Mucopolysaccharidosis type VII in a German Shepherd Dog

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- Mucopolysaccharidoses are a group of rare genetic disorders of glycosaminoglycan catabolism resulting in lysosomal storage.
- Mucopolysaccharidosis type VII was first identified in a mixed-breed dog 2 decades ago, and the same disease-causing mutation has been identified in a young German Shepherd Dog.
- Clinical signs of mucopolysaccharidosis type VII are corneal clouding and severe skeletal deformities; affected animals are unable to ambulate at several weeks to months of age.
- Screening tests for mucopolysaccharidoses are available; furthermore, there are specific blood enzyme and DNA-based tests to distinguish the forms of mucopolysaccharidosis in clinically affected and carrier animals.

A 12-week-old male German Shepherd Dog was evaluated at the University of Georgia Veterinary Medical Teaching Hospital because of a 3-week history of progressive inability to ambulate. According to the owner, this dog was the runt of a litter of 8 puppies; this assessment was made on the basis of its size, activity, and strength when acquired at 6 weeks of age from a breeder. The dog was fed a commercial dry diet formulated for growing puppies. At 8 weeks of age, the puppy was noted to occasionally fall over with no loss of consciousness when playing. These clinical signs gradually progressed from ataxia to hind limb and forelimb dysfunction, but the puppy remained bright and alert.

On physical examination, the puppy weighed 6.7 kg (15 lb); it was bright, alert, responsive, and sternally recumbent. The dog's rectal temperature and pulse and respiratory rates were within reference limits. Auscultation of the thorax revealed no abnormalities. The dog's head was large in proportion to its body, and prognathism was evident. The long bones of the appendicular skeleton were shortened and curved (disproportionate dwarfism), and there was excessive flexibility in all joints. There was mild to moderate atrophy of the skeletal musculature over the limbs. The dog's ears were abnormally lax, and the tip of each pinna was folded downwards. Ophthalmic examination revealed that both corneas were diffusely cloudy and there were multifocal granularities within the corneal stroma; however, the fundus of each eye appeared normal. A neurologic examination revealed normal conscious proprioception in all 4 limbs with normal spinal reflexes. However, hyperesthesia of the spinal nerves was detected in the cervical and lumbosacral regions, and the puppy resisted extension of the cervical portion of the vertebral column.

Results of a CBC and serum biochemical profile were within reference limits for a puppy. However, microscopic examination of a blood smear stained with Wright-Giemsa revealed cytoplasmic azurophilic granules in most lymphocytes and neutrophils (**Fig 1**). The granules stained dark blue with toluidine blue stain. These azurophilic granules are known as Alder-Reilly bodies and consist of **glycosaminoglycans (GAGs)** within lysosomes.[1] A sample of CSF was obtained for analysis but was contaminated with blood; the CSF specimen contained 2,376 RBCs/µL (reference limit, 0 cells/µL) and 6 WBCs/µL (reference range, 0 to 5 cells/µL) with a protein concentration of 30.1 mg/dL (reference range, 15 to 35 mg/dL). Microscopic examination of a centrifuged sample of CSF revealed 5 segmented neutrophils/µL and 46 lymphocytes/µL (azurophilic cytoplasmic granules detected in both cell types), 18 macrophages with nuclear debris/µL, and numerous RBCs.

On radiographic examination of the dog, the vertebral bodies appeared short and had irregularly shaped epiphyses (**Fig 2**). It was also noted that the bony margins of cartilaginous surfaces of the shoulder joints and the osseous tympanic bullae were misshapen. The hip joints were not examined radiographically. A urine sample was collected from the dog and

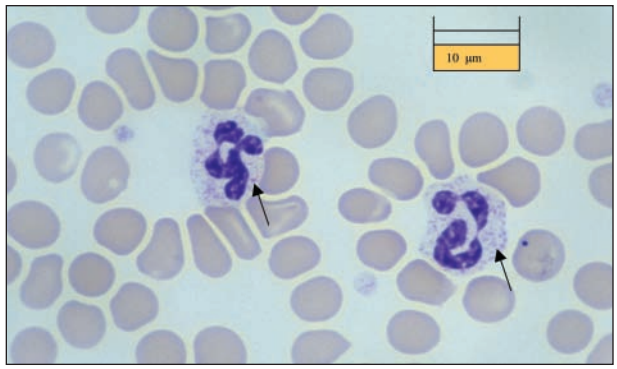

Figure 1—Photomicrograph of a blood smear of a 12-week-old dog with mucopolysaccharidosis type VII. Notice the many azurophilic granules (Alder-Reilly bodies; arrows) within the cytoplasm of neutrophils. Similar granules were also detected in lymphocytes. Wright-Giemsa stain; bar = 10 µm.

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analyzed at the Metabolic Genetic Screening Laboratory of the University of Pennsylvania. The result of a toluidine blue spot test performed with this urine sample indicated the presence of GAGs; via cellulose acetate electrophoresis, the predominant GAG identified was chondroitin sulfate with lesser amounts of dermatan sulfate. These findings were suggestive of mucopolysaccharidosis.

Blood samples were obtained from the affected puppy and a healthy control dog; for each dog, samples of blood (10 mL; in EDTA) and serum (2 mL) were shipped at 4°C overnight to the Metabolic Genetic Screening Laboratory. Leukocyte pellets were prepared from the samples of anticoagulated blood. The WBCs were separated from other blood cells by use of a dextran gradient; after washing in phosphate-buffered saline solution, the WBCs were frozen along with serum samples for later analysis of lysosomal enzyme activities. Activity of β-glucuronidase was determined in samples of serum and WBCs prepared as previously described.[2-4] Activity of β-glucuronidase was low in serum and WBCs obtained from the affected puppy, compared with activity detected in a control sample (**Table 1**). Furthermore, activities of other lysosomal enzymes that are involved in the degradation of GAGs were within or above reference range. On the basis of these results, a severe β-glucuronidase deficiency or mucopolysaccharidosis type VII disorder was diagnosed.

The DNA segment around the mutation in the β-glucuronidase gene described previously in a mixed-breed dog[5] was amplified by **polymerase chain reaction (PCR)** and subjected to restriction enzyme digestion. Genomic DNA was extracted from 200 µL of blood for PCR assay. The reactions were carried out in 50-µL volumes with 0.5µM of each primer, 0.8mM dNTPs, and 1.25 units of platinum *Taq*. The primer sequences were (sense) 5′-GGGGTCCATGTGGCAGAGCA-3′ and (antisense) 5′-CGTGTTGTTGATGGCAAGGGTAGTA-3′. The reactions were performed in 1X buffer with the following conditions: an initial denaturation at 94°C for 5 minutes, then 35 cycles of 94°C for 30 seconds, 61°C for 30 seconds, and 72°C for 30 seconds, followed by a 10-minute final extension. The antisense primer contained a mismatch that introduced a thymine to cytosine substitution at a position that corresponded to nucleotide position 602 in the amplified PCR products. This change created an Rsa I recognition site 5′-GTAC-3′ in PCR products from clinically normal dogs only. The missense mutation (a guanine to adenine transition) at nucleotide position 599 prevents the creation of an Rsa I site in products amplified from affected dogs. Restriction digests were performed in 20-µL volumes with 1X buffer and 250 U Rsa I at 37°C for 1 hour. Digested products were analyzed on 8% polyacrylamide gels.[6,7] Results indicated that the dog was homozygous for the mutant allele (**Fig 3**). Sequencing analysis confirmed a guanine to adenine substitution at nucleotide position 559 of the β-glucuronidase cDNA. The base change causes an arginine to histidine substitution at amino acid position 166 of β-glucuronidase and results in a nonfunctional enzyme.

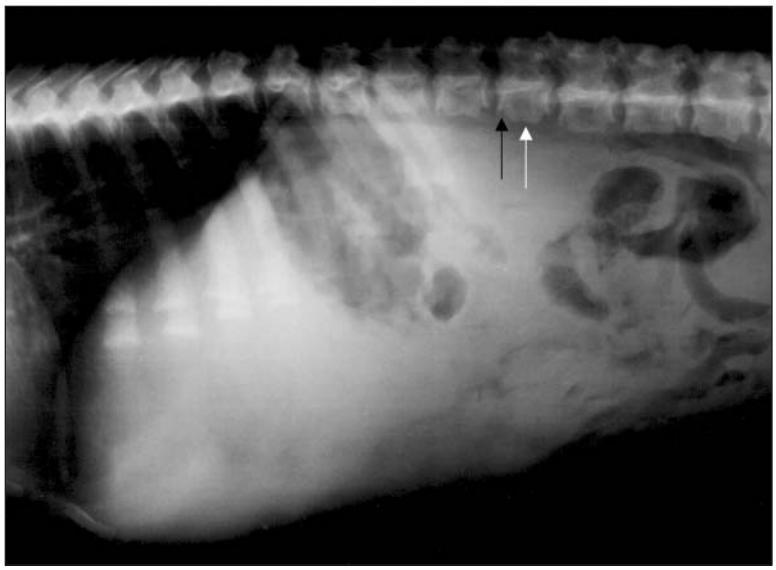

Figure 2—Lateral radiographic view of the vertebral column of a 12-week-old dog with mucopolysaccharidosis type VII. Notice that shortened vertebral bodies (white arrow) and irregularly shaped epiphyses (black arrow) are present.

Because of the progressive nature of the disease and poor prognosis, the puppy was euthanized at 13 weeks of age and a postmortem examination was performed. Compared with a clinically normal dog of the same age, the puppy was small and had a large head. Gross examination revealed

Table 1—Lysosomal enzyme activities in the serum, WBCs, and liver of a dog with mucopolysaccharidosis type VII and a healthy control dog

	Specimen					
	Serum (nmol/mL/h)		WBC (nmol/mg of protein/h)		Liver (nmol/mg of protein/h)	
Enzyme	Affected dog (%)	Control dog	Affected dog (%)	Control dog	Affected dog (%)	Control dog
β-glucuronidase	35 (13.5)	259	2 (0.7)	297	8 (3)	264
α-mannosidase	1,700 (108)	1,572	746 (153)	489	746 (117)	640
β-galactosidase	26 (153)	17	253 (356)	71	253 (116)	219
β-hexosaminidase	730 (178)	409	690 (330)	209	1,209 (110)	1,100

Numbers in parentheses represent the value for the affected dog as a percentage of the value for the control.

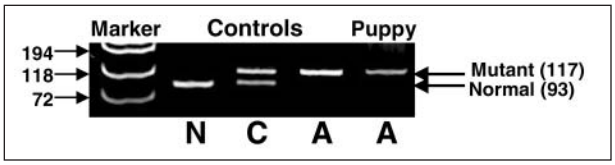

Figure 3—Comparison via a polymerase chain reaction (PCR) and restriction enzyme digestion of DNA segments around the mutation of the β-glucuronidase gene from a dog with mucopolysaccharidosis type VII (A), a carrier of the disease mutation (C), and a clinically normal dog with no mutation of the gene (N). Products were digested and analyzed on an 8% polyacrylamide gel. Numbers to the left of the figure and in parentheses indicate size of DNA segments (bp).

angular limb deformities of the appendicular skeleton with increased joint laxity. There was mild kyphosis and other abnormalities of the vertebral column (including misshapen vertebrae, abnormal dorsoventral flattening and roughened articular surfaces of the vertebral bodies, and wide intravertebral spaces). Articular surfaces of the appendicular skeleton were within normal limits. Both corneas were diffusely cloudy with multifocal granular deposits within the stroma. The size and weight of the liver were within normal limits, but the surface of the liver was slightly nodular. The atrioventricular valves of the heart were thickened and slightly nodular bilaterally. No additional gross abnormalities were noted.

In all tissues examined histologically, there were numerous cytoplasmic vacuoles that resulted in marked cellular distention; affected cells included dermal fibroblasts, hepatocytes, Kupffer cells, macrophages in tissues of the small intestine, myeloid precursor cells in the bone marrow, cells of the corneal stroma, cells in the media of the aorta and pulmonary trunk, retinal pigment epithelial cells, and chondrocytes in the vertebral growth plates. There were irregular cartilage columns in the pinnae, third eyelid, and vertebral bodies. Abnormal nonmineralized cartilage columns extended from the growth plates of the vertebrae into the metaphyses (**Fig 4**). The heart valves had increased amounts of basophilic mucinous ground substance, and there was vacuolation of the mesenchymal cells within the valves. There was mild swelling of the Purkinje cells in the cerebellar cortex of the brain and Wallerian degeneration involving both the dorsal and ventral white matter of the spinal cord. In addition, there was mild macrophage infiltration of the peripheral and dorsal nerve roots.

Lysosomes are essential cytoplasmic organelles delineated by a single lipoprotein membrane in all nucleated mammalian cells. The degradation of macromolecules is mediated by many acid hydrolases in these lysosomes.[1,8,9] Deficiency in activity of any hydrolase may lead to the accumulation of substrate within the lysosomes and thereby cause a lysosomal storage disease.[1,8-12] These disorders are examples of inborn errors of metabolism, and more than 40 lysosomal storage diseases have been identified in humans. Lysosomal storage diseases are defined by the specific enzyme deficiency and the accumulation of specific substrates (eg, GAGs) within cells. Mucopolysaccharides or GAGs such as chondroitin, dermatan, heparan, and keratin sulfates are long chains of repeating disaccharide units. Degradation of these large complex polymers requires

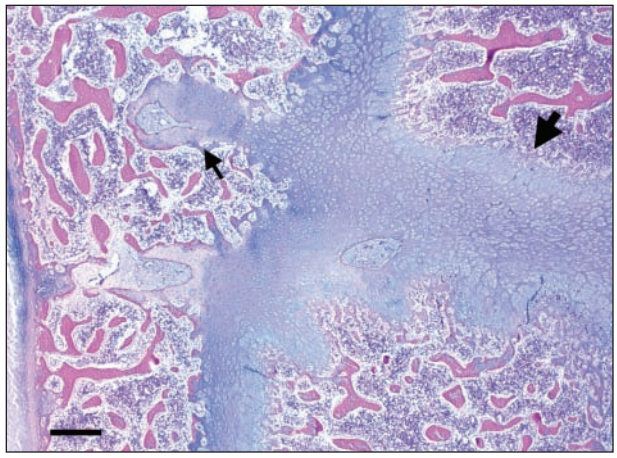

Figure 4—Photomicrograph of a section of a misshapen vertebral body from a dog with mucopolysaccharidosis type VII. Notice the irregular cartilage columns in the epiphysis (small arrow) and abnormal, nonmineralized cartilage columns extending from the growth plates of the vertebra into the metaphysis (large arrow). H&E stain; bar = 500 μm.

the coordinated activity of many lysosomal hydrolases.[13] Deficient activity of any enzyme involved in the degradation of GAGs results in mucopolysaccharidosis.

Deficiency of β-glucuronidase affects the catabolism of chondroitin, dermatan, and heparan sulfates[2] and results in mucopolysaccharidosis type VII (also known as Sly syndrome). Mucopolysaccharidosis type VII becomes evident early in life and has been identified in humans, domestic shorthair cats, a mixed-breed dog (and a subsequently derived experimental animal colony), and mice.[5,14-19] The clinical signs commonly involve the eyes (diffuse corneal opacities), skeleton (facial dysmorphia and axial and appendicular skeletal deformities referred to as dysostosis multiplex), joints (excessive joint laxity and degenerative joint disease), cardiovascular system (valvular thickening that results in systolic murmurs), and the CNS (mental retardation).[1,5,9,12,14,15,17,18,20,21] Most of these clinical features were detected in the mixed-breed dog described previously and the dog of this report.

Multiple skeletal abnormalities and corneal opacities in a young dog are suggestive of a lysosomal storage disease. Results of diagnostic assessments indicating the presence of WBC inclusions that stain metachromatically with toluidine blue and high urinary GAG excretion support a diagnosis of mucopolysaccharidosis, although growing puppies with any skeletal disease may have a slightly high concentration of chondroitin sulfate in urine because of bone growth and remodeling.[9,12,20,21] Thus, it is important to confirm a specific diagnosis by either enzyme analysis (detection of low activity of 1 lysosomal enzyme and concurrent increased activities of others in that metabolic pathway) or mutation analysis (if known for the breed of animal). In the dog of this report, both approaches were used because, to the authors' knowledge, mucopolysaccharidosis type VII has not been reported in a purebred German Shepherd Dog. Mucopolysaccharidosis type VII was first identified in a male mixed-breed dog from California 20 years ago[5]; the clinical and laboratory abnormalities noted in that dog were similar to those detected in the puppy of this report.

Autosomal recessive disorders are more likely to be detected in purebred animals in which homozygosity occurs as a result of inbreeding. Occasionally, mixed-breed dogs have been identified as homozygous for a disease-causing mutation; this homozygosity likely occurs as a product of a mother-son or sire-daughter mating, as observed in Irish Setter mixed-breed dogs with canine leukocyte adhesion deficiency[22] and English Springer Spaniel mixed-breed dogs with phosphofructokinase deficiency.[23] Similar to the dog of this report, those dogs had some relationship to the breed in which the defect had been previously reported. Affected animals are typically born to healthy parents but may have littermates with the disorder. Carrier animals (heterozygotes) have approximately half of the normal lysosomal enzyme activity, which is adequate for standard cellular function; thus, carrier animals do not develop clinical signs. The genotype of suspected carriers may be determined by identification of affected offspring or from results of enzyme analyses (ie, detection of half the activity of clinically normal animals), planned matings to known carrier animals (which would be ethically questionable), or molecular genetic testing.

Spontaneous mutations occur very rarely, and it is unlikely that the exact same mutation would occur twice within the same breed. Therefore, it is highly probable that the mutation identified in the puppy of this report originally occurred in a German Shepherd Dog. To our knowledge, a report of another dog affected by mucopolysaccharidosis type VII has not been published in the 20-year interval since the first report of this mutation; therefore, it is plausible that either affected dogs were euthanatized at a young age without a definitive diagnosis or that the dog with the original mutation and its relatives were rarely used for breeding. Nevertheless, carriers of this mutation were relocated from California[24] to Georgia where the dog of this report was discovered. Unfortunately, pedigree information pertaining to the dog with the original mutation as well as that of the dog of this report was not available for review.

As with many other naturally occurring hereditary diseases in animals, dogs with mucopolysaccharidosis type VII may serve as an animal model for the disease in humans. Therefore, a colony of dogs was established from the dam of the originally affected mixed-breed dog, and studies have been performed on that group of dogs to further our understanding of the pathophysiologic features of this disease. The molecular defect resulting in mucopolysaccharidosis type VII and associated pathologic changes in various tissues have been examined.[2,6,16,24] In addition, this animal model has been used to develop and assess novel treatment strategies such as bone marrow transplantation, enzyme replacement, and gene therapy.[3,4,6,7,25-29,a,b] Because of the progressive nature of mucopolysaccharidosis type VII, therapeutic interventions must be instituted early in the disease process to be of use. Interestingly, one of the first successful examples of gene therapy in animals has been accomplished in puppies with neonatal mucopolysaccharidosis type VII by use of a retrovirus vector containing the normal canine β-glucuronidase cDNA. Without treatment, affected puppies were recumbent and unable to ambulate by 6 months of age, as observed in the dog of this report. However, puppies that received gene therapy remained ambulatory until they were > 2 years old (the current length of the study).[7] Although applications of gene therapy to many diseases in humans are being developed, the efficacy and safety of the treatments must be evaluated further. The expectation is that advances in experimental medicine will also become available for companion animals.

Despite advances in treatment, control and prevention of lysosomal storage diseases in animals remain to be achieved. To reduce the number of animals affected by lysosomal storage diseases, it is critical that veterinarians have knowledge of the associated clinical signs and commonly affected breeds, are able to perform the appropriate diagnostic investigations, and can educate pet owners and breeders concerning the importance of breeding animals that are free of the disease-causing mutation. The carrier status of siblings, sire, and dam of an animal with a lysosomal storage disease should be ascertained prior to subsequent breeding. Two animals that are heterozygous for the mutation should not be bred to avoid producing clinically affected individuals; however, a heterozygous animal may be bred to an animal without the mutation (ie, an homozygous clinically normal individual) to retain desirable characteristics, as long as the genotype of each of the progeny is determined before its use as a breeding animal. In this way, the mutation can eventually be eliminated from the breed population, which could be done in a single generation if all breeding animals were tested and heterozygotes not mated.

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[b]Xu LF, Ponder KP, Melniczek JR, et al. Neonatal delivery of a retroviral vector expressing canine beta-glucuronidase results in long-term gene expression and marked improvement in the clinical manifestations in mucopolysaccharidosis VII dogs. *Blood* 2001;98:693A.

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