Water avoidance stress (WAS) represents a potent psychological stressor, and it is associated with visceral hyperalgesia, such as irritable bowel syndrome (IBS). In this WAS rat model, it has been reported that erosive changes at the bladder mucosa as well as the bowel mucosa.

### Purpose
We investigated the histological and genetic changes in the WAS rat’s bladder to find out the process between psychological stress and urinary frequency.

### Materials & Methods

<1> Analysis of the voiding pattern of the WAS rats

- **Animals**: 7-week-old male Sprague-Dawley rats (BW: 200 – 250 g)
- **Groups**: 1. WAS (n=8): water avoidance stress
  2. Control (n=8): sham stress

**Stress Procedure**
- 2 hours/day X 10 consecutive days
- WAS: on a glass platform in the center of a plastic container filled with 25°C water up to 1 cm below the top of the platform.
- Sham stress: a glass platform in the waterless container.

**Assessment of Voiding Behavior**
- Metabolic cage analysis: 24-hour measurement of voided urine volume.
- Intercontractile Interval (ICI)
- Voided urine volume (VV)
- 24-hr urine volume
- CMG: performed under the anesthesia of urethane (1.0 g/kg s.c.)
  - Intravesical pressure (IP): basal, threshold and maximum voiding pressure
  - ICI, VV, post-void residual urine volume (PVR)

<2> Detection of the gene changes
- Comparison between the gene expression in the bladders from WAS / control rats by…
- cDNA microarray analysis
- Quantification of mRNA expression by…
- Real time RT-PCR
- Assessment of protein expression by…
  - Immunohistochemistry
  - Western blotting

<3> The inhibition effect of a candidate among the up-regulated genes on the WAS rats
- To 8 rats, oral administration of the inhibitory agent in parallel with WAS for 10 days.
- Assessment of voiding pattern by…
  - CMG
- Assessment of protein expression by…
  - Immunohistochemistry
  - Western blotting

### Results

<1> In the metabolic cage, ICI was significantly shorter in WAS rats than in normal controls (Table 1). VV was significantly less in WAS rats than in normal controls. A total of 24-hr urine volume revealed no significant differences between the two groups.

In CMG, ICI was significantly shorter and VV was significantly less in WAS rats than in normal controls (Fig. 1, A and B, Table 2). IP and PVR revealed no significant differences between the two groups.

<2> The cDNA microarray analysis showed higher expression of cyclooxygenase-2 (COX-2) and interleukin-1 (IL-1) beta in the WAS rat bladder (Table 3).

Using RT-PCR, significant up-regulation of COX-2 was detected in the bladder of WAS rats (Fig. 2). Although IL-1 beta was up-regulated in the bladder of WAS rats, the increase in gene expression was not significant. We continued to investigate the expression of COX-2.

The immunohistochemistry of COX-2 in the WAS rats bladders showed intensive expression in the smooth muscle layer compared with normal controls (Fig. 3, A, B and D).

Western blotting confirmed that COX-2 protein expression was significantly increased in the bladders of WAS rats (Fig. 4).

<3> As a COX-2 inhibitor, etodolac was used. It was suspended in 0.5% methylcellulose solution, and administered orally at a dose of 10mg/kg in parallel with WAS. (COX-21 treatment group)

In CMG, ICI was significantly longer and VV was significantly greater in COX-2 treatment rats than in WAS rats (Fig. 3, B, C and D). IP and PVR revealed no significant differences between the two groups.

Western blotting confirmed that COX-2 protein expression was significantly decreased in COX-21-treated bladders compared with WAS bladders (Fig. 4).

### Conclusions
The WAS rats showed voiding frequency in both the metabolic cage and CMG. High expression of COX-2 on the bladder of WAS rats were seen. It was found that voiding frequency of the WAS rats was improved with the treatment of COX-2 inhibitor.

COX-2 may have some important role to mediate voiding frequency due to psychological stress. COX-2 inhibitor could be a useful treatment of the stress-induced voiding frequency.

### References
3. 39th annual meeting of ICS 2009.