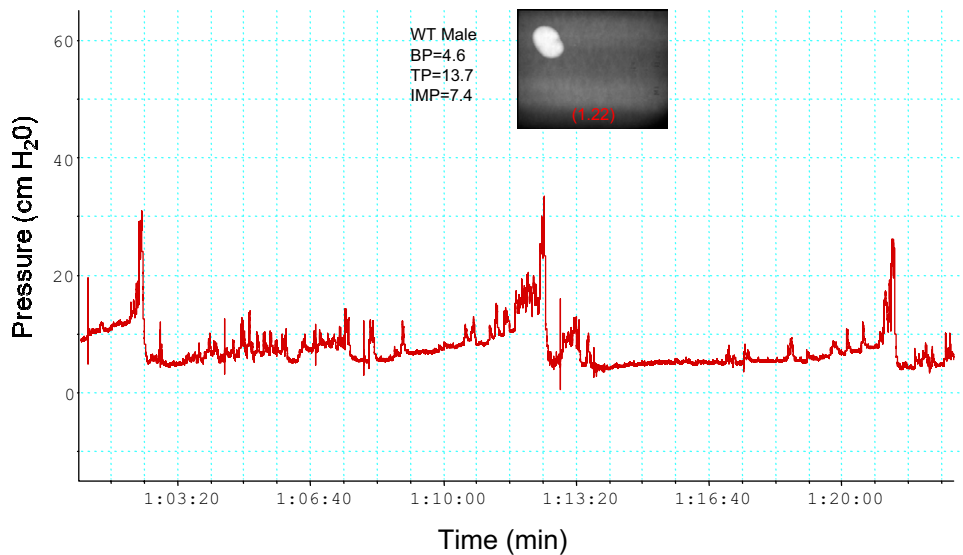


FIG. 2. Representative cystometric record of WT male mouse with corresponding pressure measurements that significantly correlated with voiding pattern scores (see table). Inset, grading score and voiding pattern. Pressure measurements were derived from analysis of entire record of this animal and correspond to table values.

determine the relationship, if any, between well established cystometric measures of bladder function and noninvasive evaluation of voiding patterns in the same mouse.

There have only been a few attempts to develop noninvasive cystometric evaluations of bladder function. For example, Stewart used methods similar to those that we report to study voiding frequency in mice exposed to radiation and cytostatic drugs.¹⁹ In that study a filter paper assay was used to estimate voided volume by counting the number of urine spots and calculating their respective areas. These measurements were compared to standard calibration curves created from known urine volumes on the same paper. Urinary frequency was determined by the number of different urine spots. Although this provided a simple measure of urine volume and frequency, there was no evaluation of interobserver variability in the interpretation of the void-

ing patterns. Moreover, results were not compared with urodynamic studies in the same animals.

Horvath et al described their results using ultrasonography to estimate bladder volume using an ellipsoid equation in rats following pharmacological manipulation.¹⁸ While this method is labor-intensive and impractical in large cohort studies, it nonetheless correlated well with in vitro measurements of bladder volume and provided a simple, noninvasive method of monitoring rat bladder function.

Wood et al developed an automated and noninvasive measurement of mouse voiding frequency and volume.²⁰ Mice were housed in cages above electronic balance pans, which measured the time and weight of each void. This technique provided a sensitive measure of mouse voiding patterns without the need for surgical manipulation but it

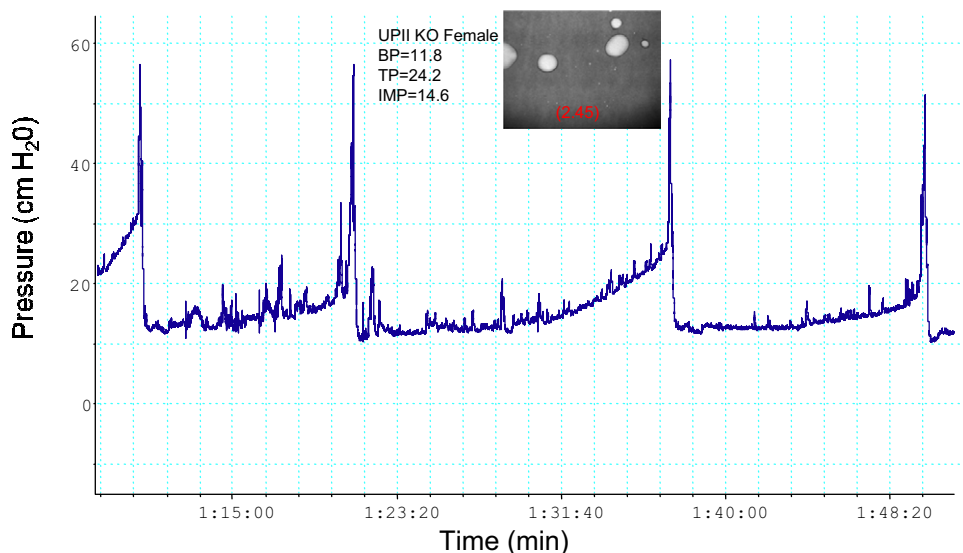


FIG. 3. Representative cystometric record of UPII KO female mouse with corresponding pressure measurements that significantly correlated with voiding pattern scores (see table). Inset, grading score and voiding pattern. Pressure measurements were derived from analysis of entire record of this animal and correspond to table values.

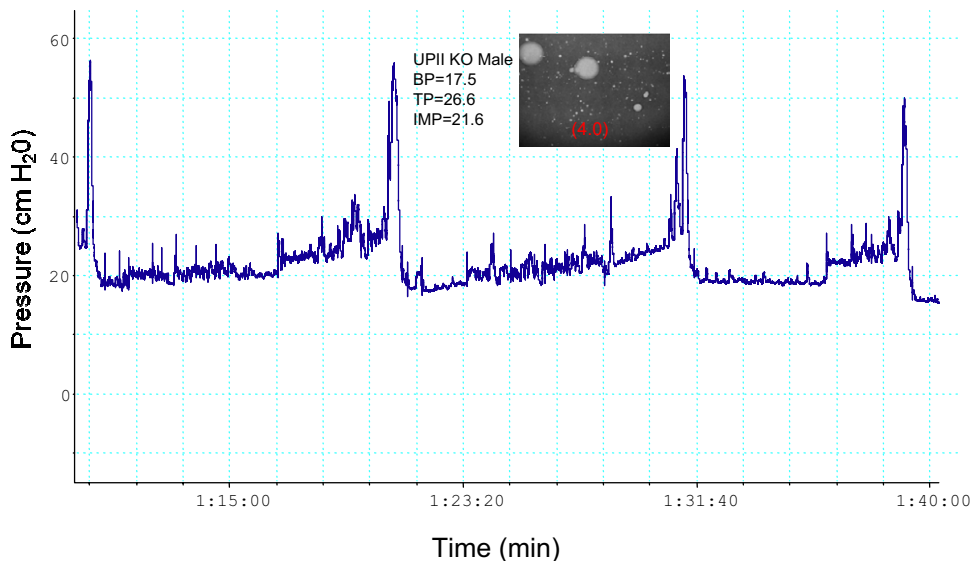


FIG. 4. Representative cystometric record of UPII KO male mouse with corresponding pressure measurements that significantly correlated with voiding pattern scores (see table). Inset, grading score and voiding pattern. Pressure measurements were derived from analysis of entire record of this animal and correspond to table values.

required computer monitoring to calculate weight changes and account for evaporative loss. Again, there was no provision for cystometric analysis in this study.

Our model extends the series by Stewart¹⁹ to include a proven correlation of the voiding patterns with measures of bladder function derived from cystometric studies. In fact, the correlations between voiding analysis, and BP, TP and IMP correspond to intuition with respect to the pathophysiology of DO. Although evaluating a larger number of animals and different DO models may reveal new relationships/correlations, the voiding pattern analysis reported is still clearly indicative of DO, as determined using traditional cystometric measures in the same animals.

As such, this noninvasive method may provide a high throughput assay of bladder function, permitting the rapid assessment of novel therapies. This in turn would allow one to more rapidly and efficiently gain mechanistic insight into bladder dysfunction/disease. Subsequent to more rigorous validation the ability to accurately predict DO from voiding pattern analysis would be a significant improvement in pre-

clinical rodent models of human bladder physiology/disease. Automation of this method of voiding pattern analysis would confer additional advantages.

The main weakness in the proposed visual analysis of voiding patterns is the labor intensive nature of the image analysis process. Therefore, we plan to automate the process using image analysis software to record, measure and further validate the voiding patterns characteristic of various rodent models of bladder disease. The overriding goal of this approach is that after it is established such an automated analytic method would rapidly and easily transform voiding pattern markings into functional bladder data that likely could be interpreted in a simple and straightforward manner, comparable to traditional cystometry studies. Such an approach has the potential to vastly improve the efficiency and ease of preclinical studies of bladder disease. In conclusion, the ultraviolet visualization of urinary voiding patterns of mice correlated well with certain measures of standard cystometric evaluations and provides a simple, noninvasive method of evaluating mouse detrusor overactivity.

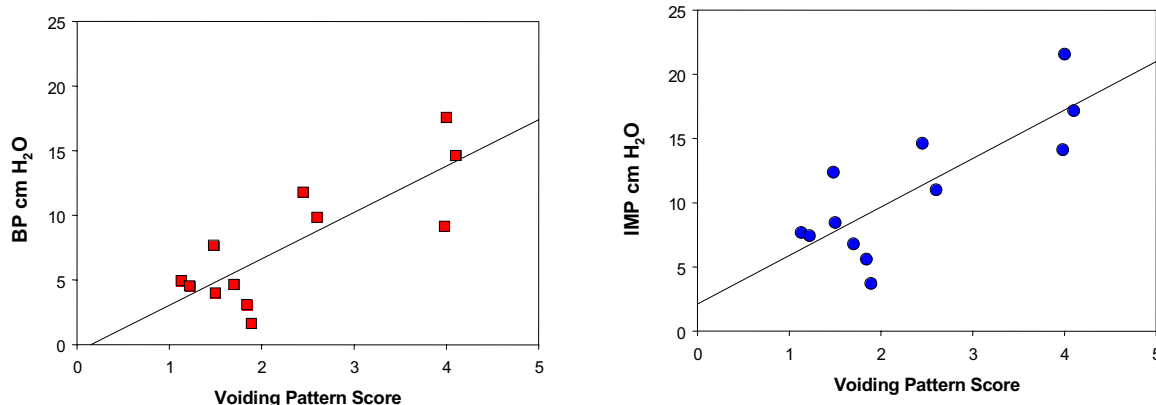


FIG. 5. Statistically significant positive correlation between BP and IMP in all study animals

Abbreviations and Acronyms

BP	=	baseline pressure
DO	=	detrusor overactivity
IMP	=	intermittent pressure
KO	=	knockout
OAB	=	overactive bladder
SA	=	spontaneous activity
TP	=	threshold pressure
UP	=	uroplakin

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