Published in final edited form as:
Int Urol Nephrol. 2010 March ; 42(1): 57–63. doi:10.1007/s11255-009-9597-y.

Bladder outlet obstruction in male cystinuria mice

Mathew Ercolani,
Department of Surgery, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ, USA

Amrik Sahota,
Department of Genetics, Rutgers University, Piscataway, NJ, USA

Catherine Schuler,
Albany College of Pharmacy and Health Sciences, 106 New Scotland Avenue, Albany, NY 12208, USA. Stratton VA Medical Center, Albany, NY, USA

Min Yang,
Department of Genetics, Rutgers University, Piscataway, NJ, USA

Andrew P. Evan,
Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA

David Reimer,
Laboratory Animal Services, Rutgers University, Piscataway, NJ, USA

Joseph G. Barone,
Department of Surgery, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ, USA

Jay A. Tischfield, and
Department of Genetics, Rutgers University, Piscataway, NJ, USA

Robert M. Levin
Albany College of Pharmacy and Health Sciences, 106 New Scotland Avenue, Albany, NY 12208, USA. Stratton VA Medical Center, Albany, NY, USA

Robert M. Levin: robert.levin@acphs.edu

Abstract

Background—Cystinuria is the most common inherited cause of urinary tract stones in children. It can lead to obstructive uropathy, which is a major cause of renal failure. Genetic studies have identified two genes, *SLC3A1* and *SLC7A9*, to be directly involved in cystine stone formation. *Slc3a1* knockout male mice develop cystine stones in the bladder and, to a lesser extent, in the kidney. *Slc3a1* knockout female mice also develop cystinuria, but they do not form stones. The specific aim of this study was to characterize bladder function in cystinuria mice.

Methods—Eight control (4 male, 4 female) and 16 *Slc3a1* knockout (9 male, 7 female) mice of mixed strain background (C57B/129, age 4–5 months) were evaluated. Each mouse was anesthetized and the bladder dome catheterized for cystometry. Immediately following cystometry, the bladder was excised, weighed, and separated into three full thickness strips for contractile studies.

Correspondence to: Robert M. Levin, robert.levin@acphs.edulevinr@acp.edu.

Results—Bladders from cystinuria male mice had significantly increased weight, all of them had stones, decreased compliance, and decreased contractile responses to field stimulation, ATP, carbachol, and KCl. Compared with controls, female knockout mice showed normal bladder weight, decreased voiding pressure, slightly decreased compliance, and slightly decreased contractile responses.

Conclusions—These studies clearly demonstrate that the bladder stones that developed in the male cystinuria mice resulted in a partial outlet obstruction. Although the female cystinuria mice did not have bladder stones, bladder function was mildly impaired; presumably by the presence of cystine crystals.

Keywords

Bladder; Cystinuria; Stones; Mouse; Obstruction

Introduction

There are multiple etiologies of bladder obstruction in humans. Congenital causes of obstruction include posterior urethral valves (PUVs), which can have profound detrimental effects on bladder, ureter, and kidney function in male children [1, 2]. About 10–15% of children undergoing renal transplantation have PUVs as the cause of their renal insufficiency through high intravesical pressure which can damage the kidneys and ureters. Cystinuria, due to cystine accumulation, can lead to obstruction, re-current infections, and renal impairment that may require transplantation [3, 4]. Partial bladder outlet obstruction (PBOO) is a common lower urinary tract disorder in elderly males. The primary cause is compression of the urethra due to benign prostatic hyperplasia. PBOO can also occur in post-menopausal women as a result of estrogen deficiency [5, 6]. Other causes of bladder obstruction include bladder and pelvic tumors and spinal cord injury.

Numerous animal models of PBOO have been described. The most widely used animal is the rabbit, but the pig, rat, and mouse also have been used [7–11]. Although there are significant differences among animal models of PBOO, there are many similarities among these models and the obstructive dysfunction seen in humans. These similarities include denervation, mitochondrial damage, dysregulation of intracellular calcium storage and release, smooth muscle hypertrophy, mucosal hyperplasia and damage, susceptibility to infection and inflammation, and decreased compliance. Animal models also have a number of limitations, including the fact that the prevalence of bladder obstruction is higher in males than in females, but female rodents have been used in several studies because of ease of manipulation.

Cystinuria is an autosomal recessive disorder caused by mutations in the renal and intestinal transporter system (rBAT/b0, +AT) for cystine and the dibasic amino acids [12–14]. Cystine is relatively insoluble at physiological pH and it accounts for approximately 10% of kidney stones in children. Unless treated, patients with cystinuria will form stones throughout their life [4, 15]. Cystinuria is more common in males and recurrent infections and bladder stones (after which the disease is named) can be a significant cause of renal impairment in these patients. Cystinuria is classified as Type I or Type II. Type I is caused by mutations in *SLC3A1* (encoding rBAT), and Type II by mutations in *SLC7A9* (encoding b0, +AT). Knockout mouse models for *Slc3a1* and *Slc7a9* have been described [16–18]. We created *Slc3a1* knockout mice to investigate the molecular and cellular basis of stone disease in cystinurial. Cystinuria male mice develop bladder stones which can lead to obstruction, infection, and renal damage. These observations provide the rationale for using cystinuria mice as models for bladder obstruction.

Methods

All procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Stratton VA Medical Center. Three groups of mice were evaluated: 8 control (4 male, 4 female) and 16 *Slc3a1* knockout (9 male, 7 female) mice. The mice were of mixed strain background (C57B/129) and 4–5 months old. Each mouse was anesthetized with 0.1 ml of pentobarbital (50 mg/ml). The bladder was exposed through a midline incision and the bladder dome catheterized with PE-50 tubing with a 23 gauge needle connected to the bladder [19, 20]. The PE tubing was connected to a Harvard infusion pump with a Statham pressure transducer in line for monitoring bladder pressure and recording the pressure on a Grass Polygraph. Saline was infused at the rate of 0.59 μl/min until overflow incontinence occurred. At this point, bladder volume was recorded. Cystometric curves are presented, normalized to 100% of bladder volume at leakage. From the cystometric curve (increase in intravesical pressure with volume), bladder compliance was calculated as the mean rise in pressure between 0 and 20% capacity. This represents the initial rise in pressure with increasing volume and thus is an excellent representation of initial compliance of the bladder. In this way, we normalize for differences in bladder capacity among the groups.

Immediately following cystometry, the bladder was excised, opened longitudinally and washed free of stones with oxygenated cold Tyrodes solution, and weighed. The mouse was then euthanized with 0.1 ml Fatal Plus euthanasia fluid, i.p. The bladder was separated into three full thickness strips from dome to base. Each strip was mounted in isolated baths containing 15 ml oxygenated Tyrodes solution (37°C) with glucose (1 mg/ml) and 1 g of tension was placed on each strip. Preliminary studies demonstrated that at this passive tension, maximal active tension was generated for all groups. These strips were then stimulated with field stimulation at 2, 8, and 32 Hz. Following field stimulation, the strips were sequentially stimulated with carbachol (20 μM), ATP (1 mM), phenylephrine (100 μM), and KCl (120 mM) with three washes with fresh oxygenated buffer between agents at 10-min intervals. We use only one concentration of drug to avoid fatigue of the strips due to continued stimulation by dose response studies, which occurs frequently in strips isolated from obstructed bladders.

Statistics

Analysis of variance followed by the Bonferonni test for individual differences was used. A $P < 0.05$ was required for statistical significance.

Results

The control mice had no bladder stones. There were no significant differences between the control male and female mice in any parameter, and thus we combined them into one control group. All male cystinuria mice had stones whereas the female cystinuria mice did not. Figure 1 shows a micro computed tomography scan of the bladder from a male mouse containing stones, and Fig. 2 shows the stones present in another male mouse when the bladder was opened. 2. The number of stones ranged from a few to over 20. There was also a wide variation in stone size (and hence weight), with the largest stones approaching 4 mm in diameter.

An increase in bladder mass was clearly evident when this organ and the kidneys were removed from cystinuria male mice, whereas bladders from cystinuria female mice were of normal appearance. In paraffin sections, cystinuria male mice showed clear evidence of smooth muscle hypertrophy and basement membrane thickening of varying degrees. Females cystinuria mice of this age group showed no gross lesions (data not shown).

The mean weight of the recovered stones per male mouse was 227 ± 98 mg. There was no difference in the body weight among the three groups, however, the bladder weight of the cystinuria male mice was over fourfold higher than the bladder weight of the control mice [control = 25 ± 1 mg; male cystinuria = 114 ± 12 mg*; female cystinuria = 22 ± 0.5 mg^{X} (*, significantly different from control; x, significantly different from male cystinuria; $P < 0.05$)]. The large stones in the male bladder relative to the kidney are not surprising, since urine accumulation in the bladder can provide an environment for further crystal precipitation and stone growth.

Although the cystinuria male mice had large heavy bladders, the volume at capacity was significantly lower than the capacity of the control mice (Fig. 3), probably due to the presence of large stones. Cystinuria female mice however showed a significantly lower pressure at leakage and an increased volume compared with the male cystinuria mice (Fig. 3). This finding could be related to the anatomy of the female urethra and the lower outlet resistance, it provides compared with the male urethra.

Cystometric curves for the control and cystinuria male and female groups are presented in Fig. 4. The intravesical pressure at capacity was similar for the control and male cystinuria groups, but significantly lower in cystinuria female mice (Fig. 4). The quantitative determination of compliance (rate of intravesical pressure rise over the first 20% bladder volume) is shown in Fig. 5. Cystinuria male and female mice showed poor compliance compared with controls, with the males being significantly worse than the females (Fig. 5). The contractile responses to field (neurogenic) stimulation are shown in Fig. 6. For both maximal contractile response, and the rate of tension generation (data not shown), the male cystinuria mice responded between 20 and 30% of the response of the control mice. Similar decreases were observed for the responses to ATP (purinergic receptor agonist), KCl (smooth muscle membrane depolarization), and carbachol (muscarinic agonist) (Fig. 7). Interestingly, the contractile response to phenylephrine (alpha-adrenergic agonist) was similar for all groups (Fig. 7). In general, for all four stimuli female cystinuria mice showed either the same contractile responses as the control mice, or the response was slightly lower (Figs. 6, 7).

Discussion

The mutational bases of cystinuria have been deciphered to a large extent in recent years [12–14], and the pathologic bases are beginning to be defined [21, 22]. What effects cystine crystals or stones have on bladder contractility is not known. This question is clinically significant since poor bladder contractility can result in an increase in post-void residual urine volume, inefficient bladder emptying, and further stone growth in the bladder. Thus, we examined the relationship between cystinuria and bladder contractility in our cystinuria mouse model. The use of *Slc3a1* and *Slc7a9* knockout mice has resulted in significant advances in our understanding of the pathogenesis of cystinuria [16–18].

One interesting observation from our study is that the incidence of stones in the knockout mice is significantly greater in males than in females. However, the compliance of the female cystinuria mice was significantly poorer than the compliance of the control mice. The reason for the sexual bias in stone formation is not known, but it has been observed in a number of other knockout mouse models for stone disease, including the *Aprt* (adenine phosphoribosyltransferase) knockout mouse model developed in our laboratory [23–26]. APRT catalyzes the synthesis of AMP from adenine and 5-phosphoribosyl-1-pyrophosphate. In the absence of APRT, 2,8-dihydroxyadenine is produced from adenine by xanthine dehydrogenase and it can precipitate in the renal interstitium, resulting in kidney disease

[23]. Similar to the cystinuria mice, *Aprt* knockout mice show a marked sexual bias in that males form stones to a significantly higher degree than females [25, 26].

In addition to the renal and ureteral damage caused by the stones, cystinuria male mice show all the classic urodynamic and contractile dysfunctions of obstructive bladder disease. These include increased bladder weight, poor compliance, and decreased contractile responses to field stimulation, ATP, carbachol, and KCl. The contractile response to phenylephrine was similar for all groups, suggesting that the alpha-adrenergic receptor density may be unchanged by obstruction and/or the urethral smooth muscle component of the male cystinuria mice was not affected by cystine crystals or stones.

Often in animal models of partial outlet obstruction, the increase in bladder mass is accompanied by an increase in bladder capacity; however, in these studies, the increase in bladder mass was accompanied by a decrease in capacity. This is likely due to the increased thickness of the bladder wall, increased stiffness, and presence of bladder stones, all of which would limit bladder capacity. These studies clearly demonstrate that the bladder stones in the male cystinuria mice result in a partial outlet obstruction of the bladder, characterized by increased bladder weight and wall thickness, decreased compliance, and decreased contractile responses to all agonists except for phenylephrine. The presence of large stones in the bladder from cystinuria male mice is consistent with the observation that some children with cystinuria are able to pass relatively large stones [27].

The histological findings of thickened basement membranes and muscular hypertrophy in bladders from cystinuria male mice suggest that stones caused outlet obstruction, either directly or by affecting detrusor muscle contractility. The finding of decreased compliance in the female cystinuria mice compared with controls supports the concept that cystine crystals, without frank stone formation in the bladder, can cause decreased bladder compliance by negatively affecting detrusor contractility.

Acknowledgments

This work was supported in part by the Office of Research and Development Medical Research Service, Department of Veterans Affairs; and in part by the Capital Region Medical Research Foundation.

References

1. Bomalaski, MD. Posterior urethral valves. 2008. http://emedicine.medscape.com/article/1016086-overview
2. Roth KS, Koo HP, Spottswood SE, Chan JC. Obstructive uropathy: an important cause of chronic renal failure in children. Clin Pediatr. 2002; 45:309–314.10.1177/000992280204100503
3. Ahmed K, Dasgupta P, Khan MS. Cystine calculi: challenging group of stones. Postgrad Med J. 2006; 82:799–801.10.1136/pgmj.2005.044156 [PubMed: 17148700]
4. Knoll T, Zöllner A, Wendt-Nordahl G, Michel MS, Alken P. Cystinuria in childhood and adolescence: recommendations for diagnosis, treatment, and follow-up. Pediatr Nephrol. 2005; 20:19–24.10.1007/s00467-004-1663-1 [PubMed: 15602663]
5. Lemack GE. Urodynamic assessment of bladder-outlet obstruction in women. Nat Clin Pract Urol. 2006; 3:38–44.10.1038/ncpuro0378 [PubMed: 16474493]
6. Schwartz BF, Stoller ML. The vesical calculus. Urol Clin North Am. 2000; 27:333–346.10.1016/S0094-0143(05) 70262-7 [PubMed: 10778475]
7. Amaro JL, Balasteghin KT, Padovani CR, Montenegro R. Structural alterations of the bladder induced by detrusor instability. Experimental study in rabbits. Int Braz J Urol. 2005; 31:579–585. [PubMed: 16386129]
8. Austin JC, Chacko SK, DiSanto M, Canning DA, Zderic SA. A male murine model of partial bladder outlet obstruction reveals changes in detrusor morphology, contractility and Myosin isoform

expression. J Urol. 2004; 172:1524–1528.10.1097/01.ju.0000138045.61378.96 [PubMed: 15371884]

9. Chevalier RL. Pathogenesis of renal injury in obstructive uropathy. Curr Opin Pediatr. 2006; 18:153–160.10.1097/01.mop.0000193287.56528.a4 [PubMed: 16601495]
10. Grases F, García-Ferragut L, Costa-Bauzá A. Study of the early stages of renal stone formation: experimental model using urothelium of pig urinary bladder. Urol Res. 1996; 24:305–311.10.1007/BF00304781 [PubMed: 8931296]
11. Schröder A, Chichester P, Kogan BA, Longhurst PA, Lieb J, Das AK, Levin RM. Effect of chronic bladder outlet obstruction on blood flow of the rabbit bladder. J Urol. 2001; 165:640–646.10.1097/00005392-200102000-00087 [PubMed: 11176451]
12. Font MA, Feliubadaló L, Estivill X, Nunes V, Golomb E, Kreiss Y, Pras E, Bisceglia L, d'Adamo AP, Zelante L, Gasparini P, Bassi MT, George AL Jr, Manzoni M, Riboni M, Ballabio A, Borsani G, Reig N, Fernández E, Zorzano A, Bertran J, Palacín M. International cystinuria consortium: functional analysis of mutations in SLC7A9, and genotype-phenotype correlation in non-Type I cystinuria. Hum Mol Genet. 2001; 10:305–316.10.1093/hmg/10.4.305 [PubMed: 11157794]
13. Botzenhart E, Vester U, Schmidt C, Hesse A, Halber M, Wagner C, Lang F, Hoyer P, Zerres K, Eggermann T. Cystinuria in children: distribution and frequencies of mutations in the SLC3A1 and SLC7A9 genes. Kidney Int. 2002; 62:1136–1142.10.1111/j.1523-1755.2002.kid552.x [PubMed: 12234283]
14. Goodyer P. The molecular basis of cystinuria. Nephron Exp Nephrol. 2004; 98:e45–e49.10.1159/00008 0255 [PubMed: 15499206]
15. Dello Strologo L, Laurenzi C, Legato A, Pastore A. Cystinuria in children and young adults: success of monitoring free-cystine urine levels. Pediatr Nephrol. 2007; 22:1869–1873.10.1007/s00467-007-0575-2 [PubMed: 17694338]
16. Feliubadaló L, Arbonés ML, Mañas S, Chillarón J, Visa J, Rodés M, Rousaud F, Zorzano A, Palacín M, Nunes V. Slc7a9-deficient mice develop cystinuria non-I and cystine urolithiasis. Hum Mol Genet. 2003; 12:2097–2108.10.1093/hmg/ddg228 [PubMed: 12915471]
17. Peters T, Thaete C, Wolf S, Popp A, Sedlmeier R, Grosse J, Nehls MC, Russ A, Schlueter V. A mouse model for cystinuria type I. Hum Mol Genet. 2003; 12:2109–2120.10.1093/hmg/ddg189 [PubMed: 12923163]
18. Font-Llitjós M, Feliubadaló L, Espino M, Clèries R, Mañas S, Frey IM, Puertas S, Colell G, Palomo S, Aranda J, Visa J, Palacín M, Nunes V. Slc7a9 knockout mouse is a good cystinuria model for antilithiasic pharmacological studies. Am J Physiol Renal Physiol. 2007; 293:F732–F740.10.1152/ajprenal.00121.2007 [PubMed: 17596531]
19. Levin RM, Haugaard N, Levin SS, Buttyan R, Chen MW, Monson FC, Wein AJ. Bladder function in experimental outlet obstruction: pharmacologic responses to alterations in innervation, energetics, calcium mobilization, and genetics. Adv Exp Med Biol. 1995; 385:7–19. [PubMed: 8571847]
20. Levin RM, Monson FC, Haugaard N, Buttyan R, Hudson A, Roelofs M, Sartore S, Wein AJ. Genetic and cellular characteristics of bladder outlet obstruction. Urol Clin North Am. 1995; 22:263–283. [PubMed: 7762110]
21. Evan AP, Coe FL, Lingeman JE, Shao Y, Matlaga BR, Kim SC, Bledsoe SB, Sommer AJ, Grynpas M, Phillips CL, Worcester EM. Renal crystal deposits and histopathology in patients with cystine stones. Kidney Int. 2006; 69:2227–2235.10.1038/sj.ki.5000268 [PubMed: 16710357]
22. Worcester EM, Coe FL, Evan AP, Parks JH. Reduced renal function and benefits of treatment in cystinuria vs other forms of nephrolithiasis. BJU Int. 2006; 97:1285–1290.10.1111/j.1464-410X.2006.06169.x [PubMed: 16686727]
23. Engle SJ, Stockelman MG, Chen J, Boivin G, Yum MN, Davies PM, Ying MY, Sahota A, Simmonds HA, Stam-brook PJ, Tischfield JA. Adenine phosphoribosyltransferase-deficient mice develop 2,8-dihydroxyadenine nephrolithiasis. Proc Natl Acad Sci USA. 1996; 93:5307–5312.10.1073/pnas.93.11.5307 [PubMed: 8643571]
24. Stockelman MG, Lorenz JN, Smith FN, Boivin GP, Sahota A, Tischfield JA, Stambrook PJ. Chronic renal failure in a mouse model of human adenine phosphoribosyltransferase deficiency. Am J Physiol. 1998; 275:F154–F163. [PubMed: 9689017]

25. Wang L, Raikwar N, Deng L, Yang M, Liang L, Shao C, Evan AP, Stambrook PJ, Sahota A, Tischfield JA. Altered gene expression in kidneys of mice with 2, 8-dihydroxyadenine nephrolithiasis. Kidney Int. 2000; 58:528–536.10.1046/j.1523-1755.2000.00199.x [PubMed: 10916076]

26. Evan AP, Bledsoe SB, Connors BA, Deng L, Liang L, Shao C, Fineberg NS, Grynpas MD, Stambrook PJ, Youzhi S, Sahota A, Tischfield JA. Sequential analysis of kidney stone formation in the *Aprt* knockout mouse. Kidney Int. 2001; 60:910–923.10.1046/j.1523-1755.2001.06000 3910.x [PubMed: 11532086]

27. Schwentner C, Oswald J, Lunacek A, Bartsch G, Radmayr C. Giant cystine stone in an infant bladder with no evidence of cystinuria—valence of possible pathomechanisms. Urol Int. 2005; 75:285–287.10.1159/000087810 [PubMed: 16215321]

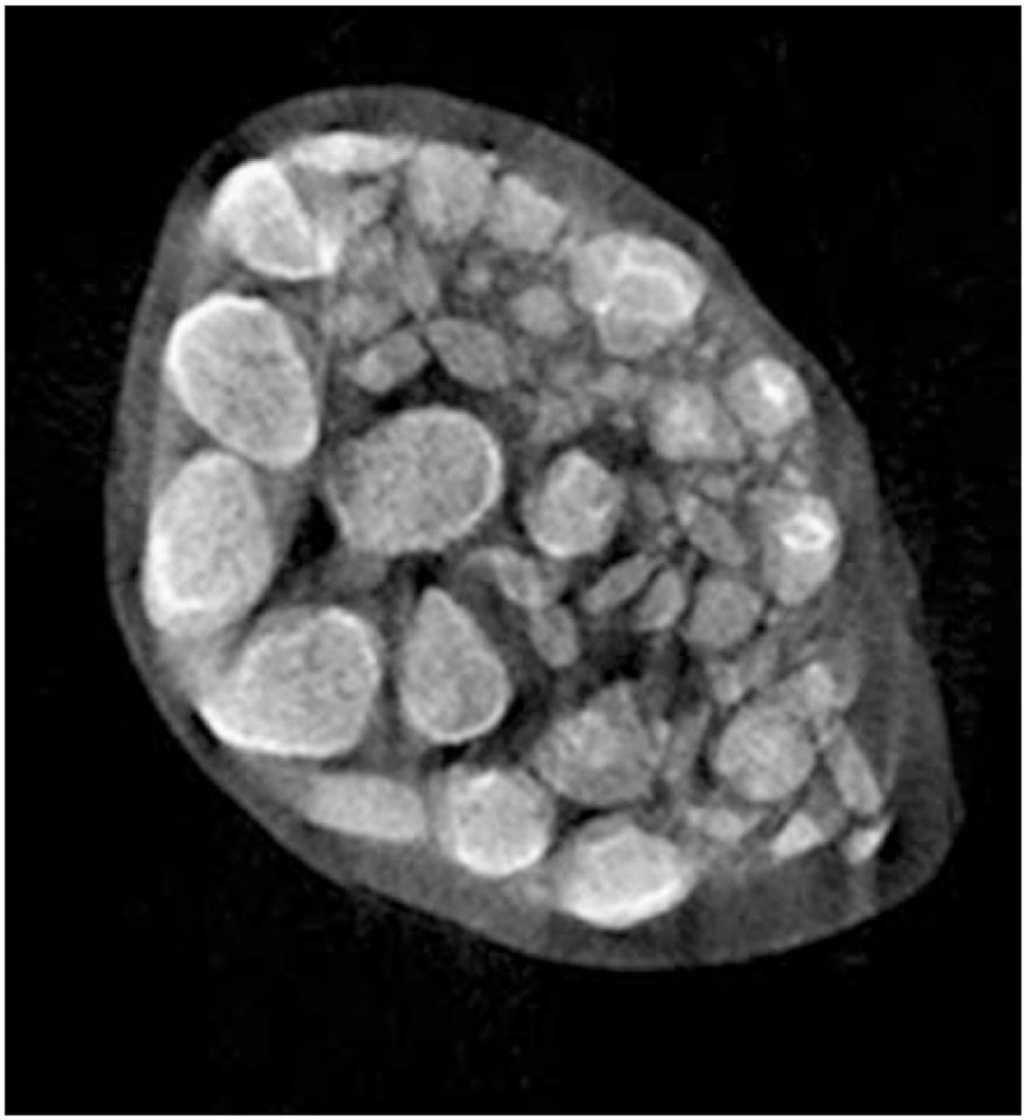

Fig. 1.
Representative micro computed tomography scan of bladder from a cystinuria male with stones

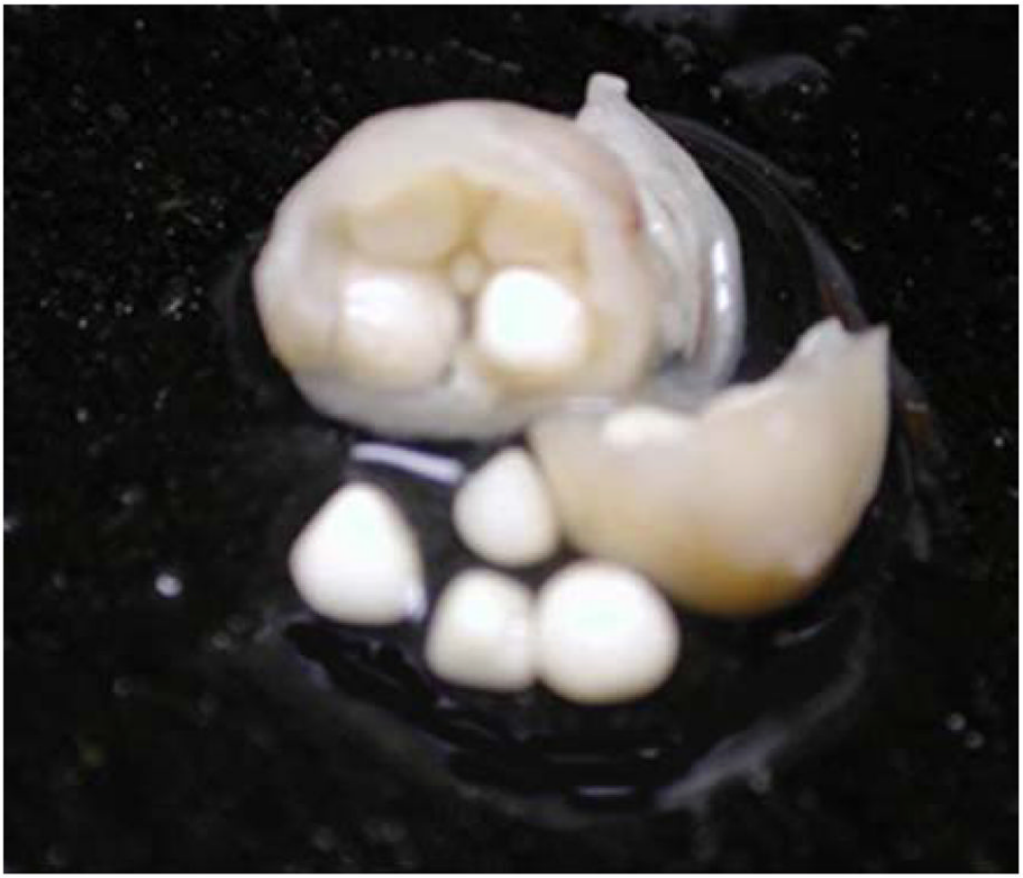

Fig. 2.
Representative view of open bladder from a cystinuria male showing stones

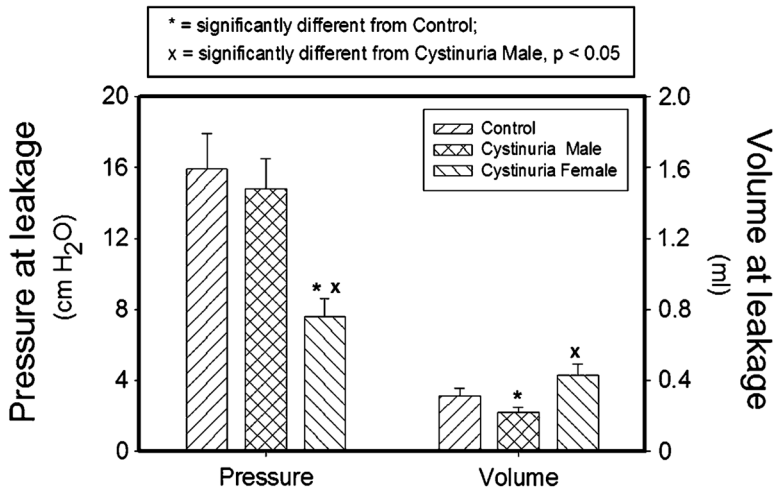

Fig. 3.

Cystometric values: pressure at leakage and volume at leakage. *Each bar* is the mean ± SEM of 7–9 mice. *Significantly different from control; x, significantly different from male cystinuria mice ($P < 0.05$)

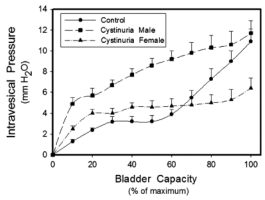

Fig. 4.

Cystometric *curves* for the control, male, and female cystinuria mice. *Each point* is the mean of 7–9 individual values

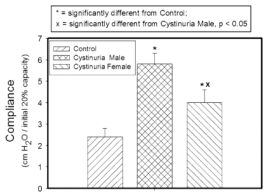

Fig. 5.

Bladder compliance: *each bar* is the mean ± SEM of 7–9 mice. *Significantly different from control; x, significantly different from male cystinuria mice ($P < 0.05$)

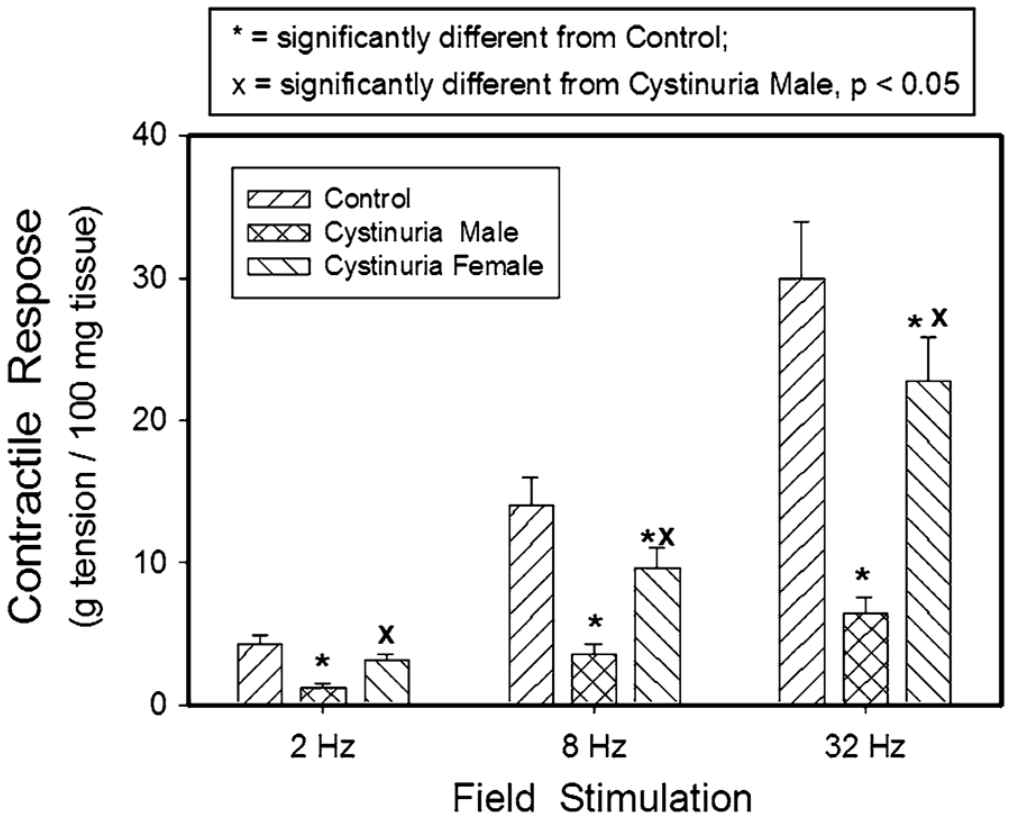

Fig. 6.

Contractile response to field stimulation, maximal response. *Each bar* is the mean ± SEM of 7–9 mice. *Significantly different from control; x, significantly different from male cystinuria mice ($P < 0.05$)

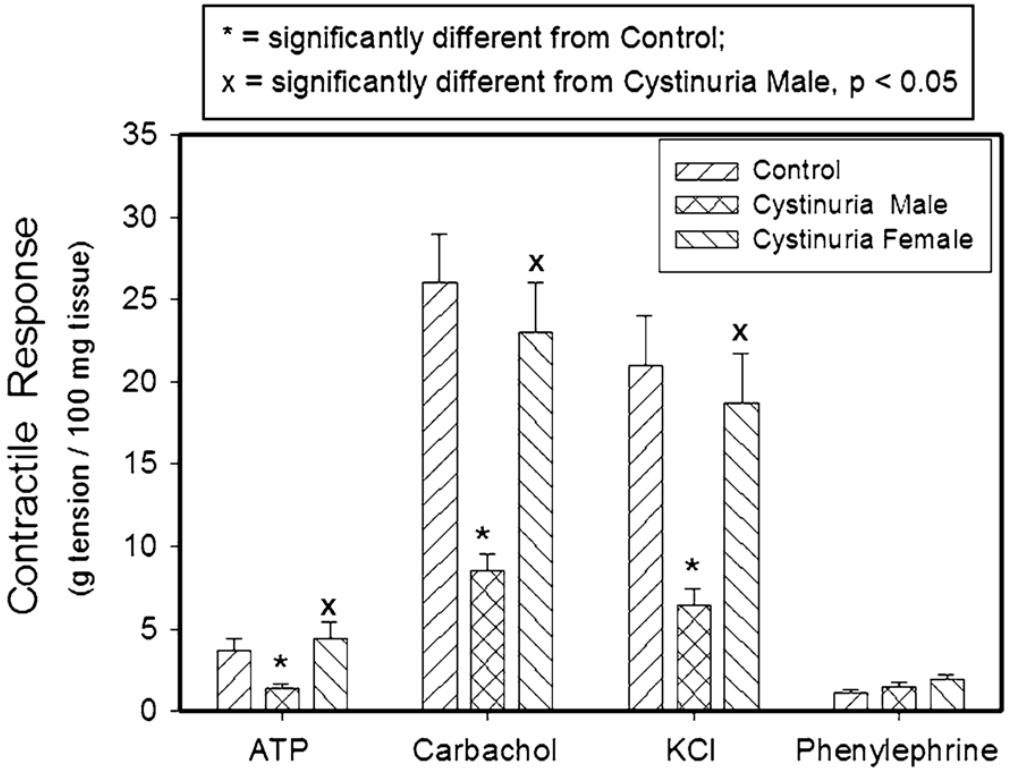

Fig. 7.

Contractile response to ATP, carbachol, KCl, and phenylephrine. *Each bar* is the mean ± SEM of 7–9 mice. *Significantly different from control; x, significantly different from male cystinuria mice ($P < 0.05$)