Gene Therapy for Inherited Metabolic Disorders in Companion Animals

Dwight D. Koeberl, Carlos Pinto, Talmage Brown, and Y.T. Chen

Abstract

Scientists first described inborn errors of metabolism, also termed inherited disorders of metabolism, early in the 20th century and since then have determined the biochemical and genetic bases of a great number of these disorders both in humans and in an increasing number of companion animals. The availability of metabolic screening tests has advanced the biochemical and genetic characterization in affected breeds of companion animals of inherited metabolic disorders involving amino acid, carbohydrate, fatty acid, and metal metabolism. Advances in gene therapy have led to the development of new treatments for inherited disorders of metabolism, and animal models have played a critical role in this research. For example, glycogen storage disease type Ia in dogs was highly responsive to adeno-associated viral vector–mediated gene therapy, which prolonged survival and for more than a year prevented hypoglycemia during fasting. Gene therapy for other glycogen storage diseases and metabolic disorders will also be feasible. The establishment of a breeding colony and the ability to sustain affected animals are critical steps toward evaluating the safety and efficacy of gene therapy with clinically relevant endpoints. The further development of gene therapy for inherited disorders of metabolism could lead to curative therapy for affected humans and animals alike.

Key Words: amino acid metabolism; large animal model; gene therapy; glycogen storage disease; inborn errors of metabolism; inherited metabolic disorders; mitochondrial disorder; organic acidemia

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Inherited Metabolic Disorders in Companion Animals

Sir Archibald Garrod first described the concept of inborn errors of metabolism in the early 20th century (Garrod 1902). This model has since been expanded to encompass over 5000 metabolic disorders described in the *Metabolic and Molecular Bases of Inherited Disease* (Scriver et al. 2001); however, if the definition is strictly limited to disorders involving intermediates of cellular energy metabolism with known biochemical bases, more consistent with Garrod's examples, the number is much lower. Each inborn error of metabolism is linked to an enzyme deficiency, typically due to autosomal recessive inheritance. The pathophysiology of each disorder has typically been attributed to the accumulation of a substrate or a toxic metabolite derived therefrom, or to the deficiency of a downstream product from the enzyme's activity. The concept of therapy for inborn errors of metabolism has essentially focused on compensating for the underlying enzyme deficiency through dietary or palliative therapies, although enzyme replacement and gene therapies are increasingly being developed to more effectively treat individual metabolic disorders.

Metabolic disorders can be classified according to the involvement of an enzyme belonging to a metabolic pathway, which consists of a series of enzymes acting sequentially on a specific metabolite and which is itself classifiable according to the type of metabolite. Thus, amino acid disorders affect metabolic pathways involved in the metabolism of amino acids, and the same is true with carbohydrate disorders, fatty acid oxidation disorders, mitochondrial oxidative phosphorylation disorders, purine/pyrimidine disorders, and metal disorders; organic acid disorders affect the deaminated products of amino acid metabolism. Lysosomal storage disorders, a unique group that encompasses many metabolic pathways localized to the lysosomes, are discussed elsewhere in this issue (Haskins 2009).

The diagnosis of metabolic disorders requires laboratory testing, either screening tests or disease-specific tests (for a recent review, see Sewell et al. 2007). Screening tests quantify either elevated compounds related to tissue dysfunction or metabolites reflecting a disorder of metabolism, and disease-specific tests demonstrate the enzyme deficiency or associated gene mutation(s). Biochemical screening tests are highly effective, because elevation of a metabolite or a pattern of metabolites often indicates a specific enzyme deficiency and gene defect (or possibly several related gene defects in the setting of genetic heterogeneity). Screening tests facilitate an initial evaluation, when a metabolic disorder is suspected,

Table 1 Inherited metabolic disorders in companion animals

Disorder	Enzyme defect	Breed	Tissue involvement
Carbohydrates			
Type I; von Gierke	Glucose-6-phosphatase	Maltese terrier	Liver, kidney
Type II; Pompe	Acid α-glucosidase	Lapland dog	Heart, skeletal muscle
Type IIIa; Cori	Glycogen debranching	Curly-coated retriever	Liver, skeletal muscle
Type IV; Andersen	Glycogen branching	Norwegian forest cat	Skeletal muscle, neurons
Type VII; Tarui	Phosphofructokinase	English springer spaniel	Erythrocytes, skeletal muscle
Amino acids			
Cystinuria	rBAT	Newfoundland dog	Kidney
Organic acids			
L-2-hydroxyglutaric aciduria	L-2-hydroxyglutarate dehydrogenase	Staffordshire bull terrier	Brain
Primary hyperoxaluria	D-glycerate dehydrogenase[a]	Cat	Kidney, liver, neuronal
Cobalamin deficiency; methylmalonic aciduria	Intrinsic factor-cobalamin receptor	Border collie, giant schnauzer	Blood
Mitochondrial oxidative phosphorylation and pyruvate defects: primary lactic acidosis			
Canine spongiform leukoencephalopathy	Mitochondrial cytochrome *b*	Australian cattle dogs and Shetland sheepdogs	Brain
Pyruvate dehydrogenase complex deficiency	Pyruvate dehydrogenase phosphatase deficiency	Clumber spaniel	Skeletal muscle
Metals			
Copper toxicosis	COMMD1	Bedlington terrier	Liver

[a] Presumed defect, based on metabolic testing.

as in the case of poor growth and low activity. Organic acid analysis of urine specimens is highly effective for the detection of organic acid disorders, whereas plasma amino acid quantification identifies most amino acid disorders. A combination of the acylcarnitine profile and plasma carnitine levels detects most disorders of fatty acid oxidation. Plasma lactate levels can indicate mitochondrial oxidative phosphorylation disorders, albeit with low sensitivity. Once a preliminary diagnosis has been established through a screening test, enzyme analysis and/or mutation detection enables confirmation of the underlying enzyme/gene defect(s).

Several inherited metabolic disorders that affect companion animals have been well characterized, and these animal models of genetic disease have been highly valuable to medical research with regard to the elucidation of pathobiology and development of therapy. Table 1 shows a comparison of inherited disorders in companion animals involving different metabolic pathways. As in humans, consanguinity increases the likelihood of rare autosomal recessive disorders. Thus, the consanguineous mating of dogs, especially, has led to the appearance and recognition of inherited disorders of metabolism in several breeds. Although the goal of selective animal breeding is to eliminate genetic disease, investigators have established colonies of the dog breeds that feature genetic disease to support genetic disease research. These colonies are critical to the evaluation of the safety and efficacy of new therapies for genetic disease.

Pathways Implicated in Metabolic Disorders of Companion Animals

Amino Acid Disorders

Cystinuria, one of the original inborn errors described by Sir Archibald Garrod (1902), results from a defect in the transporter of dibasic amino acids in the renal tubules. Although clinically mild in its clinical presentation, cystinuria is associated with nephrolithiasis in humans and dogs. It has been identified in Newfoundland dogs, in association with a nonsense mutation, R198X, in the *SLC7A9* gene encoding for the rBAT transporter (Henthorn et al. 2000).

Carbohydrate Disorders

Carbohydrate disorders, and specifically glycogen storage diseases (GSDs[1]), are among the most prolific areas of metabolic disease research in companion animals. Glycogen storage

[1] Abbreviation used in this article: GSD, glycogen storage disease

disease types Ia (GSD Ia), GSD II, GSD III, GSD IV, and GSD VII have been reported in dogs and/or cats.

GSD Ia (von Gierke disease; OMIA[2] 000418) has been reported in Maltese dogs, when littermates exhibited growth failure and early demise in a pattern consistent with autosomal recessive inheritance (Brix et al. 1995). The presence of hypoglycemia and hepatomegaly suggested the diagnosis of GSD I, further supported by the presence of glycogen accumulations in the liver and kidney. Nearly undetectable levels of glucose-6-phosphatase in the liver and kidney confirmed the diagnosis. The genetic basis was an M121I missense mutation in the gene encoding the catalytic α-subunit of glucose-6-phosphatase (Kishnani et al. 1997).

GSD II (Pompe disease; OMIA 000419) has been demonstrated in Lapland dogs, through fibroblast complementation studies in which human Pompe disease cells failed to complement acid α-glucosidase activity in heterokaryon cells (Walvoort et al. 1984). Clinical presentation included megaesophagus, exercise intolerance, and recurrent emesis (Walvoort et al. 1985). Glycogen accumulations consisting of membrane-bound vacuoles were present in cardiac, skeletal, and smooth muscle. Further genetic characterization of the affected dogs has not been reported.

GSD III (Cori disease) has been characterized in curly-coated retrievers that presented with exercise intolerance and lethargy once the dogs reached 12 months of age (Gregory et al. 2007). Liver transaminases and creatine kinase were elevated in serum by 6 months of age. Accumulations of nonmembrane-bound glycogen in liver and skeletal muscle, which featured short outer chains of α1,4-linked glucose, were accompanied by the absence of glycogen debranching enzyme. A deletion of an adenine in exon 32 of the canine AGL gene predicted a truncation of the debranching enzyme by 126 amino acid residues (Gregory et al. 2007).

GSD IV (Andersen disease; OMIA 000420) affects Norwegian forest cats, which exhibit hypoglycemia and demise in the first weeks of life, although surviving cats appeared normal until the onset of progressive neurological decline at 5 months of age (Fyfe et al. 2007). Glycogen accumulations in skeletal muscle and neurons prompted the analysis of glycogen branching activity in skeletal muscle, which was severely deficient. An underlying mutation in the GBE1 consisted of a 6.2 kilobase pair (kbp) deletion and 332 bp insertion that altered splicing of the mRNA and decreased GBE cross-reactive material in liver and muscle (Fyfe et al. 2007). Glucose administration in the neonatal period promoted the survival of affected kittens to adulthood.

GSD VII (Tarui disease; OMIA 000421) occurs in English springer spaniels in association with hemolytic crises and a metabolic myopathy that severely limited exercise (Giger et al. 1988). Phosphofructokinase deficiency in muscle caused adenosine triphosphate (ATP) and phosphocreatine depletion during exercise, which revealed the basis for rhabdomyolysis related to the block in glycolysis. The mutation in the M-PFK gene is a nonsense mutation that caused premature termination with the final 40 amino acid residues missing and protein instability (Smith et al. 1996).

[2] The Online Mendelian Inheritance in Animals (OMIA; http://omia.angis.org.au) is a database of genes, inherited disorders, and traits in more than 135 animal species (other than human and mouse, which have their own resources).

Organic Acidemias

L-2-hydroxyglutaric acidemia in Staffordshire bull terriers causes seizures, ataxia, dementia, and tremors (Abramson et al. 2003). L-2-hydroxyglutaric acid was markedly elevated in the animals' urine, plasma, and cerebrospinal fluid (CSF). Magnetic resonance imaging revealed widespread intensities involving the cerebral cortex, thalamus, cerebellum, and brainstem in T2 weighted images, which correlated with neuropathological findings (Abramson et al. 2003; Scurrell et al. 2008). Underlying mutations in the *L2HGDH* gene caused two missense changes, L433P and H434Y (Penderis et al. 2007).

Primary hyperoxaluria and L-glyceric aciduria occur in cats in association with nephrolithiasis, weakness, and muscular atrophy (Blakemore et al. 1988; De Lorenzi et al. 2005). Necropsy revealed motor neuron degeneration and accumulation of neurofilaments, which contrasted with the absence of neuropathy in humans with this disorder. Although the molecular defect is unknown, the presence of L-glyceric aciduria indicates that the underlying cause is alanine-glyoxalate aminotransferase deficiency as seen in type I primary hyperoxaluria.

Imerslund-Gräsbeck syndrome affects border collies and giant schnauzers in association with erythroblastic anemia and methylmalonic aciduria (Fyfe et al. 1991; He et al. 2005). The underlying deficiency of the intrinsic factor-cobalamin receptor caused cobalamin deficiency (Fyfe et al. 1991), and the genetic defect has been delineated in the amnionless gene (He et al. 2005).

Primary Lactic Acidemia: Disorders of Mitochondrial Oxidative Phosphorylation and Pyruvate Metabolism

Mitochondrial cytochrome *b* deficiency has been demonstrated in association with canine spongiform leukoencephalomyelopathy in Australian cattle dogs (Li et al. 2006). Affected dogs developed tremors at 1 to 2 months of age, followed by progressive neurological degeneration. Widespread vacuolation of the brain and spinal cord were present. CSF and urine analysis revealed elevated lactate, 3-hydroxybutyric, and 3-hydroxybutyric/acetoacetic ratio, consistent with a defect of mitochondrial oxidative phosphorylation. Sequencing of the mitochondrial cytochrome *b* gene revealed an underlying V98M missense mutation in affected dogs.

Pyruvate dehydrogenase phosphatase 1 deficiency causes severe exercise intolerance in Clumber and Sussex spaniels (Cameron et al. 2007). Lactic acidosis prompted analysis of pyruvate dehydrogenase complex activity, which was deficient in the affected dogs. A unique nonsense mutation (Q252X) in the *PDP1* gene was delineated. Dietary therapy consisting of L-carnitine and thiamine supplementation, in addition to a ketogenic diet, has been suggested due to the

benefit of this intervention in human patients with pyruvate dehydrogenase deficiency. The ketogenic diet improved the exercise capacity of two of the affected dogs, but did not prevent the early demise of one of them at the time of publication.

Metal Metabolism

COMMD1 deficiency has been implicated as the cause of canine copper toxicosis in Bedlington terriers (van de Sluis et al. 2002). Biliary excretion of copper is markedly decreased, leading to liver cirrhosis and chronic hepatitis in affected dogs. A deletion of exon 2 in the *COMMD1* gene was the cause of copper toxicosis in this breed, differentiating it from other forms of copper overload, including Wilson disease.

Fatty Acid Oxidation

There are no reports of disorders of fatty acid β-oxidation in dogs; however, this absence may be attributable to the fact that screening tests for these disorders have only recently become available.

Gene Therapy for Inherited Metabolic Disorders

The biochemical and genetic characterization of inherited metabolic disorders in companion animals will facilitate the development of novel treatments, such as gene therapy, for these disorders. The progress toward gene therapy for GSD Ia provides an example of the steps necessary to develop this treatment in companion animal models of metabolic disorders. Establishing a breeding colony and sustaining affected animals, before evaluating the safety and efficacy of gene therapy with clinically relevant endpoints, are critical steps in this development.

Establishing a Breeding Colony of GSD Ia Dogs

The initial characterization of affected puppies with GSD Ia revealed that their clinical abnormalities most closely resemble those of severe, neonatal onset GSD Ia in humans (Chen 2001; Perlman et al. 1979). The original three Maltese puppies with GSD Ia died between 5 and 8 weeks of age; they exhibited poor growth and lethargy, and had hepatomegaly and nephromegaly (Brix et al. 1995). Histology of the liver and kidney revealed vacuolation consistent with glycogen storage, and liver glycogen was 9.4% (normal levels are 0 to 2.7%). Liver glucose-6-phosphatase was only 5-10% of normal levels and, in conjunction with delineation of the causative mutation, confirmed the diagnosis of GSD Ia (Kishnani et al. 1997). Therefore, a colony of GSD Ia carrier (Maltese x Beagle) dogs was established through a collaboration between the Division of Medical Genetics at Duke University and North Carolina State University College of Veterinary Medicine.

We confirmed the presence of biochemical and morphological abnormalities typical of severe GSD Ia. The animals required frequent feeding as hypoglycemic episodes followed fasts of only 2 to 4 hours. As in human GSD Ia, nutritional therapy is inadequate to prevent growth failure (Chen 2001), which began in the neonatal period for the affected puppies and continued throughout life. Birthweight averaged 200 grams in affected, carrier, and normal puppies, but the unaffected puppies weighed twice as much as the affected puppies by 2 weeks of age. Postmortem examination of affected puppies treated only with nutritional therapy revealed massive vacuolation of liver and kidney cells as well as focal glomerular sclerosis (Kishnani et al. 2001), a pathologic change in the kidney that also occurs in humans with GSD Ia (Chen et al. 1988). Affected puppies were homozygous for the M121I mutation, while parents were heterozygous carriers. The underlying G to C transversion at nucleotide position 450 of the glucose-6-phosphatase cDNA resulted in loss of an *NcoI* restriction site that facilitated genotyping of newborn puppies.

Monitoring for estrus and artificial insemination facilitated the breeding of carrier dogs. After delivery, we performed jugular venipuncture within 24 hours to determine blood glucose concentration and collect samples for DNA analysis of glucose-6-phosphatase status. We also obtained daily body weights. After identification by genetic screening, affected dogs routinely received glucose in the form of 10% dextrose by subcutaneous injection twice daily (1 gm/kg/dose) to treat symptoms of hypoglycemia. If indicated, the affected puppies received more intensive nutritional support via nasoesophageal tube feeding to promote their survival.

Therapeutic Hepatic Expression of Human Glucose-6-Phosphatase in GSD Ia Dogs

The canine GSD Ia model has features similar to human GSD Ia that are highly relevant to the development of a new therapy, including profound hypoglycemia upon fasting and other biochemical parameters, glucose-6-phosphatase deficiency in the liver and kidney accompanied by glycogen accumulation in those tissues, and reduced survival (Brix et al. 1995; Chen 2001; Kishnani et al. 2001). Treatment with an adeno-associated virus (AAV) type 8 pseudotyped vector (10^{13} vector particles/kg) has prolonged the survival of all three GSD Ia dogs to more than 1 year of age, in contrast to the abbreviated survival of untreated GSD Ia dogs (Koeberl et al. 2008). As shown in Figure 1, vector-treated GSD Ia dogs thrived, no longer requiring carbohydrate supplementation after the neonatal period, and ate only every 6 to 10 hours, whereas untreated GSD Ia dogs required very frequent feedings of a high-carbohydrate diet (Beaty et al. 2002; Kishnani et al. 2001).

Fasting of treated GSD Ia dogs has demonstrated normal blood glucose levels, significantly elevated in comparison to untreated GSD Ia dogs starting at 1 month of age (Koeberl et al. 2008). Carrier littermates of the GSD Ia dogs have no hypoglycemia or other complications of GSD Ia, similar to human carriers for this disorder, and constituted a normal control

group (Chen 2001; Kishnani et al. 2001). Lactic acidosis reflects poor metabolic control and a risk for renal complications in GSD Ia (Wolfsdorf et al. 1997), and therefore its correction is an important endpoint for therapy. Plasma lactate during fasting was lower for vector-treated GSD Ia dogs at 1 month of age, equivalent to the level observed for carrier dogs (Koeberl et al. 2008). Cholesterol and triglyceride levels during fasting did not reveal significant differences between vector-treated GSD Ia dogs, untreated GSD Ia dogs on carbohydrate supplementation, or carrier dogs at 1 month of age (Koeberl et al. 2008). Urinalysis at 10 months of age revealed no proteinuria in vector-treated GSD Ia dogs, indicating that chronic renal failure had not occurred.

In summary, the prolonged survival to to more than 1 year of age and the sustained correction of hypoglycemia have demonstrated the efficacy of AAV vector–mediated gene therapy for the first time in canine GSD Ia.

Figure 1 Efficacy of adeno-associated viral (AAV) vector–mediated gene therapy in a Maltese x beagle dog with glycogen storage disease (GSD) Ia. A homozygous affected GSD Ia dog at 1 week of age, just after vector administration (top panel), and at 27 months of age (bottom panel). In the absence of efficacious gene therapy the GSD Ia puppies grew poorly and required very frequent feeding (Brix AE, Howerth EW, McConkie-Rosell A, Peterson D, Egnor D, Wells MR, Chen YT. 1995. Glycogen storage disease type Ia in two littermate Maltese puppies. Vet Pathol 32:460-465). At 21 months of age, the GSD Ia dog was thriving and required only routine care after the administration of gene therapy at 3 days of age (Koeberl DD, Pinto C, Sun B, Li S, Kozink DM, Benjamin DK Jr, Demaster AK, Kruse MA, Vaughn V, Hillman S, Bird A, Jackson M, Brown T, Kishnani PS, Chen YT. 2008. AAV vector-mediated reversal of hypoglycemia in canine and murine glycogen storage disease type Ia. Mol Ther 16:665-672). The original figure is in color and is available in the online posting of this article at www.ilarjournal.com.

Conclusion

Inherited disorders of metabolism commonly present with chronic growth failure and recurrent episodes of decompensation. Metabolic testing consisting of screening tests and specific enzyme analyses implicate a specific enzyme deficiency, enabling the identification of a causative mutation in related, affected animals. Gene therapy can be efficacious if the gene defect is complemented through gene replacement in the involved tissues. The development of gene therapy will benefit affected individuals if preclinical experiments and clinical trials follow these proof-of-principle experiments.

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