Published in final edited form as:

Female Pelvic Med Reconstr Surg. 2014 ; 20(5): 287–292. doi:10.1097/SPV.0000000000000104.

Validation of Genetically Matched Wild Type Strain and Lysyl Oxidase- Like 1 Knockout Mouse Model of Pelvic Organ Prolapse

Bruna M. Couri, MD^{1,2}, Ali Borazjani, BS^{1,3}, Andrew T. Lenis, BS^{1,4}, Brian Balog, BS^{1,6}, Mei Kuang, PhD^{1,5}, Dan Li Lin, MD^{1,6}, and Margot S. Damaser, PhD^{1,2,3,4,6,7}

¹Dept of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, OH

²Dept of Obstetrics & Gynecology, Cleveland Clinic, Cleveland, OH

³Dept. of Chemical and Biomedical Engineering, Cleveland State Univ., Cleveland, OH

⁴Case Western Reserve University School of Medicine, Cleveland, OH

⁵Dept of Cancer Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH

⁶Advanced Platform Technology Center, Louis Stokes Veterans Affairs Medical Center, Cleveland, OH

⁷Glickman Urological & Kidney Institute, Cleveland Clinic, Cleveland, OH

Abstract

Objectives—Lysyl oxidase-like 1 knockout (*Lox11^{-/-}*) mice demonstrate deficient elastin homeostasis associated with pelvic organ prolapse (POP). To further investigate the pathophysiology of POP in these animals, a genetically-matched homozygous positive (*Lox11^{+/+}*) or wild type strain is needed. This study sought to create and validate genetically-matched *Lox11^{+/+}* and *Lox11^{-/-}* strains.

Methods—Female *Lox11^{-/-}* mice were backcrossed with male wild-type mice. The resultant heterozygous mice were bred to produce *Lox11^{+/+}* and *Lox11^{-/-}* mice, whose genotype was confirmed by RT-PCR. Multiparous female *Lox11^{-/-}* (n=7) and *Lox11^{+/+}* (n=9) mice were assessed for POP weekly for 12 weeks after their first vaginal delivery. POP was compared between groups using a Kaplan Meier survival curve with p<0.05 indicating a significant difference. Vaginal connective tissue (CT) histology was assessed qualitatively and quantitatively.

Results—There were no significant differences between the groups in age or parity. Four of 7 *Lox11^{-/-}* mice developed prolapse by 8 weeks and 6 of 7 by 12 weeks post-partum. No *Lox11^{+/+}* mouse prolapsed. *Lox11^{-/-}* mice had significantly larger vaginas as determined by area within the lumen and total cross-sectional tissue area. Striated muscle fibers of the urethra in *Lox11^{-/-}* mice were less organized, shorter, and thinner than in *Lox11^{+/+}* mice.

Corresponding author: Margot S. Damaser, Ph.D., Associate Professor, Dept of Biomedical Engineering, The Cleveland Clinic, 9500 Euclid Ave. ND20, Cleveland, OH 44195, Tel. 216-444-1103, Fax. 216-444-9198, damasem@ccf.org.

Disclosures

The authors have no real or potential conflict of interest with the results of this study.

Conclusions—Genetically-matched *Lox11*^{-/-} and *Lox11*^{+/+} strains can be reliably created by a backcross method and differentiate in their prolapse phenotype. *Lox11*^{-/-} mice demonstrate pathology primarily characterized by enlargement of the vagina. Further studies are needed to elucidate the cause of this finding.

Keywords

Animal model; LOXL1 protein; pelvic organ prolapse; elastin; mouse

Introduction

Pelvic organ prolapse (POP) is a bulge of one or more of the vaginal compartments (anterior, posterior, or apical/uterus) created by abnormal descent of the pelvic organs. POP is one of the major indications for benign gynecological surgery.¹ It accounts for more than 300,000 inpatient procedures and costs more than \$1 billion per year in the US alone.^{1,2} Consequently, treatment of POP is a significant financial burden. Women with POP have reduced quality of life secondary to sexual dysfunction, urinary and fecal obstruction, and social embarrassment.¹

Lysyl oxidase like-1 homozygous negative or knockout (*Lox11*^{-/-}) mice are a valuable and well-established animal model of POP.³ Without the copper-dependent enzyme LOXL1, cross-linking of tropoelastin into a functional elastin polymer is incomplete.⁴ This genetic predisposition for abnormal elastin repair combined with an appropriate environmental trigger (pregnancy and vaginal delivery), as well as aging, leads to POP in *Lox11*^{-/-} mice.^{5,6,7} In these animals, as in humans, Cesarean-section reduces but does not eliminate the risk of POP.⁸ In humans, similar epidemiology and abnormalities in elastin homeostasis have been associated with POP.^{9,10} Therefore, *Lox11*^{-/-} mice can be considered a pathophysiology-driven animal model of POP.

Current treatment of POP does not address our evolving understanding of its pathophysiology. To better understand this pathophysiology and develop targeted therapeutics, genetically-matched strains of wild type and knockout mice are required. However, the *Lox11*^{-/-} strain of mice has been bred from brother-sister pairings without a genetically matched wild type strain. Since the genetic background can affect phenotype or even make the knockout incompatible with life,¹¹ it is necessary to characterize and validate each strain of modified animal model. The goal of this study was to create genetically matched *Lox11*^{+/+} and *Lox11*^{-/-} mice, and to compare the phenotypic and histologic outcomes in a multiparous breeding colony.

Materials and Methods

Breeding protocol and genotyping

All animal protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Cleveland Clinic. Female *Lox11*^{-/-} mice, from a colony originally obtained from Tiansen Li, Ph.D. of the Massachusetts Eye and Ear Infirmary (Boston, MA, USA), were backcrossed with male hybrid C57BL/6J and 129S1/SvImJ

Lox11^{+/+} wild-type mice (The Jackson Laboratory Stock#101043, Bar Harbor, ME, USA). The resultant heterozygous (*Lox11*^{-/+}) animals were bred to produce *Lox11*^{+/+}, *Lox11*^{-/-}, and *Lox11*^{-/+} mice (Figure 1). Genotype identification of these animals was done by PCR analysis as described previously^{5,8} and *Lox11*^{-/+} mice were euthanized. Briefly, tail clipping was performed between 3–6 weeks of age under isoflurane anesthesia. DNA extraction was performed using a DNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Primers used were: S32 5'-ACA CGT CGG TGC TGG GAT CA-3'; D5 5'-CTT TCGTAA ACC AGT ATG AGA ACT ACG ATC-3'; and N5 5'-CGA GAT CAG CCT CTG TTC CAC-3' (IDT, Coralville, IA, USA). The resultant *Lox11*^{-/-} and *Lox11*^{+/+} mice were set in genetically matched brother/sister breeding pairs at 8–9 weeks old to generate multiparous females for investigation (Figure 1). Animals were housed as single pairs and allowed to breed *ad libitum*. Males were not removed from the cages and females achieved multiparity, delivering every 3–4 weeks until they were euthanized.

Phenotypic assessment

Multiparous female *Lox11*^{-/-} (n=7) and *Lox11*^{+/+} (n=9) mice were assessed for POP weekly via the mouse pelvic organ prolapse quantification (MOPQ) scoring system, which was initially developed for assessment of POP in mice deficient in fibulin-5 (*Fbln5*^{-/-}).¹² Prolapse grade 0 indicates no perineal bulge; grade 1 indicates a mild perineal bulge, increasing gradually with increasing grades until grade 4 which indicates a complete vaginal eversion. This grading system was validated by Lee *et al.* in *Lox11*^{-/-} mice.⁵ In the current study animals were considered prolapsed when presenting with MOPQ grade 2 or 3. No mice with grade 4 prolapse were observed. Weekly MOPQ assessment was started one week after first delivery, and kept throughout multiparity, until the animal died or was euthanized for histology. The first 12 weeks of MOPQ assessments were graphed as time-to-prolapse and used for Kaplan Meier survival curves, as done previously.⁵ In addition to MOPQ, perineal length, having as reference points the middle of the vaginal introitus (upper limit) and middle of the anus (lower limit) was measured using a digital caliper. The perineal length assessment was also done weekly, and started one week after first delivery; as the MOPQ results, only the first 12 assessments are showed here.

Histological preparation

For histological analyses, randomly selected *Lox11*^{-/-} (n=3) and *Lox11*^{+/+} (n=3) female mice were euthanized using CO₂ asphyxiation followed by cervical dislocation. The vagina and the urethra, were fixed in 10% formalin, paraffin embedded en bloc, and sectioned (5 µm) transversely at the mid-urethral level. Samples were stained with elastic Van Gieson (EVG) stain for quantitative assessment of elastic fibers and collagen. Masson's Trichrome (MT) stain was used for qualitative assessment of smooth and striated muscle.

Image acquisition and analysis

Images of each EVG stained slide were acquired at a resolution of 0.185 µm/pixel using a Leica DMR 6000 microscope (Leica Microsystems, Heidelberg, Germany), a 40x objective, an 8-slide x-y-z motorized stage (Prior Scientific, Rockland, MA) and a Retiga 2000R CCD camera (Q-Imaging, B.C., Canada). Raster-scanned image tiles across each tissue cross-

section were background corrected and stitched together to form a single high resolution, large field-of-view image (HR-FOVI; Figure 2a).

Each HR-FOVI was imported into Image J (v1.46r National Institutes of Health, Bethesda, MA, USA), and regions-of-interest (ROI) were manually drawn by an investigator blinded to the genetic group (Figure 2b). The cross-sectional area of each ROI was calculated along with the average thickness of the vaginal muscularis and submucosa. Total vaginal area was defined as the area contained within the vaginal muscularis perimeter (Figure 2b). Tortuosity of the vagina was calculated by tracing a line segment on the submucosa-epithelium boundary and dividing the length by the corresponding muscularis-submucosa boundary (Figure 2c). Four randomly selected segments of the muscularis-submucosa boundary and corresponding submucosa-epithelium boundary were utilized, with each segment containing 2–4 mm of the muscularis-submucosa boundary (Figure 2c). A ROI consisting of a perimeter drawn around the external urethral sphincter was used to calculate urethral circumference and diameter (Figure 2b).

Quantitative image analysis was performed using customized visual basic macros in Image-Pro Plus (v7.0 Media Cybernetics, Silver Spring, MD, USA) for each HR-FOVI and each ROI. For each tissue cross-section, an RGB (red, green, blue) color space segmentation was performed by adjusting RGB settings until pixels of interest for collagen (pink) were optimally segmented from background tissue by visual confirmation by an expert (Figure 2d). Similarly, elastic fibers were segmented using a series of spectral and morphological filters on the value channel of the HSV (hue, saturation, value) color space (Figure 2d). All segmented image overlays were validated by an expert blinded to genetic group. For each vaginal muscularis and submucosa ROI, total collagen area and the lengths of each segmented elastic fiber were determined. Total collagen area was divided by total area of the corresponding ROI to determine collagen density (percent area fraction of collagen). Only detected elastic fibers longer than 10 μm (range 10–15 μm) were counted to minimize nuclei artifact since EVG stains both nuclei and elastic fibers black. The total number of 10–15 μm long elastic fibers was normalized to the total area of the corresponding ROI to determine the number of elastic fibers per mm^2 .

Statistical Analyses

Differences in time-to-prolapse between the two groups were compared using a Kaplan Meier survival analysis and log-rank test. The Mann-Whitney Rank Sum Test was used to compare age and parity between the two groups. Mann-Whitney Rank Sum or Student's T-test was used to compare morphometric outcomes as determined by normality assessment. $p < 0.05$ was used to indicate a statistically significant difference between groups in all cases. Quantitative values are presented as mean $\pm$ standard error of the mean.

Results

Initially 4 of the 7 *Lox11^{-/-}* mice developed POP 8 weeks after the first delivery, and 2 more animals developed POP 12 weeks afterward (Figure 3). Parity of the *Lox11^{-/-}* mice that developed POP varied from 1–3 (2 ± 0.87) at the time of POP development. *Lox11^{+/+}* mice did not develop phenotypic evidence of POP at any time point, creating a statistically

significant difference between the two groups ($p < 0.001$). *Lox11^{-/-}* mice had longer perineal length 12 weeks after first delivery compared to *Lox11^{+/+}* mice (8.7 ± 0.7 mm and 7.6 ± 0.20 mm, respectively). However, this difference did not reach statistical significance ($p = 0.10$).

The mean age of mice selected for histological assessment was 42.7 ± 3.1 weeks (*Lox11^{-/-}*) and 40.5 ± 1.4 weeks (*Lox11^{+/+}*) at the time of tissue harvest. Parity varied from 5 to 6 litters (5 ± 0.58) in the *Lox11^{-/-}* mice, and 6–7 (6.25 ± 0.50) litters in the *Lox11^{+/+}* mice at the time of tissue harvest. There were no significant differences between the two groups in age ($p = 0.4$) or parity ($p = 0.11$). At the time of organ harvest (~30 weeks postpartum), all *Lox11^{-/-}* mice selected for histological assessment presented with MOPQ grade 3 (Figure 4), but no *Lox11^{+/+}* female mice had developed prolapse.

Upon gross examination, *Lox11^{-/-}* mice had wider vaginal diameters than *Lox11^{+/+}* mice, and this was confirmed with morphometric measurements taken at histology, which demonstrated that total vaginal area was significantly greater in *Lox11^{-/-}* than in *Lox11^{+/+}* mice (Table 1 and Figure 5). Similarly, there was a trend toward larger urethral circumference and diameter in *Lox11^{-/-}* mice, although this difference was not statistically significant. Cross-sectional area of vaginal muscularis and submucosal tissue were significantly greater in *Lox11^{-/-}* mice (Table 1). There was a statistical trend ($p = 0.051$) toward increased thickness of the vaginal submucosa in *Lox11^{-/-}* compared to *Lox11^{+/+}* mice. There was no significant difference in vaginal collagen density between the groups (Table 1). Although *Lox11^{-/-}* mice had 41% and 47% fewer elastic fibers in the submucosa and muscularis, respectively, this difference did not reach statistical significance (Table 1). On MT staining, striated muscle fibers of the external urethral sphincter, were less organized, shorter, and thinner in *Lox11^{-/-}* than in *Lox11^{+/+}* mice (Figure 5).

Discussion

POP is a widespread and prevalent disorder, affecting nearly 50% of postmenopausal women.^{1,13} Although rarely life-threatening, POP significantly burdens the lives of millions of women, decreasing their quality of life and leading to social isolation and depression.¹ Current treatments for POP do not target the pathophysiology of the disease, which could partially explain the relatively high revision and complication rates.^{14,15} Recent research suggests that POP is a disorder of CT homeostasis, particularly of elastin.^{9,10} A woman's predisposition to abnormal elastin metabolism may combine with the triggers of pregnancy and traumatic childbirth to lead to POP. Therapeutic strategies that aim to correct abnormal elastin homeostasis are currently under investigation^{16,17} and could potentially be applied to prevent or reverse POP. Genetically-matched animal models of abnormal elastin homeostasis that demonstrate POP are needed to investigate the pathophysiology of POP and provided the impetus for the current study. Once validated, these animal models could be utilized for preclinical testing of potential therapeutics.

Lox11^{-/-} mice have proven to be a reliable and reproducible model of human POP, and mimic a dependence on similar risk factors, molecular markers, and presumed pathophysiology.^{5,6,7,8} While larger mammals have more similar pelvic anatomy to humans, using larger species is costly, labor intensive, and less predictable given that no genetically

manipulated strains exist.³ Several genetic knockout rodent strains develop POP, including elastin metabolism-based models such as mice deficient in *Loxl1*, *Fbln5*, and fibulin-3 (*Fbln3*).^{3,5,18,19} On a molecular level, the enzyme LOXL1 and the scaffold protein FBLN-5 both participate in elastogenesis and are critical for post-partum restructuring of elastic fibers in mice.^{6,18} Deficiency of either gene leads to POP in mice.^{6,20} Deficiency of *Loxl1* however, usually, requires the addition of an environmental trigger, pregnancy and vaginal delivery, to induce POP development,⁸ making this a more clinically applicable model.

In the current study, we show that a backcrossed colony of *Loxl1*^{+/+} mice can be created from *Loxl1*^{-/-} mice. We have demonstrated via MOPQ assessment that the backcrossed *Loxl1*^{+/+} mice do not develop phenotypic evidence of prolapse, even after multiple vaginal deliveries. The natural history of POP in *Loxl1*^{-/-} mice observed in our colony is similar to that described previously. Lee et al. found that approximately 50% of *Loxl1*^{-/-} mice prolapse by 15 weeks after first delivery,⁵ while we found similar findings at 12 weeks after first delivery. POP in *Loxl1*^{-/-} mice is an epidemiological phenomenon that occurs at variable times after delivery, as in humans. Therefore, the smaller size of our colony compared to that used by Lee et al. may have skewed our results.

Alperin et al. in a previous study with *Loxl1*^{-/-} mice, found that some females presented with POP despite being nulliparous.²¹ This is different from our experience and that of Lee et al.⁵ These discrepancies may be explained by differences in the housing environment and/or genetic background.^{11,22}

Weislander et al. showed a significance difference between the perineal length of *Fbln5*^{-/-} and wild type mice.¹² We stopped assessing the perineal length when 6 of our 7 *Loxl1*^{-/-} mice achieved POP, which could have reduced the power to detect a statistical significance in perineal length between *Loxl1*^{-/-} and *Loxl1*^{+/+} mice. Alternatively, this difference in outcomes could be due to environmental or genetic background differences between the colonies.

Histologically, our findings in *Loxl1*^{-/-} mice may perhaps best be described as an abnormality characterized by enlargement of the vagina. The cross-sectional area of the vaginal muscularis and submucosal layers in *Loxl1*^{-/-} mice were significantly increased by 62% and 101%, respectively, compared to *Loxl1*^{+/+} mice. Additionally, the total vaginal area was over 150% larger in *Loxl1*^{-/-} mice compared to *Loxl1*^{+/+} mice. This enlargement could be due to stretching of the vagina due to biomechanical instability of the surrounding pelvic organs caused by more prominent abnormalities in elastic fibers in pelvic support tissues other than the vagina. Alternatively, the enlargement could be due to abnormalities in vaginal elastic fibers that provide pathophysiological environmental cues to cells within the vagina. Although we did not find any statistically significant differences in the total number of elastic fibers, this may be due to the small sample size used for histological analysis. Moreover, the total number of elastic fibers may not be as relevant as other characteristics of elastic fibers, such as shape and arrangement, which we did not assess.

POP grading with the MOPQ scale is not based on quantitative measurements of the bulge, and therefore may have limited sensitivity. However, MOPQ as used in our study has been

tested previously for inter- and intra-observer reliability in *Fbln5*^{-/-} mice.¹² MOPQ grading has also been previously used in *Lox11* deficient mice.^{5,8} It is therefore possible that mild prolapse could have occurred in *Lox11*^{+/+} animals that was not detected by MOPQ. However, these colonies consisted of offspring of sibling pairs, providing highly similar genetics. The descriptive scale of MOPQ was sufficient to demonstrate significant differences between the two colonies of mice. Magnetic resonance imaging could potentially be used to provide greater precision of POP grading when needed.⁵

Genetic evaluation of animals was limited to PCR detection of the *Lox11* gene. Therefore, other and potentially associated genetic differences may exist between the two colonies. Nonetheless, the difference in prolapse rates between the two colonies of mice was statistically significant and definitive.

Differences in lower urinary tract function were not assessed, although these dysfunctions have been associated with *Lox11*^{-/-} mice and POP.^{5,23} Measurement of lower urinary tract function, such as with urodynamics, could have corroborated differences in *Lox11*^{-/-} and *Lox11*^{+/+} mice in this study. The finding of abnormal voiding in backcrossed *Lox11*^{+/+} mice would suggest inheritance of susceptibility, exclusive of the gene *Lox11*, that does not significantly affect histology or physiologic pelvic support.

In conclusion, we have demonstrated that a genetically similar backcrossed colony of *Lox11*^{+/+} and *Lox11*^{-/-} mice can be created from the original *Lox11*^{-/-} colony. We have also validated the durability of this backcross as a reliable animal model of POP with differences in histological findings. Additionally, we demonstrated that re-introduction of the *Lox11* gene back into *Lox11*^{-/-} mice restores normal histology and rescues the POP phenotype. A *Lox11*^{+/+} colony is valuable for experiments using *Lox11*^{-/-} mice to study pathogenesis or test novel therapeutics.

Acknowledgments

Funding Sources:

NIH R01 HD059859, Cleveland Clinic

NSF GRFP Grant # 1000139839

The authors would like to thank Bruce Kinley and the Cleveland Clinic Biological Resource Unit for expert advice and assistance.

Reference List

1. Jelovsek JE, Maher C, Barber MD. Pelvic organ prolapse. Lancet. Mar 24; 2007 369(9566):1027–1038. [PubMed: 17382829]
2. Barber MD. Incontinence: should mesh be used to correct anterior vaginal prolapse? Nat Rev Urol. 2011; 8(9):476–478. [PubMed: 21844904]
3. Couri BM, Lenis AT, Borazjani A, et al. Animal models of female pelvic organ prolapse: lessons learned. Expert Rev Obstet Gynecol. May 1; 2012 7(3):249–260. [PubMed: 22707980]
4. Mecham RP. Elastin synthesis and fiber assembly. Ann N Y Acad Sci. 1991; 624:137–146. [PubMed: 1648323]

5. Lee UJ, Gustilo-Ashby AM, Daneshgari F, et al. 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.* 2008; 295(2):F545–F555. [PubMed: 18495804]
6. Liu X, Zhao Y, Gao J, et al. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. *Nat Genet.* 2004; 36(2):178–182. [PubMed: 14745449]
7. Liu X, Zhao Y, Pawlyk B, et al. Failure of elastic fiber homeostasis leads to pelvic floor disorders. *Am J Pathol.* 2006; 168(2):519–528. [PubMed: 16436666]
8. Gustilo-Ashby AM, Lee U, Vurbic D, et al. The impact of cesarean delivery on pelvic floor dysfunction in lysyl oxidase like-1 knockout mice. *Female Pelvic Med Reconstr Surg.* 2010; 16(1): 21–30. [PubMed: 22453086]
9. Jung HJ, Jeon MJ, Yim GW, et al. Changes in expression of fibulin-5 and lysyl oxidase-like 1 associated with pelvic organ prolapse. *Eur J Obstet Gynecol Reprod Biol.* 2009; 145(1):117–122. [PubMed: 19450918]
10. Zhao BH, Zhou JH. Decreased expression of elastin, fibulin-5 and lysyl oxidase-like 1 in the uterosacral ligaments of postmenopausal women with pelvic organ prolapse. *J Obstet Gynaecol Res.* 2012; 38(6):925–931. [PubMed: 22487196]
11. Kozel BA, Knutsen RH, Ye L, et al. Genetic modifiers of cardiovascular phenotype caused by elastin haploinsufficiency act by extrinsic noncomplementation. *J Biol Chem.* Dec 30; 2011 286(52):44926–44936. [PubMed: 22049077]
12. Wieslander CK, Rahn DD, McIntire DD, et al. Quantification of pelvic organ prolapse in mice: vaginal protease activity precedes increased MOPQ scores in fibulin 5 knockout mice. *Biol Reprod.* 2009; 80(3):407–414. [PubMed: 18987327]
13. MacLennan AH, Taylor AW, Wilson DH, et al. The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of delivery. *BJOG.* 2000; 107(12):1460–1470. [PubMed: 11192101]
14. Diwadkar GB, Barber MD, Feiner B, et al. Complication and reoperation rates after apical vaginal prolapse surgical repair: a systematic review. *Obstet Gynecol.* 2009; 113(2 Pt 1):367–373. [PubMed: 19155908]
15. Olsen AL, Smith VJ, Bergstrom JO, et al. Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. *Obstet Gynecol.* 1997; 89(4):501–506. [PubMed: 9083302]
16. Venkataraman L, Lenis A, Couri B, et al. Induced Regenerative Elastic Matrix Repair in LOXL1 Knockout Mouse Cell Cultures: Towards Potential Therapy for Pelvic Organ Prolapse. *J Tissue Sci Eng.* Sep 28; 2012 3(120)
17. Losy F, Dai J, Pages C, et al. Paracrine secretion of transforming growth factor-beta1 in aneurysm healing and stabilization with endovascular smooth muscle cell therapy. *J Vasc Surg.* 2003; 37(6): 1301–1309. [PubMed: 12764279]
18. Drewes PG, Yanagisawa H, Starcher B, et al. Pelvic organ prolapse in fibulin-5 knockout mice: pregnancy-induced changes in elastic fiber homeostasis in mouse vagina. *Am J Pathol.* 2007; 170(2):578–589. [PubMed: 17255326]
19. Rahn DD, Acevedo JF, Roshanravan S, et al. Failure of pelvic organ support in mice deficient in fibulin-3. *Am J Pathol.* 2009; 174(1):206–215. [PubMed: 19095964]
20. Budatha M, Roshanravan S, Zheng Q, et al. Extracellular matrix proteases contribute to progression of pelvic organ prolapse in mice and humans. *J Clin Invest.* 2011; 121(5):2048–2059. [PubMed: 21519142]
21. Alperin M, Debes K, Abramowitch S, et al. LOXL1 deficiency negatively impacts the biomechanical properties of the mouse vagina and supportive tissues. *Int Urogynecol J Pelvic Floor Dysfunct.* 2008; 19(7):977–986. [PubMed: 18265927]
22. Abramov U, Raud S, Innos J, et al. Different housing conditions alter the behavioural phenotype of CCK(2) receptor-deficient mice. *Behav Brain Res.* Nov 3; 2008 193(1):108–116. [PubMed: 18541314]
23. Liu G, Daneshgari F, Li M, et al. Bladder and urethral function in pelvic organ prolapsed lysyl oxidase like-1 knockout mice. *BJU Int.* 2007; 100(2):414–418. [PubMed: 17555473]

Figure 1.

Schematic chart showing the backcross breeding program with circles indicating females, and squares indicating males. Diagonal lines through individuals indicate that they were euthanized and were not utilized in the experiment.

Figure 2.

Quantitative histological image analysis. **A.** Unmodified high resolution field of view image; **B.** Schematic illustrating the regions of interest (ROI) including the perimeter around the vaginal muscularis (green line), vaginal muscularis (blue), vaginal submucosa (yellow), and external urethral sphincter (red line), and as well as the area that includes both, vaginal epithelium and vaginal lumen (pink); **C.** Vaginal tortuosity was calculated by tracing a line segment on the submucosa-epithelium boundary (black line) and dividing the length by the corresponding muscularis-submucosa boundary (red line). **D.** Example containing an image field within an example ROI (red line) with collagen segmented in green and elastic fibers segmented in blue.

Figure 3.

Kaplan-Meier survival curve showing the percentage of mice in the wild type (*Lox11*^{+/+}; solid line) and *Lox11* knockout (*Lox11*^{-/-}; dashed line) colonies that were not prolapsed up to 12 weeks after first delivery, as defined by a mouse pelvic organ prolapse quantification (MOPQ) score of 0 or 1.

Figure 4.

Examples of pelvic organ prolapse in **A.** a *Loxl1* knockout (*Loxl1*^{-/-}) mouse with mouse pelvic organ prolapse quantification (MOPQ) grade 3 showing a distinct pelvic bulge demonstrating the prolapse (indicated by the arrows). The anal prolapse (indicated by *) is not part of the MOPQ assessment and was not evaluated in this study, and **B.** a wild type mouse (*Loxl1*^{+/+}) with MOPQ grade 0, showing no prolapse.

Figure 5.

Example of a high-resolution field-of-view, EVG stained, image (HR-FOVI) of a cross-sections of the vagina and urethra from **A.** an example wild-type (*Lox11*^{+/+}) mouse and **B.** an example *Lox11* knockout mouse (*Lox11*^{-/-}) vagina and urethra, and examples of Masson's trichrome stained section of the external urethral sphincter from **C.** *Lox11*^{+/+} mouse and **D.** a example *Lox11*^{-/-} mouse.

Table 1

Mice morphometrics measurements of urethra and vagina of *Lox11^{-/-}* and *Lox11^{+/+}*; percentage is calculated by subtracting mean values of *Lox11^{-/-}* from those of *Lox11^{+/+}* mice, and normalized to the near value of *Lox11^{+/+}* mice.

	<i>Lox1^{-/-}</i>	<i>Lox1^{+/+}</i>	Percent Change	P-Value
Length and Area Measurements				
Vagina	(n=3)	(n=3)		
Total Vaginal Area (mm ²) *	5.48 ±0.91	2.1 ±0.12	161%	0.01
Vaginal Muscularis Perimeter (mm)	12.04 ±1.37	9.04 ±0.65	33%	0.12
Total Muscularis Tissue Area (mm ²) *	0.51 ±0.03	0.31 ±0.02	62%	0.01
Total Submucosa Tissue Area (mm ²) *	1.08 ±0.15	0.54 ±0.06	101%	0.03
Vaginal Submucosa Thickness (µm)	92 ±9	50 ±5	84%	0.05
Vaginal Muscularis Thickness (µm)	45 ±7	36 ±4	29%	0.26
Vaginal Tortuosity	1.82 ±0.14	1.61 ±0.05	13%	0.32
Urethra	(n=2)	(n=3)		
Urethral circumference (mm)	3 ±0.33	2.25 ±0.13	33%	0.09
Urethral Diameter (mm)	0.96 ±0.1	0.72 ±0.04	33%	0.09
Extracellular Matrix Measurements				
Vaginal Collagen Density	(n=3)	(n=3)		
Submucosa (Area Fraction, %)	58.8 ±3.9	59.1 ±2.3	-0.51%	0.96
Muscularis (Area Fraction, %)	50.3 ±3.7	46.6 ±5.5	8%	0.62
Vaginal Elastic Fibers	(n=3)	(n=3)		
Submucosa (number of fibers/mm ²)	92 ±45	156 ±52	-41%	0.40
Muscularis (number of fibers/mm ²)	47 ±7	89 ±22	-47%	0.14

Note:

* indicates statistical significance difference.