Canine Muscular Dystrophy: Case Presentation and Pathophysiology

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- Canine muscular dystrophy is a rare, inherited disorder.
- Thus far, all of the mutations characterized in dogs with muscular dystrophy have been in the dystrophin gene.
- This paper presents a case of muscular dystrophy in a Cocker Spaniel. Preliminary studies suggest that her genetic defect is in one of the sarcoglycan genes.

The muscular dystrophies are a heterogeneous group of rare, inherited disorders. Clinical signs include muscular weakness and atrophy. Histologically there is a characteristic pattern of chronic degeneration and regeneration of muscle fibers accompanied by progressive fibrosis.[1] Because the clinical signs and histopathological characteristics observed in the muscular dystrophies are all similar, historically they had been grouped together as one disease entity. In the last fifteen years novel biotechniques have led to the identification of specific mutations associated with what is now recognized as a spectrum of different myopathies, and the human muscular dystrophies have subsequently been classified into nearly thirty different muscle disorders based on genetic etiology.[1]

In humans, Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) together comprise approximately two-thirds of all muscular dystrophy cases. Duchenne muscular dystrophy is both the most common and the most severe form of the disease.[2] Both DMD and BMD are caused by mutations in the gene that codes for a sarcolemmal muscle protein called dystrophin. The dystrophin gene is located on the X chromosome; DMD and BMD are X-linked recessive traits. As expected these two

diseases are almost always manifested in males. Female carriers are usually asymptomatic but often have elevated blood creatine kinase (CK) levels.[2] Autosomal recessive and autosomal dominant forms of muscular dystrophy have also been recognized, and in the past some attempt was made to classify the muscular dystrophies based on mode of inheritance.[2,3,4]

Boys with DMD usually show clinical signs by age five and are wheelchair dependent by 10-12 years of age. Most patients develop a cardiomyopathy, as cardiac muscle is affected along with skeletal muscle. Death usually occurs in the early twenties due to respiratory failure or cardiac decompensation. In BMD the disease has a much later onset, is much less severe, and has a slower and more variable rate of progression. Cardiac disease is less common in BMD.[2]

Dystrophin is located directly adjacent to the sarcolemmal membrane in myocytes. It plays a role in maintaining the integrity of the myocyte membrane during shape changes associated with contraction by mechanically linking the myofiber cytoskeleton and contractile apparatus to the extracellular matrix.[2,3] Dystrophin is a very large, 427 kD protein. Its large size likely explains why dystrophinopathies are the most common forms of muscular dystrophy, since the probability of a spontaneous mutation occurring increases in direct proportion to the size of a gene.[3] As such, there are many independent mutations that result in dystrophinopathies. Most commonly they are deletions, with or without frameshifts, or point mutations that lead to a premature stop codon and consequently a dysfunctional protein.[2]

The differences in the DMD and BMD phenotypes are a direct result of the type of mutations involved in each disease. Duchenne muscular dystrophy most often results

from a DNA deletion which leads to a shift of the translational reading frame, leading to a nonsense mutation and an unstable protein product. The dystrophin protein is many times entirely absent in DMD patients. In BMD the mutation is a typically a DNA deletion without a frameshift, leading to a truncated but partially functional protein product.[3,4,5] It is possible to retain a partially functional protein if the deletion spares enough of the important binding sites of the protein.[2,5]

Dystrophin is just one component in a group of proteins that work as a unit to stabilize the myofiber membrane during muscle contraction. This group of proteins is known collectively as the dystrophin glycoprotein complex (DGC), and it includes transmembrane and cytoskeletal proteins as well as their extracellular ligands.[4] Because these proteins together form a mechanical link between the actin cytoskeleton and the extracellular matrix, a disruption at any level in the chain results in a muscular dystrophy phenotype, and abnormalities in any one of the DGC proteins often leads to decreased amounts or compromised function of the others.[5] Furthermore, it is now known that mutations in various proteins of the DGC can lead to a muscular dystrophy phenotype. This explains the different modes of inheritance (autosomal recessive and autosomal dominant) that had been noted in some cases of muscular dystrophy, since most of the genes for these proteins are not located on the X chromosome. Some DGC proteins have not yet been associated with a dystrophic phenotype because their impairment or absence leads to early embryonic death.[5] For example, dystroglycan is a ubiquitously expressed protein that is crucial to basement membrane assembly; therefore dystroglycan deficient embryos are not viable.[5]

There is increasing evidence that the DGC has more than a structural function and in fact plays an important role in cell signaling.[5,6] For example, the DGC proteins syntrophins are critical in the neuronal nitric oxide synthase signaling pathway as well as in serine/threonine kinase pathways.[2,5] Some of these pathways are believed to involve giving the cell survival vs. apoptosis signals, or to control and regulate synthesis and repair of damage to the contractile apparatus from normal daily activity.[1,5] So although the principal defect in muscular dystrophy is a mechanical one, it appears that disruption of cell signaling pathways also contributes to the disease process.

The canine form of muscular dystrophy was initially identified in the Golden Retriever. The causative mutation was determined to be in the dystrophin gene, as in humans with DMD. Since then specific mutations have been identified in the Rottweiler and the German Shorthaired Pointer.[3] The defects in the latter two breeds are also in the dystrophin gene. And just as in human DMD, these diseases are X-linked recessive traits that are seen primarily in male dogs. Female dogs often show no clinical signs or have a very mild form of the disease.[3]

Dogs with dystrophinopathies begin to show clinical signs at about eight weeks of age.[7] Initially they have muscular weakness, exercise intolerance and a stiff gait. Within a few weeks diffuse muscle atrophy becomes apparent. These signs are accompanied by hypertrophy of specific muscles, including the semimembranosus, semitendinosus, diaphragm, tongue and esophageal muscle.[3] The hypertrophy is initially caused by an increase in the size of individual muscle fibers. Later, as the muscle begins to atrophy, it is due to an increase in fat and connective tissue. Involvement of the diaphragm can lead to respiratory problems; involvement of the tongue and esophageal muscle can lead to

dysphagia and regurgitation. By about six months of age movement becomes restricted as the muscles begin to fibrose. Dogs will often exhibit neck ventroflexion and may walk palmigrade and/or plantigrade. Life expectancy depends on the severity of clinical signs. Death may occur within the first few days in the case of severe diaphragmatic necrosis, or dogs can survive for up to several years. Like people, dogs with muscular dystrophy often develop a cardiomyopathy, and their heart disease is often what eventually leads to death.[3,7]

Case Synopsis

In August of 2002, an 11-month-old, female, spayed Cocker Spaniel named Brandy presented to the Cornell University Hospital for Animals with a chief complaint of exercise intolerance. Brandy's clinical signs were subtle: her owner reported that she was unwilling to jump on or off of furniture, and that she became tired quickly and dramatically when on walks or during obedience class. On physical exam the only abnormalities noted were epiphora and brachygnathism. Neurologic exam revealed mild diffuse lower motor neuron signs including a short-strided gait and bunny hopping while running. There were also equivocal findings of slow withdrawals and decreased tone in all four limbs.

Bunny hopping is most often caused by diffuse neuromuscular disease, a musculoskeletal problem, or myelodysplasia. Bloodwork is the first step towards distinguishing between these disease processes, since clinical chemistry changes are unexpected in the latter two. Complete blood count, chemistry panel and urinalysis were submitted and the major findings were a markedly elevated ALT (994 U/L, normal 25-

106 U/L), AST (1347 U/L, normal 16-50 U/L) and CK (51,210 U/L, normal 58-241 U/L). The elevated ALT was first noted by the referring veterinarian on pre-anesthetic bloodwork before a routine ovariohysterectomy when Brandy was about 7 months old. Creatine kinase was not included on this screening panel. The referring veterinarian repeated the ALT a month later and the was still elevated. Creatine kinase is one of the most organ specific enzymes on the chemistry panel and it is cytosolic; therefore an elevated CK is indicative of muscle damage or necrosis. Aspartate aminotransferase is found in almost all tissues, but muscle and liver are considered the major sources. Alanine aminotransferase is generally considered liver specific but increased activity is often seen with severe muscle necrosis in the dog. In this case the most provocative laboratory finding is the CK. Creatine kinase has a very short half-life in the plasma, and will return to normal in 2-3 days following a single episode of muscle damage. Many things can cause such a transient increase in CK (e.g. traumatic venipuncture, restraint, surgery etc.), but a persistent, dramatic elevation such as was seen in Brandy suggests an active muscular disease and is an indication for further diagnostics such as muscle biopsy +/- electromyography. Other findings on the chemistry panel were a slightly low creatinine and a slightly high iron level, both of which could be referable to ongoing muscle damage.

In light of Brandy's clinical signs and persistently elevated CK, a primary myopathy was suspected. Myopathies can be classified into inflammatory (e.g. infectious, immune-mediated), degenerative (e.g. toxic, endocrine, exertional/traumatic, or ischemic. Muscular dystrophy or polymyositis are the top two differentials for such a dramatically high CK. Brandy had no history of trauma or exertion, no evidence of

endocrine disease, and no toxic exposure. The inflammatory diseases could be ruled down since they are often (but not always) painful, and Brandy did not show any signs of discomfort. Diseases that cause predominantly atrophic changes (e.g. endocrine myopathies) typically do not cause an elevated CK level.

Electromyography is a nonspecific test that can confirm the presence of either a myopathy or a neuropathy, but cannot distinguish between the two. When an animal is under general anesthesia the resting muscle does not show any electrical activity. If a neuropathy or myopathy is present then spontaneous electrical activity can occur. In Brandy's case spontaneous activity was noted in the form of fibrillation potentials and positive sharp waves. When combined with the marked elevation in Brandy's blood CK level these findings were highly suggestive that her clinical signs were due to a primary myopathy rather than a neuropathy.

Because muscular dystrophy was considered a likely differential, an echocardiogram was performed to look for dystrophic changes in the cardiac muscle. Multifocal hyperechoic lesions were seen both in the papillary muscles and the myocardium. Such lesions are often seen in muscular dystrophy as a consequence of degeneration and fibrosis of the myocardium. Heart function based on fractional shortening was found to be normal.

A biopsy was taken from Brandy's biceps femoris muscle. Histopathology showed a classic picture of dystrophic muscle: muscle necrosis with multifocal clusters of myofiber degeneration and regeneration. Regenerating myofibers were recognized due to their basophilic, mRNA-rich cytoplasm. There was tremendous variability in the sizes of the myofibers, which are very uniform in normal muscle tissue. Many of the

necrotic myofibers were engulfed in macrophages. Several of the myofibers had centralized nuclei, which are often found in diseased muscle. Scattered calcific deposits were also seen. Once dystrophic muscle has exhausted its supply of satellite cells, regeneration will cease and muscle tissue will be replaced by fat and connective tissue.[2,3] Increased connective tissue was present in Brandy's muscle.

Brandy's clinical picture at that point was strongly suggestive of muscular dystrophy. However she is a female, and therefore not representative of the typical dog with muscular dystrophy. There are two possible explanations for this: 1) She has an autosomal form of the disease, or 2) she is a rare manifesting carrier of DMD. The latter scenario could be possible in the case of skewed X-inactivation, X-autosomal translocation, or Turner's syndrome (XO female).[8] Immunohistochemistry was performed in order to further elucidate the etiology of Brandy's disease. This is a sensitive test in which fresh frozen biopsy specimens are stained using antibodies against various proteins of the DGC.[3] Interestingly, the results of Brandy's immunohistochemistry showed that her muscle was positive for dystrophin, but negative for a DGC protein called alpha-sarcoglycan, arguing for an autosomal form of muscular dystrophy rather than DMD.

Alpha-sarcoglycan is one component of the four-protein sarcoglycan complex, which was discovered in 1994. Its exact function is still unknown. In humans, mutations in the sarcoglycan genes cause a group of muscular dystrophies that have been referred to as the "limb girdle" muscular dystrophies, because the hip girdle is more severely affected than the shoulder girdle.[4] For many years, the limb girdle muscular dystrophies were considered by some to be a distinct myopathy based on the unique

clinical phenotype as well as the autosomal recessive mode of inheritance. The limb girdle muscular dystrophies are now referred to as sarcoglycanopathies, and can be caused by a defect in any one of the four different sarcoglycans genes.[9,10,11] Typically a mutation in one of the genes will lead to significantly reduced levels of all of the sarcoglycans, as they appear to be synthesized and assembled as a unit.[8,11,12] There is some evidence that the sarcoglycans have a non-structural role in maintaining muscle membrane integrity, perhaps related to cell signaling.[2,5,9] Sarcoglycan deficiency in mice appears to cause greater cardiac involvement than dystrophinopathies, and some vascular involvement as well.[5]

Until Brandy's case, sarcoglycanopathies had not been reported in dogs. Results are still pending for western blot and genetic analysis studies, which will further elucidate the etiology of her disease. There is currently no naturally occurring animal model for alpha-sarcoglycanopathy, so the discovery of a canine form could have ramifications for the future of research into the pathophysiology of the human condition and the canine correlate. The availability of an animal model is also critical if studies on the safety and efficacy of various therapies are to be performed.[9] Although there are genetically engineered murine models for all of the known sarcoglycanopathies, a canine model would be more attractive due to size alone, and even more so if its disease phenotype turned out to have a greater resemblance to the human form of the disease.[2,12,13,14]

Unfortunately at this time there is neither a cure nor a specific therapy for muscular dystrophy of any genetic etiology. Anabolic supplements and/or growth factors may help slow the progression of the disease, but their efficacy is unproven, and they are expensive and can quickly become cost prohibitive for some owners. Brandy was sent

home on daily riboflavin, coenzyme Q10 and carnitine, which are components of oxidative enzyme systems and are thought to enhance muscle anabolism and slow muscle degeneration.[3] Brandy is now 1½ years old and has had no progression of her clinical signs.

Obtaining a diagnosis in canine muscular dystrophy is important for a number of reasons. Because it occurs most commonly in purebred animals it is important for breeders to be aware of a genetic defect in their animals, and furthermore to know the mode of inheritance. Diagnosis is also important to owners since the various muscular diseases call for different treatments and carry vastly different prognoses. Although the clinical course of canine dystrophinopathy is well characterized, one can only attempt to predict Brandy's prognosis based on what is known about the human disease.[3]

Current research into therapies for muscular dystrophy proceeds on several fronts. One of the most widely studied is gene therapy using viral vectors. Sarcoglycans are interesting candidates for viral gene transfer because of their size. Sarcoglycan cDNAs are less than 1.5 kilobases (considerably smaller than the dystrohin cDNA, which is approximately 14 kilobases) and so are small enough to be accomodated by adenovirus and adeno-associated vectors. And although a complete dystrophin gene transfer is not yet possible, there has been limited success in the *mdx* mouse using a "mini-dystrophin" gene, which has improved the phenotype from a DMD-like to a BMD-like disease. The biggest obstacle to vector-based gene therapy is the large volume and wide distribution of affected tissue. Muscle tissue can comprise close to 50% of the body. Furthermore muscles such as the diaphragm and heart are not easily accessed for intramuscular injections of the viral vectors. So a method of systemic delivery will likely have to be

found before this treatment could be successful.[1,13] The immunogenicity of the adenoviral vectors has also presented problems, and various methods of modifying them to reduce host immune response are being investigated. Isolation, culture and administration of muscle stem cells are also being explored, as well as novel pharmacological therapies aimed at preventing muscle degeneration, promoting muscle regeneration and preventing fibrosis.[3] In the *mdx* mice, researchers have also been able to stimulate upregulation of utrophin, an autosomal homologue of dystrophin, thereby reducing the dystrophic phenotype.[13] Although these therapies are all experimental at this time, they show promise. And when one considers how far our understanding of the muscular dystrophies has advanced in the last 15 years, there is hope that the future may indeed hold a cure.

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[1]Biggar W, Klamut H, Demacio P, et al. Duchenne muscular dystrophy: current knowledge, treatment, and future prospects. Clin Orth Rel Res; 2002; 88-106.

[2] Blake D, Weir A, Newey S, et al. Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol Rev 2002; 291-329.

[3] Shelton GD, Engvall E. Muscular dystrophies and other inherited myopathies. Vet Clin 2002; 103-125.

[4]Duggan D, Hoffman E. Autosomal recessive muscular dystrophy and mutations of the srcoglycan complex. Neuromusc Disord 1996; 475-482.

[5]Spence H, Chen Y, Winder S. Muscular dystrophies, the cytoskeleton and cell adhesion. BioEssays 2002; 542-552.

[6]Rando T. The dystrophin-glycoprotein complex, cellular signaling, and the regulation of cell survival in the muscular dystrophies. Muscle & Nerve 2001; 1575-1594.

[7] Valentine B, Cooper B, de Lahunta A, et al. Canine x-linked muscular dystrophy. Jour Neur Sci 1988; 69-81.

[8] Ozawa E, Noguchi S, Mizuno Y, et al. From dystrohinopathy to sarcoglycanopathy: evolution of a concept of muscular dystrophy. Muscle & Nerve 1998; 421-438.

[9] Bushby K. The limb-girdle muscular dystrophies—multiple genes, multiple mechanisms. Hum Mol Gen 1999; 1875-1882.

[10] Eymard B, Romero NB, Leturcq F, et al. Primary adhalinopathy (alpha-sarcoglycanopthy): clinical, pathologic, and genetic correlation in 20 patients with autosomal recessive muscular dystrophy. Neurology 1997; 1227-1234.

[11] Shelton GD, Liu L, Guo L, et al. Muscular dystrophy in female dogs. J Vet Intern Med 2001; 240-244.

[12] Durbeej M, Campbell K. Muscular dystrophies involving the dystrophin glycoprotein complex: an overview of current mouse models. Curr Opin Gen & Dev 2002; 349-361.

[13] Allamand V, Campbell K. Animal models for muscular dystrophy: valuable tools for the development of therapies. Hum Mol Genet 2000; 2459-2467.

[14] Nonaka I. Animal models of muscular dystrophies. Lab Animal Sci 1998; 8-17.