Pelvic venous congestion induces lower urinary tract dysfunction in rats

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ABSTRACT
Pelvic venous congestion (PC) is thought to be related to several diseases of the lower urinary tract (LUT). We examined the characteristics of the LUT in rats with PC. To create PC, female rats were anesthetized with isoflurane, and the bilateral common iliac veins and bilateral uterine veins were ligated. At 1–8 weeks after either ligation or sham surgery, we performed cystometry with or without administration of carbazochrome sodium sulfonate hydrate or propiverine hydrochloride, histologic examination of the bladder, blood flow imaging, assessment of locomotor activity, measurement of urinary 8-hydroxydeoxyguanosine (8-OHdG) and nitric oxide metabolites (NOx), and the Evans blue dye extravasation test. PC elevated frequency of urination after 2–6 weeks, and caused a decrease of spontaneous locomotor activity. In addition, there was a decrease of bladder blood flow, an increase of bladder vascular permeability, an increase of urinary 8-OHdG, a decrease of urinary NOx, and mild inflammatory changes of the bladder. In rats with PC, frequency of urination was normalized by administration of propiverine or carbazochrome. Rats with PC may be used as a model of PC associated with high frequency of urination, and this model may be useful when developing treatment for LUT symptoms associated with PC.

The association between metabolic syndrome and lower urinary tract symptoms has been attracting enormous interest and attention (11, 20). Hypertension, one of the diseases constituting to metabolic syndrome, deserves to be mentioned with relevance to lower urinary tract symptoms. Spontaneously hypertensive rats are known to show a significantly lower bladder capacity and voiding volume (26). This rat also shows low blood flow of the prostate and increases prostate size (22). A rat model of atherosclerosis-induced chronic bladder ischemia shows detrusor overactivity manifested as an increase in voiding frequency (16). Atherosclerosis, also one of the diseases constituting to metabolic syndrome, is thought to be a risk factor for benign prostatic hyperplasia (BPH), and the relationship between high vascular resistance (resistive index on Doppler ultrasonography) and the development of BPH is suggested (11, 22). Therefore, it is thought that hypertension and/or arteriosclerosis reduce arterial blood flow and induce BPH or overactive bladder by the ischemia.

On the other hand, the nutcracker phenomenon of the left renal vein involves compression of this vein between the abdominal aorta and the superior mesenteric artery (21, 34), which has been reported to cause varicocele, left renal congestion inducing hematuria (4), and pain and dysfunction of the pelvic organs (23). Unilateral or bilateral ovarian vein incompetence also can cause the pelvic congestion syndrome, which induces chronic pain, irritable bladder, or vulval varices and varicose veins in the lower limbs (1, 5, 8, 10). We previously reported a female patient with pelvic congestion syndrome (27). She had inferior vena cava reflux associated with tricus-
pid regurgitation which caused pelvic congestion. In addition, we identified many persons with inferior vena cava reflux on color Doppler ultrasonography, and reported that chronic prostatitis in men and stress urinary incontinence in women are significantly related to inferior vena cava reflux (28). Moreover, chronic prostatitis in men and overactive bladder in women are related to the poor flow in the common iliac vein (31). Therefore, not only the ischemia by hypertension and/or arteriosclerosis but also pelvic venous congestion is thought to be related to several lower urinary tract symptoms/diseases. In this study, we devised a model of pelvic venous congestion in female rats and examined the characteristics of the lower urinary tract in this model.

MATERIALS AND METHODS

Materials. The protocol for this study was approved by the President of University of the Ryukyus based on the judgment of our institutional Animal Care and Use Committee. To create a pelvic venous congestion (PC) model, 8 or 9 weeks old female Sprague-Dawley rats were anesthetized with 2% isoflurane. After an incision was made in the lower abdomen, the bilateral common iliac veins were ligated with metal clips, and the bilateral uterine veins with uterine arteries and uterine horn were ligated en bloc at a site near the ovaries (32). The distal common iliac veins became dilated after ligation of these veins. Then the abdomen was closed and an antibiotic (30 mg of ampicillin) was injected subcutaneously. In the sham operation group, 8 or 9 weeks old female rats were anesthetized with isoflurane and the bilateral common iliac veins were dissected free of the common iliac arteries.

Cystometry and histologic examination of the bladder. In this experiment, 34 rats with PC were studied at 1 week (1w-PC, n = 8), 2 weeks (2w-PC, n = 6), 4 weeks (4w-PC, n = 8), 6 weeks (6w-PC, n = 8), and 8 weeks (8w-PC, n = 4) after the creation of PC. In addition, 34 sham rats were studied at 1 week (1w-Sham, n = 8), 2 weeks (2w-Sham, n = 6), 4 weeks (4w-Sham, n = 8), 6 weeks (6w-Sham, n = 8), and 8 weeks (8w-Sham, n = 4) after sham surgery. PC rats and sham rats were anesthetized with urethane (0.6 g/kg subcutaneously) and placed in a restraining cage (NAIGAI-CFK-1P; NMS, Tokyo, Japan), after which a polyethylene catheter (PE50; Clay Adams, USA) was inserted transurethrally into the bladder. This catheter was connected to an infusion pump via polyethylene tubing and the bladder was filled with physiological saline at a rate of 0.05 mL/min. Cystometry was performed for at least 90 min and the changes of bladder activity were recorded. Then the bladders of 4w-PC and 4w-Sham rats were harvested and were fixed in 20% formalin for embedding in paraffin. Sections were cut from the paraffin blocks for histological examination.

Blood flow imaging. In this experiment, two intact female rats and two 2w-PC rats were studied. Rats were anesthetized with urethane (0.8 g/kg subcutaneously and 0.4 g/kg intraperitoneally), a midline abdominal incision was made to expose the anterior bladder, and physiological saline (0.5 mL) was infused transurethrally into the bladder. Bladder blood flow was measured in intact rats immediately after creation of PC and in 2w-PC rats. Bladder blood flow was assessed with a Laser Speckle Blood Flow Imager (Omegazone OZ-1; Omegawave, Inc., Tokyo, Japan) (6), which provides high resolution 2-dimensional color images of blood flow.

Assessment of locomotor activity. In this experiment, eight 4w-PC rats and eight 4w-Sham rats were used for monitored locomotor activity to evaluate the pelvic pain and bladder pain. Rats were housed individually in plastic cages, and locomotor activity was measured for a 24-h-period by using a digital counter and an infrared sensor (NS-ASS01; Neuroscience, Inc., Tokyo, Japan) (35). Lights were on from 8:00 A.M. to 8:00 P.M. Locomotor activity during the dark period was calculated as the total number of movements from 8:00 P.M. to 8:00 A.M., while that during the light period was calculated as the total from 8:00 A.M. to 8:00 P.M. Animals were housed at 23°C and had free access to standard rat chow and water.

Measurements of urinary 8-hydroxydeoxyguanosine (8-OHdG) and nitric oxide metabolites (NOx). After assessment of locomotor activity, the same sham rats and PC rats were placed in individual cages one by one, and 24-h urine was collected for measurements of 8-OHdG (a marker of oxidative stress) (6), NOx (nitrate and nitrate) and creatinine. Measurements of 8-OHdG and creatinine were performed by Cosmobio Co. Ltd. (Tokyo, Japan). NOx were measured by using the Griess method with an automated NO detector high-performance liquid chromatography system (ENO-20; Eicom, Kyoto, Japan), after a urine sample was deproteinized by addition of an equal volume of methanol (35).
Evans blue dye extravasation test. After urine was collected for the measurement of 8-OHdG and NOx, the same sham rats and PC rats were employed to measure the vascular permeability of the bladder wall under urethane anesthesia. Physiological saline (2.5 mL) was infused transurethrally into the bladder. After 1 min, Evans blue dye solution (50 mg/kg/0.3 mL) was injected intravenously, and then bladder distension was performed for 5 min. Next, physiological saline was removed from the bladder and the same volume of saline was re-infused to observe leakage of Evans blue dye into the bladder wall. Bladder tissues were harvested immediately and fixed in 20% formalin. At 24 h later, the amount of Evans blue dye leaking from the bladder tissues into the formaldehyde fixative solution was measured by a spectrophotometer with the absorbance being set at 620 nm (14).

Effects of carbazochrome and propiverine on frequency of urination. In this experiment, sixteen PC rats and eight 4w-sham rats were studied. Immediately after surgery, 16 PC rats were divided into a control PC group (n = 8) and a PC + carbazochrome group (n = 8). Rats in the PC + carbazochrome group were given carbazochrome sodium sulfonate hydrate (carbazochrome), a hemostatic drug with a capillary stabilizing action (7, 24, 25), in the drinking water at 0.25 mg/mL to reduce vascular permeability (14). The control PC group was given normal tap water. Carbazochrome was provided by Mitsubishi Tanabe Pharma Corporation (Tokyo, Japan), and administered at about 20 times the clinical dose level. All rats were allowed free access to drinking water and cystometry was performed after 4 weeks. Other 4w-sham rats were used as the sham group for cystometry. Rats in the sham, PC, and PC + carbazochrome groups were anesthetized with urethane (0.6 g/kg subcutaneously), and a polyethylene catheter (PE50) was inserted transurethrally into the bladder. Then cystometry was performed for at least 90 min, as described above, and the changes of bladder activity were recorded. In the PC group, the antimuscarinic drug propiverine hydrochloride (propiverine, 1 mg/kg) was injected intravenously (PC + propiverine group). Propiverine was provided by TAIHO Pharmaceutical Co., Ltd. (Tokyo, Japan). The dosage (1 mg/kg) of intravenous propiverine was sufficient to prolong the interval between bladder contractions in rats (30). Changes of bladder activity were recorded for longer than 60 min.

Statistical analysis. Results are reported as the mean ± standard error of the mean (SEM). Student’s t-test for paired or unpaired data was used for statistical analysis as appropriate, and P < 0.05 was considered to indicate statistical significance.

RESULTS

Continuous cystometry
There were no significant changes of the interval between bladder contractions, the maximum bladder contraction pressure, or the baseline intravesical pressure among the 5 groups of sham rats (1w-, 2w-, 4w-, 6w-, and 8w-Sham rats). The interval between bladder contractions was significantly (P < 0.05) shorter in PC groups than Sham groups at 2 weeks (9.0 ± 0.9 min vs 15.6 ± 1.6 min), 4 weeks (10.0 ± 0.9 min vs 14.4 ± 1.3 min), and 6 weeks (11.5 ± 1.2 min vs 15.4 ± 1.3 min), but not at 1 week (14.0 ± 1.5 min) and 8 weeks (14.4 ± 1.1 min) compared with that (14.4 ± 0.9 min and 15.5 ± 2.6 min, respectively) in each corresponding sham group (Fig. 1). There was no significant change of the maximum bladder contraction pressure or baseline intravesical pressure in each PC group compared with each sham group. Thus, pelvic venous congestion maneuver induced high frequency of urination after 2–6 weeks.

Pathological findings
In both 4w-Sham and 4w-PC rats, the bladder had partial adhesions with the surrounding organs. In 4w-PC rats, but not 4w-Sham rats, hematoxylin and eosin staining revealed mild inflammatory changes of the bladder wall, such as an increase of collagen fibers in the subserosa, as well as infiltration of neutrophils and lymphocytes into the submucosa and muscle layers (Fig. 2).

Blood flow imaging
There was a decrease of mean bladder blood flow from 32 and 28 arbitrary units per field before pelvic congestion to 26 (81%) and 25 (89%) units after surgery in 2 intact rats. In 2w-PC rats, bladder blood flow was 22 and 25 units, being only 78% of the flow in the intact bladder before pelvic congestion was created (Fig. 3). Thus, pelvic venous congestion maneuver decreased bladder blood flow.

Locomotor activity
In 4w-Sham rats, locomotor activity was significantly higher (increased by 350%) during the dark period compared with that during the light period (17,456 ± 695 vs. 5,043 ± 596 movements, P < 0.001). Locomotor activity during the dark period was significant-
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Neuver decreased locomotor activity during the dark period which is active phase of the rat.

Urinary 8-OHdG and NOx

The urinary level of 8-OHdG (a marker of oxidative stress) corrected for creatinine (urinary 8-OHdG/Cre ratio) was significantly higher in 4w-PC rats than in 4w-Sham rats (37.2 ± 17.1 vs. 21.8 ± 5.5 ng/mg, P = 0.029). The urinary level of NOx corrected for creatinine (urinary NOx/Cre ratio) was significantly lower in 4w-PC rats than in 4w-Sham rats (0.72 ± 0.56 vs. 2.63 ± 0.53 μM, P = 0.016) (Fig. 4).

Evans blue dye extravasation

Macroscopically, bladder wall edema and infiltration of Evans blue dye into the bladder wall were observed in 4w-PC rats, but not in 4w-Sham rats (Fig. 5). The amount of Evans blue dye extravasated from the bladder was significantly greater (increased by 23%) in 4w-PC rats compared with 4w-Sham rats (1.35 ± 0.05 vs. 1.10 ± 0.03 mg/L, P = 0.001). Thus, pelvic venous congestion maneuver increased bladder vascular permeability.

Effects of carbazochrome and propiverine

During continuous cystometry, the interval (9.3 ± 0.9 min) between bladder contractions was significantly (P = 0.008) shorter in the PC group compared with the sham group (13.0 ± 0.8 min) (Figs. 6 and 7). In the PC+carbazochrome group, the interval (13.8 ± 0.6 min) between bladder contractions significantly (P = 0.003) longer than that in the PC group, and did not differ significantly from that in the sham group. In the PC group, intravenous injection of propiverine (PC+propiverine) significantly (P = 0.049) prolonged the interval (13.8 ± 2.0 min) between bladder contractions, and the interval was not significantly different from that in the sham group. There were no significant differences of the maximum bladder contraction pressure or baseline intravesical pressure among the sham, PC, PC+propiverine, and PC+carbazochrome groups. Thus, frequency of urination in PC rats was normalized by carbazochrome or propiverine.

 DISCUSSION

The present study revealed that pelvic venous congestion induced high frequency of urination after 2–6 weeks, as well as a decrease of spontaneous locomotor activity that might have resulted from pelvic pain or bladder pain, suggesting that our rat model of pelvic venous congestion had the same

![Fig. 1](image_url) The interval between bladder contractions (A), the maximum bladder contraction pressure (B), and the baseline intravesical pressure (C) obtained by continuous cystometry in 1w-, 2w-, 4w-, 6w-, and 8w-Sham rats (open rectangle), and 1w-, 2w-, 4w-, 6w-, and 8w-PC rats (closed rectangle). The interval was significantly (*: P < 0.01) shorter in 2w-PC rats than in 2w-Sham rats, and was also significantly (*: P < 0.05) shorter in 4w- and 6w-PC rats than in 4w- and 6w-Sham rats, respectively.

ly lower (decreased by 16%, P = 0.013) in 4w-PC rats (14,667 ± 810 movements) than in 4w-Sham rats, but there was no significant difference during the light period. Thus, pelvic venous congestion ma-
Fig. 2 Photomicrographs show mild inflammatory changes, such as an increase of collagen fibers in the subserosa, as well as infiltration of neutrophils (arrow) and lymphocytes (arrowheads) into the submucosa and the muscle layer of the bladder wall in a 4w-PC rat (A: ×12.5, B: ×100, hematoxylin and eosin stain).

Fig. 3 Blood flow imaging shows bladder blood flow before pelvic congestion (A) and in a 2w-PC rat (B). The mean blood flow per field in the bladder (center of each photograph) was automatically calculated as 32 arbitrary units (A) and 22 arbitrary units (B). The bladder margin was marked with a line.

Fig. 4 The urinary levels of 8-OHdG (A) and NOx (B) corrected for creatinine in 4w-Sham rats (Sham) and 4w-PC rats (PC). The urinary 8-OHdG and NOx levels were significantly (*: $P < 0.05$) higher and lower in 4w-PC rats than in 4w-Sham rats, respectively.
Fig. 5 Macroscopic findings of the bladder after bladder distension in a 4w-Sham rat (A-1 and B-1) and a 4w-PC rat (A-2 and B-2) (representative images). The bladder wall is edematous in the 4w-PC rat (A-2) but not in the 4w-Sham rat (A-1) before injection of Evans blue dye. After injection of the dye, infiltration into the bladder wall is observed in the 4w-PC rat (B-2), but not in the 4w-Sham rat (B-1).

symptoms as patients with pelvic congestion syndrome. In pelvic venous congestion rats, there was a decrease of bladder blood flow, increase of vascular permeability, increase of urinary 8-OHdG, decrease of urinary NOx, and mild pathological inflammatory changes of the bladder. Cystometry showed that frequency of urination of pelvic venous congestion rats was normalized by administration of carbazochrome (a vascular permeability inhibitor) or propiverine (an antimuscarinic and overactive bladder therapeutic agent). Therefore, pelvic venous congestion rats may be used as a model of pelvic venous congestion with high frequency of urination, and this model may be useful when developing treatment for lower urinary tract symptoms associated with pelvic venous congestion.

The causes of pelvic venous congestion include compression of the left renal vein (nutcracker phenomenon) (21, 34) into which the left gonadal vein flows, stenosis or obstruction of the internal pudendal vein (33), and tricuspid regurgitation (27, 28). The prevalence of tricuspid regurgitation is very high, reaching 70% among adults who have undergone mass screening in Japan (12), or 17–68% among persons with otherwise “normal” hearts in the United State of America (2, 9). Our previous ultrasonographic study revealed that the prevalence of inferior vena cava reflux associated with tricuspid regurgitation in the supine position was significantly higher in women (68%) than men (49%) attending our urological outpatient clinic (28). Chronic prostatitis in men and stress urinary incontinence in women are related to inferior vena cava reflux, while there may also be a relationship of urethral syndrome and cystocele to inferior vena cava reflux in females (28). In addition, the prevalence and severity of microscopic hematuria show an increase in both males and females as inferior vena cava reflux becomes more severe (29). Therefore, pelvic or renal venous congestion secondary to inferior vena cava reflux may be one of the factors involved in the development of lower urinary tract symptoms or
hematuria.

Carbazochrome is a stable oxyepinephrine derivative that acts as a capillary stabilizer and is used to treat hemorrhage due to capillary fragility (7). Although its mechanism of action is unknown, recent studies have suggested that it reverses the thrombin-, tryptase-, and bradykinin-induced increase of endothelial cell permeability by inhibiting intracellular actin stress fiber formation and restoring intercellular tight junctions (24, 25). Accordingly, carbazochrome may reduce the extravascular leakage of blood. In a clinical study, we recently showed that carbazochrome achieved marked improvement of pain in patients with refractory chronic prostatitis, as well as improving storage symptoms and post-micturition symptoms (19). Because neither diuretic nor anti-inflammatory effects have been reported for carbazochrome (25), its mechanism of action may possibly be related to a decrease of capillary permeability. In a rat model of tranilast-induced interstitial cystitis with increased vascular permeability in the bladder, the interval between bladder contractions was short and locomotor activity was reduced. Administration of carbazochrome decreased bladder vascular permeability, prolonged the interval between bladder contractions, and improved locomotor activity, suggesting that carbazochrome inhibits the extravascular leakage of a substance acting on the bladder (14, 19). Therefore, an increase of vascular permeability due to pelvic venous congestion and consequent extravascular leakage of a stimulatory substance might
be one of the factors contributing to the occurrence of lower urinary tract symptoms.

In a rat model of atherosclerosis-induced chronic bladder ischemia, high frequency of urination is associated with an increase of oxidative stress markers in the urine or bladder tissue (15, 17). Hypertension reduces blood flow in the prostate and increases the levels of oxidative stress markers in prostatic tissues (22). On the other hand, it was reported that clamping of an intestinal segment and its draining veins to produce congestion led to more severe tissue damage than ischemia of the same segment for the same clamping time (13). In the present study, pelvic venous congestion reduced bladder blood flow and increased the urinary level of an oxidative stress marker, which suggests that pelvic venous congestion inducing bladder tissue hypoxia is one of the factors contributing to lower urinary tract symptoms. The atherosclerosis-induced chronic ischemic bladder also decreases constitutive nitric oxide synthase (NOS) (18). Since reports have shown both relaxatory and facilitatory effects of nitric oxide, as well as direct effects on the detrusor and modulatory effects via afferent nerves, nitric oxide has been shown to affect afferent nerve signalling, mainly in an inhibitory fashion (3). In the present study, therefore, the decrease of urinary NOx due to bladder tissue hypoxia might become one of causes of urinary frequency.

Because the prevalence of tricuspid regurgitation is very high, as mentioned above, pelvic venous congestion secondary to inferior vena cava reflux associated with tricuspid regurgitation may have a role in the development of lower urinary tract symptoms. Prolonged walking and sitting worsen the symptoms of chronic non-bacterial prostatitis/chronic pelvic pain syndrome, while lying down improves these symptoms, and these clinical features also suggest involvement of pelvic venous congestion (28). Therefore, lower urinary tract symptoms with pelvic venous congestion may be related to the upright posture of humans. In our present pelvic venous congestion model, high frequency of urination was improved by an anticholinergic drug and only mild inflammation of the bladder wall was observed. These findings suggest that the model reproduces the state of many elderly patients with urinary frequency and/or urge incontinence. In the present study, high frequency of urination (a short interval between bladder contractions) resolved at 8 week after creation of pelvic venous congestion, possibly due to the development of venous collaterals. In contrast, pelvic venous congestion tends to progress with aging in humans and as lower urinary tract symptoms also increase and/or worsen with aging.

In conclusion, pelvic venous congestion induced high frequency of urination after 2–6 weeks, and also caused a decrease of spontaneous locomotor activity. In addition, there was a decrease of bladder blood flow, an increase of bladder vascular permeability, an increase of urinary 8-OHdG, a decrease of urinary NOx, and mild inflammatory changes of the bladder. Our rat model of pelvic venous congestion with high frequency of urination may be useful for the development of new treatments for lower urinary tract symptoms associated with pelvic venous congestion.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

REFERENCES

1. Asciiuto G, Mumme A, Marpe B, Köster O, Asciiuto KC and Geier B (2008) MR venography in the detection of pelvic venous congestion. Eur J Vasc Endovasc Surg 36, 491–496.
2. Choong CY, Abascal VM, Weyman J, Levine RA, Gentile F, Thomas JD and Weyman AE (1989) Prevalence of valvular regurgitation by Doppler echocardiography in patients with structurally normal hearts by two-dimensional echocardiography. Am Heart J 117, 636–642.
3. Daly DM, Collins VM, Chapple CR and Grundy D (2011) The afferent system and its role in lower urinary tract dysfunction. Curr Opin Urol 21, 268–274.
4. de Schepper A (1972) Nutcracker phenomenon of the renal vein and venous pathology of the left kidney. J Belge Radiol 55, 507–511.
5. Ganeshan A, Upponi S, Hon LJ, Uthappa MC, Warakaulle DR and Uberoi R (2007) Chronic pelvic pain due to pelvic congestion syndrome: the role of diagnostic and intervention radiology. Cardiovasc Intervent Radiol 30, 1105–1111.
6. Goy Y, Tomiyama Y, Nomiy M, Sagawa K, Aikawa K and Yamaguchi O (2013) Effects of silodosin, a selective α1A-adrenoceptor antagonist, on bladder blood flow and bladder function in a rat model of atherosclerosis induced chronic bladder ischemia without bladder outlet obstruction. J Urol 190, 1116–1122.
7. Hayakawa M and Gando S (2009) Systemic and local hemostatic agents. Jpn J Thromb Hemost 20, 278–280.
8. Hobbs JT (2005) Varicose vein arising from the pelvis due to ovarian vein incompetence. Int J Clin Pract 59, 1195–1203.
9. Lavie CJ, Hebert K and Cassidy M (1993) Prevalence and severity of Doppler-detected valvular regurgitation and estimation of right-sided cardiac pressure in patients with normal two-dimensional echocardiograms. Chest 103, 226–231.
10. Liddle AD and Davies AH (2007) Pelvic congestion syndrome: chronic pelvic pain caused by ovarian and internal iliac varices. Phlebology 22, 100–104.
11. Matsumoto S and Kakizaki H (2012) Causative significance of bladder blood flow in lower urinary tract symptoms. Int J Urol 19, 20–25.
12. Masuda K, Sekine T, Toide H, Doi T and Toda E (2001)
Points of diagnosis of valve diseases. *Jpn J Med Ultrasound Technol* **26**, 101–134.

13. Moriwaki Y, Katamura H, Ichikawa Y, Yamamoto T and Sugiyama M (1992) The intestinal tissue damages and the changes of reactive oxygen species due to ischemia, congestion and reperfusion. *Jpn Soc Gastroenterol Surg* **25**, 2938–2943.

14. Nishijima S, Sugaya K, Kadekawa K, Ashitomi K, Ueda T and Yamamoto H (2013) High-dose tranistal administration to rats creates interstitial cystitis-like symptoms with increased vascular permeability. *Life Sci* **93**, 897–903.

15. Nomiya M, Sagawa K, Yazaki J, Takahashi N, Kushida N, Haga N, Aikawa K, Matsui T, Oka M, Fukui T, Andersson KE and Yamaguchi O (2012) Increased bladder activity is associated with elevated oxidative stress markers and proinflammatory cytokines in a rat model of atherosclerosis-induced chronic bladder ischemia. *Neuromodulation* **31**, 185–189.

16. Nomiya M, Yamaguchi O, Andersson KE, Sagawa K, Aikawa K, Shishido K, Yanagida T, Kushida N, Yazaki J and Takahashi N (2012) The effect of atherosclerosis-induced chronic bladder ischemia on bladder function in the rat. *Neuromodulation* **31**, 195–200.

17. Nomiya M, Burmeister DM, Sawada N, Campeau L, Zarifpour M, Keys T, Peyton C, Yamaguchi O and Andersson KE (2013) Prophylactic effect of tadalafil on bladder function in a rat model of chronic bladder ischemia. *J Urol* **189**, 754–761.

18. Nomiya M, Burmeister DM, Sawada N, Campeau L, Zarifpour M, Yamaguchi O and Andersson KE (2013) Effect of melatonin on chronic bladder-ischaeemia-associated changes in rat bladder function. *BJU Int* **112**, E221–230.

19. Oh-oka H, Yamada T, Noto H, Umeyama T, Kadekawa K, Ashitomi K, Nishijima S and Sugaya K (2014) Effect of carbazochrome sodium sulfonate on refractory chronic prostatodynia. *Eur Urol* **65**(3), E686 (abstract in Japanese).

20. Ponholzer A, Temml C, Wehrberger C, Marszalek M and Madersbacher S (2006) The association between vascular risk factors and lower urinary tract symptoms in both sexes. *Eur J Urol* **50**, 581–586.

21. Russo D, Minutolo R, Iaccarino V, Andreucci M, Capuano A and Savino FA (1998) Gross hematuria of uncommon origin: the nutcracker syndrome. *Am J Kidney Dis* **32**, E3.

22. Saito M, Tsunapi P, Oikawa R, Shimizu S, Honda M, Sejima T, Kinoshita Y and Tomita S (2014) Prostatic ischemia induces ventral prostatic hyperplasia in the SHR; possible mechanism of development of BPH. *Sci Rep* **22**, 3822.

23. Scholbach T (2007) From the nutcracker-phenomenon of the left renal vein to the midline congestion syndrome as a cause of migraine, headache, back and abdominal pain and functional disorders of pelvic organs. *Med Hypotheses* **68**, 1318–1327.

24. Sendo T, Goromaru T, Aki K, Sakai N, Itoh Y and Oishi R (2002) Carbazochrome attenuates pulmonary dysfunction induced by a radiographic contrast medium in rats. *Eur J Pharmacol* **450**, 203–208.

25. Sendo T, Itoh Y, Aki K, Oka M and Oishi R (2003) Carbazochrome sodium sulfonate (AC-17) reverses endothelial barrier dysfunction through inhibition of phosphatidylinositol hydrolysis in cultured porcine endothelial cells. *Naunyn Schmiedebergs Arch Pharmacol* **368**, 175–180.

26. Steers WD, Clemow DB, Persson K, Sherer TB, Andersson KE and Tuttle JB (1999) The spontaneously hypertensive rat: insight into the pathogenesis of irritative symptoms in benign prostatic hyperplasia and young anxious males. *Exp Physiol* **84**, 137–147.

27. Sugaya K, Miyazato T, Koyama Y, Hatano T and Ogawa Y (2000) Pelvic congestion syndrome caused by inferior vena cava reflux. *Int J Urol* **7**, 157–159.

28. Sugaya K, Matsumura E, Tasaki S, Kimura R, Kiyuna A, Nishijima S and Kadekawa K (2011) Relationship between urological disease and inferior vena cava reflux on color doppler ultrasonography. *Low Urin Tract Symptoms* **3**, 94–98.

29. Sugaya K, Nishijima S, Kadekawa K and Ashitomi K (2013) Relationship between microscopic hematuria and inferior vena cava reflux on color Doppler ultrasonography. *Open J Urol* **3**, 299–303.

30. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T and Yamamoto H (2014) Intravenous or local injections of flavoxato into the rostral pontine reticular formation inhibit urinary frequency induced by activation of medial frontal lobe neurons in rats. *J Urol* **192**, 1278–1285.

31. Sugaya K, Unten Y, Shinzato N, Gushi K, Heshiki Y, Tatwada S, Yokoya F, Hyakuna M, Uezu A and Nishijima S (2014) Relationship between the lower urinary tract symptom and the blood flow of the common iliac vein. *Jpn J Med Ultrasonics* **41**, 8686 (abstract in Japanese).

32. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T and Yamamoto H (2016) Naftopidil improves locomotor activity and urinary frequency in rats with pelvic venous congestion. *Biomed Res (Tokyo)* **37**, 221–226.

33. Terasaki T, Watanabe H, Saitoh M, Uchida M, Okamura S and Shimizu K (1995) Magnetic resonance angiography in prostatectomy. *Eur Urol* **27**, 280–285.

34. Venkatachalam S, Bumpus K, Kapadia SR, Gray B, Lyden S and Shishebhor MH (2011) The nutcracker syndrome. *Ann Vasc Surg* **25**, 1154–1164.

35. Yamashiro S, Noguchi K, Matsuzaki T, Miyagi K, Nakasone J, Sakashashi M, Koja K and Sakashashi M (2002) Beneficial effect of tetrahydrobiopterin on ischemia-reperfusion injury in isolated perfused rat hearts. *J Thorac Cardiovasc Surg* **124**, 775–784.