PIEZO2 in sensory neurons and urothelial cells coordinates urination

Henry Miller stated that “to relieve a full bladder is one of the great human joys”. Urination is critically important in health and ailments of the lower urinary tract cause high pathological burden. Although there have been advances in understanding the central circuitry in the brain that facilitates urination1–3, there is a lack of in-depth mechanistic insight into the process. In addition to central control, micturition reflexes that govern urination are all initiated by peripheral mechanical stimuli such as bladder stretch and urethral micturition reflexes. We show that PIEZO2 acts as a sensor in both the bladder urothelium and innervating sensory neurons. Humans and mice lacking functional PIEZO2 have impaired bladder control, and humans lacking functional PIEZO2 report deficient bladder-filling sensation. This study identifies PIEZO2 as a key mechanosensor in urinary function. These findings set the foundation for future work to identify the interactions between urothelial cells and sensory neurons that control urination.

The mechanotransduction channel necessary for urinary reflexes remains unknown. Several ion channels have been implicated in urinary tract function in vivo4–7, but none have been shown to be required for micturition reflexes. Moreover, it is not clear which cells are the primary sensors: umbrella cells of the innermost layer in the urothelium have been proposed to be mechanosensory8–10, but the bladder is also innervated by mechanically sensitive afferents from dorsal root ganglia (DRG)11,12. PIEZO2 is the primary mechanosensor that mediates touch, proprioception and mechanical allodynia in mice11–15. Loss-of-function mutations in PIEZO2 also resulted in complete deficits in these senses in humans13,16. Furthermore, PIEZO2 mediates interoceptive processes such as lung-stretch sensing and baroreception in mice12,13, but interoceptive deficits have not been studied in humans who are deficient in PIEZO2. As urination is driven by mechanical interoceptive reflexes, we investigated whether PIEZO2 is important for urination.

To understand how PIEZO2 contributes to urination in humans, PIEZO2-deficient individuals (n = 12; 5–43 years of age) answered questionnaires designed to capture pathology and validated against healthy control individuals to screen for voiding and elimination dysfunction19 (Fig. 1). We also assessed urological history, previous medical evaluations and non-invasive bladder ultrasound scans (Supplementary Table 1). All patients reported decreased voiding frequency, as low as once or twice daily, regardless of hydration status. Notably, the majority of individuals reported that they could spend an entire day without feeling the need to void and therefore followed a voiding schedule. A healthy frequency is defined as five to six voids per day. Despite the lack of normal sensory feedback, all patients had achieved continence at the time of evaluation except for one nine year old. However, many patients reported sudden urge incontinence, where any delay in voiding resulted in urinary accidents. Two individuals reported occasional nocturnal enuresis, and four had stress incontinence caused by laughter, cough and/or postural changes, with one case being severe enough to require treatment. Several patients had a sensation of incomplete voiding and an irregular urinary stream. Three adults described a sensation of pelvic heaviness when their bladder was full, and all three independently reported voiding by leaning over or using their hands to apply pressure to their lower abdomen. Overall, these data suggest that PIEZO2 has a key functional role in human urination.

We next carried out studies in mice to understand where and how PIEZO2 functions in the urinary tract. To test whether Piezo2 is present in bladder sensory neurons, we used RNA fluorescent in situ hybridization (FISH) in DRG tissue taken from three mice after injection of cholera toxin B–Alexa Fluor 488 (CTB), a neuronal tracer, into the bladder wall (Fig. 2a). Out of 92 bladder-innervating neurons labelled with CTB, 75 expressed Piezo2 transcript (81.5%). Piezo2 transcript was also detected in a subset of bladder urothelial cells expressing Krt20 (Fig. 2b), a marker of umbrella cells that line the bladder lumen and have been proposed to contribute to detection of bladder filling20. Seventy-four per cent of...
Mechanically-evoked micturition reflexes coordinate the bladder stretch responses in sensory neurons were dependent on Piezo2. Whether neurons detect bladder stretch directly or downstream of urothelial cell activation, we expect this stimulus to cause calcium influx. We injected a viral vector carrying Cre recombinase into postnatal day 0 (P0) to P3 pups carrying the Cre-dependent calcium indicator GCaMP6f and a conditional Piezo2-knockout allele, Piezo2fl/fl (Piezo2flox/flox;GCaMP6f+/−;control were GCaMP6f+/−). Thus, Piezo2 was deleted anywhere GCaMP6f was expressed. We observed rapid, robust responses in control sacral level 1 (S1) DRG neurons in response to manual, high-pressure bladder filling with saline (Fig. 2c), but these responses were markedly attenuated in Piezo2−/− DRG cells (Fig. 2d). Notably, cells responding to low-pressure stimuli were completely absent in Piezo2-knockout DRG (Fig. 2e). Calcium traces for cells in wild-type DRG revealed graded responses to pressure stimuli, with many cells responding to low and high pressures, but Piezo2−/− cells were silent at low pressures. Piezo2−/− DRG also had fewer cells responding to bladder stretch (Fig. 2f–m), but normal numbers of cells responding to painful pinch (Extended Data Fig. 1). This suggests that PIEZO2 is a key sensor of bladder stretch.

We next used calcium imaging to determine whether bladder-stretch responses in sensory neurons were dependent on Piezo2. Whether neurons detect bladder stretch directly or downstream of urothelial cell activation, we expect this stimulus to cause calcium influx. We injected a viral vector carrying Cre recombinase into postnatal day 0 (P0) to P3 pups carrying the Cre-dependent calcium indicator GCaMP6f and a conditional Piezo2-knockout allele, Piezo2fl/fl (Piezo2flox/flox;GCaMP6f+/−;control were GCaMP6f+/−). Thus, Piezo2 was deleted anywhere GCaMP6f was expressed. We observed rapid, robust responses in control sacral level 1 (S1) DRG neurons in response to manual, high-pressure bladder filling with saline (Fig. 2c), but these responses were markedly attenuated in Piezo2−/− DRG cells (Fig. 2d). Notably, cells responding to low-pressure stimuli were completely absent in Piezo2-knockout DRG (Fig. 2e). Calcium traces for cells in wild-type DRG revealed graded responses to pressure stimuli, with many cells responding to low and high pressures, but Piezo2−/− cells were silent at low pressures. Piezo2−/− DRG also had fewer cells responding to bladder stretch (Fig. 2f–m), but normal numbers of cells responding to painful pinch (Extended Data Fig. 1). This suggests that PIEZO2 is a key sensor of bladder stretch.

We next investigated individual micturition events to determine whether the bladder pressure required to sustain micturition was abnormal in these mice. We observed consistent pressure increases within and among wild-type mice (Fig. 3e, Extended Data Fig. 2d), but bladder pressure traces in Piezo2-knockout mice were highly variable (Fig. 3f, Extended Data Fig. 2e). The knockout mice exhibited higher peak bladder pressures (Fig. 3g), and required significantly more pressure during contractions, suggesting that the detrusor muscle must work harder to accomplish micturition (Fig. 3h, Extended Data Fig. 2f). We also assessed whether sensory input via PIEZO2 is important for urethral reflexes, which sustain efficient urination. During bladder contractions in wild-type mice, there was coordinated engagement of the urethra muscle (Fig. 3i). This reliable urethral activity was markedly attenuated in Piezo2-knockout mice (Fig. 3j, k, Extended Data Fig. 2k). Knockout urethral responses varied from silent or weak coordination to inappropriately timed hyperactivity (Extended Data Fig. 2c). Hyperactivity is a sign of detrusor–sphincter dyssynergia, a condition involving uncoordinated communication between the muscle groups responsible for urination. This indicates that the urethra was not receiving the appropriate sensory input to govern its activity during micturition. Mice lacking PIEZO2 also had more variable, larger void volumes, as longer periods between contractions allowed more bladder filling (Fig. 3l). Together, these data indicate that PIEZO2 sets stretch sensitivity in the lower urinary tract and initiates appropriately timed reflexes that contribute to efficient urination.

Next, we tested whether urination behaviour is altered in Piezo2-knockout mice. We placed mice on filter paper for 3 hours and

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**Fig. 1** Urinary dysfunction in individuals deficient in PIEZO2. Patient numbers correspond to those in Supplementary Table 1. Grey indicates a neutral answer or one not indicating pathology. Urinary frequency information is scored differently to the other questions, and is colour-coded according to the pathological score assigned to the answer in the questionnaire. Unless otherwise noted, the colour code indicates the following: grey, never; yellow, half of the time (pathology score of 2); red, every day or every night if night time is indicated in question (pathology score of 4). Dagger indicates an unanswered question. Daggert indicates that the individual answered twice per day during clinical interview.
imaged the resulting urination patterns with UV illumination. We used only female mice to preclude territorial scent-marking behaviour. Wild-type mice typically urinated in the corners and edges of the cage in large spots (Fig. 3m). Piezo2-knockout mice had a variety of urination patterns, and some displayed urine leaking (small spots) or large voids towards the cage centre (Fig. 3n, o). This phenotype was not attributed to the knockout mice spending more time in the middle of the cage (Fig. 3p). Thus, knockout mice have abnormal urination behaviour, including some apparent incontinence.

We next studied whether this observed urinary dysfunction in Piezo2-knockout mice led to long-term consequences. Chronic urinary tract dysfunction typically causes tissue remodelling as the bladder wall grows thicker to compensate for inefficient voiding. This remodelling can eventually result in ‘decompensation’, which is marked by a flaccid, ineffective bladder with sequelae of incomplete voiding, vesicoureteral reflux and increased frequency of urinary tract infections. Bladder-wall thickening was observed by haematoxylin and eosin staining in Piezo2-deficient mice (Fig. 3q–s). The weight of freshly excised bladders also revealed bladder-wall remodelling, as bladders from the knockout mice were significantly heavier than those from wild-type littermates (Fig. 3t, Extended Data Fig. 2m). Thus, impaired bladder filling stimuli in wild-type (WT) (n = 3 mice) and Piezo2<sup>−/−</sup> (KO) (n = 4 mice) DRGs. f, g, Example pressure trace from wild-type (f) and Piezo2<sup>−/−</sup> (g) DRG. Stimuli were interleaved during recording, but are shown sorted low to high, hence the discontinuous line. Data below these graphs are sorted together with the respective pressure peaks. h, i, Average per cent change in calcium fluorescence for all responding cells during the pressure peaks shown in f, g, respectively. j, k, Calcium traces for individual wild-type cells that responded to pressure stimuli in f (n = 17) (j) and for Piezo2<sup>−/−</sup> cells responding to pressure stimuli shown in g (n = 6) (k). Each cell’s responses are shown on the same horizontal line. Cells are sorted by cumulative response to the four lowest-pressure stimuli. l, m, Maximum calcium response for the corresponding cells in j, k, 1 s after pressure peak.

We observed similar phenotypes in mice that lacked PIEZO2 only in sensory neurons (Fig. 4i–p). Deleting PIEZO2 in all sensory neurons is lethal, so we used Scn10a<sup>-/-</sup> mice<sup>26</sup> (Scn10a encodes the voltage-gated sodium channel Na<sub>A</sub>,L) to delete PIEZO2 in the A<sub>δ</sub>- and C-fibre subsets, which are the primary sensory neuron types described in Piezo2-knockout mice to lead to detrusor hypertrophy, an indicator of chronic voiding dysfunction.

We next investigated the cell types in which PIEZO2 was required. We tested whether PIEZO2 deficiency in urothelial cells changed the pressure threshold that is required to initiate micturition. We used the Upk2<sup>-/-</sup> allele to abrogate PIEZO2 activity in urothelial cells (Extended Data Fig. 3a), which have been proposed to act as stretch sensors and communicate to underlying neurons using ATP<sup>27,28</sup>. We found that Upk2<sup>-/-;Piezo2<sup>fl/fl</sup></sup> knockout mice displayed similar phenotypes to the Hoxb8<sup>-/-</sup>;Piezo2<sup>fl/fl</sup> knockout mice, with higher bladder stretch thresholds, increased bladder pressure during micturition and attenuated urethral reflexes (Fig. 4a–h). In combination with expression data from FISH (Fig. 2b), these data indicate that PIEZO2 acts in umbrella cells to help set bladder-stretch sensitivity and initiate appropriate micturition reflexes. These results confirm the proposed role for umbrella cells as mechanosensory cells that participate in initiating micturition.<br><br>Fig. 2 | Piezo2 is expressed in the lower urinary tract, and sensory neurons require PIEZO2 to detect low-pressure bladder filling. a, DRG neurons were retrogradely labelled using CTB (cyan, left) and fluorescent in situ hybridization (FISH) of DRGs with probes targeting Piezo2 (magenta, middle). Arrowheads point to Piezo2-expressing bladder neurons. Scale bar, 50 μm. The tracing experiment was repeated using three mice, n = 22–36 cells analysed per mouse. b, FISH for Krt20 (green) and Piezo2 (magenta) in bladder. Arrowheads point to Piezo2-expressing umbrella cells. Scale bar, 50 μm. FISH was performed on three bladders, with two technical replications. Analysis was performed on 80–117 nuclei per bladder. c, d, Image z-stack from GCaMP6f<sup>+</sup> control mouse (c) and Piezo2<sup>fl/fl</sup> mouse (d) SI DRG during bladder filling. e, Count of cells responding to low-pressure (black) and high-pressure (red)

We observed similar phenotypes in mice that lacked PIEZO2 only in sensory neurons (Fig. 4i–p). Deleting PIEZO2 in all sensory neurons is lethal, so we used Scn10a<sup>-/-</sup> mice<sup>26</sup> (Scn10a encodes the voltage-gated sodium channel Na<sub>A</sub>,L) to delete PIEZO2 in the A<sub>δ</sub>- and C-fibre subsets, which are the primary sensory neuron types described...
in the bladder\textsuperscript{10,26}. This mouse line does not induce recombination in urothelial cells (Extended Data Fig. 3b–e). Sensory-neuron-specific Piezo2-knockout mice displayed longer intervals between contractions (Fig. 4i), but the pressure before contractions was not different from that in wild-type mice, as it was in urothelial-specific- and full caudal-knockout mice (Fig. 4j). This implies that mechanosensory stimuli activate PIEZO2 in umbrella cells to initiate bladder relaxation during filling (Fig. 4b) and that neuronal mechanosensing is dispensable for this process. Alternatively, it is possible that bladders become fibrotic and less compliant in Upk2-cre; Piezo2\textsuperscript{fl/fl} and Hoxb8-cre; Piezo2\textsuperscript{fl/fl} mice, but not in Sca10a-cre; Piezo2\textsuperscript{fl/fl} mice. Neuronal PIEZO2-knockout mice do require more bladder pressure for micturition and have highly attenuated urethral reflex responses (Fig. 4o, p). These data implicate PIEZO2 in mediating neuronal stretch responses that are critical for downstream urethral reflexes. Of note, mice with Piezo2 knockout in individual tissues did not display the marked bladder remodelling that was observed in full caudal-knockout mice (Fig. 3s, t, Extended Data Fig. 3f, g), suggesting that urothelial or neuronal PIEZO2 alone could still contribute to urinary function. These results indicate that there is a two-part signalling mechanism involving PIEZO2 in umbrella cells and
sensory neurons that set bladder sensitivity and promote micturition reflexes. Further investigations are required to address how these cell types communicate.

We have used evidence from mice and humans to identify the mechanotransduction channel PIEZO2 as a critical mediator of urinary tract function. Absence of Piezo2 in mice does not result in urinary tract paralysis and death, and PIEZO2-deficient humans are still able to urinate. This indicates that there are mechanotransduction proteins other than PIEZO2 in the urothelium and lower urinary tract sensory neurons.

For example, the mechanotransduction ion channels TMEM63B and PIEZO1 are widely expressed in the urothelium, and PIEZO1 partially mediates urothelial stretch responses in vitro.

Our results suggest a two-part model of mechanosensory signalling in the urinary tract, which is reminiscent of epithelial cell–neuronal sensory machinery in the skin (Merkel cell–neurite complexes), lung (neuroepithelial bodies) and intestine (enterochromaffin cells). Our results also implicate umbrella cells in mediating bladder relaxation during filling, perhaps by signalling to bladder muscle and/or...
through stretch-induced cellular changes. Future studies will address how urothelial cells and sensory neurons cooperate to control urinary function.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2830-7.

1. de Groat, W. C. & Yoshimura, N. Afferent nerve regulation of bladder function in health and disease. *Handb. Exp. Pharmacol.* 154, 91–138 (2009).

2. Keller, J. A. et al. Voluntary urination control by brainstem neurons that relax the urethral sphincter. *Nat. Neurosci.* 21, 1229–1238 (2018).

3. Hou, X. H. et al. Central control circuit for context-dependent micturition. *Cell* 167, 73–86 (2016).

4. Garry, R. C., Roberts, T. D. & Todd, J. K. Reflex responses of the external urethral sphincter of the cat to filling of the bladder. *J. Physiol.* 139, 13–14 (1957).

5. Cockett, D. A. et al. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* 407, 1011–1015 (2000).

6. Andersson, K. E., Gratzke, C. & Hedlund, P. The role of the transient receptor potential (TRP) superfamily of cation-selective channels in the management of the overactive bladder. *BJU Int.* 106, 1114–1127 (2010).

7. Mochizuki, T. et al. The TRPV4 cation channel mediates stretch-evoked Ca2+ influx and ATP release in primary urothelial cell cultures. *J. Biol. Chem.* 284, 21257–21264 (2009).

8. Merrill, L., Gonzalez, E. J., Girard, B. M. & Vizzard, M. A. Receptors, channels, and chemosensitivity of two major classes of bladder afferents with endings in the vicinity to the urothelium. *J. Physiol.* 587, 3523–3538 (2009).

9. Murthy, S. E. et al. The mechanosensitive ion channel Piezo2 mediates sensitivity to mechanical pain in mice. *Sci. Transl. Med.* 10, (2018).

10. Ranade, S. S. et al. Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature* 516, 121–125 (2014).

11. Szczot, M. et al. PIEZO2 mediates injury-induced tactile pain in mice and humans. *Sci. Transl. Med.* 10, (2018).

12. Zagorodnyuk, V. P., Brookes, S. J., Spencer, N. J. & Gregory, S. Mechanotransduction and disease. *Handb. Exp. Pharmacol.* 194, 91–14 (1957).

13. Zeng, W. Z. et al. PIEZOs mediate neuronal sensing of blood pressure and the baroreceptor reflex. *Science* 362, 464–467 (2018).

14. Afshar, K., Mirbagheri, A., Scott, H. & Maclellay, A. E. Development of a symptom score for dysfunctional elimination syndrome. *J. Urol.* 182, 1939–1944 (2009).

15. Ehrhardt, A. et al. Urinary retention, incontinence, and dysregulation of muscarinic receptors in male mice lacking Mras. *PloS ONE* 10, e0141493 (2015).

16. Takezawa, K. et al. Authentic role of ATP signaling in micturition reflex. *Sci. Rep.* 6, 19585 (2016).

17. Takezawa, K., Kondo, M., Nonomura, N. & Shimada, S. Urothelial ATP signaling: what is its role in bladder sensation? *Neuroreport.* 36, 966–972 (2017).

18. Ferguson, D. R., Kennedy, I. & Burton, T. J. ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes—a possible sensory mechanism? *J. Physiol.* 505, 503–511 (1997).

19. Agarwal, N., Offermanns, S. & Kuner, R. Conditional gene deletion in primary nociceptive neurons of trigeminal ganglia and dorsal root ganglia. *Genesis* 38, 122–129 (2004).

20. Sengupta, J. N. & Gebhart, G. F. Mechanosensitive properties of pelvic nerve afferent fibers innervating the urinary bladder of the rat. *J. Neurophysiol.* 72, 2420–2430 (1994).

21. Miyamoto, T. et al. Functional role for Piezo1 in stretch-evoked Ca2+ influx and ATP release in urothelial cell cultures. *J. Biol. Chem.* 289, 16565–16575 (2014).

22. Takezawa, K. et al. Development of a symptom score for dysfunctional elimination syndrome. *Sci. Transl. Med.* 10, (2018).

23. Alcaino, C. et al. A population of gut epithelial enterochromaffin cells is mechanosensitive and requires Piezo2 to convert force into serotonin release. *Proc. Natl Acad. Sci. USA* 115, E7632–E7641 (2018).

24. Wang, E. C. et al. ATP and purinergic receptor-dependent membrane traffic in bladder umbrella cells. *J. Clin. Invest.* 115, 2412–2422 (2005).

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All experiments were performed within the protocols and guidelines approved by the Institutional Animal Care and Use Committees of The Scripps Research Institute in compliance with regulatory standards established by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Statistics

Unless otherwise noted in the legends, groups were compared using two-tailed Student’s t-test with Welch’s correction, as groups did not have equal variances. For comparisons of groups with an n less than 15, we used the non-parametric Mann–Whitney test to assess differences, as we could not assess distributions. These tests were indicated in the figure legends. No statistical test was used to pre-determine sample size. Instead, sample size was determined by animal availability and previous studies in the field, which found these sample sizes sufficient to detect deficits.

Study design

We established exclusion criteria before collecting cystometry data: data from the first 30 min of cystometry recording was not used because bladder muscle activity has often not stabilized. Moreover, animals that displayed bladder leaking during recording were excluded from analysis, as leaking indicated a flawed seal and thus inaccurate filling responses. To verify the reproducibility of experimental findings, we restricted the time of day that cystometry recordings were done (Zeitgeber 8–14) and we performed every experiment in a cohort of male and female mice to compare to their wild-type littersmates. The order of recordings for different genotypes was randomized. The experimenter was blind to genotype when possible. Hoxb8-Cre+;Piezo2−/− knockout mice have obvious motor impairments, so the experimenter was never blind to genotype for these groups.

Mice

Male and female mice were anesthetized by isoflurane (5% induction, 1–2% maintenance, Kent Scientific SomnoSuite) and the bladder was catheterized and connected to saline lines (described above). Tungsten electrodes were inserted directly into the urethral muscle (A-M systems: C795500). Saline lines were connected to a pressure sensor (Biopac: RX104A-MRI) which connected via pressure transducer (TSD104A) to an MP160 Biopac system amplifier (DA100C). Electromyography electrodes were connected to a differential amplifier (EMG100C: gain 1,000, sample rate 10 kHz, low-pass filter 5 kHz, 60 Hz notch filter and 100 Hz high-pass filter). Bladder was continuously filled at 20 μl min−1, set on filter paper (Fisher Scientific: 05-714-4) and left in a darkened room for 4 h. Mice did not have access to water to prevent the bladder and test for leaking. The bladder was again set on filter paper to fill the bladder and test for leaking. The animal was sewn shut with the catheter extending out. The animal was flipped to prone position, and the vertebral column was exposed. A microdrill was used to open a window in the bone above the first sacral DRG. Epifluorescence imaging was performed using an upright microscope (FVMP-E, Olympus) equipped with a 4×, 0.28–numerical aperture air objective. Illumination was provided with a 130-W halogen light source (U-HGLGPS, Olympus), using a standard green excitation/emission filter cube. Images were acquired using an ORCA-Flash 4.0 CMOS camera (Hamamatsu) at a frame rate of 3 Hz using MetaMorph (Molecular Devices). Analysis was previously described11.

Retrograde labelling of sensory neurons

Mice were anesthetized with isoflurane (5% induction, 1–2% maintenance, Kent Scientific SomnoSuite) and the bladder was catheterized and connected to saline lines (described above). Tungsten electrodes were inserted directly into the urethral muscle (A-M systems: C795500). Saline lines were connected to a pressure sensor (Biopac: RX104A-MRI) which connected via pressure transducer (TSD104A) to an MP160 Biopac system amplifier (DA100C). Electromyography electrodes were connected to a differential amplifier (EMG100C: gain 1,000, sample rate 10 kHz, low-pass filter 5 kHz, 60 Hz notch filter and 100 Hz high-pass filter). Bladder was continuously filled at 20 μl min−1 using a syringe pump until regular urination cycles began. Data was not collected until the mouse had been stably cycling (30–40 min after beginning of recording), at which point filling rate was increased to 30 μl min−1. Data were logged with Acqknowledge software (v.4.4.2) and processed in MATLAB (v.2018b).

Behavioural assays

Female mice were placed in normal home cages that were bottomless, set on filter paper (Fisher Scientific: 05-714-4) and left in a darkened room for 4 h. Mice did not have access to water to prevent the water leaks from disturbing urine marks. Paper was imaged using a widefield camera (Logitech C930e) while illuminated by UV light. Images were thresholded and converted to B&W binary in ImageJ (v.2.0.0-rc-49/1.51d), and total number of black pixels was counted.
A region of interest corresponding to the middle 50% of the image area was used to count the number of black pixels in the middle of the cage.

**Bladder histology**

Bladders used for histology were the same bladders used for the bladder weight measurements. Wild-type and knockout littermate male bladders were collected, opened and blotted on kimwipes before weighing. After recording their freshly excised weight, they were fixed in 10% Neutral Buffered Formalin for 24 h, and then stored in 70% ethanol. Bladders were paraffin embedded, and central cross-sections were used for haematoxylin and eosin staining. Processing and staining was performed by Scripps Histology Core services. Muscle layer thickness was measured in ImageJ at 7–11 different places along the muscle wall per animal perpendicular to the muscle surface, and an average thickness value is shown per mouse.

**Clinical assessment**

Twelve patients with PIEZO2 loss-of-function mutations from 11 families (n = 4 males and 8 females, ranging in age from 4 to 43) were evaluated at the National Institutes of Health (NIH) under research protocol approved by the Institutional Review Boards of National Institute of Neurological Disorders and Stroke (NINDS, protocol 12-N-0095) between April 2015 and May 2020. Written informed consent and/or assent (for minor patients) was obtained from each participant in the study. All of the patients had biparentally inherited bi-allelic homozygous or compound heterozygous nonsense variants that are expected to result in a ‘null’ status for protein expression. Patients with PIEZO2 loss-of-function either found us or were referred to our group through our network of international collaborators. Genotype information can be found in Supplementary Table 1, along with past treatments and diagnoses. One patient, P10, carried a nonsense and a deleterious splice site variant in compound heterozygosity. Also as stated above, all patients presented with a profound congenital ubiquitous lack of proprioception, vibration, and specific loss of touch discrimination on glabrous skin. Detailed history, clinical evaluation and testing were conducted including an in-depth review of urinary function, urological history, review of previous evaluations and non-invasive bladder ultrasound. Patients were recruited from all over the world and their age ranged between 5 to 43 years (see table). Four adult patients (3 females and 1 male) provided their own history. None of the patients were taking any medications that could affect urinary function at the time of the questionnaire. Parents assisted with information gathering from their children.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

The raw data that support the findings of this study are available from the corresponding authors upon reasonable request.

**Code availability**

Code for calcium imaging analysis is previously published13. MATLAB (v.2018b) code used for cystometry analysis is available at https://github.com/PatapoutianLab/cystometry.

31. Chen, T. W. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300 (2013).

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**Author contributions**

K.L.M. designed and performed all mouse cystometry, behavioural experiments and tissue histology, analysed data and, together with A.P., wrote the manuscript. D.S., T.O., C.G.B. and A.T.C. designed and performed the human clinical assessments. Calcium imaging and analysis was performed by N.G., K.L.M. and M.S. Retrograde labelling and FISH experiments were performed by K.L.M., A.M.C. and I.D. J.K. and L.T.S. contributed analytical tools for data analysis, technical support and conceptual project design. C.G.B, A.T.C. and A.P. contributed to project design and supervision. All authors discussed results and contributed to manuscript editing.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | The bladder urothelium expresses multiple mechanosensitive proteins, and PIEZO2 is not required for sensory neuron pinch responses. 

**a**, FISH in bladder tissue with probes against *Krt20* (green) and *Piezo1* (white). DAPI in blue. 

**b**, FISH in bladder tissue with probes against *Krt20* (green) and *Tmem63b* (white). DAPI in blue. 

**c**, Z-projection of the standard deviation of responses from genital pinch in WT and **d**, *Piezo2*KO DRG. 

**e**, Quantification of peak responses during pinch shown as percent of baseline (each data point is one cell). *n* = 3 DRGs, 40 cells for WT, 4 DRGs and 69 cells for *Piezo2*KO DRGs.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | PIEZO2 is required for efficient micturition reflexes in male mice. a, Hoxb8-cre;Ai9 bladder tissue, fixed, frozen and mounted to show tdTomato (red) throughout the tissue, labelled with DAPI (blue). Scale is 100 μm. Expression was evaluated in two mice. b, Example pressure and urethra activity traces from three wild-type males and c, three Hoxb8-cre;Piezo2fl/fl knockout male littermates. d, Heat map of individual bladder contraction events in wild-type and e, knockout male mice, with corresponding urethra activity below in f and g respectively. h, Bladder contraction intervals for males. i, Bladder pressures five seconds before peak contraction for males. j, Bladder pressures five seconds before peak contraction for males. n = 6 males per group. P < 0.0001 for graphs in h, i, j and k, two-sided Student’s t-test with Welch’s correction. l, Body weights from a subset of mice whose bladder weights are shown in Fig. 2t, and m, bladder weights from animals in l, shown as a percentage of body weight. Red horizontal lines indicate means, vertical red bars indicate +/- standard deviation (shown where possible).
Extended Data Fig. 3 | Upk2- and Scn10a-cre expression and bladder weights. a, Upk2-cre;Ai9 bladder tissue fixed, frozen and mounted to show tdTomato (red) throughout the urothelium, labelled with DAPI (blue). Expression was evaluated in two mice. b, Scn10a-cre;Ai9 bladder tissue fixed, frozen and mounted to show tdTomato (red) is not present. Expression was evaluated in two mice. Thin cryosections made neuronal endings difficult to visualize. Scale: 200 μm, applies to a and b. c, Scn10a-cre;Ai9 DRG tissue showing tdTomato (red) in the majority of neurons, and d, a cell backlabelled with CTB-Alexa 488 injected into bladder. e, Merge of c and d, DAPI in blue. 9/9 backlabelled bladder cells analysed from two mice were tdTomato positive. f, Quantification of freshly excised bladder weights from four Upk2-cre;Piezo2<sup>fl/fl</sup> knockout and wild-type littermates. Age-matched littermates were 10–11 months old, which could account for greater variability. g, Bladder weights from age-matched Scn10a-cre;Piezo2<sup>fl/fl</sup> knockout mice and wild-type littermates, 7–8 months old. Red lines indicate mean values.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Acqknowledge software was used for data collection (version 4.4.2)

Data analysis

ImageJ (version 2.0.0-rc-49/1.51d), and Acqknowledge software (version 4.4.2) were used, and versions added to the supplementary information. Code availability statement in manuscript: “Code for calcium imaging analysis is previously published13. Matlab (R2018b) code was used for cystometry analysis and is available at: https://github.com/PatapoutianLab/cystometry.”

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The raw data that support the findings of this study are available from the corresponding author upon reasonable request.
Field-specific reporting

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- Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
No statistical test was used to pre-determine sample size. Instead, sample size was determined by animal availability and previous studies in the field, which found these sample sizes sufficient to detect deficits during cystometry (Keller et al., 2018 PMID: 30104734) and histological differences in remodeling and behavior (Everaerts and Zhen, 2010, PMID: 20956320).

**Data exclusions**
We established exclusion criteria prior to collecting cystometry data: data from the first 30 minutes of cystometry recording was not used because bladder muscle activity has often not stabilized. Moreover, animals that displayed bladder leaking during recording were excluded from analysis, as leaking indicated a flawed seal and thus inaccurate filling responses.

**Replication**
FISH experiments were independently replicated 2-3 times with the same results. Cystometry recordings were performed in independent male and female cohorts, and results were replicated. To verify the reproducibility of experimental findings, we restricted the time of day that cystometry recordings were done (Zeitgeber 8-14) and we performed every experiment in a cohort of male and female mice to compare to their wildtype littermates.

**Randomization**
The order of recordings for different genotypes was randomized. Beyond this, assigning animals to experimental groups is not relevant to this study, as the groups are defined by genotype. Animals of different sexes were analyzed independently to remove this covariate.

**Blinding**
The experimenter was blind to genotype when possible for all experiments. HoxB8Cre+;Piezo2f/f knockout mice have obvious motor impairments, so it was impossible to keep the experimenter blind for these groups.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

**Materials & experimental systems**

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology and archaeology |
| ☒ | Animals and other organisms |
| ☒ | Human research participants |
| ☒ | Clinical data |
| ☒ | Dual use research of concern |

**Methods**

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**
Mice were kept in standard housing with 12 h light/dark cycle set with lights on from 6 AM to 6 PM, with room temperature kept around 72 degrees Fahrenheit, with humidity between 30-80 % (not controlled). Adult male and female mice were used as indicated in the text. Age-matched knockout and wildtype littermates were tested at the same age in each cohort, but ages tested ranged from 5-12 months. The HoxB8Cre;Piezo2f/f mouse line has been previously described11. GCaMP6f/+ mice (B6:129S-Gt(Rosa)26Sortm9(CAG-GCaMP6f)Hze/J, Jackson Laboratory: Ai95, #024105) were bred to Piezo2f/f mice as described previously13,35. Piezo2f/f mice were mated with SNSCre mice29 or UPK IICre mice (B6(129)-Tg(Upk2-cre)1Rkl/WghJ, Jackson Laboratory: #029281) to create sensory-neuron specific and urothelial specific Piezo2 knockout animals, respectively. Each of these Cre lines was also crossed with Ai9 mouse (B6.Cg-Gt(Rosa)26Sortm9(CAG-ttdTomato)Hze/J, Jackson Laboratory: # 07909) to assess Cre expression.

**Wild animals**
No wild animals were used in this study

**Field-collected samples**
No field collected samples were used in this study.
Ethics oversight

All experiments were performed within the protocols and guidelines approved by the Institutional Animal Care and Use Committees of The Scripps Research Institute in compliance with regulatory standards established by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Twelve patients with PIEZO2 loss-of-function mutations from 11 families (N=4 males and 8 females, ranging in age from 4 to 43) were evaluated at the National Institutes of Health (NIH) under research protocol approved by the Institutional Review Boards of National Institute of Neurological Disorders and Stroke (NINDS, protocol 12-N-0095) between April of 2015 and May of 2020. Written informed consent and/or assent (for minor patients) was obtained from each participant in the study. Genotype information can be found in Extended Data Table 1, along with past treatments and diagnoses.

Recruitment

Patients were recruited on the basis of their biparentally inherited bi-allelic homozygous or compound heterozygous nonsense variant mutations in the Piezo2 gene. Patients with PIEZO2 loss of function either found us, or were referred to our group through our network of international collaborators. The nature of this group means that we are only analyzing patients without functional Piezo2, which is the goal of the study.

Ethics oversight

Research protocol approved by the Institutional Review Boards of National Institute of Neurological Disorders and Stroke (NINDS, protocol 12-N-0095)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

This is not a clinical trial, but approval was through: NINDS, protocol 12-N-0095

Study protocol

NINDS, protocol 12-N-0095

Data collection

Data was collected at the NIH between April of 2015 and May of 2020.

Outcomes

No outcomes measured.