Lower urogenital tract anatomical and functional phenotype in lysyl oxidase-like-1 knockout mice resembles female pelvic floor dysfunction in humans

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†Glickman Urological and Kidney Institute; ‡Division of Urogynecology and Reconstructive Pelvic Surgery, Department of Obstetrics and Gynecology, and ∥Department of Biomedical Engineering, Cleveland Clinic, Cleveland; ‡Research Service, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland; ∥Department of Radiology, Case Western Reserve University, Cleveland, Ohio; and ∥∥Department of Ophthalmology (Neuroscience), Massachusetts Eye and Ear Infirmary, Boston, Massachusetts

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Lee UJ, Gustilo-Ashby AM, Daneshgari F, Kuang M, Vurbic D, Lin DL, Flask CA, Li T, Damaser MS. Lower urogenital tract anatomical and functional phenotype in lysyl oxidase-like-1 knockout mice resembles female pelvic floor dysfunction in humans. Am J Physiol Renal Physiol 295: F545–F555, 2008. First published May 1, 2008; doi:10.1152/ajprenal.00063.2008.—Female pelvic floor dysfunction (FPFD) is a complex group of conditions that include urinary incontinence and pelvic organ prolapse (POP). In humans, elastin homeostasis has been implicated in the pathophysiology of FPFD. Lysyl oxidase-like-1 knockout (LOXL1-KO) mice demonstrate abnormal elastic fiber homeostasis and develop FPFD after parturition. We compared the lower urogenital tract (LUT) anatomy and function in LOXL1-KO mice with and without POP. LUT anatomy was assessed in LOXL1-KO mice over 28 wk. Pelvic visceral anatomy in LOXL1-KO was evaluated with a 7-Tesla magnetic resonance imaging (MRI) scanner. LUT function was assessed using conscious cystometry and leak point pressure (LPP) testing. Quantitative histological analysis of elastic fibers was performed on external urethral sphincter (EUS) cross sections. By 25 wk of age, 50% of parous LOXL1-KO mice developed POP. LOXL1-KO mice with POP had greater variability in the size and location of the bladder on MRI compared with mice without POP. Parity and POP were associated with lower LPP. Elastin clusters were significantly increased in the EUS of LOXL1-KO mice with POP. Because parity triggers POP in LOXL1-KO mice, LOXL1-KO mice with POP have variable internal pelvic anatomy, and both parity and POP are associated with a decrease in LPP, we conclude that LOXL1 LUT anatomical and functional phenotype resembles FPFD in humans. The increase in elastin clusters in the urethra of LOXL1-KO mice with POP suggests that elastin disorganization may lead to functional abnormalities. We conclude that LOXL1 warrants further investigation in the pathophysiology of FPFD.

Lower urogenital tract dysfunction (FPFD) is a complex and often debilitating group of conditions that include urinary incontinence, voiding dysfunction, and pelvic organ prolapse (POP). These disorders often coexist and have common risk factors. Vaginal childbirth is one of the risk factors associated with urinary incontinence and POP. Childbirth can result in pelvic floor laxity as a consequence of stretching and weaken-
and FBLN5 KO mice develop uterine and rectal prolapse. Unlike LOXL1 KO mice, however, FBLN5 KO mice primarily demonstrate FPFD as young nulliparous females. Both genetic mouse models are important tools in the investigation of the pathophysiology of FPFD. However, because LOXL1 KO mice primarily develop POP postpartum, and not as nulliparous mice (17), they are a better representation of the human clinical situation.

Creation of a mouse model for POP and its associated functional disorders, such as urinary incontinence or other bladder dysfunction, has raised the prospects of animal models in which genetic dependence as well as treatments and preventative measures can be tested. The answer to the question of which of the created animal models (the LOXL1 KO mouse, the FBLN5 KO mouse, or newer animal models, yet to be described) is a better model for human FPFD can only be found by detailed studies of various phenotypes, including functional and anatomical characterizations assessing similarity to the humanoid condition. The objectives of this study were to determine the effects of POP on the anatomy and function of the lower urogenital tract of LOXL1 KO mice. We describe the development of POP over time and in relation to parity using high-resolution magnetic resonance imaging (MRI) to characterize gross anatomy and morphometric histopathology to assess the urethra after prolapse. We also use urodynamic methods to determine the effect of POP on bladder and urethral function.

**Materials and Methods**

**Animals.** Parous LOXL1 KO mice on a mixed C57Bl/6 and Sv129 background were maintained in the Louis Stokes Cleveland Veterans Affairs Medical Center Animal Research Facility under standard 12:12-h light-dark cycles with free access to food and water. Mice were housed using single-pair or harem breeding conditions. Mice were weaned at 21 days and bred with littermates at 6–8 wk of age. Breeding pairs were housed together in cages and allowed to breed ad libitum. The null mutation in the LOXL1 gene in the breeding pairs of mice was confirmed by genotype analysis. Helicobacter testing was obtained and negative for *Helicobacter hepaticus*, the strain of Helicobacter associated with rectal prolapse (9). All studies were conducted in accordance with the standards of humane animal care, using protocols approved by the institutional animal care and use committee of the Louis Stokes Cleveland Department of Veteran’s Affairs Medical Center in Cleveland, Ohio.

**Prolapse Quantification.** Degree of POP was quantified using the mouse pelvic organ prolapse quantification (MOPQ) staging system (24). The MOPQ staging system was developed and validated in FBLN5 KO mice. The described MOPQ system was adapted for use with the LOXL1 KO mice, as shown in Fig. 1. Grade 0 POP is a normal perineum without evidence of POP; grade 1 represents mild POP; grade 2 represents moderate POP; and grade 3 represents severe POP. The perineum is protuberant and bulging. The arrow points to the relevant perineal bulge in each frame. Both C and D also have concurrent rectal prolapse. Rectal prolapse is not considered in the MOPQ staging system.

**Fig. 1.** Mouse pelvic organ quantification (MOPQ) system in lysyl oxidase-like 1 knockout (LOXL1) knockout (KO) mice. A: grade 0 represents no prolapse and a normal perineum. B: grade 1 represents a mild prolapse of the perineum. C: grade 2 represents moderate prolapse. D: grade 3 represents severe prolapse. The perineum is protuberant and bulging. The arrow points to the relevant perineal bulge in each frame. Both C and D also have concurrent rectal prolapse. Rectal prolapse is not considered in the MOPQ staging system.
moderate to severe prolapse, grade 2 or grade 3 POP. Nonprolapsed mice were defined as having no prolapse to mildevidence of prolapse, grade 0 or grade 1 POP. Mice were examined weekly, and grade of prolapse was recorded. Time-to-prolapse Kaplan Meier curves were used to assess the development of POP over time. Chi square statistical analysis was used to determine significance. P < 0.05 was considered statistically significant.

**Genotyping confirmation.** To confirm the lack of the LOXL1 gene in the knockout mice, total DNA was extracted from mouse tail samples using a Dneasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. The following three primers were used: S32 5′-ACG CGT CGG TGC TGG GAT CA-3′; D5 5′-CGT TCG TAA ACC AGT AGA ACT ACG ATC-3′; and N5 5′-CGA GAT CAG CAG CCT CTG TTC CAC-3′.

One unit of Platinum Taq DNA Polymerase was added (10966-018; Invitrogen) with 20 ng total DNA in each 25-μl reaction. PCR was carried out in the Mastercycler Gradient (Eppendorf) at 94°C for 4 min, 35 cycles for 45 s at 94°C, 45 s at 62°C, and 2 min for 30 s at 72°C. The reaction was reinitiated at 72°C for 10 min. DNA ladder (100 bp) was used as standard in 1.5% agarose gel (GIBCO-BRL, Gaithersburg, MD) to determine the PCR product. A band at 400 bp was identified for the wild type and at 300 bp for the mutant, LOXL1 KO.

**MRI.** To assess gross anatomy of the pelvic organs in live mice, select animals were anesthetized with isoflurane inhalation anesthesia and placed in a 7-Tesla Bruker Biospec MRI scanner within the Case Center for Imaging Research. High-resolution (600 × 200 × 200 μm) T1-weighted, fat-suppressed images were obtained with a turbospin echo sequence (TR/TE = 1,500 ms, 20 ms, 4 echoes). Age-matched, 3- to 6-mo-old, female LOXL1 KO mice with (n = 2) and without (n = 2) POP were used in the experiment. Prolapsed mice were parous and had stage 3 POP, and nonprolapsed mice were nulliparous and had stage 0 POP by the MOPQ staging system. Images were viewed serially using VolSuite 3.3.20 image processing software (Ohio Supercomputer Center). Three-dimensional (3-D) reconstructions of selected images were created using Amira 4.1.1 image segmentation software. Bladder volumes and vaginal volumes, in animal examples, were calculated using the 3-D segmented images. Mice were killed immediately after imaging.

**Suprapubic tube implantation.** A suprapubic catheter was implanted in 11 nonprolapsed and 6 prolapsed age- and parity-matched LOXL1 KO mice for urodynamic assessment of lower urinary tract function, as follows (5). Under ketamine (100 mg/kg body wt) and xylazine (10 mg/kg body wt) 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 6-0 silk suture. The catheter was tunneled subcutaneously and externalized at the nape of the neck, out of reach of the animal. The catheter was closed with a plug and secured to the animals’ dorsal skin with 4.0 vicryl suture. The animals’ abdomen was closed in two layers with 4.0 vicryl suture. Each mouse underwent cystometry (CMG) and leak point pressure (LPP) testing 2 days after suprapubic tube implantation (4).

**CMG.** 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/h while bladder pressure was recorded. Voided urine was collected by pipette, and the volume was measured using a calibrated beaker on a force transducer. Over 90% of the infused volume was collected via this method. The pressure and force transducers were connected to an amplifier, polygraph (model MT9500; Astro-Med, West Warwick, RI), and computer. Data were digitized at 10 samples/s by an analog-to-digital conversion system. After an initial stabilization period including at least one spontaneous void, the data on at least six representative micturition cycles were collected. Mean peak bladder pressure, baseline bladder pressure, fill time, fill volume, void volume, peak voiding pressure, voiding frequency, and frequency of nonvoid contractions were calculated from CMG data, as has been described previously (1).

**LPP testing.** After CMG, mice were anesthetized with urethane (1.2 g/kg ip) for LPP testing, as has been described previously (16). The bladder catheter was connected to a pressure transducer and flow pump as described above. Each mouse was placed supine and underwent an accommodation period of filling (1 ml/h) and spontaneous voiding. Immediately after spontaneous voiding, the bladder was emptied using a Crede maneuver. The bladder was filled to two-thirds of the estimated bladder capacity. While bladder pressure was recorded and digitized, gentle manual 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 was visualized from the urethra, the externally applied pressure was removed rapidly. LPP testing was conducted at least six times in each mouse. Mean peak bladder pressure, fill volume, and abdominal LPP (peak baseline pressure) were calculated from LPP data. LPP was defined as peak bladder pressure minus baseline bladder pressure.

**Image acquisition and analysis of elastin in mouse urethra.** Images of histological cross sections of mice midurethras stained with von Gieson (elastin) stain were acquired using a Leica DMR upright microscope (Heidelberg, Germany), a ×40 (dry) objective, an RGB color filter, and a Retiga 2000R charge-coupled device digital camera (Q-Imaging; Burnaby, BC, Canada). Image acquisition was fully automated for up to eight slides using an eight-slide, linearly encoded X, Y, Z motorized stage (Prior Scientific, Rockland, MA) managed by Objective Imaging’s Oasis 4i controller (Kansaville, MI), TurboScan software (Objective Imaging), and Image-Pro 6.1 (Media Cybernetics, Silver Spring, MD). During acquisition, high-magnification (auto-focused) image fields were acquired across the entire urethra and stitched together to form a single high-resolution, large field-of-view (FOV) image (~10 × 10 tiles/mosaic). Each image field was background corrected before stitching to ensure continuity and minimize chromatic variability. For quantitative analysis of elastin content and morphology, large FOV images were batch processed using customized macros and algorithms generated for Image-Pro Plus 6.1 (Media Cybernetics). In each large FOV image, a user-defined region of interest (ROI) was delineated around the urethra by an investigator blinded to mouse strain, prolapse status, and parity, using muscle boundaries visible in corresponding cross sections stained with Masson’s Trichrome. Images were then sharpened using an unsharp mask filter (7 × 7 kernel) and converted to the hue, saturation, value color space. To generate a binary mask of elastin fibers, the value channel of the sharpened image was extracted, inverted, processed using a large spectral edge filter (10 × 10 pixel window) to enhance the appearance of elastin fibers, and thresholded by intensity (>100 gray levels, area (>20 pixels), length (>3 pixels), and aspect ratio (>1.5). Because intensely stained brown nuclei, tissue boundaries (interface between tissue and muscle background), and dark-brown mucosa are possible sources of artifact, a series of processing steps was applied to minimize their relative contributions to the computer-generated elastin mask. Briefly, a mask of the mucosa was created by extracting the saturation channel of the original image, blurring this image using a low-pass filter, applying an intensity threshold, and dilating the resultant mask. A mask of interface or background boundary pixels was generated by applying a color-cube-based profile that segmented white pixels in the original image and dilating the resultant binary image. A mask of brown nuclei (and tissue) was generated by converting the original image to the YIQ color space, extracting the in-phase channel (red-brown pixels), removing noise (median filter), and applying an intensity threshold. These masks were then subtracted from the above elastin mask, and parameters, including elastin area, length, and number, were calculated.
For analysis of elastin fiber thickness, the Euclidean Distance Map (EDM) and skeletonized representation of the elastin mask were multiplied together to produce an image containing elastin fiber medial lines (1 pixel wide) with pixel gray values corresponding to fiber width (distance of medial pixel to fiber edge). These pixel values were summed, divided by the total number of skeletal pixels, and multiplied by two to report mean elastin fiber thickness. Similarly, to determine prevalence and size of elastin “clusters” (focal regions where elastin fibers are in close proximity), the skeletonized image of the elastin mask was inverted and used to generate an EDM image of nonelastin, background pixels. This EDM image was subsequently thresholded using an upper intensity value corresponding to a maximal fiber separation of 1 μm; cluster number and area in the resultant mask were reported for segmented regions 50 μm². Last, to provide a means to normalize elastin data between images, total tissue (excluding white background pixels) and ROI areas were also calculated.

Statistical analysis. Results are presented as means ± SE of each experimental group. Univariate analysis was performed using t-test on normally distributed data and Wilcoxon rank sum test on nonnormally distributed data. Multivariate analysis was performed using two-way ANOVA. Pairwise multiple comparisons were performed using the Tukey test. P ≤ 0.05 was considered statistically significant.

RESULTS

Natural history of POP in LOXL1 KO mice. Female LOXL1 KO mice (n = 62) were observed for POP for a mean time of 28 wk (minimum 15 wk to maximum 67 wk). Of the 62 mice observed for prolapse, 13 were nulliparous and 49 were parous. Overall, 31 (50%) developed grade 2 or 3 prolapse. Of the 13 nulliparous mice, 2 (15%) developed POP. Of the 49 parous prolapsed mice, 10 mice developed POP after one litter (32%), 10 after two litters (32%), 5 after three litters (17%), and 4 after four litters (14%). The effect of age and parity on prolapse was demonstrated by Kaplan-Meier curves (Fig. 2). By 25 wk of age, 50% of parous LOXL1 KO mice had developed POP. Parity significantly affects the age at which prolapse develops (chi square; P = .016). For each unit increase in parity, the estimate for the age for grade 2 or 3 decreases by 25%. Therefore, with increasing parity, POP develops at a younger age.

MRI. Although the externally visible phenotype of the two animals with POP was the same (severe POP), high-resolution MRI imaging revealed high variability in the location and size of the bladder and the vagina in LOXL1 KO mice with POP. Qualitative MRI analysis determined that LOXL1 KO mice without POP had uniform bladder sizes and locations. In these mice, the vagina, uterus, cervix, and rectum were also observed in consistent anatomical positions. In the nonprolapsed LOXL1 KO mice, the bladder was located above the pubic symphysis, the vagina was clearly visualized, and the rectum did not protrude beyond the perineum (Fig. 3A).

In LOXL1 KO mice with POP, qualitative MRI analysis revealed increased variability in the size and location of the bladder in prolapsed mice compared with nonprolapsed mice.
with the bladder below the pubic symphysis and distending the perineum in one case (Fig. 3, B and C). In another prolapsed mouse, the bladder was located normally; however, the vagina was markedly distended, leading to perineal distension (Fig. 3D). The rectum could be seen protruding outside the body in the prolapsed animals. The maximum bladder diameter was 14 mm, with noticeable bladder wall thickening of 0.4 mm. Bladder and vaginal volumes were calculated by 3-D reconstruction. There was marked variation in bladder volume between the LOXL1 KO mice with POP and LOXL1 KO mice without POP. For example, the bladder volume in one nonprolapsed mouse was 0.094 ml, whereas the bladder volume of a prolapsed mouse was 0.334 ml (Fig. 4, A and B). Similarly, marked variation was noted in vaginal volumes of LOXL1 KO mice with and without POP. The vaginal volume was calculated to be 0.013 ml in a nonprolapsed mouse, whereas, in a mouse with prolapse, the vaginal volume was 0.161 ml (Fig. 4, C and D).

Functional experiments. LOXL1 KO mice that underwent urodynamics were prolapsed or nonprolapsed as determined by physical exam. The age of the mice ranged from 19 to 67 wk, and parity ranged from one to five. There were no significant differences between the mean age in prolapsed mice (32 ± 4 wk) and nonprolapsed mice (29 ± 8 wk; P = 0.75). There was no significant difference in parity between the groups. Median parity was two (range 1–4) in prolapsed mice vs. a median parity of two (range 1–5) in nonprolapsed mice (P = 0.66). LOXL1 KO mice, with and without POP, voided spontaneously and at regular intervals. There was no evidence of chronic bladder outlet obstruction based on regular voiding intervals, volumes voided, and calculated postvoid residuals. CMG parameters between the two groups were not significantly different on univariate analysis (Table 1).

LPP testing demonstrated a slow rise to peak and rapid drop. Prolapsed mice had a significantly increased fill volume compared with nonprolapsed mice (P = 0.015). No other differences in LPP parameters were identified between prolapsed and nonprolapsed groups on univariate analysis (Table 2). However, multivariate analysis revealed lower LPP in the prolapsed group vs. nonprolapsed group when controlled for parity (23.06 ± 3.23 vs. 37.08 ± 4.06 cmH2O; P = 0.021). Parity significantly contributed to reduced LPP, both within groups and between groups (Fig. 5). After one delivery, prolapsed mice had significantly lower LPP than nonprolapsed mice (33.6 ± 5.0 vs. 62.6 ± 7.0 cmH2O; P = 0.006). No significant differences were identified between the two groups after the second and third deliveries. Among mice without POP, LPP was significantly lower after the second delivery (32.6 ± 7.0 cmH2O) and after the third delivery (16.0 ± 7.0 cmH2O) compared with after the first delivery (62.6 ± 7.0 cmH2O; P = 0.03 and P = 0.021, respectively). Among mice with POP, LPP was lower after the third delivery (9.5 ± 7.0 cmH2O) compared with after the first delivery (33.6 ± 5.0 cmH2O; P = 0.042).

Quantitative histological analysis. Quantitative histological analysis of elastic fibers in the external urethral sphincter demonstrated an increased number of elastin clusters in the external urethral sphincter of LOXL1 KO mice with prolapse (28.7 ± 3.9) compared with LOXL1 KO mice without prolapse (17.8 ± 1.8) and nulliparous C57Bl/6 mice (9.6 ± 2.9, P = 0.002), as shown in Fig. 6. Pairwise comparisons revealed significant differences in the number of elastin clusters be-
between LOXL1 KO mice with and without prolapse (P = 0.002) and between LOXL1 KO mice with prolapse and C57Bl/6 mice (P = 0.05), but not between LOXL1 KO mice without prolapse and C57Bl/6 mice (P = 0.176). Elastin fiber density was significantly lower in the LOXL1 KO mice with prolapse compared with the C57Bl/6 controls (257 ± 4 vs. 356 ± 19 fibers/100 mm², P = 0.001) and LOXL1 KO mice without prolapse compared with C57Bl/6 mice (267 ± 19 vs. 356 ± 19 fibers/100 mm², P = 0.003). However, no difference in elastin fiber density was identified between LOXL1 KO mice with and without prolapse (P = 0.889). No significant differences were identified between groups in the mean elastin fiber thickness or mean elastin fiber length.

In the quantitative histological analysis, all of the C57Bl6 mice were nulliparous, and all LOXL1 KO mice with POP were parous. Of the LOXL1 KO mice without POP, four were parous and two were nulliparous. No differences were identified in the number of elastin clusters when analyzed by parity in either the prolapsed (P = 0.448) or the nonprolapsed (P = 0.325) group. The numbers in each group are small, however; therefore, we cannot conclude that parity does not affect elastin disorganization.

**DISCUSSION**

FPFD is a complex and costly group of related conditions that include urinary incontinence, voiding dysfunction, and POP. Based on a questionnaire administered to a community population, the prevalence estimates for each of these disorders are as follows: 7% prolapse, 15% SUI, 13% overactive bladder, 25% anal incontinence, and 37% for any two or more pelvic floor disorders (19). However, in office-based examinations, POP has been found to affect up to 50% of women over 50 years of age, with a lifetime prevalence of 30–50% (7). It is estimated that 11.1% of all women will require surgical treatment for urinary incontinence, POP, or both during their...
demonstrating a fivefold reduction in LOXL1 in the uterosacral ligaments of women with POP and differential expression in the cardinal/uterosacral ligaments of women with POP when compared with controls (25). These findings have been independently confirmed in histopathological analyses (8, 14). The inherent limitations of clinical studies and experimentation on human subjects do not allow investigation of cause-and-effect relationships. In addition, investigation into theories into the role of risk factors in susceptibility to FPFD is limited in human studies. Thus availability of an animal model in which more than one element of FPFD could be replicated would prove to be invaluable for future investigations.

In 2004, Liu et al. (18) demonstrated that mice lacking the protein LOXL1 routinely develop POP postpartum. LOXL1 is a copper-dependent monoamine oxidase required to synthesize elastin polymers from tropoelastin monomers in adult tissues. LOXL1 KO mice develop POP after pregnancy and delivery or with aging, presumably due to an inability to maintain homoeostasis of mature elastic fibers. In 2006, Liu et al. (17) further characterized the FPFD seen in these mice. In this report, LOXL1 KO mice developed overt prolapse 1–3 days postpartum (Acute Stage), which retracted over 1–2 wk, leaving a persistent perineal bulge with internal pelvic organ descent (Stable Stage). One-third of mice developed POP after one delivery, and all mice developed POP after two deliveries. No mice delivered after the development of POP. Gross dissection of LOXL1 KO mice with POP demonstrated that the vaginal wall was markedly distended with uterine descent into the upper vagina. Bladder hypermobility was also noted in these mice. These anatomical and functional differences were thought to be the result of abnormal elastin remodeling in the postpartum period (17).

Our efforts to further characterize the natural history and anatomy of POP in LOXL1 KO mice demonstrated significant differences in the incidence of and time to development of POP from those described in earlier studies (17, 18). Furthermore, the anatomical variation that we observed in LOXL1 KO mice with POP has not been reported previously. Although our data mirror Liu et al.’s observation that one-third of LOXL1 KO mice develop POP after one delivery, it differs from their finding in that only one-half of the remaining mice in our population developed POP after their second delivery, and approximately one-third of mice in our population developed POP after their third or fourth delivery. In addition to differ-

<table>
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<th>CMG Variable</th>
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Values are means ± SE; n, no. of mice. CMG, conscious cystometry; LOXL1, lysyl oxidase-like 1; KO, knockout.

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Additional risks for POP are related to FPFD, including POP and lower urinary tract dysfunction, including urinary incontinence in women is 12.4 billion, and the estimated national annual direct costs of POP surgery adds an additional one billion 1997 U.S. dollars (23). Risk factors for FPFD include vaginal delivery, age, and obesity. Furthermore, evidence suggests that heritable or genetic factors play an important role in the pathophysiology of POP (11). Despite the finding in that only one-half of the remaining mice in our population developed POP after their second delivery, and approximately one-third of mice in our population developed POP after their third or fourth delivery. In addition to differ-

Table 1. CMG results in prolapsed vs. nonprolapsed LOXL1 KO mice

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The inherent limitations of clinical studies and experimentation on human subjects do not allow investigation of cause-and-effect relationships. In addition, investigation into theories into the role of risk factors in susceptibility to FPFD is limited in human studies. Thus availability of an animal model in which more than one element of FPFD could be replicated would prove to be invaluable for future investigations.
ences in the incidence of POP, the mean time to develop grade 2 or 3 POP after delivery was 4.6 ± 1.5 wk (range 0.0–22.6 wk). This differs from the 2- to 3-wk postpartum time course for the development of stable POP described by Liu et al. (17). Despite the differences in the incidence and timing of POP in LOXL1 KO mice between our study and Liu’s, statistical analysis of our data confirms their conclusion that pregnancy and delivery are the significant triggering factors that lead to POP. As in humans, significant variability exists in the number of deliveries required to trigger POP. Further investigation is required to determine the underlying pathophysiology behind these variations.

The LOXL1 KO mouse not only parallels human variability in its association with pregnancy, it also mirrors human anatomical variation. POP in humans is characterized by the downward decent of the pelvic organs. These organs include the bladder (cystocele), the rectum (rectocele), small bowel (enterocele), or the uterus or vaginal apex (uterine/apical prolapse) (11). However, clinical examination does not reliably predict the location of pelvic viscera in women with POP due to anatomical variability (12). Qualitative MRI analysis of LOXL1 KO mice with and without POP also demonstrated significant anatomical variability in LOXL1 KO mice with POP. Despite similar appearance of the perineal bulge, MRI revealed that pelvic viscera underlying the bulge might include vagina, bladder, or both. This finding further supports the value of the LOXL1 KO mice as an animal model of POP. Although the MRI in this study identify qualitative differences in the size and location of the female pelvic viscera in LOXL1 KO mice with POP, quantitative differences in mice with and without POP cannot be determined due to the limited number of animals in this portion of the study. Additional studies are required to determine what percentage of LOXL1 KO mice with POP demonstrate bladder, rectal, and/or uterine descent and to correlate these findings with their corresponding disorders in humans. The use of MRI to identify in vivo anatomical differences between mice with and without POP will not only accurately assess internal pelvic anatomy, it will allow investigators to perform longitudinal anatomical studies with animals serving as their own controls.

In addition to anatomical changes, LOXL1 KO mice also demonstrate evidence of voiding dysfunction. Using 24-h voiding studies, Liu et al. (17) showed a 10-fold increase in voiding frequency and a corresponding decrease in the volume of voids in prolapsed LOXL1 KO mice compared with wild-type controls. Without bladder pressure measurements, it could not be determined if the voiding dysfunction resulted from bladder or urethral pathology. Liu et al. (16), therefore, performed CMG and LPP on LOXL1 KO mice with stable POP after vaginal delivery and nulliparous wild type (LOXL1+/−) without POP (16). CMG studies demonstrated increased voiding frequency, small volume voids, and lower voiding pressure in LOXL1 KO mice.

Fig. 6. Elastin fiber clusters in the urethra of LOXL1 KO mice with and without POP and C57Bl/6 control mice. A: urethra of C57Bl6 control mouse. B: urethra of LOXL1 KO mouse without POP. C: urethra of LOXL1 KO mouse with POP. The urethra is stained with a modified von Gieson stain and shown at ×40 magnification. Elastin is stained black, and elastin clusters are highlighted in blue in A–C. D: expanded view of the area bounded by the rectangle in C. Elastin fibers are black. Representative clusters of elastin fibers are indicated by black arrows, and representative nonclustered elastin fibers are indicated by white arrows. E: no. of elastin clusters in the external urethral sphincter. *Significant difference compared with C57Bl6 control mice. †Significant difference compared with LOXL1 KO mice without POP.
prolapsed mice compared with wild-type nonprolapsed mice. No difference in LPP or in vitro bladder contractility was noted in this study. However, several potential confounders are acknowledged, including multiple factors that were not controlled. First, the experimental group was comprised of primiparous mice, whereas mice in the control group were nulliparous. Second, mice in the experimental group had severe stable POP, whereas the control group consisted of mice without POP. Last, mice with null mutations in the LOXL1 gene were compared with wild-type, or homozygous LOXL1+/−/− mice. Although this study supports the previous findings of Liu et al. (17), one limitation is that it is unclear whether the voiding dysfunction is due to the physiological changes associated with POP or due to functional changes associated with altered elastin homeostasis as a result of LOXL1 deficiency.

In our study, we compared age- and parity-matched LOXL1 KO mice with and without POP to determine the effect of POP on bladder and urethral function. POP in LOXL1 KO mice was associated with lower LPPs when controlled for parity. Prolapsed mice had consistently lower LPPs than nonprolapsed mice of the same parity. Although our findings are not entirely consistent with the findings of Liu et al.’s study (16), the conclusions of the earlier study are difficult to interpret because the findings are confounded by both the effects of POP and LOXL1 status. In our study, we investigated the functional effects of POP by studying only animals that were LOXL1 deficient. Our findings suggest that POP leads to decreased LPP and decreased bladder capacity in this mouse model.

In humans, the association between POP and SUI is complex. The relationship between POP and SUI can be variable, depending on the degree of POP. Severe POP is associated with mechanical obstruction of the urethra and therefore higher LPP (15). Only 5% of LOXL1 KO mice have been reported to have evidence of obstruction (17). No mice in our study had evidence of bladder outlet obstruction; therefore, our urodynamic findings are likely not attributable to a mechanical obstruction.

In humans the incidence of SUI in women with severe POP is low. However, when the POP is reduced, urethral dysfunction is frequently unmasked, and occult SUI is demonstrated (24). Accordingly, abdominal LPP, and maximum urethral closure pressure decrease after reduction of POP compared with LPP values before POP reduction (10). In addition, no difference in levator ani muscle electromyogram activity has been demonstrated before and after reduction of prolapse (20). These findings suggest that there is an association between POP and SUI that is likely due to urethral dysfunction as opposed to levator ani dysfunction. In light of the above study, the decreased LPP associated with POP seen in our study may be due to intrinsic urethral dysfunction, likely from abnormal elastin homeostasis, as demonstrated by increased clusters of elastin fibers. Because the association of POP and SUI is poorly understood, this animal model may give us insight into the pathophysiology of urinary incontinence in patients with POP.

Furthermore, mean LPP values declined with increasing parity in both prolapsed and nonprolapsed mice. The difference between LPPs in prolapsed and nonprolapsed mice is larger after the first delivery compared with after the second and third. This decrease in LPP could be a result of the need for elastin remodeling after delivery, which LOXL1 KO mice are unable to complete. The largest decrease in LPP in LOXL1 KO mice with POP occurs primarily after the first delivery, suggesting that the majority of the urethral damage occurs after the first delivery in mice with POP. Subsequent deliveries had a smaller effect in this group. The nonprolapsed mice demonstrated decreased LPP with increasing parity, which could be due to several potential factors: altered elastin homeostasis, age-related changes to pelvic floor function, or soft tissue or nerve injury associated with pregnancy and delivery. Both elastin and POP and the interaction of elastin with POP likely affect LPP. Our CMG studies between prolapsed and nonprolapsed LOXL1 KO mice of varying parity did not demonstrate evidence of increased voiding frequency, as previously reported (17). Our findings suggest that the previously reported increased voiding frequency and decreased voided volume may be the result of an incompetent urethral outlet in these mice, as evidenced by lower abdominal LPPs. Because all animals in this study were LOXL1 KO mice, and therefore had altered elastic fiber homeostasis, we hypothesize that POP contributes to reduced LPP in LOXL1 KO mice. It is unclear whether POP causes additional damage to the external urethral sphincter or is simply a marker of more severe pelvic floor damage.

One difference between our study and that of Liu et al. (16) is seen in the bladder filling volume of both LOXL1 KO mice with and without POP during LPP testing. In the earlier study, the bladder filling volume of LOXL1 KO mice with POP was 0.37 ± 0.06 ml. The bladder filling volumes in our study were significantly smaller. We found that bladder filling volume in LOXL1 KO mice with POP was 0.09 ± 0.004 ml. The reason for this discrepancy is unclear. Bladder capacity measurements during awake CMG testing of LOXL1 KO mice with POP were similar in the two studies, with bladder capacities of 0.10 ± 0.01 vs. 0.08 ± 0.01 ml in the Liu et al. study vs. our current report, respectively. Given that testing conditions were similar in the two studies, the reason for the difference in bladder filling volume during LPP testing is unclear. Future studies are required to clarify these findings.

One possible confounder of LPP testing was the supine position of the animals. Although the supine position of the animals during LPP testing does not reflect the natural position of the animal’s bladder when ambulatory, performing LPP in the supine position may better simulate LPP testing in humans with the POP reduced. The supine position may allow for relief of any potential obstruction by the hypermobile bladder of the LOXL1 KO mice with POP.

Another possible confounder of LPP testing may be the effect of anesthesia on bladder and urethral function. The bladder filling volume during LPP testing was significantly greater in LOXL1 KO mice with POP than in mice without POP. Therefore, the bladder volume threshold that induced a spontaneous void was greater in prolapsed LOXL1 KO mice than in nonprolapsed LOXL1 KO mice. This difference was not identified on cystometry, as might be expected; therefore, it may be due to differential effects of anesthesia in the two groups. Although urethane anesthesia has been used in LPP testing in rats for some time now, and was determined to be the optimal anesthetic agent for LPP testing in rats (1), studies to clarify the effects of different anesthetic agents on LPP values have not been performed in mice. It is possible that anesthesia may reduce or eliminate the compensatory effect of the pelvic floor musculature that may be present in the awake animal.
Additional studies are required to explain the physiology underlying this finding.

Histological differences between LOXL1 KO mice with and without POP were limited. Elastin fiber length and width were similar between the groups, as was elastin fiber density. However, there were significantly more elastin fiber clusters in the LOXL1 KO mice with POP compared with those without POP. This suggests increased disorganization in the elastin fibers in LOXL1 KO mice with POP. Because LOXL1 plays an important role in elastin remodeling, it is possible that POP is associated with increased elastin disorganization in the urethra of LOXL1 KO mice and leads to a loss of urethral elasticity, resulting in urethral dysfunction. Because LOXL1 wild-type (+/+) mice were not included in the LPP assessments, we are unable to fully determine the specific effects of altered elastin homeostasis on LPP. Our study was designed to isolate the effect of POP on LPP. We conclude that POP is associated with decreased LPP. To determine whether altered elastic fiber homeostasis independently leads to a decrease in LPP, testing would need to be performed in nulliparous, nonprolapsed LOXL1 KO mice and nulliparous nonprolapsed LOXL1 wild-type (+/+) mice.

In describing the anatomy and function of the lower urogenital tract of LOXL1 KO mice with POP, we demonstrated that parity is a significant trigger for development of prolapse over time. We used MRI to characterize the variable gross anatomy of the mouse pelvis with prolapse. We used light microscopy techniques to demonstrate increased elastin disorganization in the LOXL1 KO mouse urethra after prolapse. In addition, we used urodynamic methods to show decreased LPP in parous LOXL1 KO mice with POP compared with LOXL1 KO mice without POP.

As in humans, POP in LOXL1 KO mice develops and progresses over time and is associated with one or multiple deliveries. The pelvic anatomy of LOXL1 KO mice with POP can be characterized in vivo using MRI. Pelvic visceral anatomy is variable in LOXL1 KO mice with POP, and physical examination may not accurately characterize anatomical abnormalities seen in prolapsed LOXL1 KO mice. Both parity and POP are associated with a significant decrease in LPP in LOXL1 KO mice. This may be due to intrinsic sphincter deficiency related to POP, the deficiency of LOXL1, or both. The increase in elastin clusters in the urethra of LOXL1-KO mice with POP suggests that elastin disorganization may lead to functional abnormalities. Our findings support prior research that abnormal elastic fiber homeostasis plays an important role in the pathophysiology of FPFD. We conclude that LOXL1 lower urogenital tract anatomical and functional phenotype resembles FPFD in humans and that LOXL1 warrants further investigation in the pathophysiology of FPFD.

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REFERENCES


14. Kokcu A, Yanik F, Cetinkaya M, Alper T, Kandemir B, Malatyalioglu F. Histological differences between LOXL1 KO mice with and without POP were limited. Elastin fiber length and width were similar between the groups, as was elastin fiber density. However, there were significantly more elastin fiber clusters in the LOXL1 KO mice with POP compared with those without POP. This suggests increased disorganization in the elastin fibers in LOXL1 KO mice with POP. Because LOXL1 plays an important role in elastin remodeling, it is possible that POP is associated with increased elastin disorganization in the urethra of LOXL1 KO mice and leads to a loss of urethral elasticity, resulting in urethral dysfunction. Because LOXL1 wild-type (+/+) mice were not included in the LPP assessments, we are unable to fully determine the specific effects of altered elastin homeostasis on LPP. Our study was designed to isolate the effect of POP on LPP. We conclude that POP is associated with decreased LPP. To determine whether altered elastic fiber homeostasis independently leads to a decrease in LPP, testing would need to be performed in nulliparous, nonprolapsed LOXL1 KO mice and nulliparous nonprolapsed LOXL1 wild-type (+/+) mice.

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