White matter changes associated with feline GM2 gangliosidosis (Sandhoff disease): correlation of MR findings with pathologic and ultrastructural abnormalities.

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White Matter Changes Associated with Feline G_{M2} Gangliosidosis (Sandhoff Disease): Correlation of MR Findings with Pathologic and Ultrastructural Abnormalities

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PURPOSE: To establish changes on MR of the brain in a feline model of Sandhoff disease in order to develop standards by which this model may be used in future noninvasive studies. **METHODS:** Five affected felines and six age-matched, littermate controls were evaluated. T1- and T2-weighted images were obtained once or twice for each of four affected and five control animals at 4½ to 12 weeks of age, for a total of 15 MR examinations. Images were evaluated qualitatively for the pattern of myelination and the size of the ventricular system. After the animals were killed, pathologic specimens of the brain were examined with light and electron microscopy, and pathologic changes were correlated with MR. **RESULTS:** Compared with control animals, affected animals showed MR evidence of delayed myelination, manifested by white matter signal hypointensity on T1-weighted images and signal hyperintensity on T2-weighted images. This finding was corroborated by histopathologic findings of decreased myelin in the subcortical and internal capsule regions. White matter abnormalities were not detected ultrastructurally in the animals examined. **CONCLUSION:** Although G_{M2} gangliosidosis is primarily a neuronal disease, MR imaging can show changes in myelination of white matter tracts that may be secondary to the neuronal damage. This provides a noninvasive method of in vivo monitoring as therapeutic strategies are developed in this animal model.

Index terms: Gangliosidoses; Brain, magnetic resonance; Animal studies

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The lysosomal storage diseases are inherited metabolic disorders in which lysosomal enzyme substrates accumulate and cannot be eliminated from the cells in which they are produced. The G_{M2} gangliosidoses are a group of autosomal recessive inherited disorders in which the ganglioside G_{M2} and other glycolipids accumulate intracellularly within lysosomes. This occurs most significantly within neurons. The associated lysosomal swelling and cellular destruction lead to neurologic dysfunction and frequently death. The two most common forms are Tay-Sachs disease, which results from a deficiency of the lysosomal isoenzyme β-hexosaminidase A, and Sandhoff disease, which is attributable to a deficiency of both the β-hexosaminidase A and B isoenzymes. Clinically, the G_{M2} gangliosidoses in humans are classified into acute, subacute, or chronic forms, with the acute, infantile-onset form being a rapidly progressive neurodegenerative process culminating in death by four years of age (1). Animal models of G_{M2} gangliosidosis have been described for dogs, cats, and swine (2–7). Feline models of early-onset Sandhoff disease have been recognized in both domestic and Korat cats (2, 3, 5, 8).

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Characterizing the natural history of disease in an animal model is necessary to demonstrate its similarity to the human disease and to establish a baseline for future therapeutic intervention. Magnetic resonance (MR) is useful to study animal models, because it is noninvasive and can be performed at multiple time points to assess the natural history of a disease process as well as response to therapy. The neuroradiologic interpretation of MR images can be correlated with gross or microscopic pathology as well as molecular analyses to provide a rigorous scientific basis for the clinical practice of neuroradiology. A disadvantage is that general anesthesia is required for animal MR imaging.

This study was undertaken to determine and characterize the MR abnormalities in white matter in feline G_{M2} gangliosidosis and to correlate the MR findings with the histopathologic and ultrastructural changes observed. MR was used to develop standards by which this model may be used in future noninvasive studies.

Methods

A colony of inbred Korat cats, carrying an autosomal recessive gene for G_{M2} gangliosidosis, was established in 1984 as an animal model for Sandhoff disease in humans. These animals are housed and studied in an institution accredited by the American Association for Accreditation of Laboratory Animal Care, following National Institutes of Health guidelines for animal care. All procedures were performed as part of protocols approved by the Institutional Animal Care and Use Committee.

Identification of Affected/Normal Animals

Five affected Korat kittens and six age-matched littermate control kittens were studied. The diagnosis of infantile-onset G_{M2} gangliosidosis was made based on characteristic clinical signs beginning between 2 and 3 weeks of age, with an abnormality in the facies as the first observable sign. A fine head tremor and mild ataxia were generally noted by 4 weeks of age. To confirm their affected/normal status, an assay for total β-hexosaminidase activity in leukocytes was performed for each kitten at 6 weeks of age. Two kittens killed at 4 weeks of age had assays performed at that time. Leukocytes were prepared from heparinized whole blood and analyzed following the method of Kaback et al (9, 10), as previously reported (6). Clinically affected animals had less than 5% of the normal β-hexosaminidase enzyme activity in their peripheral blood leukocytes.

MR Imaging

Animals were anesthetized with intramuscular ketamine (22 mg/kg), midazolam (0.22 mg/kg), acepromazine (0.1 mg/kg), and atropine (0.04 mg/kg). A loop gap resonator coil was used on a 1.5-T GE Signa unit (General Electric, Milwaukee, Wis). Transaxial T1-weighted images with a 3-mm section thickness, 256 × 192 matrix, 9-cm field of view, and pulse sequence of 300/16/4 (repetition time/echo time/excitations) and T2-weighted images with a fast spin-echo technique and pulse sequence of 2500/105/4 were obtained. Four affected felines and five age-matched normal controls were scanned at 4½ to 12 weeks of age as shown in the Table. One affected and one normal animal were not scanned but were killed at 4 weeks of age for histopathologic evaluation. MR scans were evaluated visually by three of the authors (R.A.K., S.R.G., and A.J.B.) and, after independent review, consensus was established by discussion. The white matter signal intensities of T1- and T2-weighted images were compared between age-matched affected and healthy subjects. Scans also were evaluated for ventriculomegaly. Normal subjects were necessary for controls, because no data were available for the normal sequence of myelination in the feline.

MR findings in Korat cats with G_{M2} Gangliosidosis

I.D. No.	Age at MR, wk	Change in WM Signal*		Age at Death, wk
		T1	T2	
9307†	4.5	↓	0‡	12
9283†	6	0§	0§	8
9293†	7.5	↓	↑‖	12
9300	7.5	↓	↑	8
9283	8	↓	↑	8
9293	10.5	↓	↑	12
9307¶	12	↓	↑	12
9284†	. . .	. . .	. . .	4

* Change in white matter (WM) signal of affected animals is represented as either an increase (↑), decrease (↓), or no discernable change (0) in signal relative to age-matched healthy controls.

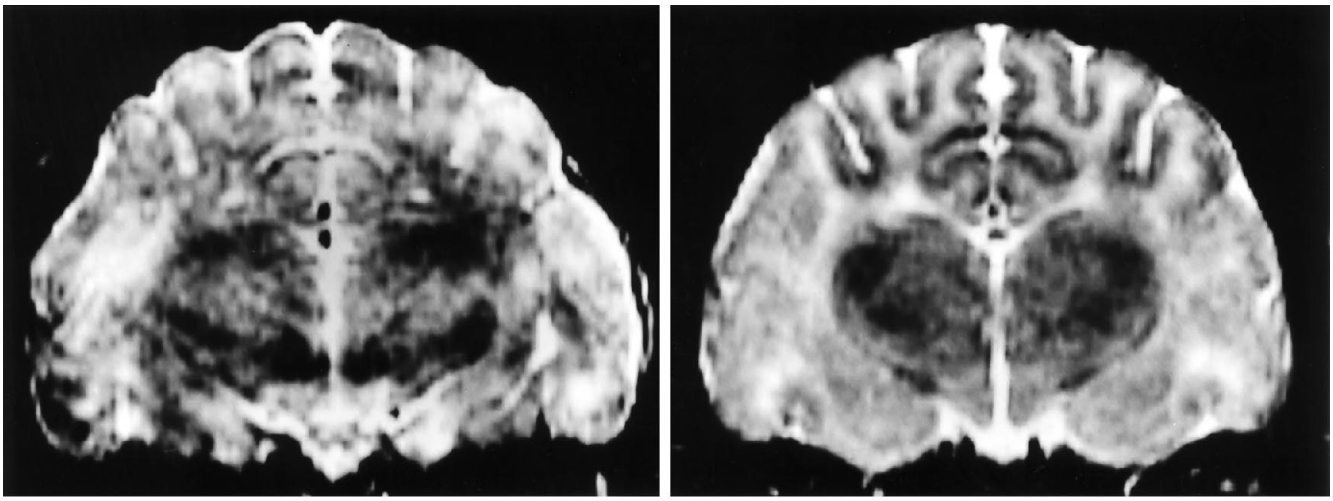
† Three of the five affected animals (9307, 9283, and 9293) were MR imaged at two different times. Data are presented in the sequence of ages evaluated. One affected animal (9284) was not MR imaged but was killed at 4 weeks of age to provide tissues for histopathologic evaluation.

‡ Poor T2-weighted image quality made assessment of these images difficult.

§ A different coil was used to image this animal at 6 weeks of age versus its age-matched control; therefore, no true comparisons could be made.

‖ Decreased signal in the thalami was noted on T2-weighted images.

¶ The control animal, to which this animal was compared, was clinically normal, but vacuolated neurons typical of those seen in affected animals were identified on histopathologic examination of brain.



A B

Fig 1. Transaxial T2-weighted (2500/105) MR images from a 7½-week-old healthy Korat cat (*A*) and its age-matched littermate with G_{M2} gangliosidosis (*B*). When compared with the healthy animal, the white matter of the affected animal appears hyperintense on this T2-weighted image.

Histopathology

Most animals were killed at various ages, as indicated in the Table, by intravenous administration of pentobarbital sodium. Two healthy animals were not killed. Brains were removed immediately after death and immersion fixed in 10% neutral buffered formalin. Brains were sliced transaxially and representative sections of cerebrum, with cortical gray and white matter, and brain stem were embedded in paraffin. Paraffin-embedded sections were cut at 5 μm and adjacent sections stained with hematoxylin and eosin and Luxol fast blue, respectively. Brains of five affected subjects (at 4, 8, or 12 weeks of age) were compared with four age-matched controls. Histopathologic examination was done by one of the authors (A.N.D.). Findings were reported descriptively.

Electron Microscopy

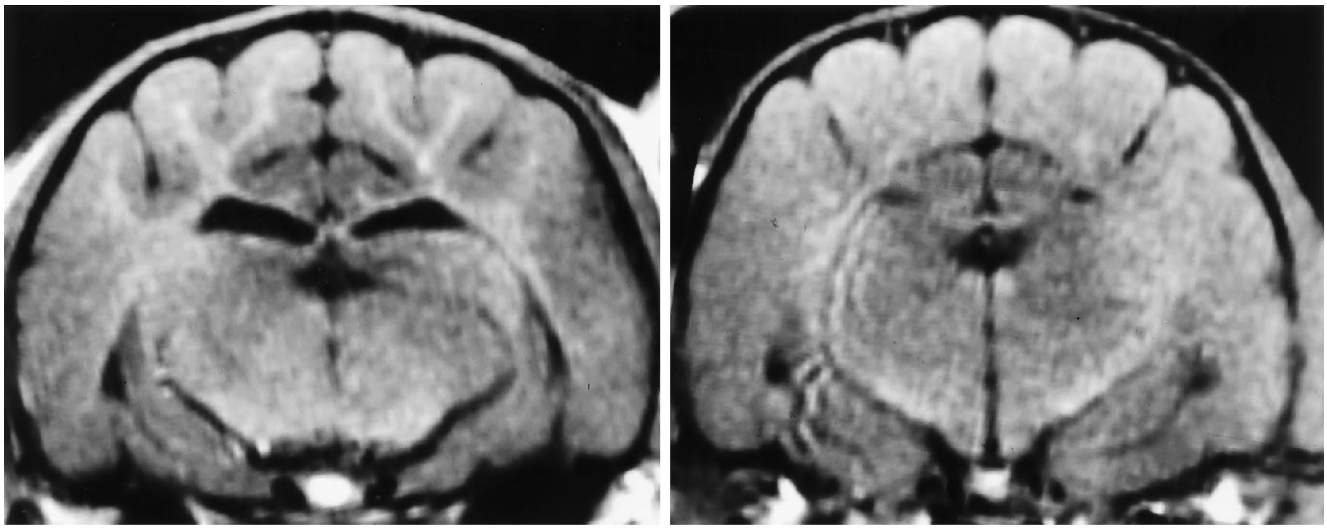
Tissues from two affected and two healthy animals, one each at 4 weeks and 12 weeks of age, were examined ultrastructurally by an experienced observer (A.N.D.). Samples were collected from the cortical gray matter of the 4-week-old animals and from the subcortical white matter of the 12-week-old animals. Tissues were cut into 1- to 2-mm pieces and fixed in 1.5% glutaral, 1.5% paraformaldehyde buffered with 0.1 mol/L sodium cacodylate (pH 7.4). After primary fixation, the sections were washed in 0.1 M sodium cacodylate buffer for 25 minutes and postfixed in 2% OsO_4 for 90 minutes. The tissues then were dehydrated through graded alcohol solutions, held for 1 hour in 100% toluene, and infiltrated and embedded in epoxy resin (LX-112, Ladd Research Industries, Vt). Sections were cut at 0.1 μm, stained with lead citrate and uranyl acetate, and examined with a Philips EMU 301 transmission electron microscope (Philips Electronic Instruments, NJ).

Results

MR Findings

Both T1- and T2-weighted brain images of Sandhoff disease–affected felines had an abnormal appearance when compared with age-matched controls. Imaging differences were subtle in the scans of 4½-week-old kittens and became progressively more striking as the animals were older. When compared with controls, the white matter of affected animals was hyperintense on T2-weighted images, a finding most apparent in animals 7½ to 8 weeks of age (Fig 1). In the animals imaged at 10½ or 12 weeks of age, this difference in T2 prolongation was limited to the internal capsule and pyramidal tracts. Decreased signal intensity was noted on the T2-weighted images in the thalami of one of the 7½-week-old affected kittens.

Hypointensity on T1-weighted images also was observed in affected animals as compared with controls. At 4½ weeks of age, this difference was primarily apparent only in the frontal periventricular region. At 7½ weeks of age, the periventricular white matter of affected animals was hypointense relative to controls. This difference was more apparent at 12 weeks of age, with evidence of myelination, manifested by increased signal on T1-weighted images, only



A B

Fig 2. Transaxial T1-weighted (300/16) MR images from a healthy, 10½-week-old Korat cat (*A*) and its age-matched littermate with G_{M2} gangliosidosis (*B*). Signal hypointensity, apparent especially in the subcortical white matter of the affected animal, relative to the control, is supportive of diminished myelination.

visible in the white matter of the internal capsule and periventricular regions of affected kittens (Fig 2A), whereas the white matter in the control subjects could be seen throughout the subcortical white matter of the cerebrum (Fig 2B) and cerebellum. One affected animal was found to have moderate ventriculomegaly and one healthy animal had mild ventriculomegaly. No other structural abnormalities were observed.

Histopathologic Findings

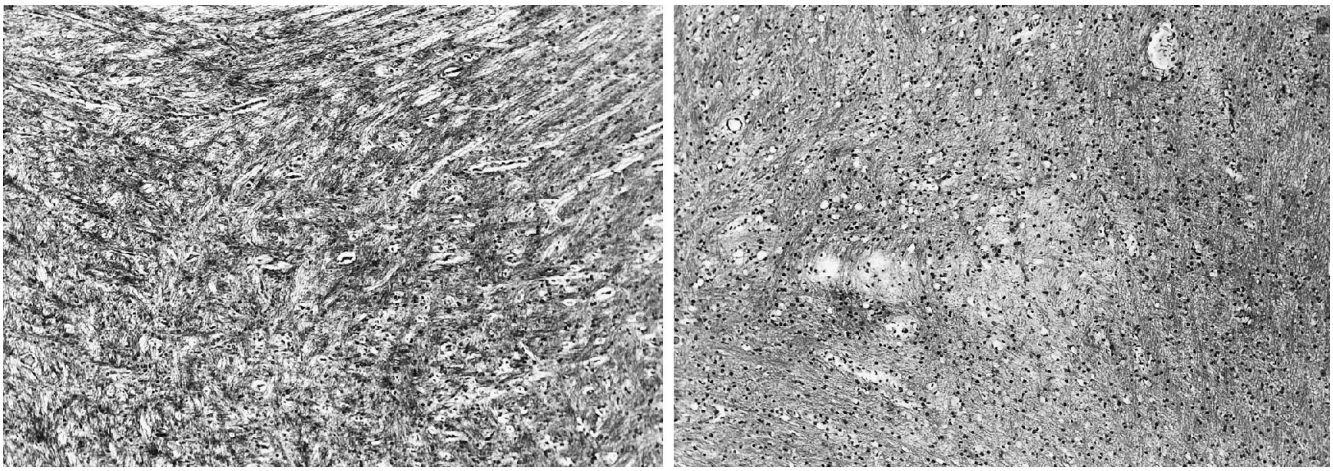
Microscopic evaluation of hematoxylin and eosin–stained brain sections from the affected kittens confirmed the presence of ballooned, vacuolated neurons containing foamy, eosinophilic material. Subcortical white matter and internal capsule appeared less wide on cross-section in affected kittens than in controls. This qualitative difference in white matter was substantiated by examination of Luxol fast blue–stained sections, on which white matter appeared pale in all regions examined (Fig 3). Focal areas of vacuolization, accompanied by macrophages, were observed in the white matter of two affected animals. Swollen axons, characterized by eosinophilic spheroids within the white matter, were seen in most affected animals. One animal with a normal phenotype and a normal appearance on MR had β-hexosaminidase activity approximately 50% of normal and was therefore classified as a carrier (6). This level of enzyme is believed adequate to prevent lysosomal storage (11), but histopathologic changes similar to those seen in affected animals were found.

Electron Microscopic Findings

Numerous membrane-bound inclusions within vacuolated cells, characteristic of lysosomal storage disease, were apparent by transmission electron microscopy. Concentrically laminated membranocytoplasmic bodies were occasionally seen within vacuoles, as has been previously reported (5, 6). When myelinated axons from subcortical white matter were examined in cross section, comparing an affected kitten with its age-matched littermate control, no difference in the number or thickness of myelin sheaths could be appreciated (Fig 4A and B). Scattered axons were distended and contained numerous laminated dense inclusions and mitochondria (Fig 4B).

Discussion

The normal brain matures in a predetermined, organized manner that corresponds to the various stages of development (12). White matter maturation, attributable to myelination of white matter tracts, has been well characterized by MR imaging in humans (12–16). The MR appearance of the pediatric brain changes sub-



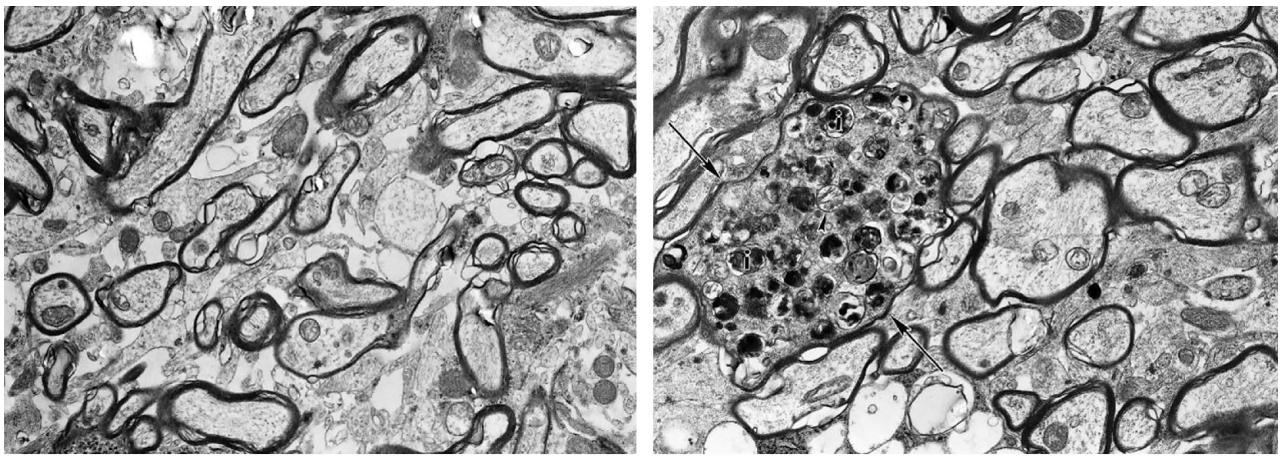
A B

Fig 3. Photomicrographs; original magnification, ×142. *A*, Subcortical white matter from a healthy, 12-week-old Korat cat. Intense staining with Luxol fast blue can be seen.

B, Corresponding subcortical white matter from the age-matched littermate affected with G_{M2} gangliosidosis. Pale staining with Luxol fast blue is evidence of diminished myelination.

stantially during the first 2 years of life, with progressive shortening of both T1 and T2 being recognized as normal myelination proceeds.

Although the gangliosidoses are thought to be a gray matter disease primarily, myelin deficiency has been recognized in cats, dogs, and humans with G_{M1} gangliosidosis (17–19), and has recently been suggested to occur in humans with G_{M2} gangliosidosis (20). In three patients with Sandhoff disease for which MR changes have been reported, signal changes have been progressive. The first patient, a 12-month-old girl, had demonstrable hypointensity in the thalami on T2-weighted images (21). The second patient, first imaged at 19 months of age, had signal hyperintensity in the thalami on T1- and hyperintensity on T2-weighted images, beginning in the occipital white matter and spreading anteriorly throughout the cerebral white matter by 27 months of age (20). The younger sibling of this second patient had no MR imaging abnormalities detected at 9 months of age; however, when imaged at 15 months of age, hyperintensity on T2-weighted images could be seen



A B

Fig 4. Electron micrograph (original magnification, ×13 500) of the subcortical white matter from a healthy, 12-week-old Korat cat (*A*) and an age-matched littermate with G_{M2} gangliosidosis (*B*). The number and thickness of myelin sheaths appears similar when the healthy and affected animals are compared. A distended axon (*arrows*), containing dense laminated inclusions (*i*) and mitochondria (*arrowhead*), is present in *B*.

in the occipital and temporal white matter, internal capsule, and left globus pallidus and on T1-weighted images in the thalami (20). Here we report the progressive MR appearance of diminished myelination in feline Sandhoff disease and demonstrate the corresponding decrease in central nervous system white matter by light microscopy.

The T1 relaxation of adult white matter is sufficiently shorter than that of gray matter to overcome the approximately 12% lesser water content of white matter, producing an image in which white is much brighter than gray (22). The differences between MR images of (unmyelinated) neonatal brains and adult brains support the suggestion that myelin accounts for this difference in T1 shortening (12, 22). The T1 shortening appears to be primarily a magnetization transfer effect between galactocerebrosides and cholesterol, which are on the outer surface of the myelin molecule, and surrounding water (22–26). The T2 shortening seems to correspond more closely to a relative dehydration secondary to the tightening of the myelin spiral by conformational changes of the myelin proteins and saturation of polyunsaturated fatty acids (27–29). The galactocerebrosides and cholesterols accumulate before any spiraling around the axons occurs: therefore, the T1 shortening occurs before the T2 shortening. Perhaps animal studies such as these can eventually shed more light on the relationship of myelin development to the MR changes that accompany it.

In a detailed study of the MR appearance of the normal maturation of neonatal and infant human brain, Barkovich and colleagues found that signal changes associated with brain maturation were apparent on T1-weighted images earlier than on T2-weighted images and, therefore, were more useful for monitoring normal development up to 6 to 8 months of age. After 6 months of age, T2-weighted images become more valuable for marking normal developmental milestones (12). In this feline model of Sandhoff disease, the difference in white matter signal between clinically normal and affected animals was more striking on T1-weighted images and became more apparent as the animals approached 12 weeks of age (the oldest subjects evaluated). However, the differences observed on T2-weighted images, although less striking, were noted at the earliest age evaluated (4½ weeks) and appeared to diminish as the animals matured. Therefore, although T1-weighted images may be more useful for monitoring early, normal development, both T1- and T2-weighted images may be useful in assessing myelination delay.

Our histopathologic findings of decreased cross-sectional width of white matter on hematoxylin and eosin–stained tissue and pale white matter on Luxol fast blue–stained tissue of affected animals, especially apparent in the internal capsule and subcortical white matter, regions known to myelinate later in development, support a developmental delay in maturation of white matter. Although definitive evidence is lacking, we believe the white matter changes are secondary to the neuronal disease and suggest that it may be related to abnormalities in the axonal substrate. Baierl et al suggest that T1-weighted images are well suited to detect delayed myelination when gray/white contrast between the patient and an age-matched control is compared (13). Our MR findings support this suggestion, with clear differences in white matter signal and gray/white contrast apparent on T1-weighted images as the healthy feline's myelination progressed more rapidly than that in affected animals. Examination of electron micrographs of subcortical white matter and corpus callosum from dogs with G_{M1} gangliosidosis revealed an apparent paucity of myelinated axons (17). What is surprising in the current study, and needs more explanation, is the normal ultrastructural appearance of the myelin sheaths in the setting of abnormal Luxol fast blue staining and MR of the white matter. In this study, only a small sample of white matter was evaluated from an affected animal, and it is possible that regional differences were not detected. Evaluation of additional samples and additional animals may be necessary to identify dysmyelinogenesis at the ultrastructural level.

Of particular interest is the carrier animal that was clinically normal but had histopathologic changes similar to homozygote affected animals. Animals and human patients that are heterozygote carriers of this autosomal recessive disorder typically are clinically and histologically normal (1, 11). Two previous animals from this Korat colony have been seen that were normal initially and had carrier levels of β-hexosaminidase activity but developed characteristic clinical signs at about 4 to 6 months of age. This may correspond to the later-onset, subacute, or chronic forms of Sandhoff disease

seen in humans, which are compatible with intermediate or long-term survival (1). We are currently investigating the possibility of a second mutation to account for the findings in these three animals.

In summary, although G_{M2} gangliosidosis is primarily a neuronal disease, abnormal myelination, presumed to be a secondary phenomenon, can be observed. The major imaging change, confirmed by pathologic examination, was abnormal signal in the white matter in affected subjects, compared with age-matched controls. This reflects that the T1 and T2 of white matter in the affected animals did not shorten as would be expected in animals with normal brain maturation. Although these signal intensity abnormalities may be attributable to either abnormal formation of myelin or destruction of normal myelin, it is most likely attributable to a defect in the formation of myelin in these animals with Sandhoff disease.

Definitive diagnosis of G_{M2} gangliosidosis requires biochemical confirmation (1). However, recognition of delayed myelination by MR may contribute to a tentative diagnosis in the human patient. Additionally, intracerebral delivery and transfer of genetic material, for the treatment of both localized and global central nervous system disorders, is currently an area of intense investigation (5, 8, 30, 31). MR may provide a method to monitor therapeutic response, in vivo, to enzyme or gene delivery in animal models of this disease.

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