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Cystinuria caused by a *SLC7A9* missense mutation in Siamese-crossbred littermates in Germany

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Summary

Cystinuria is caused by defective proximal renal tubular reabsorption of the amino acids cystine, ornithine, lysine and arginine (COLA). The low solubility of cystine in mildly acidic urine may lead to the formation of urinary cystine crystals and uroliths. Much progress has been made recently in the diagnosis and understanding of cystinuria in companion animals. In cats, cystinuria affects equally both genders independent of neutering status and, despite being rare, already more cystinuria-causing mutations have been detected in cats compared to dogs. In this study a litter of Siamese-crossbred cats in Germany was assessed clinically for cystinuria and screened for mutations known to cause cystinuria in cats. An adult male castrated cat was presented with cystine crystalluria and calculi-related urinary obstruction and treated with perineal urethrostomy, cystotomy and medical management. This cat and a neutered male littermate without evidence of urinary tract disease were found to be positive for cystine by urinary nitroprusside test, to have increased urinary COLA values and to be homozygous for the p.Val294Glu mutation in the *SLC7A9* gene coding for b^{0,+}AT subunit of the b^{0,+} renal COLA transporter. Another littermate was non-cystinuric and did not carry this mutation. The same *SLC7A9* mutation was previously found in a Maine coon, a Sphinx and a medium-haired cat in North America suggesting a common ancestor and likely first widespread *SLC7A9* mutation causing cystinuria in cats. Genetic screening for this mutation may offer a simple and precise mean to diagnose other cats for cystinuria and offer specific management.

Keywords

Feline; cystine; urolithiasis; COLA; tiopronin

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Conflict of interest

The authors confirm that they do not have any conflict of interest.

Introduction

Cystinuria was one of the first “inborn errors of metabolism” in humans coined by Sir Archibald Garrod (6) and has since been described in many other species such as dogs (2, 8, 10) cats (4, 24, 25, 34), maned wolves (3), a caracal lynx (39), servals (8), ferrets (29), and mice (37). The impaired renal reabsorption of the amino acids cystine, ornithine, lysine and arginine, combined known as COLA (normally >98%), results in increased urinary concentrations of these amino acids. Cystine has a low solubility in neutral to mildly acidic pH values, and at higher concentrations it precipitates and forms hexagonal crystals and yellow brown uroliths in the bladder, urethra and occasionally the kidneys (36).

The COLA transporter b^{0+} resides in the proximal renal tubuli and intestinal brush border and contains two subunits: The *SLC3A1* gene codes the heavy subunit rBAT a luminal globular protein with a transmembrane tail, and the *SLC7A9* gene codes the light subunit $b^{0+}AT$, an intramembrane protein (36). In humans, mice, dogs, and cats, cystinuria has been documented to be caused by mutations in either the *SLC3A1* or *SLC7A9* gene and to be inherited autosomal dominant or recessive (2, 24, 25, 36).

Cystinuria in dogs has been reported in > 70 breeds (2, 8, 9, 20, 31) and is classified into different types (I-A, II-A, and II-B) depending on an autosomal recessive (I) and dominant (II) inheritance and mutant gene involved (A for *SLC3A1* and B for *SLC7A9*) (2). There is also an androgen-dependent type III of cystinuria (as seen in Mastiff-type breeds but also Irish terriers, Kromfohrländer, Scottish Deerhounds and several other breeds), and while the molecular basis of type III cystinuria has not yet been reported, it can be cured by chemical and surgical castration (2, 8).

In contrast, cystinuria has rarely been reported in cats. In 1991, a cystinuric domestic cat was first described (4) and in 1999 eighteen cases were briefly summarized (34). According to urolith laboratories, cystine calculi represent only up to 0.5% in Germany (and neighboring countries) (7, 13) and 0.1% of all feline uroliths in North America (31) compared with 4.4% of all canine calculi in Europe (12) and 0.3–1% in North America (31). Cystinuria was reported equally in male and female cats and independently of neuter status and thus an androgen-dependent cystinuria type seems unlikely (34). Recent studies documented mutations in *SLC3A1* (24) and *SLC7A9* (25) genes to cause cystinuria in cats from North America and thereby also showed a similar genetic heterogeneity as in humans (36) and dogs (2, 9). In this report here we describe the clinicopathological features, molecular genetic diagnosis and management of a litter of cystinuric cats in Germany.

Case description

Animals and history

A 3-year-old male castrated mixed-breed cat (cat 1) was presented for signs of stranguria and hematuria to the Tierärztliche Klinik für Kleintiere, Neu-Anspach, Germany. Five weeks prior to presentation cystine calculi were surgically removed from his bladder by a primary care practitioner.

Cat 1 was a strictly indoor cat obtained as a kitten from a local rescue organization and was castrated at 10 months of age. The queen was reportedly a Siamese like phenotype, and the tom was a stray European shorthair cat, but neither parent was available for further examination. Two male castrated indoor littermates (cats 2 and 3) were still living nearby in another household, while the one female littermate was unavailable for further examination and testing. The three male cats were black with white lockets, the female was a tortoiseshell and they did not have the recessive point color (23). According to their owners all three male littermates were healthy and had never shown any signs of urinary tract disease, although cat 2 had an intermittent history of urinating at inappropriate places.

Cat 1 was fed a raw meat diet (Kitty's Barf Complete with 90% rabbit or duck, Bellami, Oldenburg, Germany [16.5% crude protein]) since he was 4 months old. Cat 2 was fed a commercial canned (Felix, Nestlé Purina Pet Care, Euskirchen, Germany [7.5% crude protein]) and a dry kibble cat diet (Happy Cat with salmon, Interquell GmbH, Wehringen, Germany [32% crude protein]).

Clinical findings, therapy and course in cat 1

On clinical examination the cat was alert and in good body condition (bodyweight 5.8 kg) but showed some distress and a distended bladder. Results of complete blood count of ethylenediaminetetraacetic acid (EDTA) anticoagulated blood and blood chemistry including blood ammonia in heparinized plasma were all within normal limits. Ultrasound examination revealed a large bladder (8.6 × 5.8 cm) with a large volume of hyperechoic debris but no urolith was identified. Microscopic examination of a urine sediment showed many erythrocytes, leukocytes, and hexagonal crystals which are characteristic for cystine. The pH value by dipstick was 7, and the specific gravity was > 1.040. A urinary bacterial culture was negative. To release recurrent dysuria and reduce risk of re-obstructing, a perineal urethrostomy was performed.

Seven weeks after first presentation the cat represented with stranguria and was catheterized via the urethrostomy site to remove debris several times with increasing frequency over the next 3 months.

Twenty weeks after first presentation, ultrasound examination showed two calculi of 0.6 × 0.3 cm and 0.6 × 0.2 cm and lots of smaller debris (Fig 1a). The urinary sediment revealed hexagonal crystalluria, composed of 100% cystine which was confirmed by infrared spectroscopy, as well as bacteriuria, hematuria, pyuria and an increased number of transitional epithelial cells (Fig 1b). Quantitative amino acid determination identified mild COLAuria (Table 1). The urinary pH was 8 (measured by dipstick which tends to overestimate pH) (19), and the specific gravity was 1.038. Major growth (4+) of *Staphylococcus aureus* sensitive to doxycycline, chloramphenicol and gentamycin was noted. The cat was treated with potassium citrate (Kaliumcitrat, Euro OTC Pharma GmbH, Bonen, Germany) at 150 mg/kg orally twice daily to alkalinize the urine and tiopronin (Captimer, Biokanol, Rastatt, Germany) at 20 mg/kg orally twice daily to chelate cystine as a long term treatment, and doxycycline-monohydrate (Doxycyclin 100, 1A Pharma, Oberhaching, Germany) at 5 mg/kg orally twice daily for 2 weeks to eliminate the bacterial

infection. Also the diet was changed to a feline prescription renal diet (Royal Canin Renal with tuna, Cologne, Germany [6.6% crude protein, 0.35% arginine]).

The cat continued to show urinary tract signs and recurrent urinary obstruction resulting in an episode of post-renal azotemia. Serum creatinine rose from 1.9 mg/dl to 3.0 mg/dl (reference range: 0.8–2.4 mg/dl). The condition was managed with intravenous isotonic fluid infusion (Ringer Lactate, B. Braun Vet Care, Melsungen AG, Germany) at 5 ml/kg/h and a urinary catheter via urethrostomy site. The serum creatinine value declined to 2.2 mg/dl within one day followed by a cystotomy at week 21 to remove four calculi (Fig. 1c) as well as debris, and flush the bladder. The catheter was removed during surgery. The removed calculi were composed of 100% cystine as determined by infrared spectroscopy. Post-operatively the isotonic fluid therapy was continued with 3.3 ml/kg/h for 5 days.

At week 22, the cat developed mild but progressive hair loss and pruritus around the temporal region and pinnae, and the skin became crusty with little pustules and erythema (Fig. 2). Histopathology of the biopsied skin lesion revealed a crusty allergic dermatitis with eosinophils, mast cells and folliculitis, consistent with a drug reaction but not typical of erythema multiforme. The urine showed a pH value of 7 and no crystals. However, the urinary nitroprusside test result was strongly positive (Fig. 1d) and quantitative amino acid determination showed continuing COLAuria (Table 1). Cystine was low in the first COLA test in an otherwise COLAuric urine sample likely due to prior cystine precipitation (many cystine crystals were seen).

Because of the likely drug reaction tiopronin was discontinued. Treatment with prednisolone (CP-Pharma, Burgdorf, Germany) at 2 mg/kg daily was initiated and rapidly tapered over the next 2 weeks as the skin lesions completely resolved within 2 weeks. However, the cat was kept on a renal diet and potassium citrate and monitored by sediment evaluation and dipstick showing no crystals and a urine pH of 8 ± 1 . At last examination at week 35, the patient displayed no signs of lower urinary tract disease and neither calculi nor crystals were seen by ultrasound or urine sediment, respectively.

Findings in cats 2 and 3

Clinical examination, ultrasound and urine sediment of the two healthy male littermates were all normal and neither crystals nor calculi were noted by sediment analysis or ultrasound, respectively. The urinary nitroprusside test result was strongly positive for cat 2 and negative for cat 3 (Fig. 1d) and quantitative amino acid determination identified mild COLAuria in cat 2 (Table 1).

Genetic Analyses for cystinuria

EDTA blood samples from the three cats were sent to the Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA (PennGen) in order to genotype them for the one reported *SLC3A1* (24) and five recently reported *SLC7A9* mutations (25) causing cystinuria in cats from North America. DNA extraction and genotyping were performed as previously described (24, 25).

The genetic screening using polymerase chain reaction (PCR)-restriction fragment length polymorphism or real-time PCR assays revealed that cats 1 and 2 were homozygous for one previously reported *SLC7A9* point mutation (c.881T>A) causing a single amino acid change (p.Val294Glu) in b⁰⁺AT subunit of b⁰⁺ COLA transporter (Fig. 3). Both cats were wild-type homozygous (normal) for the *SLC3A1* mutation and the other four *SLC7A9* mutations. Cat 3 was wild-type homozygous (normal) for all six mutations.

Discussion

This case study discovered that two Siamese-crossbreds cats with cystinuria based upon cystine calculi or urinary nitroprusside and COLA tests had the same point mutation in the *SLC7A9* gene as previously reported in cystinuric cats in North America (25). While appropriate calculi and urinary testing has been required to establish a diagnosis of cystinuric animals of any species, DNA mutation screening tests offer a method of diagnosing cystinuric animals prior to showing clinical signs and are particularly useful for cases where a relative is known to be affected by a specific mutation. In dogs, some disease-causing mutations may be seen in different breeds as well as mixed breed dogs more commonly, and across continents. This seems also to be the case for one specific *SLC7A9* mutation (c.881T>A) found in purebred and other domestic cats.

The homozygous c.881T>A *SLC7A9* point mutation found in the two cats from Germany reported here replaces a highly conserved, hydrophobic valine to a negatively charged, hydrophilic glutamic acid (p.Val294Glu) and is located in an extracellular domain of b⁰⁺AT subunit of the b⁰⁺ protein (Fig. 3a). A Maine Coon, a Sphynx, and a domestic medium-hair cat in North America were previously found to be homozygous for this same mutation (25), thereby this is the only mutation thus far reported in more than one cat and across continents. The presence of the same *SLC7A9* variant in different breeds and geographical locations in the United States and Europe suggests a more wide-spread and common distribution of this mutant allele as well as one distant common ancestor as these breeds are not closely related. Screening other cystinuric cats for this c.881T>A *SLC7A9* mutation worldwide may be helpful to better define the prevalence of the mutation and feline cystinuria.

Six cystinuria-associated DNA mutations have been discovered in cats in North America: one missense mutation in the *SLC3A1* gene and three missense mutations and two splicing-related variants in the *SLC7A9* gene (24, 25). This shows a heterogeneity in cats as reported in humans and dogs. In dogs three *SLC3A1* mutations but only one *SLC7A9* mutation have been recently documented (2, 9). In cystinuric humans 170 *SLC3A1* (Type A) and 123 *SLC7A9* (Type B) variants have been reported, and indeed cystinuria in humans is genetically very heterogeneous (HGMD database accessed on October 2016). The homologous mutation to the p.Val294Glu *SLC7A9* mutation seen in cats has not yet been reported in dogs or other species.

Depending on the *SLC3A1* and *SLC7A9* mutations the renal cystine and COLA transporter function is to various degrees impaired and causes either cystinuria as an autosomal recessive or dominant trait. The fact that all cystinuric cats with the p.Val294Glu mutation,

including the cats reported here, were homozygous suggests that this is an autosomal recessive trait although we did not find any heterozygote cats and thus could not examine them for the presence of cystine and cystine crystals in urine.

Cystinuric cats may not show cystine crystals or calculi but are typically positive by urinary nitroprusside spot as well as COLA test. These tests have proven useful in screening any dog breeds at risk for cystinuria as well as family members prior to developing calculi. Similarly, these tests offered by some larger veterinary laboratories can improve the diagnostic ability of cystinuria in cats. It should be noted that well mixed urine should be submitted to the laboratory in order to not miss precipitated cystine in urine. Furthermore, occasionally high protein diets and supplements as well as other renal tubular defects such as Fanconi syndrome may cause a false positive test result (8). It is unlikely that the nitroprusside test is falsely negative in cystinuria while the androgen-dependent type in dogs might show it because of the variety of cystine excretion (2, 8).

Cystinuric animals typically develop urinary tract signs, such as pollakiuria, stranguria and dysuria, as well as obstruction and kidney failure. The cats with this specific missense mutation in this and the previous report (25) were young adult cats, and thus this appears to be a moderately severe renal transporter defect. And while cat 1 developed recurrent calculi, the cystinuric littermate did not show obvious urinary tract signs except inappropriate voiding. The difference in manifestation is likely caused by other factors such as diet, fluid intake, urinary pH and concentration and voiding frequency. Furthermore, other factors that lead to cystine precipitation and calculi formation must play a role. In humans the excretion has a diurnal variation and crystals form in phases of super-saturation (5) but there are no studies on solubility of cystine in companion animals. Cat 1 was fed on BARF (biologically appropriate raw food), a diet rich in protein leading to high concentration of COLA and promoting urine acidification. Cat 2 was fed commercial cat food and did not show clinical signs. The feeding of methionine and cysteine rich protein sources (such as chicken and salmon) results in higher levels of cystine in the urine, while the reduced intake of cystine has no influence on the urinary cystine output (11).

A more severe form of cystinuria has been reported in kittens with also lethargy, hypersalivation and neurological signs, such as behavioral changes and seizures, probably due to hyperammonia caused by arginine deficiency (24, 32). Cats in contrast to other animals and humans depend on oral arginine intake as they cannot synthesize arginine. Cystinuric cats with impaired intestinal arginine and ornithine absorption and excessive urinary COLA excretion can become arginine deficient resulting in hyperammonemia and neurological signs (26, 27). Both cats of this report did not show any neurological signs and had normal blood ammonia concentrations when tested, but arginine intake should not be restricted.

Treatment options described for humans and dogs have not been validated in cats and only anecdotal reports exist (22, 30, 33). Urinary catheterization, surgical correction of urinary obstruction and rehydration are well established emergency measures, and were used in one of the cystinuric cats of this report which appeared to be well managed for the last 4 months as of this writing. Cystinuric animals are usually not infected but may develop secondary

infections due to the instrumental interventions as likely occurred in the obstructed cat with cystine calculi of this report and may require selective antibiotic therapy.

More long term medical efforts are directed at: (I) forced diuresis by intravenous fluids and moist diets. (II) lowering the cystine excretion by different lower protein diets (no salmon and chicken), but low protein diets may not be palatable to cats. (III) Alkalinizing urine with potassium citrate (and appropriate urinary diet) to maintain a urine pH of 7.5 to increase cystine solubility (28) and to improve the efficiency of thiol drugs (1), albeit pH monitoring in cats is not simple. (IV) Chelation of cystine with tiopronin to make cystine more soluble in the urine. All these medical efforts were applied in the symptomatic cystinuric cat of this report and seem to control recurrence of cystine calculi over the period of 4 months. However, it should be noted some cats do not develop cystine calculi and obstruction for years without special management as for instance the littermate in this report. There appear yet undefined factors that facilitate cystine precipitation.

Chelating agents, like penicillamine and tiopronin (2-mercapto-propionyl-glycine), have been used in humans and dogs, but have not been approved for use in dogs and cats. While tiopronin is an effective chelating agent, its intake may be associated with various side effects, albeit the side effects seem less than with penicillamine (18, 35). In dogs side effects like proteinuria, anemia, increased serum hepatic enzyme activities, tiredness, aggressiveness, myopathy, and skin lesions like small pustules or crusty dry nose, have been documented (14, 15). In humans fever, arthralgia, skin lesions and lymphadenopathy resembling lupus erythematosus (with a positive antinuclear antibody titer) may be seen with tiopronin therapy. Pharyngitis, oral ulcers, skin rash, ecchymosis, pruritis, urticaria and pemphigus-like lesions were reported (21, 39). Other adverse effects are hypersensitivity reactions, proteinuria and hematological abnormalities (like aplastic anemia, leukopenia, thrombocytopenia) (18, 35, 38). Cat 1 of this report developed an allergic skin reaction likely due to tiopronin as first mild lesions occurred after 2 weeks, progressed under 6 weeks of treatment (Fig. 2) and subsided after withdrawal and a short course of prednisolone within two weeks. Thus, we did not continue tiopronin treatment and cannot assure its safety in cats.

Conclusion for practice

A homozygous c.881T>A mutation in *SLC7A9* gene has been found in two cystinuric feline littermates in Germany. The same mutation has been previously found in cats of different breeds in North America. This suggests a more widespread distribution of this mutation worldwide. Screening cystinuric cats for this mutation could help to get more information about the prevalence of this mutation and of cystinuria in cats.

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References

1. Asplin DM, Asplin JR. The interaction of thiol drugs and urine pH in the treatment of cystinuria. *J Urol*. 2013; 189:2147–2151. [PubMed: 23261477]
2. Brons AK, Henthorn PS, Raj K, Fitzgerald CA, Liu J, Sewell AC, et al. *SLC3A1* and *SLC7A9* mutations in autosomal recessive or dominant canine cystinuria: a new classification system. *J Vet Intern Med*. 2013; 27:1400–1408. [PubMed: 24001348]
3. Bush M, Bovee KC. Cystinuria in a maned wolf. *J Am Vet Med Assoc*. 1978; 173:1159–1162. [PubMed: 738937]
4. DiBartola SP, Chew DJ, Horton ML. Cystinuria in a cat. *J Am Vet Med Assoc*. 1991; 198(1):102–104. [PubMed: 1995560]
5. Fjellstedt E, Denneberg T, Jeppsson JO, Christensson A, Tiselius HG. Cystine analyses of separate day and night urine as a basis for the management of patients with homozygous cystinuria. *Urol Res*. 2001; 29:303–310. [PubMed: 11762791]
6. Garrod, AE. The Croonian Lectures Delivered Before the Royal College of Physicians of London. London, England: Oxford University Press; Jun. 1909 Inborn errors of metabolism. 1908
7. Gerber B, Brandenberger-Schenk F, Müller C. Urolithen bei Katzen in der Schweiz von 2002 bis 2009. *Schweiz Arch Tierheilkd*. 2016; 158:711–716. [PubMed: 27707684]
8. Giger, U., Brons, AK., Fitzgerald, CA., Slutsky, J., Raj, K., Stora, V., et al. Updates on cystinuria and fanconi syndrome: amino acidurias in dogs. Proceedings of the 2014 American College of Veterinary Internal Medicine Forum; 2014 June 4–7; Nashville.
9. Henthorn, PS., Cystinuria, Giger U. Cold spring harbor monograph series: The dog and its genome. Ostrander, EA. Giger, U., Lindblad-Toh, K., editors. New York: Cold Spring Harbor Laboratory Press; 2006. p. 349-364.
10. Henthorn PS, Liu J, Gidalevich T, Fang J, Casal ML, Patterson DF, et al. Canine cystinuria: polymorphism in the canine *SLC3A1* gene and identification of a nonsense mutation in cystinuric Newfoundland dogs. *Hum Genet*. 2000; 107:295–303. [PubMed: 11129328]
11. Hess WC, Sullivan MX. Canine cystinuria. The effect of feeding cystine, cysteine, and methionine at different dietary protein levels. *J Biol Chem*. 1942; 143:545–550.
12. Hesse A, Hoffmann J, Orzekowsky H, Neiger R. Canine cystine urolithiasis: A review of 1760 submissions over 35 years (1979–2013). *Can Vet J*. 2016; 57:277–281. [PubMed: 26933264]
13. Hesse A, Orzekowsky H, Frenk M, Neiger R. Epidemiologische Daten zur Harnsteinerkrankung bei Katzen im Zeitraum 1981–2008. *Tierärztl Prax*. 2012; 40(K):95–101.
14. Hoppe A, Denneberg T. Cystinuria in the dog: Clinical studies during 14 years of medical treatment. *J Vet Intern Med*. 2001; 15:361–367. [PubMed: 11467594]
15. Hoppe A, Denneberg T, Jeppsson JO, Kågedal B. Canine cystinuria: an extended study on the effects of 2-mercaptopropionylglycine on cystine urolithiasis and urinary cystine excretion. *Br Vet J*. 1993; 149:235–251. [PubMed: 8334506]
16. Houston DM, Moore AE. Canine and feline urolithiasis: examination of over 50,000 urolith submissions to the Canadian Veterinary Urolith Center from 1998 to 2008. *Can Vet J*. 2009; 50:1263–1268. [PubMed: 20190975]
17. Jackson OF, Jones DM. Cystine calculi in a caracal lynx (*Felis caracal*). *J Comp Pathol*. 1979; 89:39–50. [PubMed: 422771]
18. Jaffe A. Adverse effects of sulfohydryl compounds in man. *Am J Med*. 1986; 80:471–476. [PubMed: 2937293]
19. Kwong T, Robinson C, Spencer D, Wiseman OJ, Karet Frankl FE. Accuracy of urine pH testing in a regional metabolic renal clinic: is the dipstick accurate enough? Urolithiasis. 2013; 41:129–132. [PubMed: 23435644]
20. Ling GV, Franti CE, Ruby AL, et al. Urolithiasis in dogs. II: Breed prevalence, and interrelations of breed, sex, age, and mineral composition. *Am J Vet Res*. 1998; 59:630–642. [PubMed: 9582969]
21. Lucky PA, Skovby F, Thier SO. Pemphigus foliaceus and proteinurias induced by α -mercaptopropionylglycine. *J Am Acad Dermatol*. 1983; 8:667–672. [PubMed: 6863624]

22. Lulich JP, Berent AC, Adams LG, Westropp JL, Bartges JW, Osborne CA. ACVIM small animal consensus recommendations on the treatment and prevention of uroliths in dogs and cats. *J Vet Intern Med.* 2016; 30:1564–1574. [PubMed: 27611724]
23. Lyons LA, Imes DL, Rah HC, Grahn RA. Tyrosinase mutations associated with Siamese and Burmese patterns in the domestic cat (*Felis catus*). *Anim Gent.* 2005; 36(2):119–126.
24. Mizukami K, Raj K, Giger U. Feline cystinuria caused by a missense mutation in the *SLC3A1* gene. *J Vet Intern Med.* 2015; 29:120–125. [PubMed: 25417848]
25. Mizukami K, Raj K, Osborne C, Giger U. Cystinuria associated with different *SLC7A9* gene variants in the cat. *PLoS One.* 2016:e0159247. [PubMed: 27404572]
26. Morris JG. Nutritional and metabolic responses to arginine deficiency in carnivores. *J Nutr.* 1985; 115:524–531. [PubMed: 3981273]
27. Morris JG, Rogers QR. Arginine: an essential amino acid for the cat. *J Nutr.* 1978; 108(12):1944–53. [PubMed: 722344]
28. Nakagawa Y, Asplin JR, Goldfarb DS, et al. Clinical use of cystine supersaturation measurements. *J Urol.* 2000; 164:1481–1485. [PubMed: 11025687]
29. Nwaokorie EE, Osborne CA, Lulich JP, Albasan H. Epidemiological evaluation of cystine urolithiasis in domestic ferrets (*Mustela putorius furo*): 70 cases (1992–2009). *J Am Vet Med Assoc.* 2013; 242:1099–1103. [PubMed: 23547673]
30. Osborne CA. How would you manage cystine urocystoliths in a female siamese cat? *DVM360 Magazine.* Feb 01.2003
31. Osborne CA, Lulich JP, Kruger JM, Ulrich LK, Koehler LA. Analysis of 451,891 canine uroliths, feline uroliths, and feline urethral plugs from 1981 to 2007: Perspectives from the Minnesota Urolith Center. *Vet Clin North Am Small Anim Pract.* 2009; 39:183–197. [PubMed: 19038658]
32. Osborne, CA., Lulich, JP., Lekcharoensuk, C., et al. Hyperammonemic encephalopathy in cystinuric cats. *Proc. 19th ACVI 794*; Denver, CO. 2001.
33. Osborne CA, Sanderson SL, Lulich JP, et al. Canine cystine urolithiasis: Cause, detection, treatment and prevention. *Vet Clin North Am Small Anim Pract.* 1999; 29:193–211. [PubMed: 10028158]
34. Osborne CA, Lulich JP, Sanderson S, Rogers Q, Giger U. Feline cystine urolithiasis – 18 cases. *Feline Pract.* 1999; 27:S31–32.
35. Pak CY, Fuller C, Sakhaee K, Zerwekh JE, Adams BV. Management of cystine nephrolithiasis with alpha-mercaptopyrionylglycine. *J Urol.* 1986; 136:1003–1008. [PubMed: 3534301]
36. Palacin, M., Goodyer, P., Nunes, V., Cystinuria, Gasparini P. *The Online Metabolic and Molecular Bases of Inherited Disease.* Valle, D. Vogelstein, B. Kinzler, KW., et al., editors. New York: McGraw-Hill Medical; 2013. Available from <http://ommbid.mhmedical.com/book.aspx?bookid=971>
37. 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. [PubMed: 12923163]
38. Thiola® (Tiopronin) prescribing information
39. Tordiffe AS, van der Watt GF, Reyers F. Cystine urolithiasis in a caracal (*Caracal caracal*). *J Zoo Wildl Med.* 2012; 43:649–651. [PubMed: 23082535]

Fig. 1.

Urinary tract and urine evaluation of a cystinuric cat (cat 1). a) Ultrasound image of the bladder with calculi and debris. b) Urinary sediment showing hexagonal cystine crystals (x 600). c) Four cystine calculi surgically removed from the bladder (diameter 0.3–0.4 cm). d) Urinary nitroprusside test results of cats 1, 2 and 3. Scores range from 0 to 4+; magenta color represents high concentration of cystine in urine. PC = positive control, NC = negative control.

Abb. 1 Befunde am Harntrakt und der Urinuntersuchung einer Katze mit Cystinurie (Katze 1). a) Ultraschallbild der Blase mit Steinen und Gries. b) Urinsediment mit hexagonalen Cystinkristallen (x 600). c) Vier chirurgisch aus der Blase entfernte Cystinsteine (Durchmesser 0,3–0,4 cm). d) Resultate des Urinnitroprussidtests der Katzen 1, 2 und 3. Die Skala reicht von 0 bis 4+; Magenta-färbung deutet auf eine hohe Cystinkonzentration im Urin hin. PC = Positivkontrolle, NC = Negativkontrolle.

Fig. 2.

Allergic skin lesions seen in a cystinuric cat (cat 1) following 6 weeks of tiopronin treatment.

Abb. 2 Allergische Hautveränderungen bei einer Katze mit Cystinurie (Katze 1) nach 6 Wochen Behandlung mit Tiopronin

Fig. 3.

a) Normal and mutant *SLC7A9* gene sequences around c.881T>C mutation. The point mutation changes a hydrophobic valine to a negatively charged, hydrophilic glutamic acid (p.Val294Glu). b) DNA test using a PCR-restriction fragment length polymorphism (RFLP) to screen for the c.881T>C mutation. The amplified band is 248 base pair (bp) in length, which is cleaved with the restriction enzyme *BceAI* at one site producing two bands (138 bp and 108 bp) for the normal allele. The mutant allele is missing the restriction site, and thus showing only one undigested band at 248 bp. Arrowheads indicate primers used for the PCR-RFLP assay. c) Result of the polyacrylamide gel electrophoresis of PCR-RFLP products: Cats 1 and 2 are homozygous for the mutant allele (one larger band) and cat 3 is homozygous for the normal allele (wild-type; two smaller bands). Lanes: L = 100 bp DNA ladder. PC = positive control, NC = negative control.

Abb. 3 a) Normale und mutierte Gensequenzen um die c.881T>C-Mutation im *SLC7A9*-Gen. Die Punktmutation ersetzt ein hydrophobes Valin durch eine negativ geladene, hydrophile Glutaminsäure (p.Val294Glu). b) DNA-Test unter Verwendung von PCR-Restriktionsfragmentlängen-Polymorphismen (RFLP) für das Screening auf die c.881T>C-Mutation. Die amplifizierte Bande mit einer Länge von 248 Basenpaaren (bp) wird mit dem Restriktionsenzym *BceAI* an einer Stelle geschnitten, wodurch beim normalen Allel zwei Banden (138 bp und 108 bp) entstehen. Beim mutierten Allel fehlt die Restriktionsschnittstelle, weshalb sich eine ungespaltene Bande von 248 bp ergibt. Die Pfeilspitzen markieren die für den PCR-RFLP-Ansatz genutzten Primer. c) Resultat der Polyacrylamidgel-Elektrophorese von PCR-RFLP-Produkten: Katze 1 und 2 sind homozygot für das mutierte Allel (eine größere Bande), Katze 3 ist homozygot für das normale Allel (Wildtyp; zwei kleinere Banden). Spuren: L = 100-bp-DNA-Leiter, PC = Positivkontrolle, NC = Negativkontrolle.

Table 1

Urinalysis results from two cystinuric littermate cats.
Tab. 1 Ergebnisse der Hamuntersuchung bei zwei Katzen mit Cystinurie aus einem Wurf.

	Cat 1		Cat 2	Reference range
	Before medical treatment (week 20) ¹	During medical treatment (week 23) ¹		
Specific gravity	1.038	1.016	1.076	1.016–1.060
pH (dipstick)	8	7	8	5.0–7.0
Bacterial culture	<i>S. aureus</i> (4+)	<i>E. coli</i> (1+)	negative	negative
Nitroprusside test ²	n. d.	3+	4+	negative
COLA (μmol/g creatinine)	cystine	138	102	13 ± 5
	ornithine	87	36	11 ± 4
	lysine	1517	245	88 ± 13
	arginine	285	106	18 ± 7
	total COLA	2028	488	130 ± 20
Crystals	100% cystine		none present	none present
Urolith				

¹ Weeks from the first presentation of clinical signs regarding cystinuria

² Score ranged from 0 to 4+

COLA = cystine, ornithine, lysine and arginine; n. d. = not done