Published in final edited form as:

BJU Int. 2012 August ; 110(3): 413–419. doi:10.1111/j.1464-410X.2011.10773.x.

Altered bladder function in elastin-deficient mice at baseline and in response to partial bladder outlet obstruction

Xiaogang Jiang*, Ian Luttrell*, Dean Y. Li†, Claire C. Yang*, and Kanchan Chitaley*

*Department of Urology, University of Washington, Seattle, WA

†Program in Human Molecular Biology and Genetics, University of Utah, Salt Lake City, UT, USA

Abstract

- To examine functional and molecular changes of the bladders from elastin-haploinsufficient mice (*Eln*^{+/-}) at baseline as well as in response to partial bladder outlet obstruction (pBOO).
- Female *Eln*^{+/-} and wild type (*Wt*) mice (3–4 months old) were studied.
- The bladder elastin content was quantified by measuring desmosine.
- Mice were divided into two groups to undergo surgery to create pBOO or to undergo sham surgery. Three days after surgery, bladder function was evaluated by *in vivo* cystometry, and the contractile response of bladder strips exposed to electrical field stimulation (EFS) and carbachol was examined by *ex vivo* myography.
- The *Eln*^{+/-}-sham mice had a 33.6% decrease in bladder elastin compared with *Wt*-sham mice.
- Cystometry showed significantly decreased bladder compliance and capacity in *Eln*^{+/-}-sham vs *Wt*-sham mice; pBOO increased bladder compliance and capacity to a greater extent in *Eln*^{+/-} mice compared with *Wt* mice.
- Bladder strips from *Eln*^{+/-}-sham mice showed a significantly heightened contractile response to both EFS and carbachol compared with *Wt*-sham mice.
- A significantly increased contractile response to carbachol was detected in *Wt*-pBOO vs *Wt*-sham but not between *Eln*^{+/-}-pBOO and *Eln*^{+/-}-sham mice.
- The results that elastin-deficient mice had decreased bladder compliance and capacity and increased bladder contractility; and that *Wt*-pBOO mice showed an enhanced contractile response to carbachol, but *Eln*^{+/-}-pBOO mice did not, suggest that elastin is critical for normal bladder function and is involved in bladder response to pBOO.

Keywords

matrix; bladder outlet obstruction; bladder dysfunction; animal models

INTRODUCTION

As one of the major components of the extracellular matrix (ECM), elastic fibres endow connective tissue with resilience, allowing deformability and passive recoil without energy input [1]. These properties are critical to the function of organs that undergo repeated cycles

of extension and recoil, including arteries, lungs and skin [1]. Elastic fibres are made up of about 90% tropoelastin monomers and 10% microfibril proteins [2]. Impaired elastin production has been associated with several inherited and acquired conditions, such as stenosis of large arteries (Williams syndrome) [3], aortic aneurysms [4], emphysema [5], and loose, wrinkled skin (cutis laxa) [6].

The bladder is an organ that experiences repeated cycles of extension and recoil. From empty to full states the bladder wall can be stretched several fold. The normal bladder is able to accommodate increasing volumes of urine without a significant increase in intravesical pressure, whereas the non-compliant bladder is rigid and develops high intravesical pressure in the absence of detrusor contraction at low urine volume. The mechanical properties of the bladder depend on both the detrusor muscle and the ECM. The main constituents of the latter are collagen and elastic fibres.

To date, most studies addressing bladder function and dysfunction have focused on bladder smooth muscle and its autonomic innervation, whereas the role of ECM, particularly elastic fibres, in normal bladder function and dysfunction remains speculative. Transgenic mice overexpressing rat elastin showed increased bladder compliance compared with their wild-type littermates [7], indicating that elastin content is positively correlated with bladder compliance. However, there are inconsistent findings of the levels of elastin content in patients with non-compliant bladders [8,9], highlighting the need for further investigation into the exact role of elastin in bladder function. In this study we examined the functional and molecular changes of bladders from transgenic mice carrying a heterozygous deletion of exon 1 in the elastin gene (*Eln*^{+/-}).

MATERIALS AND METHODS

C57BL/6J mice bearing a heterozygous deletion of exon 1 in the elastin gene (*Eln*^{+/-}) were obtained from Dean Y. Li (University of Utah). It should be noted *Eln*-null mice die shortly after birth as a result of uncontrolled growth of smooth muscle cells, which eventually occlude the arterial lumen [10], and could not be used for this study. Female *Eln*^{+/-} mice and their wild-type (*Wt*) littermates were housed in a temperature-controlled room with a 12 : 12-h light : dark cycle and maintained with access to food and water *ad libitum*. The mice were studied at 3–4 months of age. All procedures were conducted with the approval of the Institutional Animal Care and Use Committee of the University of Washington and in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*.

Elastin and collagen content in the bladders of the *Wt* and *Eln*^{+/-} mice were quantified by measuring concentrations of desmosine and hydroxyproline, respectively, as previously described [11,12]. Results were expressed as picomoles of desmosine or micrograms of hydroxyproline, normalized to total bladder protein.

For RNA extraction and PCR, bladder tissue was frozen in liquid nitrogen, ground to powder, and processed with Trizol (Invitrogen, Carlsbad, CA, USA) for total RNA isolation. The cDNA was synthesized with RETROscript (Ambion, Austin, TX, USA) using random hexamers. Quantitative real-time PCR employing SYBER green fluorescent reagent (Applied Biosystems, Foster City, CA, USA) was performed in a 7500 Fast Real-Time PCR System (Applied Biosystems) with the following primers: tropoelastin (5'-GCGTCTTGCTGATCCTCTTG-3'; 5'-GGGAAGTCCACCAGGAAGTC-3'); collagen-III (5'-ACCAAAAGGTGATGCTGGAC-3'; 5'-GACCTCGTGCTCCAGTTAGC-3'); smooth muscle myosin heavy chain (5'-AAGCTGCGGCTAGAGGTCA-3'; 5'-CCCTCCCTTTGATGGCTGAG-3'); acidic ribosomal phosphoprotein P0 (5'-

TCAGTGTGCCAGCTCAGAAC-3'; 5'-TCCCACCTTGTCTCCAGTCT-3'). The relative amounts of mRNA were calculated from the values of a comparative threshold cycle, using acidic ribosomal phosphoprotein P0 as a control.

To produce the partial bladder outlet obstruction (pBOO) model, mice were anaesthetized with isoflurane in oxygen. The bladder outlet was accessed through a suprapubic incision. A 22-gauge catheter was placed alongside the urethra, and both the urethra and the catheter were ligated together with a 6-0 silk suture. The catheter was then removed and the incision was closed. Sham mice underwent surgical opening but no ligation. Three days after the pBOO or sham surgery, one set of mice underwent *in vivo* cystometry, and bladder tissue from another set of mice was isolated for *ex vivo* contractility assays. For *in vivo* cystometry mice were anaesthetized with isoflurane and secured in a supine position. The abdomen was opened via the previous incision. The suture ligature around the urethra of pBOO mice was removed. A 24-gauge catheter was inserted into the bladder through the urethra, and the bladder was emptied. The depth of the catheter in the bladder was then adjusted to make sure that the tip of it did not abut the bladder wall. The catheter was then secured with tape, and the abdomen was reclosed. After a stabilization period of 10 min, the catheter was connected to a pressure transducer (Kent Scientific Corporation, Torrington, CT, USA) and an infusion pump (SP220i Syringe Pump, WPI, Sarasota, FL, USA). The bladder was infused with sterile saline (room temperature) at rate of 10 μ L/min, and the intravesical pressure–volume curve was recorded continuously until urine leaked around the catheter. The infusion volume at this point was regarded as the maximum bladder capacity. The bladder compliance was calculated as the change from baseline in infusion volume divided by the change in intravesical pressure.

For the *ex vivo* contractility assay mice were killed and the bladders were excised and put into physiological salt solution. A strip from the midline of the anterior wall of the bladder was isolated and mounted into a small vessel myograph (Multi Myography model 610M; Danish Myo Technologies, Ann Arbor, MI, USA). The bladder strips were acclimated without tension for 30 min. With the passive tension set at 4 mN, the contractile response of the bladder strips to electrical field stimulation (EFS, delivered by a Grass S88X Stimulator, Astro-Med, West Warwick, RI, USA) was recorded at 1.25, 2.5, 5.0, 10.0, 20.0 and 30.0 Hz. Bladder contraction in response to carbachol was monitored with delivery of increasing half-log doses every 2 min starting at 10^{-8} M and ending with 3×10^{-6} M. Force generation was monitored with an ADInstruments Powerlab 8/30 and interpreted by Chart 5.5.4 for Windows (ADInstruments, Colorado Springs, CO, USA), and was normalized to the tissue weight.

For Western blot analysis the complete bladders from *Wt* and *Eln*^{+/-} mice were cut into small pieces using fine scissors, put into 1 ml SDS lysis buffer containing 1 $\times$ Complete Protease Inhibitor Cocktail (Roche, Madison, WI, USA), and homogenized. Equal volumes of protein lysates from each bladder were separated by SDS–PAGE. The proteins were transferred onto Immobilon-P Transfer Membrane (Millipore, Billerica, MA, USA). Immunodetection was carried out with anti-Smooth Muscle Actin (Sigma, St Louis, MO, USA) antibody and visualized with Western Blotting Luminol Reagent (Santa Cruz Biotechnology Co. Ltd, Santa Cruz, CA, USA). Protein levels were quantified by densitometry, using the Labworks program (UVP, Upland, CA, USA).

Significance was determined using Student's *t* test, and Bonferroni correction was applied if multiple comparisons were needed. Linear regression and Pearson correlation were performed with SPSS software. For all experiments, *P* < 0.05 denoted statistical significance. Data are expressed as mean $\pm$ SEM.

RESULTS

No significant difference in body weight was detected between *Eln*^{+/-} and *Wt* mice (20.94 ± 0.41 g vs 21.48 ± 0.27 g, $P = 0.28$). There was no significant difference in bladder weights of *Eln*^{+/-}-sham vs *Wt*-sham or *Eln*^{+/-}-pBOO vs *Wt*-pBOO mice, but both *Eln*^{+/-}-pBOO and *Wt*-pBOO mice had significantly increased bladder weights compared with their respective sham counterparts (Table 1).

In a preliminary study, pBOO was created in *Wt* mice and their bladders were harvested at 1, 3 and 7 days after the surgery to assess expression of tropoelastin (the elastin precursor) and collagen III mRNAs. We found that the tropoelastin mRNA expression was significantly increased at 3 and 7 days (Fig. 1A), whereas the mRNA expression of collagen III was significantly upregulated at 7 days, but not at 1 and 3 days (Fig. 1B), postoperatively. To investigate the role of elastin in the detrusor response to pBOO without the confounding of collagen, we selected 3 days of pBOO for this study.

Bladder desmosine (a quantitative measure of elastin content) normalized to total protein was decreased by approximately 33.6% in *Eln*^{+/-} mice compared with *Wt* mice (Fig. 2A). The tropoelastin mRNA was also significantly decreased in bladder tissue from *Eln*^{+/-} compared with *Wt* mice (Fig. 2B). To examine if changes in matrix were limited to elastin in *Eln*^{+/-} mice, we measured bladder collagen by hydroxyproline analysis. We did not detect any significant difference in bladder collagen content in *Eln*^{+/-} vs *Wt* mice (Fig. 2C).

The *Eln*^{+/-}-sham mice had significantly decreased bladder compliance and capacity compared with *Wt*-sham mice (Fig. 3A,B and Table 1). No significant difference in bladder compliance and capacity was detected between *Wt*-sham and *Wt*-pBOO mice, although data from the latter group showed greater variability. *Eln*^{+/-}-pBOO mice showed an approximately 90% and 58% increase in bladder compliance and capacity, respectively, compared with *Eln*^{+/-}-sham mice (Fig. 3A,B and Table 1), although the difference did not reach statistical significance.

Bladder elastin and collagen content were determined by measuring desmosine and hydroxyproline, respectively, in all four groups of mice. As the elastin content relative to collagen is more relevant to bladder function than the absolute elastin content [13], we calculated the ratio of elastin-to-collagen (E/C). *Eln*^{+/-}-sham mice showed significantly decreased E/C compared with *Wt*-sham mice; when *Wt*-sham vs *Wt*-pBOO, and *Eln*^{+/-}-sham vs *Eln*^{+/-}-pBOO mice were compared, a trend of increase (approximately 28% and 29%, respectively) in the E/C was observed, although it did not reach statistical significance (Fig. 3C, Table 1). We next wanted to determine if there was a significant relationship between bladder compliance/capacity and E/C. A simple linear regression was performed using E/C as the independent factor and bladder compliance or capacity as the dependent factor, respectively. There was a significant positive relationship between E/C and bladder compliance, as well as between E/C and bladder capacity when all animals were included (Fig. 4A,C). As pBOO caused greater variability in all parameters, we next wanted to determine if a significant positive relationship between E/C and bladder compliance and capacity was still present when only pBOO mice were included. A significant positive relationship was observed between E/C and bladder compliance, but not between E/C and bladder capacity ($P = 0.06$) when only *Wt*-pBOO and *Eln*^{+/-}-pBOO were included (Fig. 4B,D).

In the *ex vivo* contractility assay bladder strips from *Eln*^{+/-}-sham mice exhibited a significantly augmented response to both EFS (at 1.25–30 Hz, Fig. 5A) and carbachol (at 3×10^{-8} to 3×10^{-6} M, Fig. 6A) compared with *Wt*-sham mice. To determine if the bladders from *Eln*^{+/-} mice respond to pBOO differentially, the contractility of bladder strips from

sham and pBOO mice of both *Eln*^{+/-} and *Wt* groups was compared. Bladder strips from *Wt*-pBOO mice showed significantly heightened contraction to carbachol (at 3×10^{-8} to 3×10^{-7} M, Fig. 6B) but not EFS (Fig. 5B) compared with those of *Wt*-sham mice. In contrast, *Eln*^{+/-}-pBOO mice had a significantly decreased response to both EFS (at 1.25–30 Hz, Fig. 5C) but no difference in response to carbachol compared with *Eln*^{+/-}-sham mice (Fig. 6C).

To determine if the heightened bladder contractility of the *Eln*^{+/-} mice compared with *Wt* mice was the result of an increase in smooth muscle content, we examined the expression level of smooth muscle actin in the bladder lysates of *Eln*^{+/-} and *Wt* mice. There was no significant difference in smooth muscle actin protein expression (per bladder) in *Eln*^{+/-} and *Wt* mice ($n = 8/\text{group}$, $P = 0.13$, data are not shown). We next examined the mRNA expression of smooth muscle myosin heavy chain, a more specific contractility marker, in the bladders of *Eln*^{+/-} and *Wt* mice. We did not detect a significant difference in the mRNA expression of smooth muscle myosin heavy chain between the two groups ($n = 8/\text{group}$, $P = 0.14$, data are not shown).

DISCUSSION

Elements of the ECM are believed to determine the visco-elastic properties of the bladder [14,15]. In particular, collagen is thought to provide tensile strength, whereas elastin probably enhances tissue compliance [7]. In the present study, we found that *Eln*^{+/-} mice, with approximately one-third less bladder elastin and no significant change in collagen content, had significantly decreased bladder compliance and capacity compared with *Wt* controls. Bladder tissue from these elastin-deficient mice also had a heightened contractile response to EFS and carbachol. Moreover, we found an impaired compensatory response of the bladder to pBOO in elastin-deficient mice compared with *Wt* mice.

The primary finding of the present study is that the bladders of *Eln*^{+/-} mice had significantly decreased bladder compliance and capacity compared with *Wt* mice (Fig. 3A,B). Compliance is measured during bladder filling and not during contraction, so compliance mainly reflects the passive mechanical properties of the ECM as conferred by elastin and collagen. That elastin-deficient mice had decreased bladder compliance corroborates clinical studies showing the relationship between elastin content and detrusor compliance. Djavan *et al.* [8] reported a significant decrease in elastin mRNA and elastic fibre deposition in patients with non-compliant bladders. Several studies reported that patients with Williams syndrome, an inherited disease associated with deletions of the elastin gene, had a high incidence of bladder dysfunction, including increased frequency and incontinence (symptoms and signs of reduced bladder compliance) [16–18]. In animal models, Lemack *et al.* [7] found increased bladder compliance in transgenic mice overexpressing rat-elastin compared with their non-transgenic littermates. Conversely, mice with the same genotype as in this study were found to develop hypertension [19], possibly through the mechanism that the aorta and arteries of elastin-deficient mice become less distensible (reduced compliance), leading to higher blood pressure during systole. Together with previous findings, our results support the concept that elastin can enhance tissue flexibility and extensibility, and therefore, tissue compliance.

Although in our *in vivo* cystometry study compliance probably reflects the composition of the ECM, the *ex vivo* contractile response to EFS and carbachol mainly reflects the functional state of the bladder smooth muscle and autonomic nerve terminals. We found a significantly heightened contractile response of bladder tissue from *Eln*^{+/-}-sham mice to both EFS and carbachol compared with that from the *Wt*-sham mice. Our results are also in line with previous findings in abdominal aortas and renal arteries of the *Eln*^{+/-} mice [20]. The enhanced contractility of aortas and renal arteries may be because of an increased

number of lamellar units (concentric layers of smooth muscle cells and adjacent elastic lamina in the arterial wall), assumed to be a developmental adaptation to increased stress to the vessel wall when there is a decrease in elastin content [19]. Furthermore, elastin, synthesized by smooth muscle cells, may have a negative feedback effect on smooth muscle cells. This is supported by an *in vitro* study suggesting that degraded elastin (or non-polymerized elastin) promotes smooth muscle proliferation [21]. In the bladder wall, however, we did not detect a significant alteration in smooth muscle content by measuring smooth muscle actin and myosin heavy chain expression of the bladders from *Eln*^{+/-} and *Wt* mice. It is possible that the difference in bladder smooth muscle content of *Eln*^{+/-} and *Wt* mice was too mild to be detected by the method we used, but still had a significant effect on bladder contractility. Alternatively, heightened bladder contractility in *Eln*^{+/-} mice may be caused by changes in the contractile properties of smooth muscle cells independent from hyperplasia or hypertrophy. Further studies are warranted to explore the mechanism(s) underlying heightened bladder contractility in the context of decreased elastin content.

In response to an increased workload associated with pBOO, the bladder wall undergoes a series of functional and histological changes, and experiences compensated and decompensated phases. Several studies have reported increased elastin production in response to pBOO [14,22,23], however, the possible role of elastin in the response of bladder to pBOO remains to be determined. We did not detect a statistically significant difference in bladder compliance and capacity between pBOO and sham mice of *Wt* and *Eln*^{+/-} groups. In the literature increased [7], decreased [24], or unchanged [25] bladder compliance was reported in pBOO mouse models, possibly as a result of the different severities and durations of pBOO as well as the uneven progression of the pathophysiology of mouse bladders in response to pBOO. Although in the early phase or with a less severe pBOO the mice may have a compensated bladder (a normotonic or hypertonic bladder that can adequately empty), a bladder in the late phase after pBOO or with a more severe obstruction may be decompensated (a hypotonic bladder that is unable to empty). The significantly wider ranges of bladder compliance and capacity in pBOO vs sham mice in this study (Fig. 3A,B and Table 1) may reflect the variable response of the mice to the surgically created pBOO 3 days postoperatively. Interestingly, although the bladder elastin content (shown as E/C) increased to a similar extent (approximate 28% in *Wt* and 29% in *Eln*^{+/-} mice) in response to pBOO, the *Eln*^{+/-}-pBOO mice showed a greater increase in bladder compliance (approximately 90% vs 2%) and capacity (approximately 58% vs -3%), although it did not reach significance because of the wide variability in the pBOO group (Table 1 and Fig. 3A,B). This finding indicates that the effect of increased elastin content caused by pBOO is dependent on the basal level of the bladder function: while it 'normalized' the decreased bladder compliance and capacity in elastin-deficient mice, the increase in elastin content induced by 3 days of pBOO in our study did not alter the bladder compliance and capacity to the same extent in *Wt* mice. That is, *Wt* bladders with normal function were not sensitive to a further increase in elastin content as they probably have an optimum amount of elastin. The same degree of elastin increase in *Eln*^{+/-} mice augmented bladder compliance and capacity, because they had significantly impaired basal elastin content and bladder function. Furthermore, the significant positive relationship between bladder elastin content and bladder compliance and capacity (Fig. 4A-C) provides further argument that elastin is critical for bladder function.

The bladder strips from *Wt*-pBOO mice vs *Wt*-sham mice showed a significantly heightened contractile response to carbachol but not EFS. The effect of EFS is dependent on both nerve fibres and smooth muscle while carbachol directly affects the latter [26]. In response to pBOO, the detrusor immediately undergoes hyperplasia/hypertrophy, and therefore shows enhanced contraction in response to carbachol. However, we speculate that within a period of 3 days, the sympathetic nerve fibres are unlikely to increase proportionally with the

smooth muscle, and so the EFS-induced response remained unchanged. This is in line with the findings of ‘patchy denervation’ of the detrusor smooth muscle in response to pBOO [25]. Interestingly, we did not observe a robustly enhanced contractile response to carbachol in *Eln*^{+/-}-pBOO vs *Eln*^{+/-}-sham mice (Fig. 6C), and more strikingly, *Eln*^{+/-}-pBOO mice showed a significantly decreased response to EFS compared with *Eln*^{+/-}-sham mice (Fig. 5C). These findings indicate that elastin is critical for the heightened contractile response to pBOO, although the underlying mechanisms remain unclear.

In conclusion, although previous studies have reported altered elastin content in dysfunctional human bladders [8,9,27–29], it was previously difficult to delineate a cause-and-effect relationship between changes in elastin content and changes in bladder function. The transgenic elastin-deficient mouse model used in the present study enabled us to show that sufficient elastin content is critical for healthy bladder function, and elastin is a factor in the detrusor response to pBOO. Further studies are needed to delineate the mechanisms underlying the interaction between elastin and smooth muscle as well as the relationship between elastin content and bladder contractility.

Acknowledgments

This work was supported by the American Urological Association Foundation Research Scholars Program/Astellas USA Foundation and NIH R01 DK082S60 (Chitale, PI). Desmosine and hydroxyproline quantification was performed by B. Starcher, University of Texas Health Center, Tyler, TX.

Abbreviations

ECM	extracellular matrix
Wt	wild-type
pBOO	partial bladder outlet obstruction
EFS	electrical field stimulation

References

1. Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. *J Cell Sci.* 2002; 115:2817–28. [PubMed: 12082143]
2. Goldfischer S, Coltoff-Schiller B, Schwartz E, Blumenfeld OO. The infrastructure of aortic elastic fibers. *Tissue Cell.* 1983; 15:429–35. [PubMed: 6612711]
3. Morris CA, Mervis CB. Williams syndrome and related disorders. *Annu Rev Genomics Hum Genet.* 2000; 1:461–84. [PubMed: 11701637]
4. Tanaka A, Hasegawa T, Chen Z, Okita Y, Okada K. A novel rat model of abdominal aortic aneurysm using a combination of intraluminal elastase infusion and extraluminal calcium chloride exposure. *J Vasc Surg.* 2009; 50:1423–32. [PubMed: 19958989]
5. Shi W, Bellusci S, Warburton D. Lung development and adult lung diseases. *Chest.* 2007; 132:651–6. [PubMed: 17699136]
6. Morava E, Guillard M, Lefeber DJ, Wevers RA. Autosomal recessive cutis laxa syndrome revisited. *Eur J Hum Genet.* 2009; 17:1099–110. [PubMed: 19401719]
7. Lemack GE, Szabo Z, Urban Z, et al. Altered bladder function in transgenic mice expressing rat elastin. *Neurourol Urodyn.* 1999; 18:55–68. [PubMed: 10090127]
8. Djavan B, Lin V, Kaplan EP, et al. Decreased elastin gene expression in noncompliant human bladder tissue: a competitive reverse transcriptase-polymerase chain reaction analysis. *J Urol.* 1998; 160:1658–62. [PubMed: 9783926]
9. Charlton RG, Morley AR, Chambers P, Gillespie JJ. Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. *BJU Int.* 1999; 84:953–60. [PubMed: 10571619]

10. Li DY, Brooke B, Davis EC, et al. Elastin is an essential determinant of arterial morphogenesis. *Nature*. 1998; 393(6682):276–80. [PubMed: 9607766]
11. Sato F, Wachi H, Ishida M, et al. Distinct steps of cross-linking, self-association, and maturation of tropoelastin are necessary for elastic fiber formation. *J Mol Biol*. 2007; 369:841–51. [PubMed: 17459412]
12. Lazar MH, Christensen PJ, Du M, et al. Plasminogen activator inhibitor-1 impairs alveolar epithelial repair by binding to vitronectin. *Am J Respir Cell Mol Biol*. 2004; 31:672–8. [PubMed: 15308506]
13. Osborne-Pellegrin M, Labat C, Mercier N, Challande P, Lacolley P. Changes in aortic stiffness related to elastic fiber network anomalies in the Brown Norway rat during maturation and aging. *Am J Physiol Heart Circ Physiol*. 2010; 299:H144–52. [PubMed: 20435849]
14. Kim KM, Kogan BA, Massad CA, Huang YC. Collagen and elastin in the obstructed fetal bladder. *J Urol*. 1991; 146:528–31. [PubMed: 1861293]
15. Baskin L, Meaney D, Landsman A, Zderic SA, Macarak E. Bovine bladder compliance increases with normal fetal development. *J Urol*. 1994; 152:692–5. [PubMed: 8021997]
16. Schulman SL, Zderic S, Kaplan P. Increased prevalence of urinary symptoms and voiding dysfunction in Williams syndrome. *J Pediatr*. 1996; 129:466–9. [PubMed: 8804343]
17. Pérez Jurado LA, Peoples R, Kaplan P, Hamel BC, Francke U. Molecular definition of the chromosome 7 deletion in Williams syndrome and parent-of-origin effects on growth. *Am J Hum Genet*. 1996; 59:781–92. [PubMed: 8808592]
18. Sammour ZM, Gomes CM, Duarte RJ, Trigo-Rocha FE, Srougi M. Voiding dysfunction and the Williams–Beuren syndrome: a clinical and urodynamic investigation. *J Urol*. 2006; 175:1472–6. [PubMed: 16516025]
19. Li DY, Faury G, Taylor DG, et al. Novel arterial pathology in mice and humans hemizygous for elastin. *J Clin Invest*. 1998; 102:1783–7. [PubMed: 9819363]
20. Faury G, Pezet M, Knutsen RH, et al. Developmental adaptation of the mouse cardiovascular system to elastin haploinsufficiency. *J Clin Invest*. 2003; 112:1419–28. [PubMed: 14597767]
21. Brooke BS, Bayes-Genis A, Li DY. New insights into elastin and vascular disease. *Trends Cardiovasc Med*. 2003; 13:176–81. [PubMed: 12837579]
22. Rubinstein M, Sampaio FJ, Costa WS. Stereological study of collagen and elastic system in the detrusor muscle of bladders from controls and patients with infravesical obstruction. *Int Braz J Urol*. 2007; 33:33–9. [PubMed: 17335596]
23. Holm NR, Horn T, Smedts F, Nordling J, de la Rosette J. The detrusor muscle cell in bladder outlet obstruction—ultrastructural and morphometric findings. *Scand J Urol Nephrol*. 2003; 37:309–15. [PubMed: 12944189]
24. Anumanthan G, Tanaka ST, Adams CM, et al. Bladder stromal loss of transforming growth factor receptor II decreases fibrosis after bladder obstruction. *J Urol*. 2009; 182:1775–80. [PubMed: 19692014]
25. Pandita RK, Fujiwara M, Alm P, Andersson KE. Cystometric evaluation of bladder function in non-anesthetized mice with and without bladder outlet obstruction. *J Urol*. 2000; 164:1385–9. [PubMed: 10992420]
26. Longhurst PA, Levendusky MC, Bezuijen MW. Diabetes mellitus increases the rate of development of decompensation in rats with outlet obstruction. *J Urol*. 2004; 171:933–7. [PubMed: 14713857]
27. Kim KM, Kogan BA, Massad CA, Huang YC. Collagen and elastin in the normal fetal bladder. *J Urol*. 1991; 146:524–7. [PubMed: 1861292]
28. Cortivo R, Pagano F, Passerini G, Abatangelo G, Castellani I. Elastin and collagen in the normal and obstructed urinary bladder. *Br J Urol*. 1981; 53:134–7. [PubMed: 7237045]
29. Gilpin SA, Gosling JA, Barnard RJ. Morphological and morphometric studies of the human obstructed, trabeculated urinary bladder. *Br J Urol*. 1985; 57:525–9. [PubMed: 4063733]

What's known on the subject? and What does the study add?

As one of the major components of the extracellular matrix, elastic fibres are believed to enhance tissue compliance. However, the role of elastic fibres in normal bladder function and dysfunction remains speculative. Although transgenic mice overexpressing elastin showed increased bladder compliance, the findings in patients with non-compliant bladders are inconsistent.

Using transgenic elastin-deficient mouse models, this study provides the first direct evidence that sufficient elastin content is critical for healthy bladder function, and elastin is involved in the detrusor response to partial bladder outlet obstruction.

FIG. 1.

Time-course of the expression of tropoelastin and collagen III in the detrusor after partial bladder outlet obstruction (pBOO) in wild-type (Wt) mice. **A**, Tropoelastin mRNA expression was significantly upregulated at 3 and 7 days, but not at 1 day after pBOO surgery. **B**, Collagen III mRNA expression was significantly upregulated at 7 days, but not at 1 and 3 days, postoperatively. * $P < 0.05$; $n = 6/\text{group}$.

FIG. 2.

Bladder elastin and collagen content in *Eln*^{+/-} vs wild-type (Wt) mice. **A**, Elastin content, as measured by desmosine quantification, was significantly lower in bladder tissue from *Eln*^{+/-} mice than in Wt mice. **B**, Tropoelastin mRNA was also significantly decreased in bladder tissue from *Eln*^{+/-} vs Wt mice. **C**, Hydroxyproline analysis revealed no significant difference in bladder collagen content in *Eln*^{+/-} vs Wt mice. **P* < 0.05; n = 8 to n = 12/group.

FIG. 3.

In vivo cystometry and bladder elastin content in Eln^{+/-} and wild-type (Wt) mice. **A**, Eln^{+/-}-sham mice showed significantly decreased bladder compliance compared with Wt-sham mice, but no significant difference was detected between Wt-partial bladder outlet obstruction (pBOO) vs Wt-sham or Eln^{+/-}-pBOO vs Eln^{+/-}-sham mice. **B**, Eln^{+/-}-sham mice showed significantly decreased bladder capacity compared with Wt-sham mice, but no significant difference was detected between Wt-pBOO and Wt-sham or Eln^{+/-}-pBOO and Eln^{+/-}-sham mice. **C**, Eln^{+/-}-sham mice showed significantly decreased elastin to collagen ratio (E/C) compared with Wt-sham mice, whereas Wt-pBOO and Eln^{+/-}-pBOO showed an increased trend in E/C in the bladders but did not reach significance compared with the respective sham mice. *P < 0.05; n = 7 to n = 16/group.

FIG. 4.

Relationship between bladder elastin content and bladder function. **A**, Linear regression analysis of all four groups of mice showed a significant positive relationship between bladder compliance and elastin to collagen ratio (E/C) in the bladder. **B**, Linear regression analysis including only wild-type–partial bladder outlet obstruction (Wt-pBOO) mice and $Eln^{+/-}$ -pBOO mice showed a significant positive relationship between bladder compliance and E/C in the bladder. **C**, Linear regression analysis including all four groups of mice showed a significant positive relationship between bladder capacity and E/C in the bladder. **D**, Linear regression analysis of only pBOO groups did not show a significant positive relationship between bladder capacity and E/C in the bladder. $n = 7$ to $n = 9$ /group.

FIG. 5.

Ex vivo contractility assay: response to electric field stimulation (EFS). **A**, Bladder tissue from *Eln^{+/-}-sham* mice had a heightened contractile response to EFS at 1.25–30 Hz vs wild-type (*Wt*) -sham mice. **B**, Bladder tissue from *Wt*-partial bladder outlet obstruction (pBOO) mice showed a trend of decreased contraction in response to EFS but did not reach a significant level, compared with those from *Wt*-sham mice. **C**, Bladder tissue from *Eln^{+/-}-pBOO* mice had a significantly decreased contractile response to EFS at 1.25–30 Hz compared with those from *Eln^{+/-}-sham* mice. **P* < 0.05; *n* = 7 to *n* = 10/group.

FIG. 6.

Ex vivo contractility assay: response to carbachol. **A**, Bladder tissue from Eln^{+/-}-sham mice exhibited a heightened contractile response to carbachol at 3×10^{-8} to 3×10^{-6} M compared with wild-type (Wt) -sham mice. **B**, Bladder tissue from Wt-partial bladder outlet obstruction (pBOO) vs Wt-sham mice showed a significantly increased contractile response to carbachol at 3×10^{-8} to 3×10^{-7} M. **C**, No significant difference in response to carbachol was found between bladder tissue from Eln^{+/-}-pBOO and Eln^{+/-}-sham mice. *P < 0.05; n = 8 to n = 10/group.

TABLE 1Bladder weight, compliance, capacity and E/C (mean $\pm$ SEM)

	Sham		pBOO	
	<i>Wt</i>	<i>Eln</i> ^{+/-}	<i>Wt</i>	<i>Eln</i> ^{+/-}
Bladder weight (mg)	26.91 $\pm$ 2.19 (<i>n</i> = 10)	25.12 $\pm$ 1.60 (<i>n</i> = 9)	47.74 $\pm$ 3.97 [*] (<i>n</i> = 16)	50.19 $\pm$ 3.13 [†] (<i>n</i> = 15)
Bladder compliance (μ l/cmH ₂ O)	15.83 $\pm$ 2.18 (<i>n</i> = 10)	7.51 $\pm$ 1.71 [*] (<i>n</i> = 9)	16.18 $\pm$ 3.00 (<i>n</i> = 16)	14.30 $\pm$ 2.88 (<i>n</i> = 15)
Bladder capacity (μ l)	401.8 $\pm$ 31.1 (<i>n</i> = 10)	218.0 $\pm$ 37.1 [*] (<i>n</i> = 9)	391.1 $\pm$ 65.9 (<i>n</i> = 16)	344.5 $\pm$ 62.8 (<i>n</i> = 15)
E/C	3.49 $\pm$ 0.27 (<i>n</i> = 7)	2.08 $\pm$ 0.24 [*] (<i>n</i> = 7)	4.46 $\pm$ 0.64 (<i>n</i> = 9)	2.69 $\pm$ 0.48 (<i>n</i> = 9)

E/C, ratio of elastin-to-collagen; pBOO, partial bladder outlet obstruction; Wt, wild-type.

^{*} Significant compared with the Wt-Sham (*P* < 0.05).[†] Significant compared with the *Eln*^{+/-}-Sham (*P* < 0.05)