UPDATES ON CYSTINURIA AND FANCONI SYNDROME: AMINO ACIDURIAS IN DOGS

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Introduction

Disorders of the renal proximal tubules can cause selective or generalized aminoaciduria and may be associated with urinary losses of other solutes such as glucose, lactate, electrolytes and bicarbonate. Two renal tubular defects involving amino acids have long been recognized in dogs, namely cystinuria, leading to cystine calculi and urinary obstruction, and Fanconi syndrome, progressing to renal failure if untreated. Both hereditary disorders have been investigated at the molecular level and are more complex than originally anticipated. Furthermore, the ingestion of Chinese jerky treats has recently been found to be associated with Fanconi syndrome in many dogs and rarely cats. The current understanding of pathophysiology, clinicopathological findings, diagnosis, and therapeutic options will be presented.

Fanconi Syndrome

Fanconi syndrome, named after the Swiss pediatrician Guido Fanconi and also known as Fanconi's syndrome or Fanconi disease, should not be confused with Fanconi anemia, a bone marrow disorder in humans. Fanconi syndrome represents a majorproximal renal tubular defect, which hampers the adequate reabsorption of glucose, amino acids, bicarbonate, sodium, calcium, phosphate, lactate, ketones, and carnitine. This rather general loss of multiple functions of the proximal renal tubules can be associated with renal tubular acidosis and lead to progressive renal failure if left untreated. In the renal tubules there are multiple co-transporters for sodium and glucose, amino acids, calcium, and inorganic phosphorus and a sodium/hydrogen ion antiporter, which, depending upon the concentration gradient established by the sodium-potassium pump, move hydrogen ions into the urine. Defects in all tubular transporters, peroxisomes, the membrane sodium/potassium ATPase and thus tubular gradient or structural membrane abnormalities may be responsible. For most types of Fanconi syndrome, the genetic defects remain unknown. In humans, Fanconi syndrome is most often associated with hereditary cystinosis (not to be confused with cystinuria), fructose intolerance, galactosemia, glycogenosis, as well as Lowe and Wilson disease. Most recently, aberrant localization of the peroxisomal *EHHADH* gene product with disruption of mitochondrial metabolism, has been found to lead to renal Fanconi syndrome in one kindred, thus indicating a central role of mitochondria in proximal tubular function. The mechanisms involved in Fanconi syndrome in dogs may include multiple genetic predispositions, acquired causes, or combinations of both. The molecular defect associated with Fanconi syndrome in Basenjis has recently been elucidated. Basenjis are genetically predisposed to Fanconi syndrome, which is inherited as an autosomal recessive trait in the breed. As many as 10% of Basenjis are affected, and they typically develop signs in middle-age (4-7 years). A mutation in the *Fan1* gene on CFA3 has recently been discovered. Norwegian Elkhounds also often develop glucosuria early in life, which may progress to Fanconi syndrome, but no molecular studies have been reported. Other breeds that may also be genetically predisposed to develop Fanconi syndrome include Labrador Retrievers, Cocker spaniels, West Highland White Terriers, Shetland Sheepdogs, and Schnauzers in which Fanconi syndrome has been secondary and related to a copper-associated hepatopathy in these breeds.

There are several acquired causes of Fanconi syndrome such as exposure to heavy metals (e.g. lead, mercury, and cadmium), drugs (gentomycin, tetracycline, and azathioprine) and infections (leptospirosis). Fanconi syndrome has also been reported in cancer and hepatic copper disease in several dogs. Particularly, recent studies indicate that Labrador Retrievers with copper-associated hepatopathy develop Fanconi syndrome. More recently Fanconi syndrome has been associated with the ingestion of chicken and duck and even veggie jerky treats of different brands that contain Chinese products. Currently no specific ingredient or contaminant responsible for the intoxication has been identified but the FDA and state governments are actively investigating the jerky treats. Six antibiotics (trimethoprim, tilmicosin, enrofloxacin, sulfaclozine, sulfamethoxazole, and sulfaquinoxaline) have been found in excessive amounts in some chicken jerky treats, however, there is no evidence to associate their presence with the observed illnesses. Nearly all the dogs affected are from North America and Australia and include toy to small breed dogs, such as Chihuahuas, Maltese, and Yorkshire, Jack Russell and West Highland White Terriers, but not brachycephalic breeds. Their predisposition may be related to the proportionally larger amount of jerky treats ingested by small breeds compared to large breed dogs, or may reflect a broader hypersensitivity of the smaller breeds.

Clinical signs of intoxication may occur within days to months of ingesting jerky treats, are unspecific and may include lethargy, inappetence, vomiting, and diarrhea. Polydipsia and polyuria are the most common clinical signs of Fanconi syndrome, while other signs of Fanconi syndrome are highly variable and may be related to the ensuing renal failure. In the past, a diagnosis of Fanconi syndrome resulted in a guarded to poor prognosis. However, in the present day, treated Basenijs may reach a near normal life expectancy. Moreover, if the trigger is removed, such as withdrawal of Chinese jerky treats, the renal damage may be more minimal and reversible. Routine serum chemistry and urinalysis as well as venous blood gas analysis are indicated to define the extent of the tubular defect and acidosis. Liver enzymes may be elevated suggesting a hepatopathy. Venous blood gases may show a metabolic acidosis (with blood pH normal to near 7), low bicarbonate concentration and a reduced base excess. A total CO_2 level on a chemistry screen is not sensitive enough to detect the metabolic acidosis due to compensatory hyperventilation. Serum chemistry also will reveal deficiencies in sodium, potassium, calcium and phosphorous. Osteopenia is apparently not a feature of Fanconi syndrome in dogs as it is in humans. Some dogs develop azotemia, which worsens over time, with renal failure being the most common cause of death.

Urinalysis typically reveals glucosuria. In fact, the marked glucosuria in light of a normal blood glucose level is generally the reason to diagnostically pursue a Fanconi syndrome. Urine metabolic screening from dogs with Fanconi syndrome shows often massive generalized aminoaciduria and lactic aciduria (and the glucosuria is also confirmed). Metabolic screening at the University of Pennsylvania (http://research.vet.upenn.edu/penngen/PennGenHome/tabid/91/Default.aspx) can document the aminoaciduria, the hallmark finding of Fanconi syndrome. Dogs with Fanconi syndrome have much higher urinary concentrations of cystine and dibasic amino acids in comparison to cystinuria, as well as exhibit massive excretion of all other amino acids. Paradoxically, despite a severe degree of cystinuria in any dog with Fanconi syndrome, they rarely if ever develop cystine calculi and urinary obstruction; the dilute and alkaline urine may prevent the crystal formation. Moreover, while there is severe aminoaciduria, there is either no or only mild proteinuria. The University of Missouri offers DNA testing for Basenijs (http://www.caninegeneticdiseases.net). However, the molecular basis in other breeds is unknown .

The therapeutic protocol (https://www.basenji.org/ClubDocs/fanconiprotocol2003.pdf) developed and modified over the years by Steve Gonto, a human anesthesiologist as well as a critical care/life support clinician and owner of Basenjis, has been used widely. While there are no formal comparative trials of different therapeutic modalities, the life expectancy and quality of life have apparently greatly improved by using this protocol. It focuses on aggressive correction of the various losses with particular attention to bicarbonate, normalizing electrolytes and minerals, and providing a high quality diet. Bicarbonate is dosed to increase pCO_2 and HCO_3 levels. Unless the animal is in renal failure, a high protein diet is recommended to replenish the amino acid (and protein) losses and to rebuild muscle mass. Renal dialysis may be considered in the acute management of severe renal failure.

In a survey of medical records, Basenjis with Fanconi syndrome received between 2.6-15.6 grams of sodium bicarbonate in tablet form divided per day. Multivitamin and calcium/phosphorus may be supplemented as needed. Treatment of Fanconi syndrome can be rewarding if the disease is recognized early. The median survival time for Basenjis was 5 years after the diagnosis was made. This medical protocol can be used to manage dogs with the acquired form, as long as the trigger is removed. The treatment must be adjusted for the breed size and severity of the metabolic derangements. Dogs that ingested Chinese jerky treats are known to have improved and even resolved their metabolic derangements within a few months after withdrawing the treats, unless they had already developed severe renal failure by the time of diagnosis. Labrador Retrievers and other dogs with copper-associated hepatopathy and Fanconi syndrome may also require copper chelation and low copper diets.

Cystinuria

In contrast to the rather broad transmembrane transport deficiency causing hereditary and acquired forms of Fanconi syndrome, cystinuria is a limited hereditary renal transport disorder involving cystine and the dibasic amino acids ornithine, lysine, and arginine, collectively known as COLA. Instead of the normal >99% reabsorption in the proximal renal tubules, these four amino acids are lost in the urine, but only cystine causes a problem. The low solubility of cystine in acidic urine predisposes the formation of cystine crystals and uroliths in the urinary tract, resulting in the typical clinical signs of cystinuria. While the transport of these amino acids also occurs in the gut and thus cystinuria also affects intestinal COLA absorption, the impaired intestinal absorption of the COLA amino acids is not associated with any clinical deficiencies in animals, with the exception of hyperammonemia due to arginine deficiency in cats.

Cystinuria is one of the first reported “inborn errors of metabolism” described in humans more than 200 years ago, and has been described in several other species including dogs, cats, ferrets, maned wolves, and servals. Two genes, *SLC3A1* and *SLC7A9* encode the polypeptide subunits of the $b^{o,+}$ basic amino acid transporter system and its dysfunction results in cystinuria. *SLC3A1* encodes a protein referred to as rBAT and *SLC7A9* encodes a protein called $b^{o,+}$AT. $b^{o,+}$AT heterodimerizes with rBAT exclusively to form the COLA amino acid transporter. Cystinuria in humans has been classified phenotypically into two types differing by mode of inheritance (autosomal recessive or incomplete autosomal dominant) are caused by mutations in the *SLC3A1* and *SLC7A9* genes which encode the $b^{o,+}$AT basic amino acid transporter system; mutations have not been identified for approximately 10% of cystinuric human patients.

Since 1823, when cystinuria was first described in dogs, dogs of many breeds have been diagnosed, but only recently have studies revealed the genetic heterogeneity. We have proposed a new classification system for canine cystinuria based upon that in humans. A severe cystinuria showing autosomal recessive inheritance was first characterized in Newfoundlands and Landseers, and has more recently been identified in additional breeds. Although both males and females are affected, males more frequently show clinical signs of urinary obstruction. Different mutations in *SLC3A1* resulting in an early stop codon and loss of $b^{o,+}$AT function have been identified in Newfoundlands and Labrador Retrievers (Type I-A). A 6 bp deletion removing 2 threonine residues in *SLC3A1* was found in autosomal-dominant (AD) cystinuria with a more severe phenotype in homozygous than in heterozygous Australian Cattle Dogs and a mixed breed dog (Type II-A). A missense mutation in *SLC7A9* was identified in autosomal dominant cystinuria in Miniature Pinschers from Europe, with only heterozygous affected dogs observed to date (Type II-B).

Cystinuria has also been described in an additional 70 canine breeds. In several of these breeds from which more data is available, such as the Mastiff, French Bulldog, Basset hound and Irish terrier, the disease predominantly occurs later in life, is less severe, more variable, and only involves males. We now refer to this as canine androgen-dependent or type III cystinuria but still do not know the specific genetic defects. Interestingly, Irish Terriers in Europe and Australia seem to be more commonly affected than in the US, where cystinuria in Irish Terriers is rarely diagnosed. Type III cystinuric dogs (with or without cystine calculi) also seem to have variable degrees of cystine and COLA in their urine. These variations could be due to cystine precipitation, diet, age-related variability, the castration effects, and genetic heterogeneity. Molecular studies in Irish Terriers, Mastiffs and several other breeds have failed to detect disease-causing mutations in the exons of either *SLC3A1* or *SLC7A9* genes, however an *SLC3A1* missense mutation

appears to be associated with cystinuria in Mastiffs and related breeds but not Irish Terriers or Scottish Deerhounds. Only mature males are cystinuric and surgical (and even medical) castration resolves excessive cystine and COLA excretion, but the mode of inheritance is still unclear. On average, dogs with type I and II cystinuria have a several fold higher urinary COLA excretion than type III cystinuric male dogs.

Cystine uroliths can form in the kidney (rarely), ureters, bladder or urethra and can lead to urinary blockage, renal failure and associated life-threatening clinical manifestations. A clinical diagnosis of a cystine uroliths can be suspected based upon the breed and yellow-brown color of the uroliths, and can be confirmed by crystallographic or chemical analysis of calculi in specialized stone laboratories such as those at the Universities of Minnesota and California, as well as commercial laboratories worldwide. While in the US approximately 1% of analyzed urinary calculi are cystine stones, in Europe the proportion appears to be as high as 3% . Cystine calculi are usually radiolucent and thus may be missed by radiography, but are readily visualized by ultrasound. Furthermore, urinalysis often reveals the presence of characteristic hexagonal cystine crystals.

A simple urinary screening test, to determine if a dog is cystinuric, is available through the Metabolic Genetic Laboratory at the University of Pennsylvania (http://research.vet.upenn.edu/penngen/PennGenHome/tabid/91/Default.aspx). This test can detect any type I and II cystinuric animal, but not necessarily all dogs with type III cystinuria. Amino acid analyzers can be used to determine the amount of cystine and the other amino acids in urine, however this quantitative amino acid analysis is currently restricted to a few laboratories and is relatively expensive. Inclusion of the dibasic amino acids (ornithine, lysine, and arginine) in the quantitation of cystine is preferred due to the possibility that urinary cystine may have precipitated, causing lower concentrations than were originally present in the urine, and therefore leading to false negative interpretations. Based upon our studies, dogs with either cystine levels of >200 μmol/g creatinine or COLA values of >700 μmol/g creatinine are considered cystinuric. Moreover, for several breeds with type I and II cystinuria, a breed-specific mutation test is available that not only detects cystinuric dogs, but also the asymptomatic carriers (for recessive traits). While in the 1990s as many as 5% and 30% of Newfoundlands were cystinuric (homozygous affected) and carriers, respectively, very few Newfoundlands are cystinuric or carriers in 2013, thanks to the implementation of this DNA screening test, which is available through our and many commercial laboratories. In contrast, the mutant alleles in Labrador Retrievers, Australian Cattle Dogs, and Miniature Pinscher dogs appear to be limited to a small subset of the breed population. Finally, in Mastiffs and closely related breeds, a DNA marker test to identify dogs that are at risk for early stone formation is being offered on a limited basis at the University of Pennsylvania (http://research.vet.upenn.edu/WSAVA-LabSearch).

Urinary tract obstructions due to cystine calculi can present as life-threatening emergency situations. If the calculi are in the urethra, catheterization and endoscopy may allow their passage, permit them to be moved back into the bladder (retrograde hydropulsion). Laser therapy as well as lithotripsy may break them up, and surgery can be used to remove them entirely. In contrast, attempts to dissolve cystine stones medically by drugs and diet have generally failed. Dogs with type I and II cystinuria are persistently strongly cystinuric from puppyhood and remain at high risk of developing cystine calculi throughout life, independent of gender. Alpha-mercaptopropionylglycine (Thiola), a chelating agent that has fewer side-effects and is more potent than D-penicillamin, may lower the risk of calculi formation in cystinuria but is very expensive. Forced diuresis, urine alkalinization (pH>7.5), and reduced protein diets (no chicken) may lower the recurrence risk, while high protein diets and protein supplements tend to increase the risk for calculi formation. Male dogs with type III cystinuria have more variable and usually lower cystine excretion. Thus, they may or may not develop calculi and their recurrence rate is lower. Moreover, castration of Mastiffs and related breeds and Irish Terriers with type III cystinuria diminishes the excretion of cystine and dibasic amino acids to the normal female range, indicating a testosterone-mediated modulating effect on the renal tubular amino acid reabsorption. In these breeds, we recommend castration to reduce the formation and risk of blockage by cystine calculi, which will also contribute to reducing the incidence of this genetic predisposition.

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