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Deletion of the lysyl oxidase-like 1 gene induces impaired elastin fiber synthesis and inefficient urethral closure in rats

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ABSTRACT

We investigated the bladder and urethral function in a rat model lacking the protein lysyl oxidase-like 1 (Loxl1). Female nulliparous rats of *Loxl1*$^{-/-}$ or age-matched wild type (WT) rats had leak-point pressure testing, cystometry, histopathological analyses of lower urinary tract, and contractile response of isolated detrusor strips to carbachol and electric field stimulation. The *Loxl1*$^{-/-}$ rats showed increased looseness and redundancy of the skin, the decreased intercontraction interval and voided volume in cystometry, the lower leak-point pressure, thinner elastic fibers of the mesentery, bladder, urethra and vagina, and smaller contractile response of detrusor strips to carbachol when compared to the WT rats. Thus, the insufficient hydrostatic mechanism of urethra via submucosal impaired elastin synthesis might reduce the resting urethral closure pressure and the diminished cholinergic contractile response of detrusor smooth muscle might be involved in bladder activity in the *Loxl1*$^{-/-}$ rats.

INTRODUCTION

Pelvic floor disorders, including stress urinary incontinence and pelvic organ prolapse, reduce quality of life (Norton *et al.* 1988). The prevalence of pelvic floor disorders in adult women has been estimated to be 23.7% and is presumed to increase with age to up to 49.7% in women aged 80 years or older (Nygaard *et al.* 2008). Stress urinary incontinence is a common condition that is defined as involuntary loss of urine secondary to an increase in abdominal pressure during events such as sneezing, coughing, or laughing (Chancellor *et al.* 2005; Melville *et al.* 2005; Kadekawa *et al.* 2020), and pelvic organ prolapse is defined as abnormal descent or herniation of the pelvic organs protruding against the vaginal wall (Barber 2016). The etiology of stress urinary incontinence and pelvic organ prolapse in women is multifactorial, and the histories of vaginal childbirth, aging, estrogen deficiency, and connective tissue deficiencies have previously been reported to contribute significantly to the emergence of both stress urinary incontinence and pelvic organ prolapse (Bump and Norton 1998; Melville *et al.* 2005; van Brummen *et al.* 2007; Nygaard *et al.* 2008; Tinelli *et al.* 2010). The tensile strength and elasticity of the pelvic floor, which holds the pelvic organs in place, is provided by the connective tissues, two important components of which are the proteins collagen and elastin (Macarak *et al.* 1995; Kadekawa *et al.* 2017). Alterations in collagen and elastin lead to morphologic changes of the bladder and have been shown to have an impact on the contractility, com-

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pliance and capacity of the bladder in rats (Kim *et al.* 2000; Kadekawa *et al.* 2017). A rat model has shown elastin's high turnover and its important role in remodeling the pelvis during pregnancy and after giving birth (Rahn *et al.* 2008). Although elastin might have important roles in stress urinary incontinence and pelvic organ prolapse, the details of its function in the lower urinary tract are still unclear.

Elastin attains its tensile strength and resilience by forming a cross-linked polymer. The lysyl oxidase-like 1 gene (*Loxl1*) is involved in forming elastin polymers: It encodes a copper-dependent monoamine oxidase that catalyzes the deamination of a lysine residue on tropoelastin monomers during the formation of crosslinks between monomers (Liu *et al.* 2004). Mice lacking the protein *Loxl1* do not deposit normal elastic fibers in the uterine tract postpartum and develop pelvic organ prolapse; enlarged airspaces in the lung; loose skin; and lower urinary tract dysfunction, with decreased bladder capacity and voiding pressure (Liu *et al.* 2004, 2007). Although mouse models have become highly useful because of the feasibility for genetic modification, it is difficult to evaluate the urethral function due to its small size. We investigated the bladder and urethral function in a rat model lacking the protein Loxl1.

MATERIALS AND METHODS

Animals. For this study, a genetically modified rat line with a Loxl1 gene knockout was developed from outbred Sprague-Dawley rats (Japan SLC inc., Shizuoka, Japan) by CRISPR/Cas9 technology. (Cong *et al.* 2013; Mali *et al.* 2013). The study used 60 *Loxl1*$^{-/-}$ ($n = 29$) and age-matched wild type (WT) female rats ($n = 31$) weighing 190 to 248 g. All animal protocols and procedures were approved by the Institutional Animal Care and Use Committee of the University of the Ryukyus.

Breeding protocol and genotyping. Loxl1$^{-/-}$ rats for use in the study were created with the CRISPR/Cas9 system, which included synthesis of sgRNA sequencing 5'-GGGCTCTGAGTACGTGCCTG-3'. The resultant heterozygous (*Loxl1*$^{-/+}$) animals were bred to produce *Loxl1*$^{+/+}$, *Loxl1*$^{-/-}$, and *Loxl1*$^{-/+}$ rats. Tails were clipped between 3 to 6 weeks of age under isoflurane anesthesia, and DNA was extracted with a DNeasy Mini Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The genotype of the animals was identified by polymerase chain reaction analysis with the primers 5'-CTGACCCCTTGGCCTCAAAA-3' and 5'-GAT GGCTGGCGATAAGTGGT-3'. At age 8 to 9 weeks, the *Loxl1*$^{-/-}$ and *Loxl1*$^{+/+}$ rats were set in genetically matched brother/sister breeding pairs to generate multiparous females for examination of external genitalia after giving birth.

Observation of external genitalia. To assess pelvic organ prolapse in the *Loxl1*$^{-/-}$ rats, we examined the appearance of the external genitalia in the male and female rats. In addition, in 6 adult nulliparous female rats (*Loxl1*$^{-/-}$: $n = 3$; WT: $n = 3$) we inserted a modified 10-Fr Foley balloon catheter (with the tip cut off) into the vagina under 2% isoflurane anesthesia and closed the vaginal orifice with a suture to prevent the catheter from coming out. Then, we inflated the balloon with 4 mL of water to distend the vagina and left the balloon in place for 3 h, after which we removed the catheter (Kamo *et al.* 2006; Nishijima *et al.* 2016). Four days later, we examined the external genitalia of the rats and infused 200 mL of air into the intra-abdominal space via a needle to increase the intra-abdominal pressure. We then evaluated the appearance of the perineum under urethane anesthesia (0.3 g/kg intraperitoneally and 0.9 g/kg subcutaneously).

Measurement of leak point pressure by electrical stimulation of abdominal muscle. After anesthetizing female rats (*Loxl1*$^{-/-}$: $n = 8$; WT: $n = 8$) with urethane, we exposed the urinary bladder and bladder neck through an abdominal incision, and ligated and bilaterally transected the bilateral branches near the bladder neck including the ureters, vessels, and pelvic nerves to prevent reflex bladder contractions. We then inserted a PE-50 catheter connected to a pressure transducer into the bladder through the bladder dome to record intravesical pressure and placed a purse suture tightly around the catheter. After closing the wound, we placed the animals in the supine position on boards and made bilateral abdominal skin incisions near the tips of the eleventh to thirteenth ribs to expose the abdominal muscles for electrical stimulation. We emptied the bladder, injected 0.3 mL saline solution containing Evans blue (100 μg/mL; Sigma, St. Louis, MO, USA) into the bladder through the intravesical catheter and recorded the intravesical pressure. The exposed abdominal muscles were stimulated with an electrical stimulator (SEN-7203; Nihon Kohden, Tokyo, Japan) and an isolator (SS-202J; Nihon Kohden). We repeated electrical stimulation (rectangular pulses with a duration of 0.5 ms at 20 ms intervals for 1 s) every

10 s and gradually increased the stimulus intensity from 2 V to a maximum of 25 V to increase the intravesical pressure in a stepwise manner. We defined leak point pressure as the intravesical pressure at which we observed fluid leakage from the urethral orifice (Kamo *et al.* 2007; Kadekawa *et al.* 2012; Nishijima *et al.* 2016).

Continuous cystometry analysis. We anesthetized a separate group of female rats (*Loxl1*$^{-/-}$: $n = 8$ and WT: $n = 8$) with urethane (0.3 g/kg intraperitoneally and 0.9 g/kg subcutaneously) and inserted a polyethylene catheter (PE-50; Clay Adams, NJ, USA) through the urethra into the bladder. Then, we monitored bladder activity via the catheter, and infused physiological saline at a rate of 0.05 mL/min. After rhythmic bladder contractions had become stable for at least 60 min, during the final 30 min of cystometry we measured the intercontraction interval, maximum contraction pressure during voiding, and intravesical baseline pressure. After measuring these parameters, we calculated the voided volume from the intercontraction interval and saline infusion speed, and drained the residual saline from the bladder through the bladder catheter by gravity to determine residual volumes. Bladder capacity was calculated as the sum of the voided and residual volumes.

Histological analyses. For histological analyses, we killed randomly selected female nulliparous rats (*Loxl1*$^{-/-}$: $n = 6$; WT: $n = 6$) by CO_2 asphyxiation followed by cervical dislocation. In 3 animals of each group, the mesentery was fixed in 10% formalin, and stained with 1% resorcin-fuchsin and 1% light green solution for quantitative assessment of elastic fibers. In other 3 rats of each group, the vagina, bladder and urethra were fixed in 10% formalin, paraffin embedded en bloc and cut into 5 μm sections. Samples were stained with elastica van Gieson stain.

Organ chamber study. The urinary bladder was excised from female rats (*Loxl1*$^{-/-}$: $n = 4$; WT: $n = 6$) under isoflurane anesthesia and rapidly placed in aerated Krebs solution to remove fat and connective tissues. The composition of the Krebs solution was as follows: 118 mM NaCl, 4.7 mM KCl, 1.9 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$ and 11.7 mM glucose. Then, the bladder was cut lengthwise from the base to the dome into 2 × 8 mm strips, and each strip was transferred to a 25 mL organ bath containing Krebs solution at 37°C with 95% O_2 and 5% CO_2. One end of each strip was fixed to a metal rod, and the other end was attached to a force transducer (MLTF050/ST, ADInstruments). Strips were subjected to 1 g of resting tension and allowed to equilibrate for at least 1 h. Data were recorded with a PowerLab 4/26 (ML846, ADInstruments).

We recorded contractile responses to 80 mM KCl, carbachol (CCh; Sigma), electric field stimulation (EFS), a purinergic receptor activator (10 μM α,β-methylene ATP; Calbiochem), and 30 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Ono Pharmaceutical, Osaka, Japan) as changes of tension from baseline and constructed concentration-response curves by cumulative addition of CCh (10 nM to 0.1 mM). For neural stimulation, we placed the smooth muscle strips between double-ring platinum electrodes in the organ bath. Then, EFS (pulse width of 0.5 ms, 10 V, 2-s duration, and 2-min interval) was delivered at increasing frequencies (1, 2, 4, 8, 16 and 32 Hz) by a stimulator with a pulse drive amplifier (SEG-3104, Nihon Kohden). Once we obtained reproducible frequency-response curves, we desensitized the purinoceptor by adding 10 μM α,β-methylene ATP (ABMA) three times and 1 μM tetrodotoxin (TTX; Wako, Osaka, Japan) once and then recorded the contractile response to EFS in the presence of 1 μM atropine. EFS-induced contractions after addition of TTX were subtracted from the other EFS-induced contractions to calculate the number of TTX-sensitive EFS-induced contractions. The area under the curve was calculated from the concentration response curves of atropine and ABMA, and the contractions were measured as a percent of atropine-sensitive (cholinergic), purinoceptor desensitization-sensitive (purinergic) and residual (noncholinergic, nonpurinergic) components (Igawa *et al.* 2014). The relaxant response to 2 μM isoproterenol (Kowa, Tokyo, Japan) was examined after precontraction of bladder muscle strips with 30 μM $PGF_{2\alpha}$, 10 μM ABMA, 30 μM CCh, 32 Hz EFS, or 80 mM KCl. All responses were normalized to the contraction evoked by 80 mM KCl.

Statistical analysis. All data are shown as the mean ± standard error (SE). Data were analyzed by Student's *t* test or ANOVA, followed by a post hoc Bonferroni's multiple comparisons test. All statistical tests were performed with Prism 7.0 (GraphPad Software, Inc., CA, USA), and $P < 0.05$ was considered to indicate statistical significance.

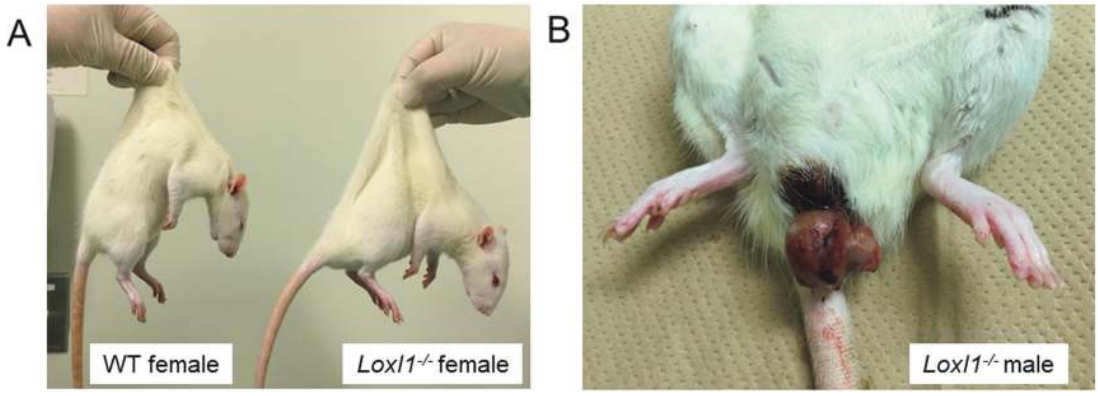

Fig. 1 Gross appearance of loose skin of *Loxl1*$^{-/-}$ rats and WT rats (A), and rectal prolapse of *Loxl1*$^{-/-}$ male rats (B). (**A**) The *Loxl1*$^{-/-}$ rats showed increased looseness and redundancy of the skin when compared with the WT rats. (**B**) The *Loxl1*$^{-/-}$ female rats show no pelvic organ prolapse and descent of pelvic floor even after childbirth, although some of the *Loxl1*$^{-/-}$ male rats showed rectal prolapse during this study.

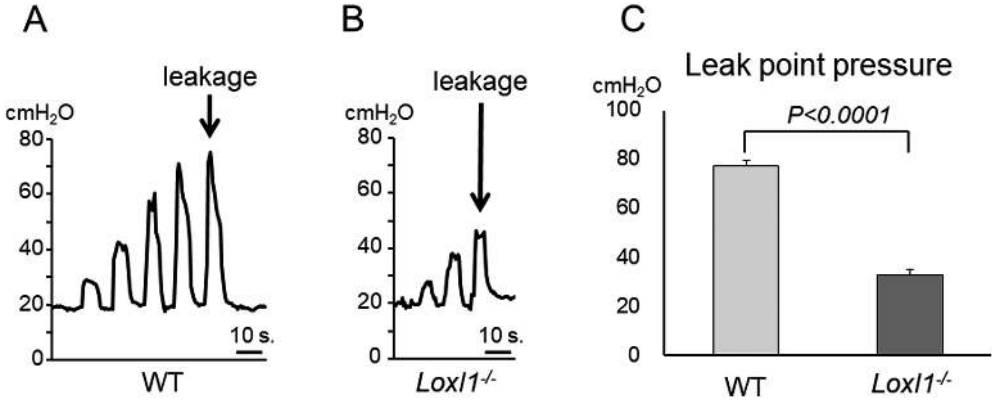

Fig. 2 Representative recording of intravesical pressure with abdominal electrical stimulation (A, B), and comparison of leak point pressure (C) between the wild type (WT) and the *Loxl1*$^{-/-}$ rats. (**A**, **B**) In leak point pressure measurements, the electrical stimulation of abdominal muscles with rectangular electric pulses induced increases in the intra-vesical pressure in a stimulus-dependent manner. Scale bar: 10 s. (**C**) The leak point pressure in the *Loxl1*$^{-/-}$ rats was significantly lower (32.62 ± 2.61 cmH_2O) than the WT rats (77.39 ± 2.17 cmH_2O).

RESULTS

Observation of external genitalia

To assess the effect of Loxl1 gene knockout on pelvic organ prolapse, we examined the appearance of the male and female rats. The *Loxl1*$^{-/-}$ male and female rats showed increased looseness and redundancy of the skin when compared with the WT rats (Fig. 1A). Although, some of the *Loxl1*$^{-/-}$ male rats showed rectal prolapse (Fig. 1B), the *Loxl1*$^{-/-}$ female rats showed no pelvic organ prolapse or descent of the pelvic floor, even after giving birth. We also saw no difference in the appearance of the external genitalia between the *Loxl1*$^{-/-}$ and the WT female rats when air was infused to increase the intra-abdominal pressure 4 days after vaginal distention.

Leak point pressure measurements

To examine the influence of Loxl1 gene knockout on urethral continence ability, we measured the leak point pressure under the situation of increased intraabdominal pressure with electric stimulation. In the leak point pressure measurements, electrical stimulation of abdominal muscles with rectangular electric pulses induced increases in the intravesical pressure in a stimulus-dependent manner (Fig. 2A, B). Fluid leakage from the external urethra after electrical stimulation was observed in all rats of both groups. The leak point pressure was significantly lower in the *Loxl1*$^{-/-}$ rats (32.62 ± 2.61 cm H_2O) than in the WT rats (77.39 ± 2.17 cm H_2O) (Fig. 2C). These results indicate that deficient elastin fiber might reduce the resting urethral closure pressure and induce the

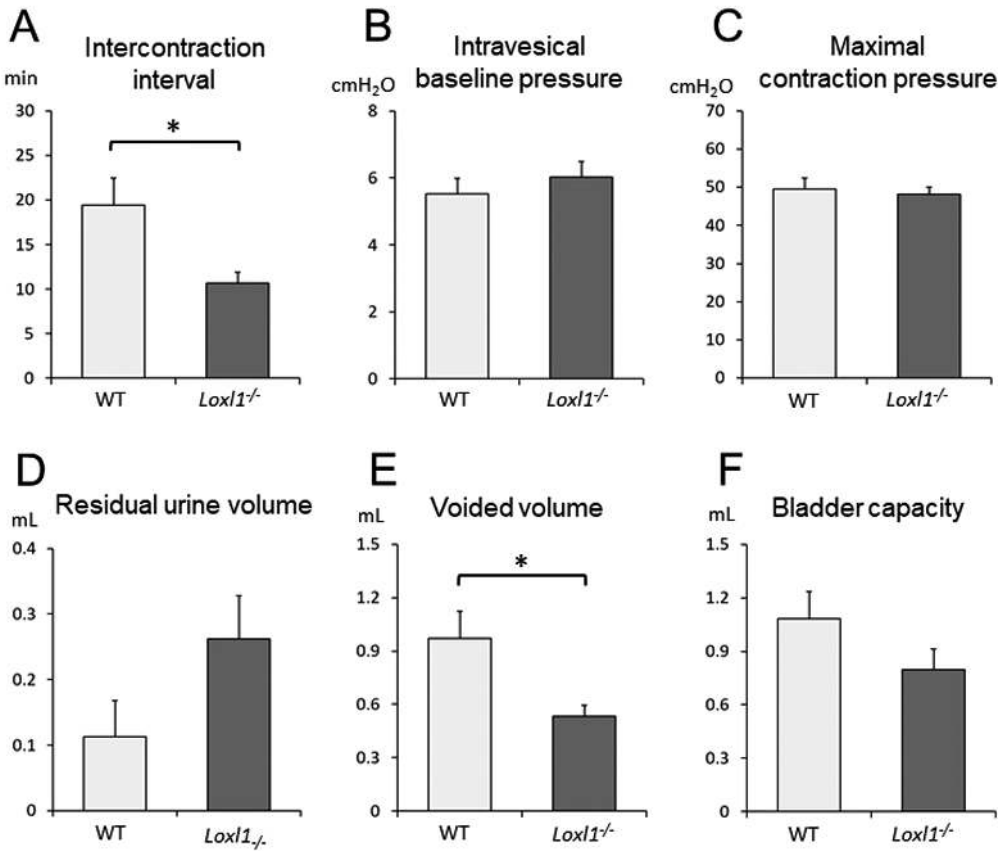

Fig. 3 Comparison of cystometric parameters between the wild type (WT) and the *Loxl1*$^{-/-}$ rats. In the *Loxl1*$^{-/-}$ group, cystometric analyses showed significant decreases in intercontraction interval (A) and voided volume (E) compared to the WT group. There were tendencies of the increase of residual urine volume (D) and the decrease of bladder capacity (F) in the *Loxl1*$^{-/-}$ rats compared with the WT rats. There was no significant difference in the intravesical baseline pressure (B) and maximal contraction pressure (C) between the two groups. *: $P < 0.05$.

lower leak point pressure in the *Loxl1*$^{-/-}$ rats compared with the WT rats.

Continuous cystometry analyses

To examine the influence of Loxl1 gene knockout on bladder activity, we measured the intravesical pressure. Cystometry analyses showed a significantly lower intercontraction interval (Fig. 3A) and voided volume (Fig. 3E) in the *Loxl1*$^{-/-}$ group than in the WT group. There were tendencies of the increase of residual urine volume (Fig. 3D) and the decrease of bladder capacity (Fig. 3F) in the *Loxl1*$^{-/-}$ rats compared with the WT rats. There was no significant difference in the intravesical baseline pressure (Fig. 3B) and maximal contraction pressure (Fig. 3C) between the two groups. These results indicate that deletion of elastin fibers might decrease the elasticity of the bladder wall in the *Loxl1*$^{-/-}$ rats, resulting in a reduced intercontraction interval and voided volume.

Histological analyses

To examine the effect of Loxl1 gene knockout on elastin synthesis, we compared the density of elastin fibers in the mesentery between 2 groups. On resorcin-fuchsin and light green staining (Fig. 4A, B), elastin fibers which were seen as strait strings were higher density in the WT group than in the *Loxl1*$^{-/-}$ group. These results indicate that Loxl1 gene knockout deleted the elastin formation effectively.

To examine the elastin deposition in the genital organs, we compared the elastin fibers in the urethra, bladder and vagina between 2 groups. On elastica van Gieson staining, massive elastic fibers were seen in lamina propria of the urethra (Fig. 5Aa, b), bladder (Fig. 5Ba, b) and vagina (Fig. 5Ca, b) in the WT rats when compared with in the *Loxl1*$^{-/-}$ rats. In particular, the median sagittal section of the proximal urethra revealed thicker elastic fibers in the WT group than in the *Loxl1*$^{-/-}$ group (Fig. 5Aa).

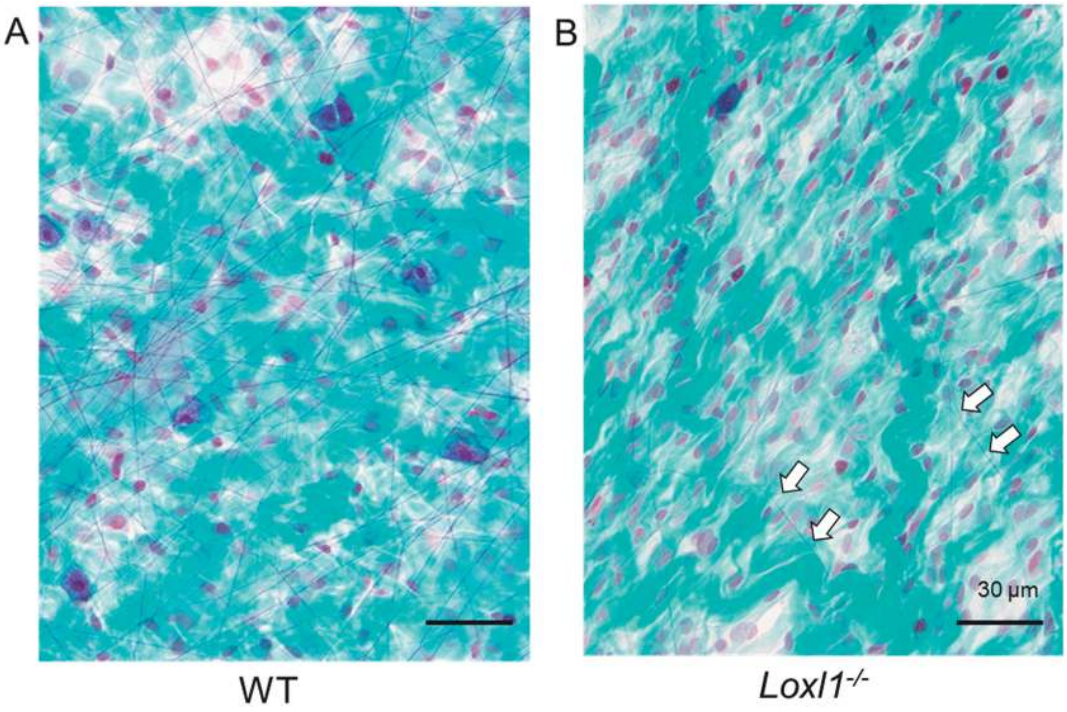

Fig. 4 Histopathological comparison of mesentery on resorcin-fuchsin and light green staining between the wild type (WT) and the *Loxl1*$^{-/-}$ rats. On resorcin-fuchsin and light green staining, elastin fibers which were seen as strait strings were higher density in the WT rats when compared with in the *Loxl1*$^{-/-}$ rats. Scale bar; 30 μm.

Organ chamber study

To assess the affinity to the muscarinic receptor in the 2 groups, we measured the contractile response of muscle strips of bladder. The contractile response of the bladder strips to CCh at concentrations of 3×10^{-7} M to 10^{-5} M was significantly weaker in the *Loxl1*$^{-/-}$ group than in the WT group (Fig. 6A). The logEC50 values for CCh contraction were significantly higher in the *Loxl1*$^{-/-}$ group than in the WT group (-5.65 ± 0.03 M vs. -5.93 ± 0.02 M, respectively). This result indicated that the affinity of muscarinic receptors in the *Loxl1*$^{-/-}$ group was smaller than in the WT group.

Although the two groups showed no significant difference in their response to EFS, the magnitude of blockade of the contraction response in the *Loxl1*$^{-/-}$ group after atropine was smaller compared to the WT group (Fig. 6Ba, Bb). In addition, the contraction response in the *Loxl1*$^{-/-}$ group was significantly smaller with atropine, and the contraction remained in the *Loxl1*$^{-/-}$ group but not in the WT group after administration of atropine and ABMA (14% and 2% at 32 Hz, respectively). To compare the components of cholinergic or purinergic effects in two groups, we calculated the percentage of each component from the results of EFS. The percentage of cholinergic component in the EFS-induced contraction was significantly decreased in the *Loxl1*$^{-/-}$ group than the WT group (Fig. 6Ca), while there was a tendency of increased noncholinergic and nonpurinergic component in the *Loxl1*$^{-/-}$ group (Fig. 6Cc). These results indicate that noncholinergic and nonpurinergic components might play a role in bladder contraction in the *Loxl1*$^{-/-}$ rats. We found no significant changes in the contraction responses to KCl, $PGF_{2\alpha}$, or ABMA or in the relaxing response to isoproterenol.

DISCUSSION

We investigated the roles of elastin in urethral continence function in a rat model lacking the protein Loxl1. We found the following in the *Loxl1*$^{-/-}$ rats compared with the WT rats: (1) male and female rats had loosening and redundancy of the skin, and some male rats showed rectal prolapse, but no female rats did; (2) the leak point pressure was significantly lower, (3) the intercontraction intervals and voided volume were significantly lower; (4) elastic fibers of the mesentery, bladder, urethra, and vagina were thinner; and (5) the contractile response of detrusor strips to CCh was significantly smaller.

Previous studies in the *Loxl1*$^{-/-}$ female mice

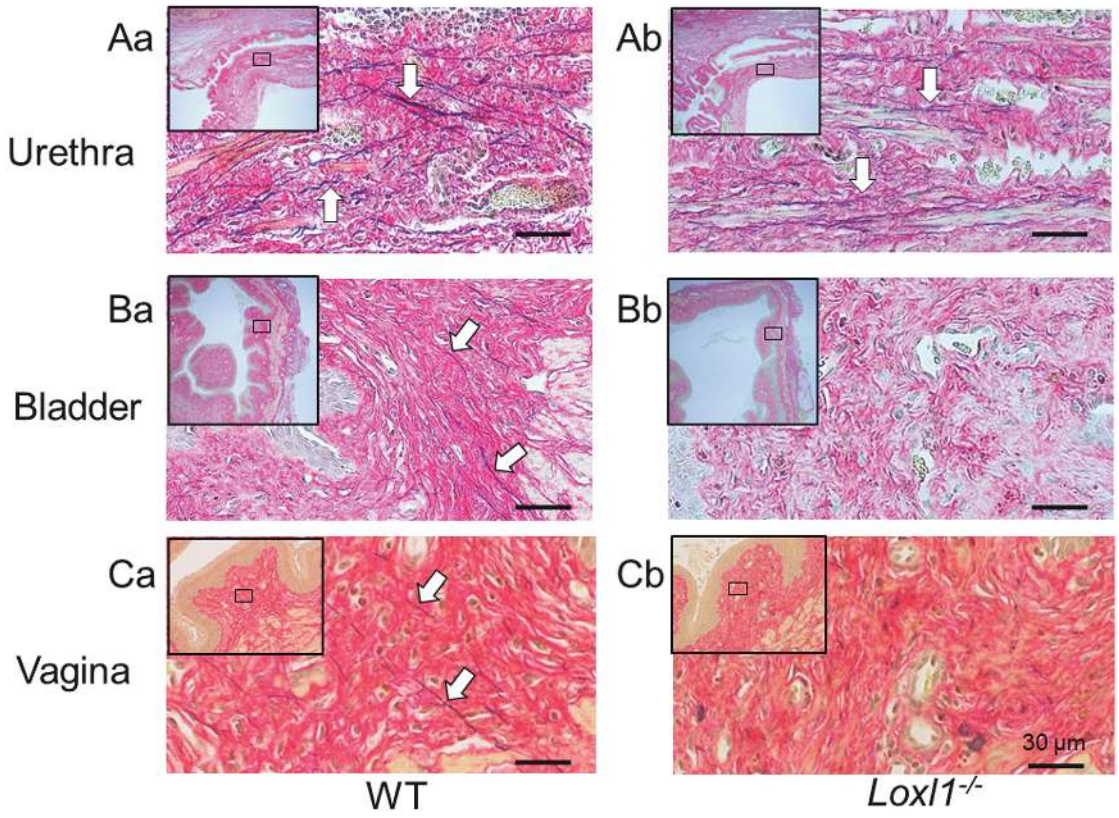

Fig. 5 Histopathological comparison of urethra (A-a, b), bladder (B-a, b), and vagina (C-a, b) on elastica van Gieson staining between the wild type (WT) and the *Loxl1*$^{-/-}$ rats. On elastica van Gieson staining, massive elastic fibers were seen in lamina propria of the urethra (Aa, b), bladder (Ba, b) and vagina (Ca, b) in the WT rats when compared with in *Loxl1*$^{-/-}$ rats. Scale bar; 30 μm.

showed that pelvic organ prolapse occurred post-partum and that permanent pelvic descent remained, with a large genitourinary bulge after the prolapse had retracted 14 days post partum (Liu *et al.* 2004, 2007). However, we did not find pelvic organ prolapse in the *Loxl1*$^{-/-}$ female rats, although, interestingly, some of the *Loxl1*$^{-/-}$ male rats showed rectal prolapse. One explanation for the discrepancy between our findings in the *Loxl1*$^{-/-}$ female rats and earlier findings in the *Loxl1*$^{-/-}$ female mice might be that the structure and strength of the pelvic floor may differ between rats and mice because of the difference in the size of their pelvis. In addition, the role of Loxl1 in elastin synthesis and the role of elastin in the pelvic floor strength may differ between the 2 animals. Further studies are needed to clarify these points.

The present study showed that urethral continence function was inefficient in the *Loxl1*$^{-/-}$ rats, as evidenced by the lower leak point pressure compared with the WT animals. The mechanism of resting urethral closure pressure is well established and is important for preserving continence. Continence is preserved by the integrity of the vesicourethral junction, the urethral and pelvic floor musculature, the hydrostatic pressure in the submucosal blood vessels, and the tension of urethral fibroelastic elements (Bump *et al.* 1988). The submucosal fibroelastic tissue has been assumed to be a major factor in maintaining resting urethral closure pressure (Dass *et al.* 1999). Our histopathological analyses, especially in the urethra, showed that the elastic fibers were thinner in the *Loxl1*$^{-/-}$ rats than in the WT rats. Previous study using mice also revealed that the submucosal elastic density was 41% fewer in the *Loxl1*$^{-/-}$ group than in the WT group (Bruna *et al.* 2014). Thus, deficient elastin fiber might reduce the resting urethral closure pressure and induce the lower leak point pressure in the *Loxl1*$^{-/-}$ rats compared with the WT rats.

In the cystometry analysis, the *Loxl1*$^{-/-}$ rats showed a significantly lower intercontraction interval and voided volume than the WT rats, as well as a tendency towards a higher residual urine volume and lower

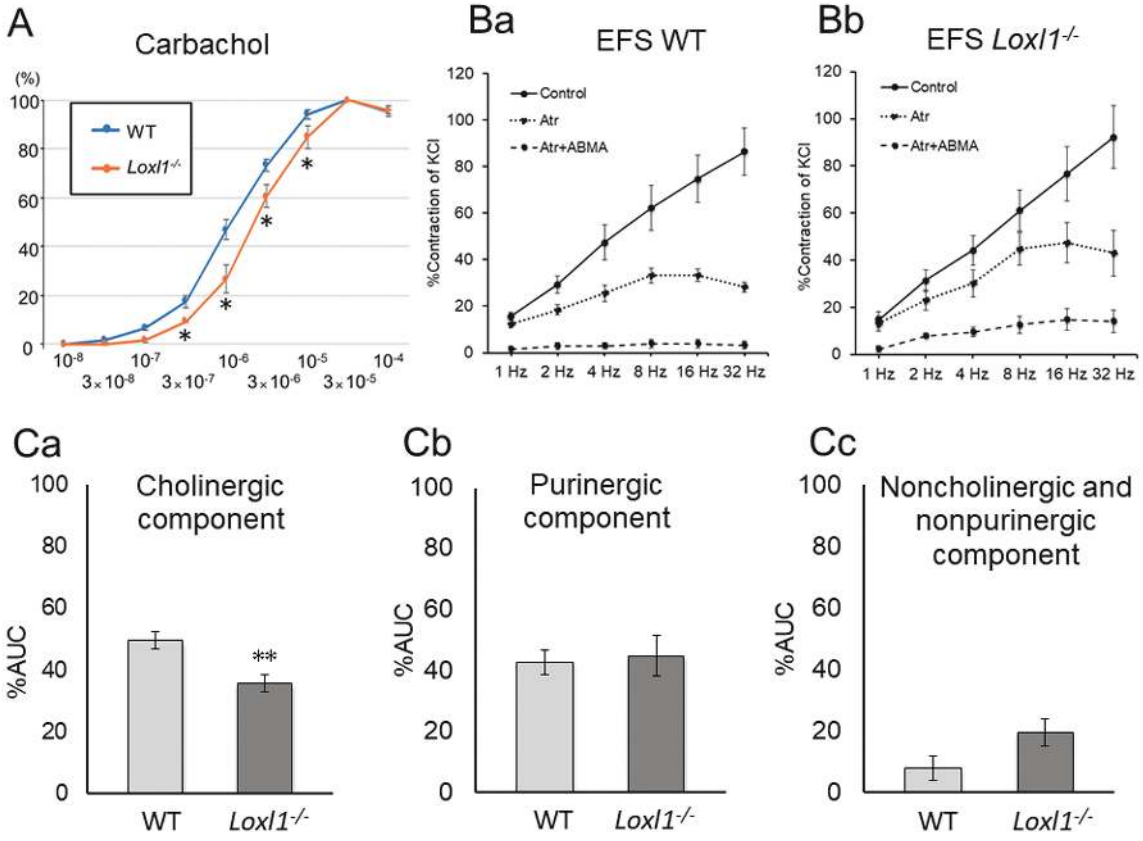

Fig. 6 Concentration-response curves for carbachol (A) and frequency-response curves for electrical field stimulation (EFS) with atropine (Atr) and α,β-methylene ATP (ABMA), and component proportion of EFS-induced contractions of detrusor strips (Ca, b, c) between the wild type (WT) and the *Loxl1*$^{-/-}$ groups. (**A**) The contractile response of detrusor strips to carbachol at concentrations from 3×10^{-7} M to 10^{-5} M was significantly smaller in the *Loxl1*$^{-/-}$ group compared with the WT group. (**Ba**, **Bb**) Although the two groups showed no significant difference in their response to EFS, the magnitude of blockade of the contraction response in the *Loxl1*$^{-/-}$ group after atropine was smaller compared to the WT group. (**Ca**, **b**, **c**) Percentage cholinergic component in the EFS-induced contraction was significantly decreased in the *Loxl1*$^{-/-}$ group than the WT group, while there was a tendency of increased noncholinergic and nonpurinergic component in the *Loxl1*$^{-/-}$ group.

bladder capacity. We hypothesize that, because elastin is one of the main components that provide tissue with elasticity, the thinner elastin fiber in the *Loxl1*$^{-/-}$ rats may decrease the elasticity of the bladder wall, resulting in a reduced intercontraction interval and voided volume (Macarak *et al.* 1995; Kadekawa *et al.* 2017). Previous studies showed that the *Loxl1*$^{-/-}$ female mice have a 10-fold higher voiding frequency than the WT control mice, with a corresponding decrease in volume in metabolic chamber analyses (Liu *et al.* 2006), and a lower bladder capacity and maximum contraction pressure during voiding in cystometric analyses (Liu *et al.* 2007), suggesting that the *Loxl1*$^{-/-}$ mice have worse urinary tract function, most likely due to urethral dysfunction. These results in mice and our findings of insufficient urethral closure pressure—shown by the low leak point pressure and deficient elastic fibers in the bladder wall— in the *Loxl1*$^{-/-}$ rats indicate that insufficient elastin synthesis might have a negative influence on bladder activity.

In the organ chamber analyses, the contractile response to CCh in detrusor strips from the *Loxl1*$^{-/-}$ groups was significantly shifted to the right compared with the WT group and the affinity of muscarinic receptors was smaller. In addition, the contraction response in the *Loxl1*$^{-/-}$ group was significantly smaller with atropine, and the contraction remained in the *Loxl1*$^{-/-}$ group but not in the WT group after administration of atropine and ABMA (14% and 2% at 32 Hz, respectively). These results indicate that noncholinergic and nonpurinergic components might play a role in bladder contraction in the *Loxl1*$^{-/-}$ rats. The low affinity for the muscarinic receptor and the effects of noncholinergic and nonpurinergic components might have a negative influence on

bladder activity in these rats. We found no significant difference between the 2 groups in the contractile response to the control stimulation by EFS. Although the reason for this result is not clear, we hypothesize that the decreased cholinergic component and increased noncholinergic and nonpurinergic component in the *Loxl1*$^{-/-}$ groups might have canceled out the difference in contractile response between the 2 groups.

In conclusion, impaired elastin synthesis in the submucosa of the urethra might reduce the resting urethral closure pressure, and a diminished cholinergic contractile response of detrusor smooth muscle might be involved in bladder activity in the *Loxl1*$^{-/-}$ rats.

CONFLICT OF INTEREST

none

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