AMP-ACTIVATED PROTEIN KINASE SUPPRESSES CONNEXIN43 IN BLADDER SMOOTH MUSCLE CELLS AND AMELIORATES VOIDING DYSFUNCTION IN CYCLOPHOSPHAMIDE-INDUCED MOUSE CYSTITIS

Hypothesis / aims of study
Lower urinary tract symptoms are common and impair Quality of life, especially in elderly population. Accumulating evidence indicate that many types of lower urinary tract dysfunction are closely related to local oxidation, inflammation, as well as overactivity of several channels, including connexin 43 (Cx43) channels (1)(2). Given that AMPK, a key regulator of cellular energy homeostasis, has anti-oxidative, anti-inflammatory, and channel-inhibiting properties, we speculated that AMPK might be exploited for treatment of refractory overactive bladder. The aim of this study was to test this hypothesis.

Study design, materials and methods
Primarily cultured bladder smooth muscle cells (BSMCs) obtained from rat bladders were exposed to AMPK-modulating agents. The protein expression, localization and function of Cx43 were analyzed by Western blot, immunofluorescent staining, scrape loading dye-transfer assay and gel contraction assay, respectively. Cx43 promoter activity and mRNA expression were assessed by measuring luciferase activity in cells transfected with Cx43 promoter plasmid and RT-PCR. The role of the specific signalling molecules in Cx43 expression and function was established through siRNA interference or chemical inhibitors or activators. Bladder activity was evaluated through contractile response of ex vivo isolated bladder strips to various stimuli and voiding pattern of mouse in metabolic cage. Mouse model of lower urinary tract dysfunction was induced by intraperitoneal injection of cyclophosphamide (CYP). Histopathological analysis was done to evaluate the extent of bladder injury.

Results
1) Activation of AMPK in BSMCs with three structurally and functionally different AMPK activators, AICAR, flufenamic acid and metformin, all suppressed Cx43 expression at both protein and mRNA levels (Fig.1). They also inhibited gap junctional intercellular communication. Consistently, downregulation or inhibition of AMPK with siRNA or chemical inhibitor enhanced Cx43 expression and function. 2) AMPK activation did not affect Cx43 degradation. However, it inhibited transcriptional factor CREB phosphorylation and CRTC2 expression, and suppressed Cx43 promoter activity. Downregulation of CREB with siRNA elevated Cx43. 3) Functional analysis revealed that the suppression of Cx43 by AMPK was associated with a reduced BSMC contraction. In vivo administration of AMPK activator AICAR resulted in a reduced urinary frequency and an increased urine volume voided per micturition (UVVM). 4) AMPK also suppressed Cx43 promoter activation, protein expression and function under the stimulation of PDGF-BB, TNFα plus IL-1β, or cAMP-elevating agent forskolin. 5) In CYP-induced mouse cystitis, AICAR treatment reduced bladder oxidation, inflammation and Cx43 overexpression, as revealed by immunoblot detection of carbonyl groups, COX-2 and iNOS, as well as Cx43 (Fig. 2). Histopathological analysis revealed that AMPK prevented CYP-induced detachment of urothelial cells and reduced subepithelial edema. Functional analysis revealed that AICAR treatment ameliorated voiding dysfunction. 6) Compared to Cx43 wild-type mouse (Cx43+/+), Cx43 heterozygous (Cx43+/-) mice had a higher UVVM and lower urinary frequency. Furthermore, Cx43+/- mice displayed much better micturition pattern under the pathological stimulation of CYP.

Interpretation of results
Our study demonstrated, for the first time, that AMPK suppressed Cx43 expression and function in BSMCs under both physiological and pathological conditions. This effect of AMPK was most probably due to its suppressive effect on transcriptional factor CREB. This is supported by the observations that AMPK inhibited Cx43 promoter activity and that downregulation of CREB with siRNA indeed resulted in a reduced expression of Cx43.

The suppressive effect of AMPK on Cx43 was associated with a reduced BSMC contraction in vitro and altered mouse voiding pattern in vivo. In CYP-induced mouse cystitis, AMPK ameliorated voiding dysfunction. This effect was, at least partially, due to its action on Cx43 because mice with lower level of Cx43 (Cx43+/-) displayed a much better voiding pattern than mice with higher level of Cx43 (Cx43+/+).

AMPK has multifaceted functions. In this study, it suppressed not only CYP-induced Cx43 elevation, but also oxidation and inflammation, two major pathological changes seen in many types of bladder dysfunction. Oxidation and inflammation are also the causes of the altered channel activities and urothelial injury. In this context, our finding about suppression of oxidation, inflammation and channel activity by AMPK could have important basic and clinical implications.

Concluding message
Collectively, our results indicate that AMPK suppresses Cx43 in BSMCs and ameliorates voiding dysfunction in mouse model of cystitis. Targeting AMPK could be developed as a novel therapeutic approach for treatment of lower urinary tract dysfunction.
References

Disclosures
Funding: None Clinical Trial: No Subjects: ANIMAL Species: Mouse and Rat Ethics Committee: All animal procedures were reviewed and approved by the University of Yamanashi Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.