442

Lee U[1], Gustilo-Ashby A M[2], Daneshgari F[1], Li T[3], Damaser M[4]
1. Glickman Urological Institute, Cleveland Clinic, 2. Department of Obstetrics and Gynecology, Cleveland Clinic, 3. Department of Opthalmology(Neuroscience),Massacussetts Eye and Ear Infirmary,Harvard Medical School, 4. Department of Biomedical Engineering, Cleveland Clinic and Lerner Research Institute

PARITY AND PELVIC ORGAN PROLAPSE LEADS TO REDUCED LEAK POINT PRESSURE IN LOXL1-DEFICIENT MICE

Hypothesis/Aims of Study

Deficiency of Lysyl Oxidase Like-1 (LOXL1) results in impaired elastin remodeling in mice and pelvic organ prolapse (POP) following pregnancy and vaginal delivery of pups. Previous studies [1,2] demonstrate that the absence of LOXL1 leads to increased voiding frequency in parous pelvic organ prolapsed mice compared to non-prolapsed nulliparous WT mice. It is unclear whether the differences in voiding pattern are due to the LOXL1 deficiency or the effect of pelvic organ prolapse. The objective of this study was to demonstrate the effect of pelvic organ prolapse on bladder function and abdominal leak point pressure in LOXL1-deficient mice with pelvic organ prolapse.

Study Design/Materials and Methods

Parous LOXL1$^{-/-}$ mice on a mixed C57Bl/6 and Sv129 background were maintained in an Animal Research Facility under standard 12 hour light-dark cycles with food and water provided *ad libitum*. Mice were housed using single pair or harem breeding conditions. Degree of POP was quantified using the MOPQ quantification system [3]. As shown in Figure 1, grade 0 POP is a normal perineum without evidence of POP and Grade 3 POP is a severe perineal bulge. Age and parity matched parous LOXL1$^{-/-}$ mice were divided into a prolapsed group (grade 3 POP) and a non-prolapsed group (grade 0 POP). Each mouse underwent conscious cystometry (CMG) and leak point pressure (LPP) testing two days after suprapubic tube implantation.

Under ketamine (100mg/kg body weight intraperitoneal) /xylazine (10mg/kg body weight intraperitoneal) anesthesia, a suprapubic bladder catheter (PE-10 tubing with a flared tip) was implanted in the dome of the bladder and secured with a circular purse string 7-0 silk suture. The catheter was tunneled subcutaneously and externalized at the nape of the neck, out of reach of the animal.

Two days after implantation of a bladder catheter, the animals were placed in modified metabolic cages for conscious CMG. The implanted bladder catheter was attached via a stopcock to both a pressure transducer (model P300, Grass instruments, West Warwick, RI) and a flow pump (model 100, KD scientific, Holliston, MA). The bladder was filled via the catheter with room temperature normal saline at a rate of 1 ml/hour while bladder pressure was recorded. Voided urine was collected and volume was measured using a force transducer. The pressure and force transducers were connected to an amplifier, polygraph (model MT9500, Astro-Med, Inc, West Warwick, RI) and computer, and digitized pressure data was recorded at a rate of 10 samples a second. After an initial stabilization period including at least one spontaneous void, the data on at least 6 representative micturition cycles were collected. The mean peak bladder pressures, baseline bladder pressures, fill time, fill volume, void volume, void pressure, voiding frequency, frequency of nonvoid contractions, and overall bladder contraction frequency were calculated from CMG data.

After CMG, mice were anesthetized with urethane (1.2 g/kg intraperitoneal) for LPP testing. The bladder catheter was connected to a pressure transducer and flow pump as described above. The mouse was placed supine and underwent an accommodation period of filling (1ml/hr) and spontaneous voiding. While bladder pressure was recorded and digitized, gentle pressure was applied externally over the bladder (using the Crede maneuver) to slowly increase pressure until the mouse leaked saline via the urethra. When leakage occurred, the externally applied pressure was rapidly removed. The LPP was conducted at least six times in each mouse. The mean peak bladder pressures, baseline bladder pressures, fill volume and abdominal leak point pressures were calculated from LPP data.

Univariate analysis was performed using t-test on normally distributed data, and Wilcoxon rank sum test on non-normally distributed data. Multivariate analysis was performed using 2 way ANOVA. Pairwise multiple comparison were performed using the Tukey test. P values less than 0.05 were considered significant.

Results

The age of the mice ranged from 19 to 67 weeks and parity ranged from 1 to 55 with no significant differences between the age and parity of the prolapsed (n=11) and non-prolapsed (n=6) LOXL1$^{-/-}$ mice. CMG parameters between the two groups was not significantly different on univariate or multivariate analysis. Significant differences were identified on LPP testing. Prolapsed mice showed a higher fill volume than non-prolapsed mice (0.086± 0.012 vs. 0.063 ± 0.022; p=0.015). Multivariate analysis revealed lower LPP in the prolapsed group versus non-prolapsed group when controlled for parity (23.06 ± 3.23 vs. 37.08 ± 4.06; p=0.021). Parity significantly contributed to reduced leak point pressures both between groups and within groups. After one delivery, prolapsed mice had a significantly lower LPP than non prolapsed mice (33.61 ± 4.98 vs. 62.60 ± 7.04; p=0.006). No significant differences were identified between groups after the 2nd and 3rd deliveries. Among mice without POP, LPP was lower after the 2nd delivery (32.66±7.04) and after the 3rd delivery (15.99±7.04) compared to after the first delivery (62.6±7.04; p=0.03 and p=0.002, respectively). Among mice with POP, LPP was lower after the 3rd delivery (9.45±7.04) compared to after the first delivery (33.61±4.98; p=0.042). Figure 2 summarizes the effect of parity and POP on LPP.

Interpretation of results

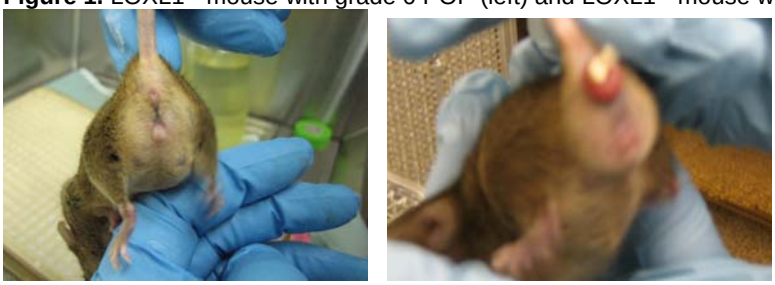
Both parity and POP significantly decrease abdominal LPP in LOXL1-deficient mice. The first delivery appears to have the most significant impact on LPP. Our CMG studies between prolapsed and non-prolapsed Loxl1 mice of varying parity did not demonstrate evidence of overactive bladder as was seen in our previous report. These findings suggest that the increased void frequency and decreased void volume may be the result of an incompetent urethral outlet in these mice, as evidence by lower LPPs. Based on these findings, we hypothesize that the altered elastic fiber homeostasis plays a role in the pathophysiology of stress urinary incontinence in women.

Concluding message

Parity and pelvic organ prolapse leads to reduced leak point pressure in LOXL1-deficient mice. Based on these findings, altered elastic fiber homeostasis may play a role in the pathophysiology of stress urinary incontinence in women.

References
(1) Am J Path 2006 Feb;168(2):519-28
(2) BJU Int 2007 accepted for publication
(3) Int Urogyn J 2006; 17,Supp 3:361-408, P13

Figure 1. LOXL1$^{-/-}$ mouse with grade 0 POP (left) and LOXL1$^{-/-}$ mouse with grade 3 POP (right).



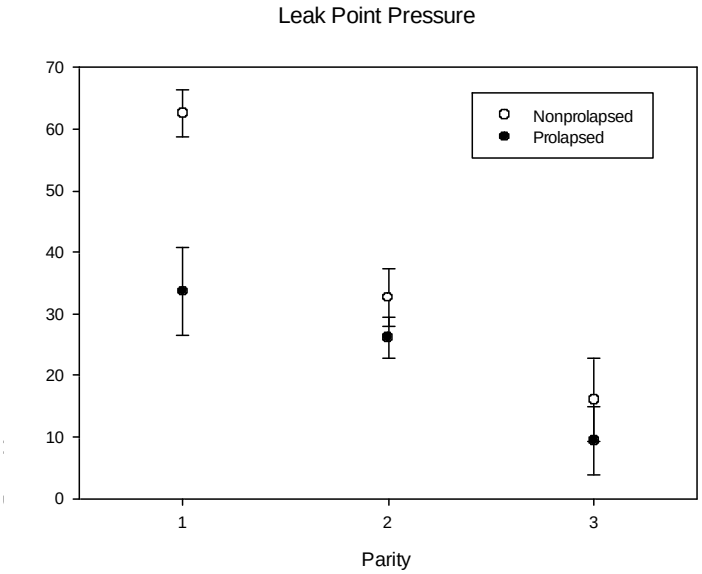


Figure 2. Abdominal leak point pressure vs. parity in Prolapsed (n=11) vs Non-prolapsed (n=6) LOXL1-deficient mice.

FUNDING: Cleveland Clinic RPC and Cleveland VA
ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Cleveland VA IACUC