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A model of mucopolysaccharidosis IIIB (Sanfilippo syndrome type IIIB): *N*-acetyl-α-D-glucosaminidase deficiency in Schipperke dogs

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Summary: Mucopolysaccharidosis III (MPS III) is characterized by lysosomal accumulation of the glycosaminoglycan (GAG) heparan sulphate (HS). In humans, the disease manifests in early childhood, and is characterized by a progressive central neuropathy leading to death in the second decade. This disease has also been described in mice (MPS IIIA and IIIB), dogs (MPS IIIA), emus (MPS IIIB) and goats (MPS IIID). We now report on dogs with naturally occurring MPS IIIB, detailing the clinical signs, diagnosis, histopathology, tissue enzymology and substrate levels. Two 3-year-old Schipperke dogs were evaluated for tremors and episodes of stumbling. Examination of the animals found signs consistent with cerebellar disease including dysmetria, hind limb ataxia and a wide-based stance with truncal swaying. There were mildly dystrophic corneas and small peripheral foci of retinal degeneration. Magnetic resonance imaging of the brain and skeletal radiographs were normal. Intracytoplasmic

granules were found in the white cells of peripheral blood and cerebral spinal fluid, and in myeloid lineages in bone marrow. Electrophoresis of urinary GAGs indicated the presence of HS, while assays of cultured fibroblasts found *N*-acetyl-α-D-glucosaminidase (Naglu) activity of between 4.3% and 9.2% of normal. Owing to neurological deterioration, both dogs were euthanized, and post-mortem examinations were performed. Biochemical studies of liver and kidney from both animals demonstrated profound deficiency of Naglu activity and abnormally high GAG levels. Pathology of the brain included severe cerebellar atrophy, Purkinje cell loss, and cytoplasmic vacuolation in neurons and perithelial cells throughout the central nervous system. Pedigree analyses and Naglu levels of family members supported an autosomal recessive mode of inheritance. Using an obligate heterozygote, a breeding colony has been established to aid in understanding the pathogenesis of MPS IIIB and testing of potential therapies.

Mucopolysaccharidosis IIIB (MPS IIIB, Sanfilippo syndrome type IIIB, McKusick 252920) is one of a group of autosomal recessive diseases caused by loss of function of one of the four lysosomal enzymes involved in heparan sulphate (HS) catabolism. The cause of MPS IIIB is deficient activity of the lysosomal glycosidase *N*-acetyl-α-D-glucosaminidase (Naglu, EC 3.2.1.50). In humans, loss of normal Naglu activity results in HS and glyosphingolipid accumulation, and is characterized clinically as a childhood-onset (3–6 years of age) progressive neuropathy of the central nervous system (CNS), leading to death usually in the second decade of life (Neufeld and Muenzer 2001). Of the four enzymopathies seen in humans (designated A to D), MPS IIIA and IIIB are the most common (Poorthuis et al 1999). The incidence of MPS III overall has been estimated to be 1 in 73 000 live births (Meikle et al 1999). The pathological lesions seen throughout the CNS primarily affect neurons and macrophage-like cells. As part of the clinical course, severe behavioural abnormalities may be seen, which can include sleep disturbance, hyperactivity and aggressive behaviour.

There is no specific primary therapy for this condition; however, the study of animal models may prove beneficial. To date, animal models exist for all forms of MPS III except type IIIC. Extant animal models include the wirehaired Dachshund and New Zealand Huntaway dog, both with MPS IIIA (Aronovich et al 2000; Fischer et al 1998; Jolly et al 2000; Yogalingam et al 2002); a spontaneous mouse model of MPS IIIA (Bhattacharyya et al 2001 Bhaumik et al 1999); a knockout mouse model of MPS IIIB (Li et al 1999); an emu model of MPS IIIB (Aronovich et al 2001; Bermudez et al 1995, 1997; Freischutz et al 1997; Giger et al 1997); and a caprine model of MPS IIID (Cavanagh et al 1995; Friderici et al 1995; Thompson et al 1992). In this report, we detail the findings of two Schipperke dogs with MPS IIIB, the first spontaneous form of MPS IIIB seen in a nonhuman mammal. An offspring from one of these animals was used in the foundation of a research colony for the study of canine MPS IIIB.

METHODS

Animals

Dog 1 was a neutered male Schipperke from New York State. The dog was the product of a breeding between half-siblings, with the dog having a grandsire common to both maternal and paternal lines. At 3.7 years of age, after a fall down stairs, the animal was presented by his owners to their veterinarian. Before this time, the animal had an unremarkable history involving only routine veterinary care, with the exception that 5 months prior to the fall the animal was noted to have a change in hair coat colour from the normal black to a deep auburn, and mental dullness. Upon presentation, complaints included mental dullness, ataxia, lethargy and anorexia. At this time the owners pursued a referral consultation. The referral veterinarians considered there to be marked cerebral and cerebellar involvement. An inherited metabolic condition was among their differential diagnoses, and urine samples were evaluated at the Metabolic Genetic Screening Laboratory at the Veterinary Hospital of the University of Pennsylvania (VHUP), at which time increased heparan sulphate was found, leading to a working diagnosis of MPS III. At the age 5.3 years the dog was examined by the Section of Medical Genetics at VHUP. Physical and neurological examination revealed a poor body condition, mental dullness and ataxia, with hypermetria, truncal swaying and fine whole-body and head tremors. Postural reactions ranged from absent to normal to slightly increased, and segmental reflexes were normal to slightly decreased. The disease was considered diffuse and central, with a prominent cerebellar component. Ophthamological examination indicated minimal corneal dystrophy and 2–6 small areas of mild peripheral retinal degeneration. A menace reflex was absent in both eyes, but this was considered to be due to the CNS component and not the observed and slight ophthalmological abnormalities. Radiographs indicated a normal skeletal system, an enlarged liver that extended beyond the costo-condral arches, and a paucity of retroperitoneal fat. A complete blood cell count was normal with the exception of azurophilic granules in many lymphocytes. Serum biochemistry showed only a slight elevation of blood urea nitrogen (BUN; 47 mg/dl, normal 5–25). At 5.6 years of age, the dog was again seen by the Section of Medical Genetics at VHUP. At this time there was a mild (II/VI) systolic heart murmur. Neurological signs had progressed, with a worsening of postural reactions, which were delayed and exaggerated. Because of the poor prognosis, the patient was euthanized at the request of the owners, and a complete necropsy was conducted. Examination of cerebrospinal fluid (CSF) detected cytoplasmic granules in WBC. Samples were processed for both routine histopathology and electron microscopy. Unfixed tissues were also taken and cultured or frozen.

Dog 2 was a sexually intact female Schipperke from North Carolina. This dog was the product of nonconsanguineous breeding as assessed using a four-generation pedigree. Dog 2 was reported by the owners to have been normal and healthy until presented by them to the local veterinarian at 3 years of age with complaints of a head tilt. Shortly after the onset of the head tilt, there was a mating between dog 2 and an unrelated Schipperke in the household. The pregnancy went to term,

and four healthy pups were subsequently whelped and weaned. During the pregnancy and weaning period, the owners noticed in dog 2 a progressive general ataxia, characterized by a high stepping gait and a propensity to fall. Vestibular episodes occurred several times a day characterized by opisthotonos lasting for approximately 20 seconds. At the age of 3.5 years the owners presented the dog to the Neurology Service at the Veterinary Teaching Hospital of the North Carolina State University. The animal was tetraparetic, with a wide-based stance and a head tilt to either direction. The dog fell to either side, had an intention tremor, was hypermetric, and had exaggerated postural reactions, bilaterally decreased menace response and vertical nystagmus. Diagnostics included serum biochemistry, a complete blood cell count, urinalysis, MRI and CSF analysis. Abnormalities included elevated BUN (55 mg/dl, normal 7–31) and serum potassium (6.2 mmol/L, normal 3.8–5.6), thrombocytosis (579 000/μl, normal 181 000–350 000), lymphocytosis (6216/μl, normal 1000–5000) and eosinophilia (1156/μl, normal 100–750). Urinalysis was consistent with a mild bacterial urinary tract infection. Although total nucleated cell count in the CSF was normal, cytospin examination identified mononuclear cells with many variably sized dark-staining cytoplasmic granules. This last finding prompted a provisional diagnosis of galactosialidosis, a lysosomal storage disease previously reported in Schipperkes (Knowles et al 1993). Samples including urine and whole blood for metabolic studies, a bone marrow aspirate for cytology and culture, and a skin biopsy for fibroblast culture were taken when the dog was 3.8 years of age and sent to the Section of Medical Genetics at VHUP. The analysis of these samples indicated that the patient had granulations in peripheral blood lymphocytes; granulation in the stromal cells, macrophages, lymphocytes and plasma cells of the bone marrow; and increased urinary heparan sulphate. Assay of cultured skin fibroblasts identified a marked Naglu deficiency (see below). At 4.2 years of age the neurological signs had progressed and the owners elected euthanasia, followed by a complete necropsy. Samples were processed for both routine histopathology and electron microscopy. Unfixed tissues were also taken for further biochemical analyses.

In addition to the patients described above, samples were also analysed from the four pups that dog 2 had whelped, as well as the sire. One of these pups, a presumptive obligate carrier, was bred to two mixed-breed bitches in order to found a breeding colony to further study this condition. Carriers were selected on the basis of Naglu activity in cultured fibroblasts. One male and two females carriers each from the two resultant litters were kept, and F_1 intercross breedings were conducted to produce affecteds.

Biochemical analysis

All the biochemical tissue analyses were performed on samples stored at $-80^{\circ}C$, with the exception of cultured fibroblasts.

Enzyme analysis: Tissues assayed included fibroblasts from the two clinically affected dogs and from a healthy unrelated dog; white blood cells (WBC) from

dog 2, four of her pups, the sire of the litter, and a normal control; and fresh frozen samples of liver, spleen, and kidney cortex and medulla from the two affected animals and four age-matched normal beagles (three female, one male). Tissues were homogenized in the appropriate buffers and supernatants were assayed for enzyme activity. Protein concentration was determined using a kit (Bio-Rad Protein Assay kit, Bio-Rad Laboratories, Hercules, CA), based on the assay of Bradford (1976). Activities were calculated as amount of substrate released/h per mg protein, and are also reported as percentage of control activity. All tissue samples were assayed for Naglu activity (Marsh and Fensom 1985). Cultured fibroblasts and organ tissue samples were further assayed for β-glucuronidase (β-gluc, EC 3.2.1.31) and total β-hexosaminidase (tβ-hex, EC 3.2.1.52) activities (Sammarco et al 2000). Cultured fibroblasts were assayed as previously described (Li et al 1999; Thompson and Nowakowski 1991; Wenger and Williams 1991) for activities of heparin sulphamidase (SGSH; EC 3.10.1.1), acetyl-CoA:α-glucosaminide *N*-acetyltransferase (GMAT; EC 2.3.1.3), *N*-acetylglucosamine 6-sulphatase (GMS; EC 3.1.6.14), α-L-iduronidase (α-idua, EC 3.2.1.76), arylsulphatase B (AsB, EC 3.1.6.12), α-mannosidase (α-mann, EC 3.2.1.24), β-galactosidase (β-gal, EC 3.2.1.23) and α-neuraminidase (α-neur, EC 3.2.1.18).

Glycosaminoglycan analysis: The analysis of urine glycosaminoglycans (GAGs) was conducted as previously described (Fischer et al 1998). Preliminary screening of urine was by a toluidine blue spot test (Berry and Spinanger 1960), which was performed on urine of clinically affected dogs and normal controls. Qualitative analysis of the urinary GAG pattern was done by cellulose acetate electrophoresis (Wessler 1968). The identity of the urine GAG was confirmed by digestion using chondroitinase ABC and AC (Taniguchi et al 1975). Liver, spleen, and kidney cortex and medulla GAG content was measured on the two affected animals and four age-matched normal beagles (three female, one male) and was performed as previously described (Sammarco et al 2000). The assay is based, with modifications, on the assay of Björnsson (1993), which relies on the specific precipitation by Alcian blue of sulphated GAGs in a low-pH, high-concentration salt solution. The GAG content is reported as μg sulphated GAG/mg protein.

Evaluation of pathology

Post-mortem samples were processed for routine histopathology and electron microscopy immediately after euthanasia. Euthanasia was performed by intravenous administration of 80 mg/kg of sodium pentobarbital (Veterinary Laboratories, Inc., Lenexa, KS, USA) in accordance with the American Veterinary Medical Association guidelines, and tissue samples were frozen immediately on dry ice. Samples for light microscopy were collected and fixed in buffered 10% formalin, paraffin-embedded, sectioned and stained with haematoxylin and eosin, periodic acid–Schiff reagent (with and without diastase digestion), Luxol fast blue, and toluidine blue. Specimens for electron microscopy were immersion fixed in Trump's universal fixative (McDowell and Trump 1976) and were post-fixed in osmium tetroxide. Semi-thin sections were stained with toluidine blue and basic fuchsin.

Pedigree analysis

Pedigrees for animals 1 and 2, as issued by the American Kennel Club, were supplied by the owners. Further analysis utilized an online database of Schipperke pedigrees (http://www.bonchien.com/schipsearch.html).

RESULTS

Biochemical analyses: Screening analysis of urine for inherited metabolic disease revealed positive MPS spot tests for both dogs 1 and 2 (Figure 1). Electrophoresis of urine GAGs identified HS, as judged by co-migration of urine GAG with the HS standard (Figure 1), and corroborated by enzyme digestion studies (data not shown). Screening of cultured fibroblasts from both dogs 1 and 2 for the activity of 11 lysosomal hydrolases either involved in GAG degradation or associated with neuropathic lysosomal storage disorders identified a profound lack of Naglu activity of 4.3% and 9.2% of control in dogs 1 and 2, respectively (Table 1). All other enzyme activities, including β-gal and α-neur, ranged from approximately 50% to 350% of control levels (Table 1).

Enzyme activities of Naglu, β-glucuronidase and β-hexosaminidase were measured in homogenates of liver, spleen, and kidney cortex and medulla from the two affected dogs, and four normal age-matched controls (Table 2). The mean Naglu activity of the affected dogs, as a percentage of normal, was 2.8%, 4.1%, 4.8% and 6.6% in kidney

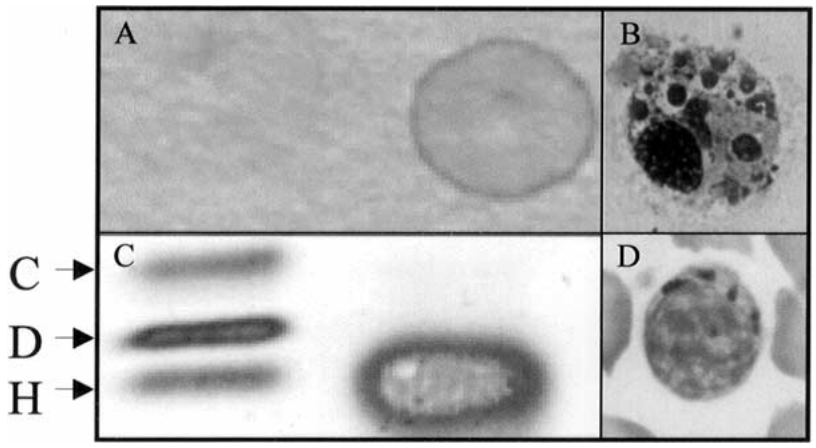

Figure 1 (A) Results of MPS spot test for dog 2, normal urine spot on the left and the urine spot from dog 2 on the right. (B) A light micrograph of a mononuclear cell, stained with Wright–Giemsa, from the CSF of dog 1. (C) Cellulose acetate electrophoresis of urinary GAGs from dog 1, demonstrating mucopolysaccharidurıa and heparansulphaturia. The arrows on the left indicate the GAG standards: C, chondroitin 4-sulphate; D, dermatan sulphate; H, heparan sulphate. (D) A light micrograph of a Wright–Giemsa-stained peripheral blood smear from dog 1 indicating granulation of lymphocytes

Table 1 Results of fibroblast enzyme assays

		Dog 1			*Dog 2*		
Enzyme	*Disease*		*Control*[c]	*%Normal*		*Control*[c]	*%Normal*
α-L-Iduronidase[a]	MPS I	16.2	14.6	111	17.4	15.4	113
Heparin sulphamidase[b]	MPS IIIA	695	208	334	330	208	159
N-Acetyl-α-D-glucosaminidase[a]	MPS IIIB	0.34	8.0	4.3	0.61	6.7	9.2
Acetyl-CoA:α-glucosaminide *N*-acetyltransferase[b]	MPS IIIC	12 800	26 000	49.0	27 400	26 000	105
N-Acetylglucosamine 6-sulphatase[c]	MPS IIID	3.2	1.3	250	4.5	1.3	350
Arylsulphatase B[d]	MPS VI	57.5	31.4	183	57.5	31.4	183
β-Glucuronidase[a]	MPS VII	71.5	111	64.4	75.3	46.6	162
β-Galactosidase[a]	GM_1 gangliosidosis	130	143	90.4	260.1	165	157
β-Hexosaminidase (total activity)[a]	GM_2 gangliosidosis	1 500	1 850	81.5	475	418	114
α-Mannosidase[a]	α-Mannosdosis	142	132	107	226	162	139
α-Neuraminidase[a]	Sialidosis	12	11	110	9.1	9.7	94

[a]nmol 4-methylumberlliferone/h per mg protein
[b]cpm/h per mg protein
[c]mg *N*-acetylglucosamine/h per mg protein
[d]nmol nitrocatechol/h per mg protein
[e]Control value for an unrelated normal dog

Table 2 Organ enzyme activity

	Kidney medulla	*Kidney cortex*	*Liver*	*Spleen*
β-Glucuronidase[a]				
Affected[b]	730	940	680	350
Normal (± standard deviation)[c]	280 ± 110	460 ± 120	260 ± 52	360 ± 37
Affected as % of normal	260	210	270	97
β-Hexosaminidase (total activity)[a]				
Affected[b]	14 000	17 000	13 000	4 400
Normal (± standard deviation)[c]	2 800 ± 430	3 800 ± 1 100	3 400 ± 1 000	3 100 ± 1 400
Affected as % of normal	520	430	390	140
N-Acetyl-α-D-glucosaminidase[a]				
Affected[b]	0.32	0.40	0.35	0.21
Normal (± standard deviation)[c]	11 ± 2.4	9.7 ± 2.6	7.3 ± 0.97	3.2 ± 1.1
Affected as % of normal	2.8	4.1	4.8	6.6

[a]nmol 4-methylumberlliferone/h per mg protein
[b]Mean of values for dogs 1 and 2
[c]Mean of values for 4 age-matched normal control beagle dogs (3 female and 1 male)

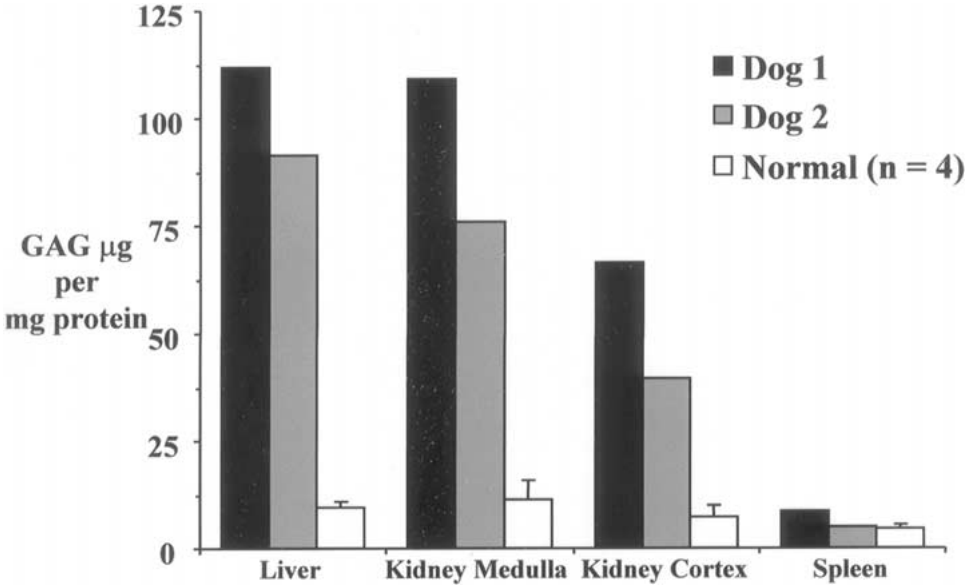

Figure 2 Total sulphated GAG values from the two MPS IIIB-affected dogs reported as μg GAG/mg protein. The GAGs were assayed in liver, kidney cortex and kidney medulla, using an Alcian blue dye precipitation method. Normal values were derived using 4 age-matched normal beagles (3 females, 1 male). Error bars on normal values represent one standard deviation

cortex, kidney medulla, liver and spleen, respectively (Table 2). The activities of reference enzymes tended to be increased in the affected dogs.

Total sulphated GAGs were measured in the liver, spleen, and kidney cortex and kidney medulla (Figure 2). Marked elevations of GAGs were seen in the liver ($\sim$11-fold increase), kidney cortex ($\sim$8-fold increase) and kidney medulla ($\sim$7-fold increase) in the affected dogs. The values of GAGs from the spleen either were not increased (dog 2) or were only marginally increased (dog 1) relative to the findings in other tissues. Values of tissue GAGs were consistently higher in dog 1 relative to dog 2, which may be related to age at euthanasia (5.6 versus 4.2 years of age, respectively).

Evaluation of pathology: Pathological findings were similar in both dogs and included grossly enlarged lateral ventricles (dog 2) and atrophied cerebellum. Histotologically, there was severe diffuse Purkinje cell loss, and the remaining Purkinje cells contained fine cytoplasmic granules, were mildly swollen and were often displaced within the granular layer. There was variable, moderate to marked, diffuse thinning of the molecular and granular layers. Neuronal and ependymal cell vacuolation in the spinal cord was diffuse and moderate, with severe lesions in the ventral horn. There was evidence of focal arachnoid proliferation (dog 1). There was diffuse, mild to severe, neuronal and glial cell vacuolation in the cerebrum and brainstem, which included cells of the spinal nucleus of the trigeminal nerve,

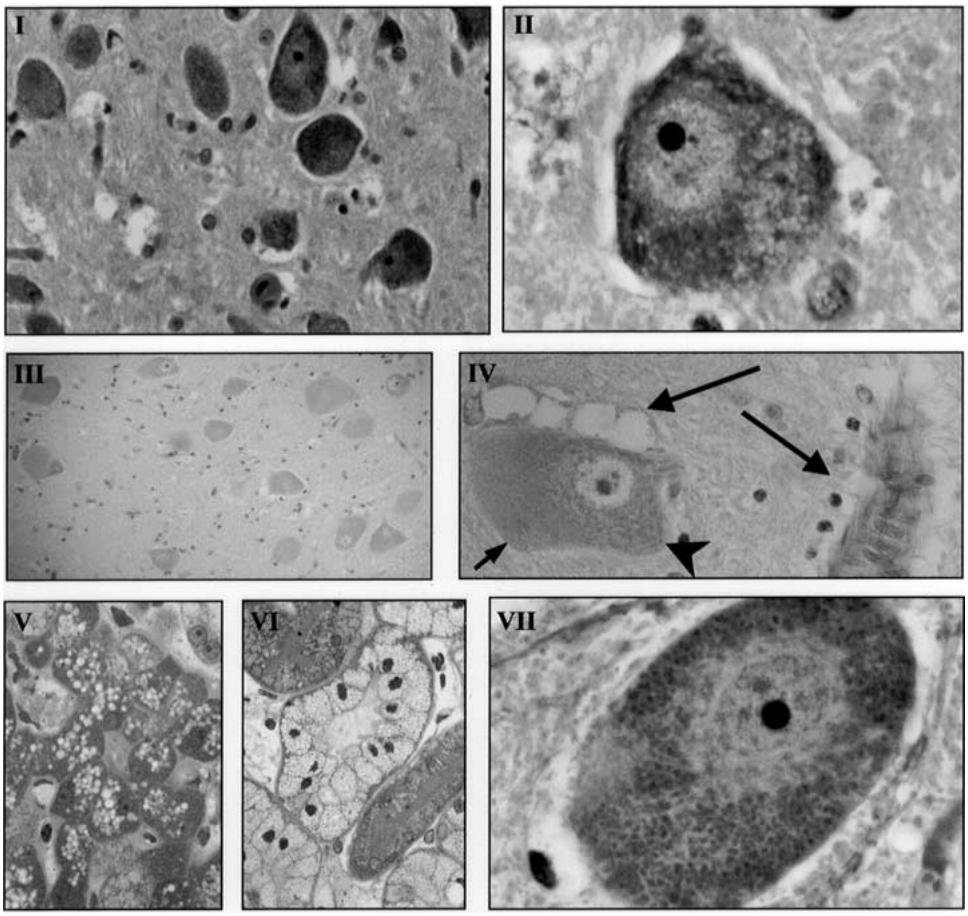

Figure 3 Photomicrographs of the histopathology of cytoplasmic storage in brain, kidney and liver of dogs 1 and 2. (I) (×200) and (II) (×500) are stained with toluidine blue and show the intracytoplasmic accumulation of acid GAGs in neurons of the midbrain of dog 2. (III) (×100) and (IV) (×400) show intracellular storage in the spinal nucleus of the trigeminal nerve of dog 1 stained with PAS. (III) shows distended neuronal cell bodies throughout the section. (IV) shows lighter-staining PAS-positive storage material (small arrow) compressing the darker-staining Nissl substance around the nucleus (arrowhead). Storage vacuoles of perikarial and perithelial microglial cells are evident (large arrows). (V) and (VI) are photomicrographs of semi-thin sections of liver (V, ×200) and kidney (VI, ×200) from dog 1, stained with basic fuchsin, indicating severe hepatocellular and renal tubular epithelial accumulation of storage material. (VII) High magnification (×500) of a neuronal cell body from the cerebrum of dog 2, stained with Luxol fast blue, indicating probable glycosphingolipid storage material. Magnifications given are the original magnifications, reproduced at 80%

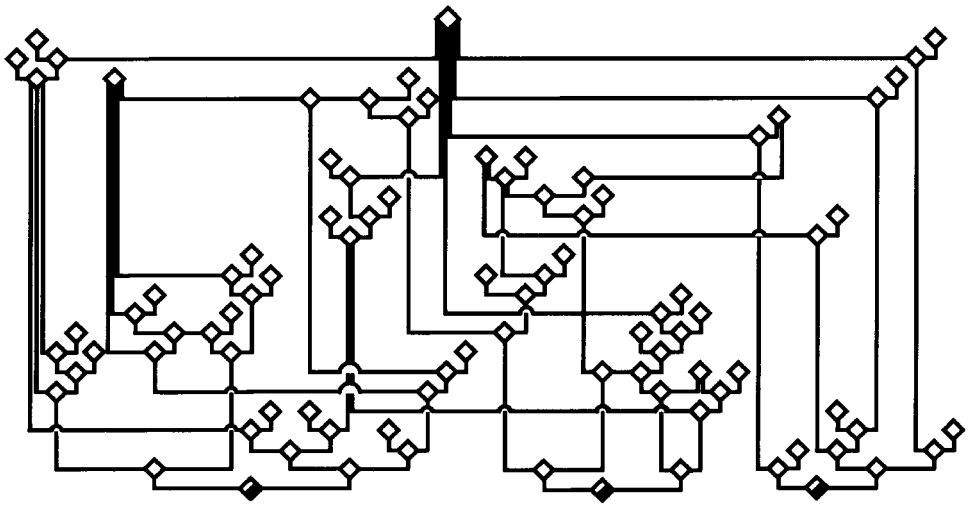

Figure 4 A pedigree showing all common ancestry found in eight-generation pedigrees of the sire and dam of dog 2 and the common grandsire of dog 1. The animal at the top center of the pedigree was the only individual common to the pedigrees of all three animals. The common grandsire of dog 1 and the sire and dam of dog 2 are shown as carriers (half-shaded symbols), to reflect their status as putative carrier and obligate carriers, respectively. The individuals are intentionally shown using the sex-unknown symbol (diamond), and carriers are not specifically identified to maintain the anonymity of the pedigree

vestibular nuclei, cerebral cortex, hippocampus, hypothalamus, thalamus, and caudate nucleus. Cytoplasmic vacuolation of perikarial and perithelial microglial cells was evident, suggesting accumulation of GAGs. The spinal nucleus of the trigeminal nerve contained periodic acid–Schiff (PAS)-positive storage material in the cytoplasm of neurons (Figure 3).

Vacuoles were also evident in perikarial and perithelial microglial cells. Intracellular neuronal accumulation of toluidine blue- and Luxol fast blue-positive material is consistent with storage of GAGs and glycosphingolipids (Figure 3). While specific immunohistochemical staining was not conducted, this toluidine blue- and Luxol fast blue-positive staining pattern of storage material in the brain would not be inconsistent with the intracellular accumulation of HS and the glycosphingolipid GM_2 ganglioside, the latter being known to accumulate in the brain of MPS III patients (Jones et al 1997; Wallace et al 1966) and in animal models of MPS I and IIID (Jones et al 1998; Shull et al 1984).

Hepatic accumulation of storage material was severe, located both in periportal and midzonal hepatocytes as well as in Kupffer cells (Figure 3). The kidney showed evidence of segmental accumulation of storage material (Figure 3) and moderate diffuse chronic pyelitis (dog 1). Mild accumulation of storage material, particularly in macrophages, was noted in nearly every tissue examined, but also included the ganglion cells of the retina, the bronchiolar epithelial cells, the ductal epithelial and islet cells of the pancreas, and the myenteric plexuses of the small and large

intestines and stomach, which also showed accumulation in the chief cells. Gall bladder, skeletal muscle, tongue, heart and synovium had no lesions.

Pedigree analysis and family studies: All parents of the affected animals were reported to be clinically normal. Analysis of the four-generation pedigrees found consanguinity in dog 1, with the existence of the one grandsire. No consanguinity was evident in the pedigree of dog 2. Further analysis of eight-generation pedigrees, back through paternal and maternal lines of dog 2, and back through lines of the common grandsire of dog 1, identified one animal who was common to the paternal and maternal lines of dog 2 and the grandsire of dog 1 (Figure 4). The analyses of pedigrees show the disease condition to be consistent with autosomal recessive inheritance, as is the case for MPS IIIB in other species. The pedigree suggests that the existence of this genetic condition in these two dogs may be the result of the popular sire/dam effect.

Dog 2, as mentioned, had whelped a litter of four pups, sired by a clinically normal Schipperke dog. The Naglu activities of white blood cells of dog 2, of the sire and of pups of the litter were assayed and indicated a pattern of enzyme activity with the levels of Naglu activity of the pups (0.86, 0.85,0.97 and 1.1 ng 4MU/h per mg protein) intermediate between the low activity of the dam (0.02 ng 4MU/h per mg protein) and the near-normal activity of the sire (1.6 ng 4 MU/h per mg protein) (4MU = 4-methylumbelliferone). This pattern is consistent with an autosomal recessive inheritance in dog 2, in which case all offspring would be obligate carriers.

A breeding colony has now produced the first series of F_1 intercrosses of the MPS IIIB carrier dogs. Of 27 offspring produced in three litters, a total of six affected pups were seen, with affecteds seen in each litter. At 3 months of life the affected pups were clinically indistinguishable from normal apart from severe cytoplasmic granulation in the mononuclear cells of the CSF.

DISCUSSION

Numerous animal models of lysosomal storage diseases have been identified and have proved invaluable for the understanding of the pathogenesis and development of novel therapies. Although a knockout murine model of MPS IIB has been created and emus with a naturally occurring form have been described, a large-animal model would be desirable for further studies. The diseased dogs presented here represent such a model.

The initial analysis of samples from these dogs identified a storage disorder, with cytoplasmic granulation in leukocytes and mononuclear cells of the CSF, heparan sulphaturia and early adult-onset of CNS signs with a prominent cerebellar component. Initial enzymological screening and a family study were highly suggestive of Naglu deficiency. This was confirmed by the assays of liver, spleen and kidney, which documented a pattern of low Naglu activity (2.8% and 6.6% of normal), with concomitant increased GAG levels. The spleen was near normal with respect to both GAG levels. The pattern seen in these tissues, of GAG accumulation and associated changes in the activity levels of other lysosomal enzymes, has been noted in other MPS disorders, including MPS I, II and III (Van Hoof and Hers 1968), although the mechanism is unknown. The tissues most affected with histopathological lesions, i.e. the liver, kidney and CNS, are consistent with those that tend to be the most

affected tissues in human cases of MPS III as well as in animal models (Bhaumik et al 1999; Fischer et al 1998; Jolly et al 2000; Jones et al 1997; 1998; Li et al 1999; Wallace et al 1966). The presence in the CNS of neurons that stain positive with Luxol fast blue or PAS with diastase treatment is indicative of storage of polar lipids or carbohydrate-containing vicinal glycal groups and saturated lipids, respectively (Lhotka 1953; Pearse 1961, 1968). These staining properties would not be inconsistent with the storage of GM_2 and GM_3 gangliosides, which are known to accumulate in the neurons of humans with MPS III as well as in animal models of MPS III (Hadfield et al 1980; Jones et al 1997, 1998; Li et al 1999; Wallace et al 1966).

While many of the CNS lesions are strikingly similar to those seen in human MPS III, such as the storage in neurons of glycosphingolipids and the vacuolation of microglial and perithelial cells, the most impressive finding was the near-complete loss of Purkinje cells. Reports describing the neuropathological lesions of MPS III in human patients are few and vary with respect to detail; they describe a broad range of involvement of the cerebellum, ranging from an early report of MPS III (type unknown) in two siblings, aged 2 and 4 years, with marked decreases in the number of Purkinje and granular cells (Wallace et al 1966), to a case of MPS IIIB in a 17-year-old patient in which little evidence of cerebellar lesions was found (Hadfield et al 1980), with more typical findings being occasional loss of Purkinje cells, ballooning of some remaining Purkinje cells and frequent expansion of their molecular layer dendrites with storage material (Dekaban and Constantopoulos 1977; Ghatak et al 1977; Jones et al 1997; Kriel et al 1978). The most likely explanation of the severity of the cerebellar signs of the canine form of MPS IIIB may be that it is a species peculiarity of HS accumulation seen in the dog. This conclusion is borne out by findings in the canine models of MPS IIIA, which have primarily cerebellar signs and which show severe, either local or widespread, loss of Purkinje cells (Fischer et al 1998; Jolly et al 2000).

Analysis of the pedigree of the two animals reported in this study suggests the existence of a popular sire/dam effect in the Schipperke breed with an individual up to eight generations back that may have carried the mutant allele. A previous report of a lysosomal storage disease in Schipperkes exists, detailing a clinical and pathological condition that in every way mirrors the cases presented here, wherein the authors posited a diagnosis of galactosialidosis (Knowles et al 1993), which unfortunately could not be confirmed with the tissues available. Given the extraordinary similarity of these cases, their occurrence in the same breed of dog, and the incomplete aetiological diagnosis of the former case, we speculate that the earlier report was in fact a case of MPS IIIB.

The importance of animal models for the study of rare disorders has prompted us to found a breeding colony to better study the pathogenesis of and treatments for MPS IIIB. Canine models of MPS I and VII have long been important tools in understanding pathological processes and evaluating treatments, and recent work in canine MPS VII has shown the dramatic potential of gene therapy (Ponder et al 2002). Thorough analyses of the first pups are underway. Areas of particular interest for study will include neuronal pathogenesis in general and the mechanism of Purkinje cell loss in particular, and evaluations of any of a number of potential therapeutic approaches

including gene therapy, brain targeted therapy, stem cell therapy and substrate reduction therapy.

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