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Inheritance, Biochemical Abnormalities, and Clinical Features of Feline Mucolipidosis II: The First Animal Model of Human I-Cell Disease

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Abstract

Mucolipidosis II (ML II), also called I-cell disease, is a unique lysosomal storage disease caused by deficient activity of the enzyme N-acetylglucosamine-1-phosphotransferase, which leads to a failure to internalize enzymes into lysosomes. We report on a colony of domestic shorthair cats with ML II that was established from a half-sibling male of an affected cat. Ten male and 9 female kittens out of 89 kittens in 26 litters born to clinically normal parents were affected; this is consistent with an autosomal recessive mode of inheritance. The activities of three lysosomal enzymes from affected kittens, compared to normal adult control cats, were high in serum (11–73 times normal) but low in cultured fibroblasts (9–56% of normal range) that contained inclusion bodies (I-cells), reflecting the unique enzyme defect in ML II. Serum lysosomal enzyme activities of adult obligate carriers were intermediate between normal and affected values. Clinical features in affected kittens were observed from birth and included failure to thrive, behavioral dullness, facial dysmorphia, and ataxia. Radiographic lesions included metaphyseal flaring, radial bowing, joint laxity, and vertebral fusion. In contrast to human ML II, diffuse retinal degeneration leading to blindness by 4 months of age was seen in affected kittens. All clinical signs were progressive and euthanasia or death invariably occurred within the first few days to 7 months of life, often due to upper respiratory disease or cardiac failure. The clinical and radiographic features, lysosomal enzyme activities, and mode of inheritance are homologous with ML II in humans. Feline ML II is currently the only animal model in which to study the pathogenesis of and therapeutic interventions for this unique storage disease.

Mucolipidosis II (ML II, OMIM 252500; McKusick 2000) in humans, also known as I-cell disease, is a rare, unique, recessively inherited lysosomal storage disease (LSD) (Kornfeld and Sly 2000). Although clinically similar to several forms of mucopolysaccharidosis (MPS), it is biochemically distinct, as it results from a problem in trafficking of enzymes to the lysosomes rather than deficiency of a specific lysosomal hydrolase. ML II is caused by deficient activity of N-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase, EC 2.7.8.17) in the Golgi apparatus, the first of two enzymes responsible for binding phosphate to mannose residues of lysosomal acid hydrolases (Canfield et al. 1998; Hasilik et al. 1981; Okada et al. 1985; Waheed et al. 1982). The resulting mannose-6-phosphate moiety of these hydrolases normally binds to specific receptors that direct the enzymes to the lysosomes. In ML II, these enzymes are not trafficked properly to the lysosomes and instead leak into the extracellular compartment. Thus affected lysosomes are secondarily deficient in most acid hydrolases (Lightbody et al. 1971) and are unable to degrade some macromolecules. In contrast, plasma levels of lysosomal hydrolases are high (Leroy et al. 1971; Sprigz et al. 1978). The resulting lysosomal storage, first reported in cultured fibroblasts (Hanai et al. 1971; Leroy and DeMars 1967), is seen as cytoplasmic inclusion bodies, particularly in the mesenchymal cells, hence the term I-cell (inclusion cell) disease (Leroy et al. 1971;

Tondeur et al. 1971). Because of the clinical similarities to MPS and sphingolipidosis, the disease is referred to as mucolipidosis II (Kaufmann et al. 1970), although there is apparently no excessive lipid storage in lysosomes (Hanai et al. 1971), and the precise nature of the accumulated materials remains elusive (Kornfeld and Sly 2000).

Human ML II is characterized by coarse facial features, severe skeletal abnormalities, psychomotor retardation, failure to thrive, growth retardation, and death within the first few years of life (Kornfeld and Sly 2000; Leroy and DeMars 1967; Leroy et al. 1969). One domestic shorthair cat with ML II has been described (Bosshard et al. 1996; Hubler et al. 1996). In this article we define the establishment of a colony of cats with ML II from a carrier male cat related to the original affected cat, characterize the clinical and biochemical features and the mode of inheritance, and document the close homology to ML II in humans.

Materials and Methods

Animals

One male (3739) and two female (3740 and 3741) phenotypically normal half siblings of the original affected domestic shorthair cat with ML II found in Switzerland (Bosshard et al. 1996; Hubler et al. 1996) were donated to the University of Pennsylvania. The male half sibling of the affected cat (no. 3739) was bred to his two female littermates as well as outcrossed to normal female domestic shorthair cats. Phenotypically normal F_1 females were backcrossed to cat 3739. F_1 females that produced affected kittens were also bred to phenotypically normal F_1 and F_2 males. Breeding records, physical examination findings, body weights, and time and cause of death were recorded for all offspring. Cats were raised and studied according to National Institutes of Health (NIH) and U.S. Department of Agriculture (USDA) guidelines for the care and use of laboratory animals in research. The animals were provided ad libitum food and water and housed with 12-hour light cycles at 21°C with 12–15 air exchanges per hour. Affected and related cats that did not die a natural death were euthanized at various ages using sodium phenobarbital in accordance with the guidelines of the American Veterinary Medical Association.

Lysosomal Enzyme Assays

The original affected cat's diagnosis was based on low leukocyte GlcNAc-phosphotransferase activity, severely increased serum activity of several lysosomal enzymes, and clinicopathologic findings (Bosshard et al. 1996; Hubler et al. 1996). Because the substrate for GlcNAc-phosphotransferase activity is not readily available and the molecular basis of feline ML II remains undefined, the ML II status of the cats in this article was determined by measuring the activity of several lysosomal enzymes in serum, as well as by the characteristic clinical signs and the typical cytoplasmic inclusions in the mesenchymal cells, as has been done in humans (Tondeur et al. 1971). Blood was collected via jugular venipuncture and serum was separated by centrifugation and stored frozen at −20°C until assayed. In addition, fibroblast cultures were aseptically prepared from tissue biopsies from the linea alba or pericardium of affected and normal cats at postmortem evaluation. Fibroblasts were grown (RPMI 1640 medium, Invitrogen Corp., Gibco, Grand Islands, NY) in T175 flasks, and at confluency, the fibroblasts were trypsinized, washed (Hanks' balanced salt solution without calcium and magnesium, Cellgro, Mediatech, Herdon, VA), and frozen at −130°C until analysis (Verma and Babu 1995). α-mannosidase (EC 3.2.1.24), β-glucuronidase (EC 3.2.1.31), and α-fucosidase (EC 3.2.1.51) activities were measured in serum and fibroblasts using the artificial substrate 4-methylumbelliferyl β-D-glucuronide (Sigma, St. Louis, MO), and activities were expressed as nmol/h/ml serum or mg protein as described (Bosshard et al. 1996; Glaser and Sly 1973; Wegner and Williams 1991). Protein concentrations were determined by the method of Lowry (Lowry et al. 1951).

Clinical and Laboratory Evaluations

Clinical, neurologic, ophthalmologic, and radiologic examinations were performed in a routine manner. Blood for complete blood cell counts (CBC) and serum chemistry determinations, as well as urine samples for urinalysis and a toluidine blue spot test (MPS spot test) for glycosaminoglycan (GAG) detection (Berry and Spinanger 1960) were obtained. Tissue samples for light microscopy were collected from cats at postmortem examination, fixed in buffered 10% formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. All clinical, laboratory, biochemical, and pathologic results of affected cats were compared to related and unrelated age-matched control cats.

Results

Breeding Studies and Pedigree Analysis

Breeding between a phenotypically normal male cat (no. 3739), a half sibling of the original cat with ML II, and his two phenotypically normal female siblings as well as to other unrelated female cats did not produce any phenotypically affected kittens. However, breeding between male 3739 and his daughters (F_1 females) and between F_1 or F_2 males and F_1 females produced phenotypically normal and affected kittens. One F_3 female was bred to an F_1 male. When outcrossing cats that produced affected kittens to unrelated normal cats, all kittens were phenotypically normal (data not shown). Litters that were born to parents that produced at least one affected cat between November 1999 and April 2002 were included in the study (Table 1). None of the affected kittens reached reproductive age. Cats were considered phenotypically affected when they exhibited typical clinical signs and had high serum lysosomal enzyme levels (see below). A total of 26 litters with 89 kittens were born to phenotypically healthy parents. There were 10 male and 9 female kittens that were affected among 14 litters with 63

kittens and at least 1 affected cat (Figure 1). A total of 7 kittens were stillborn (4 among the 63) and 1 kitten had no serum lysosomal enzymes determined. These eight kittens were excluded from further analysis. The proportion of affected kittens was 23.4%.

There were no affected kittens out of 89 in 12 litters generated from outcrossing parents that produced affected cats with normal unrelated cats, thus excluding a dominant mode of inheritance. Both genders were affected, which ruled out an X-linked recessive mode of inheritance. The affected kittens were siblings of phenotypically normal cats and were born to phenotypically normal parents. These findings support an autosomal recessive mode of inheritance. Although the population tested was biased by selecting litters that were born to parents that produced at least one affected cat, the 23.4% of affected cats is very close to the 25% expected for an autosomal recessive mode of inheritance. Chi-square analysis was 0.012 ($P > .05$; one degree of freedom), which supports the null hypothesis that the number of affected kittens observed was not different from the expected number of affected kittens for an autosomal recessive mode of inheritance.

Table 1. Breeding data of the phenotypically normal half sibling (no. 3739) of the original cat with mucolipidosis II

	Male/no. of females (generation)				
Breeding	3739/ 4 (F_1)	F_1[a]/ 2 (F_1)	F_2/ 4 (F_1)	F_1/ 1 (F_3)	Total
Litters					
Total	13	5	7	1	26
With affected kittens[b]	6	2	5	1	14
Without affected kittens	7	3	2	0	12
Affected offspring					
Total	6	4	8	1	19
Males	4	2	3	1	10
Females	2	2	5	0	9
Phenotypically normal offspring[c]					
Total	35	5	20	3	63
Males	23	2	6	2	33
Females	12	3	14	1	30
Stillborn offspring[d]					
Total	1	3	3	0	7

[a] F_1, F_2, F_3 refer to first, second, and third generations of offspring of male 3739.

[b] Affected male and female kittens had characteristic clinical signs and high serum lysosomal enzyme levels.

[c] Phenotypically normal kittens with lysosomal enzyme levels in the normal or carrier range.

[d] Stillborn kittens with no clinical examination or enzyme diagnosis available.

Biochemical Studies

Adult Cats

Because affected cats died or were euthanized before adulthood, serum activities of α-mannosidase, β-glucuronidase, and α-fucosidase were only determined in phenotypically normal adult cats that produced affected kittens, as well as normal unrelated controls. Serum enzyme activities from nine obligate carriers (including male 3739) and eight phenotypically normal unrelated healthy adult cats were measured (Table 2). All obligate heterozygotes had clearly higher serum lysosomal enzyme activities, ranging from two to three times normal. Thus a determination of α-mannosidase, β-glucuronidase, and α-fucosidase activity in serum with appropriate controls could readily differentiate carriers from normal adults. However, when the serum activities from adult cats were compared to those in kittens (see below), the range for the obligate carriers was markedly lower in the adults.

Neonatal Kittens (Less Than 1 Week)

Neonatal kittens were classified phenotypically based on the presence or absence of typical clinical signs until their death (see below). Serum lysosomal enzyme activities from 59 of 63 neonatal kittens of the 14 litters having at least one affected kitten were measured (4 were stillborn). Serum lysosomal activities from affected kittens were many-fold higher (11–73 times normal adult control) than activities of phenotypically normal littermates (Table 3), supporting the diagnosis of ML II. This study did not follow phenotypically normal kittens to adulthood and most of them were not used for breeding and enzyme analyses. Therefore their genotype could not be definitively determined. However, if one categorized phenotypically normal kittens into a group having at least two of the three measured enzymes increased by more than 1.5- but less than 6-fold above the mean of normal adult cats, 24 kittens were defined as heterozygotes and 16 as homozygous normal. Although the population tested was biased toward affected kittens, out of these 59 neonatal kittens the proportions of affected (32%), carrier (41%), and normal kittens (27%) were not statistically different from a 1:2:1 ratio and thus consistent with an autosomal recessive trait.

Microscopic examination of fibroblast cultures from kittens affected with ML II had multiple cytoplasmic granules approximately 10 μm in diameter (Figure 2), whereas fibroblasts from phenotypically normal relatives and unrelated healthy cats did not have inclusions. The activities of α-mannosidase, β-glucuronidase, and α-fucosidase were measured and were found to be reduced in fibroblasts from affected kittens as compared to normal cats (Table 4). The mean β-glucuronidase activity in affected fibroblasts was the highest, at 56% of normal, while the mean α-mannosidase activity with 9% of normal activity was the lowest.

Clinical Findings

Initial clinical features in affected kittens included failure to thrive and facial dysmorphism. Kittens with ML II had similar birth weights when compared to normal littermates,

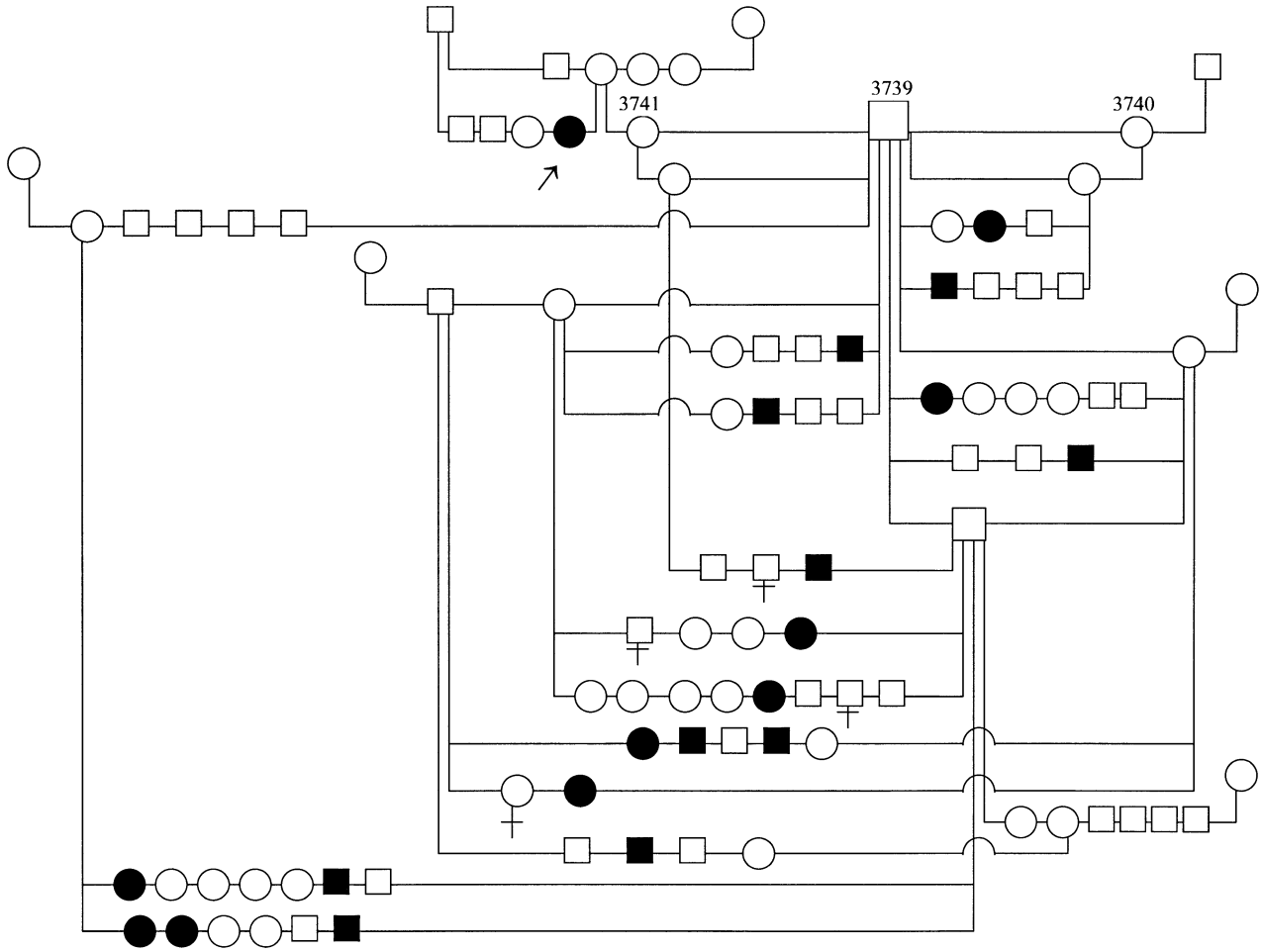

Figure 1. Pedigree of the cat family with mucolipidosis II. Litters with at least one affected ML II cat born to phenotypically normal parents between November 1999 and April 2002. The data from the litters with no affected kittens are not shown. Females are circles, males squares; affected are filled circles and squares, stillborn have a cross, and the propositus has an arrow. Affected kittens were offspring of phenotypically normal parents, they had phenotypically normal siblings, and both sexes were affected (47% females, 53% males). No affected kittens were born from outcrossing parents of an affected animal with normal unrelated cats. These findings are consistent with an autosomal recessive mode of inheritance.

but experienced significant weight and growth retardation that was evident by 3 months of age (Figure 3). Affected kittens failed to thrive and died or were euthanized due to disease progression between the first day of life and 216 days (mean 47 days, median 10 days; Figure 4), whereas morbidity and mortality in unaffected littermate kittens were negligible.

The affected cats appeared behaviorally dull and were quieter and less playful than their littermates. They showed poor muscle tone from the first week of life and developed progressive hind limb ataxia. Facial abnormalities present at birth included thickened eyelids, hypertelorism (wide-spaced eyes), frontal bossing, a depressed nasal bridge, and low-set ears (Figure 5). In addition, affected kittens had congenital carpal varus or valgus deformities, which became more pronounced as the cats grew.

Five affected kittens were auscultated by a board-certified cardiologist. Three of these kittens had normal heart sounds, while two kittens had continuous, machinery murmurs (grade 2/5) auscultated at the left and right heart base. Several affected kittens developed upper respiratory tract infections, whereas normal littermates did not. Four other affected cats developed pleural and peritoneal transudates in their terminal stages, one of them also developed a pericardial transudate. Thoracic and abdominal radiographs appeared normal, except for cardiomegaly and pleural effusions that developed as end-stage changes. Echocardiographic evaluations were performed in five of the affected kittens. Three kittens had mild to moderate cardiomegaly in an eccentric pattern with Doppler-detected continuous flow in the main pulmonary artery (although two of them had ascultatable murmurs). No significant abnormalities were detected in the other two kittens evaluated by echocardiography, although the left atrium appeared mildly enlarged in one.

Table 2. Mean lysosomal enzyme activities (± SD) in serum from adult obligate heterozygote cats for mucolipidosis II and normal unrelated cats

Lysosomal enzyme	Obligate carrier cats (n = 9)	Normal unrelated cats (n = 8)
α-mannosidase, nmol/h/ml	30,071 ± 6,340	10,564 ± 3,686
Percentage[a]	244 ± 53	95 ± 55
β-glucuronidase, nmol/h/ml	778 ± 326	265 ± 107
Percentage[a]	285 ± 162	96 ± 47
α-fucosidase, nmol/h/ml	454 ± 114	195 ± 67
Percentage[a]	218 ± 63	101 ± 51

SD = standard deviation.

[a] Percentage of normal control activity in samples run simultaneously.

Complete blood cell counts, serum chemistry values, and urinalyses of affected kittens were normal, except for a mild neutrophilia compared to healthy normal kittens and previously published values (Meyers-Wallen et al. 1984). The urine MPS spot test was slightly positive in three of seven affected kittens tested.

Radiographic abnormalities of the skeleton were noticed at an early age. Radial and ulnar bowing were evident by the first month of life (Figure 6A). Delayed mineralization of the femoral and tibial epiphyses was noticed by the second week of life (Figure 6B,C), but resolved by 2.5 months of age. Metaphyseal flaring occurred at 1 month of age. By 3 months of life, coxofemoral joint laxity and luxation of the antebrachial-carpal joints were seen (Figure 6A). Cervical vertebral fusion occurred between the fourth and fifth month of life (Figure 6D), and the lumbar vertebral bodies fused shortly thereafter. The vertebral bodies were short, and the dorsal spinal processes were misshapen. Other skeletal abnormalities such as spina bifida and hemivertebrae were observed in a few affected kittens.

On ophthalmologic examination of four affected cats followed from birth to death, absent menace and decreased pupillary light reflexes with no corneal changes were noticed after the third month of life. At 4 months of age, the pupils remained dilated in ambient light. Retinal development initially appeared normal, but at 2.5 months of age thinning of the dorsal retina venule and altered tapetal reflectivity were noticed (Figure 7). These fundic abnormalities progressed to end-stage retinal degeneration and blindness by 3.5 months of age, with optic disc pallor, marked attenuation of the retinal vessels, and tapetal hyperreflectivity (Figure 7).

As mentioned above, affected cats died naturally or were euthanized in end-stage disease. Other than the small stature and skeletal abnormalities, gross pathologic findings included bronchopneumonia (n = 1) and pleural, peritoneal, and pericardial effusions (n = 4). Cardiomegaly was noticed in two of four affected kittens that survived longer than 3 months. Histopathologic findings and biochemical analysis of tissues for enzymes and storage material will be reported elsewhere.

Discussion

Thus far, more than 40 LSDs have been described in humans, and for 21 of these, naturally occurring animal models have been reported. Of these 21, 13 have been described in cats (Haskins and Giger 1997; Haskins et al. 2002). ML II, first described as I-cell disease in humans in 1967 (Leroy and DeMars 1967), has been reported in approximately 50 patients, with a prevalence of 0.16 per 100,000 births (Poorthuis et al. 1999) and remains a rare, unique LSD that has not been completely characterized. After the initial description of one cat with ML II (Bosshard et al. 1996; Hubler et al. 1996), we describe here the establishment of a colony of cats with ML II and demonstrate its degree of homology to the disorder in humans.

A diagnosis of ML II in humans is made based on clinicopathologic findings (Leroy et al. 1971), the characteristic inclusions in fibroblasts (i.e., I-cells) (Leroy and DeMars 1967), with a secondary deficiency of fibroblast lysosomal enzyme activities (Sprigz et al. 1978; Taber et al. 1973; Whelan et al. 1983), and high activities of lysosomal enzymes in serum or plasma and cell culture media (Sprigz et al. 1978;

Table 3. Mean lysosomal enzyme activities (± SD) in serum[a] from kittens with mucolipidosis II and phenotypically normal littermate and unrelated kittens

Lysosomal enzyme	Affected kittens (n = 19)	Phenotypically normal littermate kittens[b] (n = 40)	Normal healthy unrelated kittens (n = 5)
α-mannosidase, nmol/h/ml	63,636 ± 26,185	13,590 ± 9,802	8,875 ± 1,609
Percentage[c]	1,180 ± 346	246 ± 136	122 ± 22
β-glucuronidase, nmol/h/ml	9,239 ± 2,910	670 ± 775	144 ± 80
Percentage[c]	7,369 ± 3,048	544 ± 678	84 ± 47
α-fucosidase, nmol/h/ml	3,003 ± 1,299	730 ± 773	128 ± 25
Percentage[c]	2,576 ± 972	876 ± 1,074	269 ± 53

SD = standard deviation.

[a] Serum samples taken at less than 1 week of age.

[b] Data includes heterozygote and normal kittens.

[c] Percentage of normal adult control activity run simultaneously.

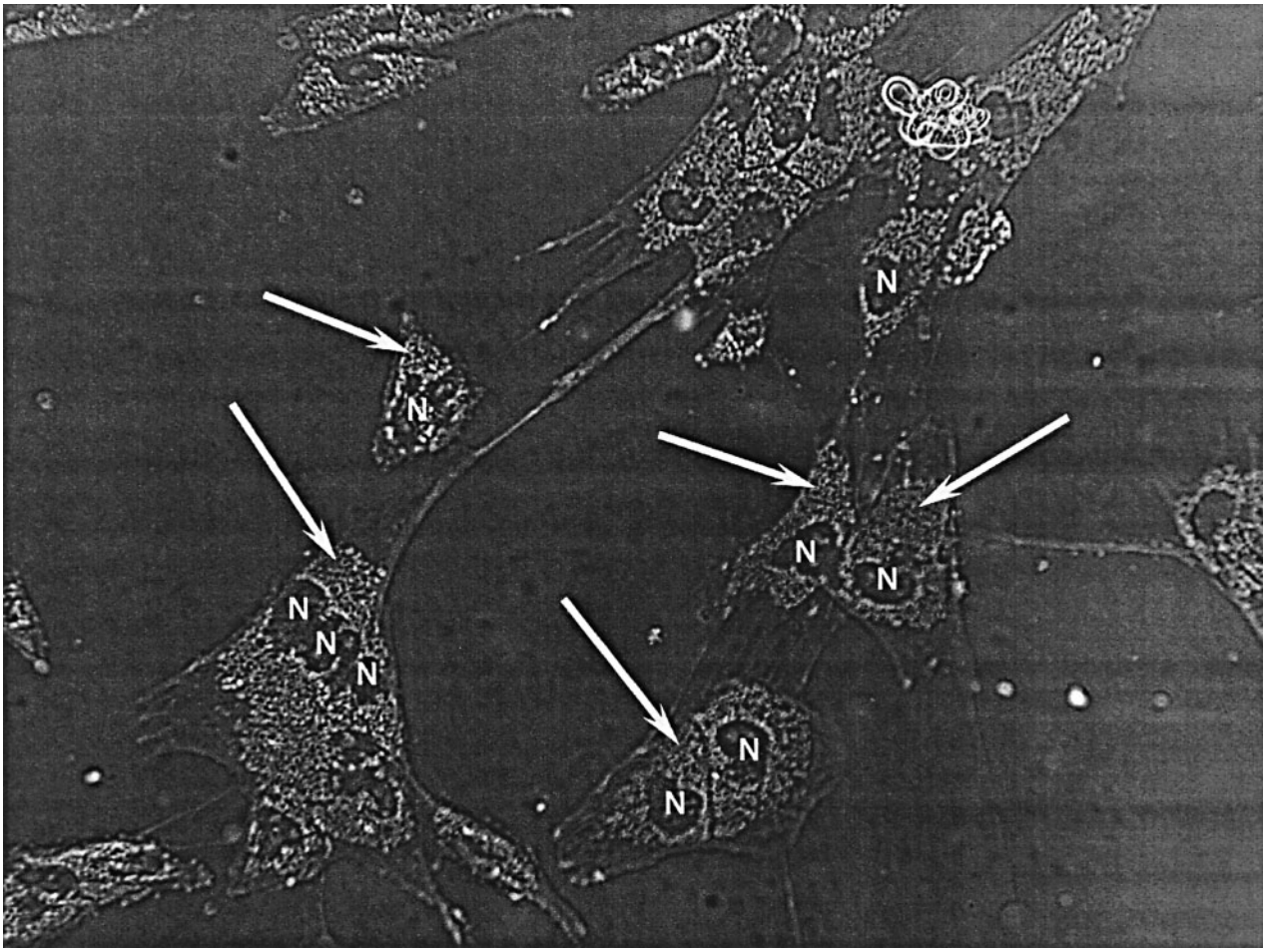

Figure 2. I-cells: Cytoplasmic inclusion bodies in fibroblasts of a kitten with mucolipidosis II. Multiple cytoplasmic granules (arrows) approximately 10 μm in diameter in fibroblasts cultured from an affected kitten shown at 100× magnification. N = nucleus.

Whelan et al. 1983). A deficiency of GlcNAc-phosphotransferase activity in fibroblasts and other organs can be demonstrated (Hasilik et al. 1981). The enzyme GlcNAc-phosphotransferase is a large multimeric protein with three subunits produced by two genes. One gene codes the α and β subunits with 21 exons spanning 92 kb and is located on human chromosome 12. The coding region for the γ subunit is on chromosome 16 (Canfield et al. 1998). However, the sequence of the GlcNAc-phosphotransferase genes and mutations causing ML II in humans has not been published (Canfield et al. 1998 and personal communication). This sequence information will permit the further molecular characterization of ML II in cats.

Table 4. Mean lysosomal enzyme activities (± SD) in fibroblasts from kittens with mucolipidosis II and normal unrelated control cats

	Affected cats (n = 7)	Normal cats (n = 3)
α-mannosidase, nmol/h/mg	41 ± 21	479 ± 156
Percentage[a]	9 ± 4	
β-glucuronidase, nmol/h/mg	308 ± 51	555 ± 45
Percentage[a]	56 ± 9	
α-fucosidase, nmol/h/mg	32 ± 44	280 ± 131.1
Percentage[a]	11 ± 15	

SD = standard deviation.

[a] Percentage of the mean of normal activity.

Fibroblasts from affected kittens contained the characteristic inclusions, which appeared identical to those seen in humans (Hanai et al. 1971; Leroy and DeMars 1967). The content of these inclusions has not been fully characterized but appears not to be GAGs or lipids (Hanai et al. 1971). Although the GlcNAc-phosphotransferase activity was measured in the related affected cat, but not in animals described here, affected kittens had extraordinarily high serum activity of the measured lysosomal enzymes; similar increases have been reported in human patients (Weismann et al. 1971). Furthermore, these enzyme activities were diminished in fibroblasts from affected kittens. The residual enzyme activities in fibroblasts may be due to passive leakage

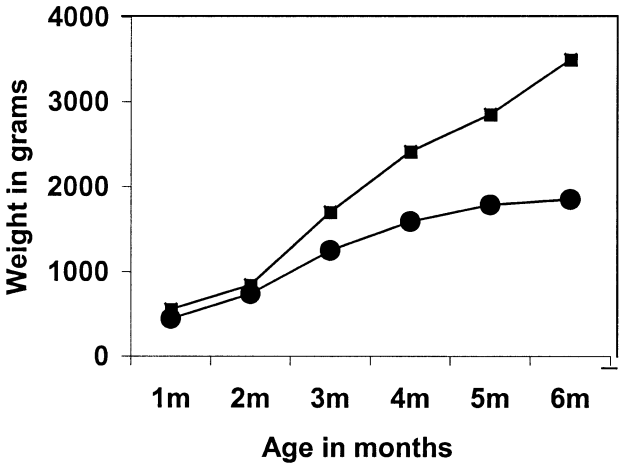

Figure 3. Body weight in grams from birth to 6 months of kittens with mucolipidosis II and unaffected littermates. Affected kittens had similar birth weights when compared to normal littermates but experienced significant retardation evident after 2 months of age. Circles represent ML II affected, squares represent phenotypically normal littermates. (*$P < .05$, single-factor ANOVA between 3 and 5 months).

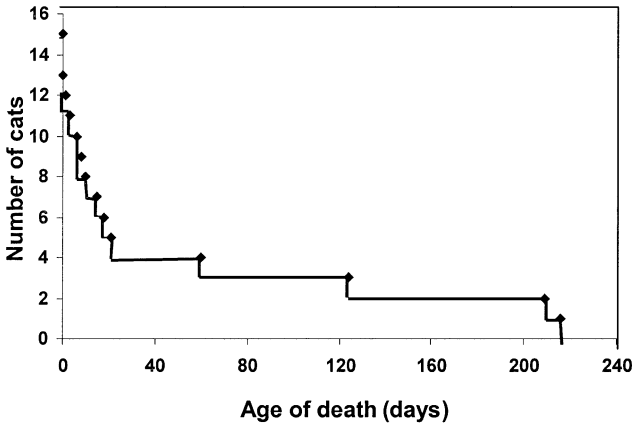

Figure 4. Survival of 15 cats with mucolipidosis II. Survival of 15 affected kittens that died or were euthanized with end-stage disease: 11 of these cats died or were euthanized before 3 weeks of age and all were dead by 7 months of age, with the longest survival being 216 days (mean 47 days, median 10 days). Four affected kittens euthanized for eye histology before end-stage disease have been excluded.

from the cell culture medium or to alternative mechanisms for trafficking enzymes to the lysosome and may be cell type specific (Dittmer et al. 1999; Glickman and Kornfeld 1993). Thus residual activity of lysosomal enzymes in tissue may explain why humans and cats affected with ML II do not show storage and urinary excretion of many lysosomal substrates such as GAG. The urine MPS spot test was slightly positive in three of seven affected kittens tested, however, this is a common finding in young animals.

Family studies in humans with ML II have been limited. One report described 21 patients in 18 families (Okada et al. 1985), while others were case reports of one to eight patients. Based on these studies, ML II in humans is thought to be an autosomal recessive disease (Kornfeld and Sly 2000; Leroy et al. 1971; Taber et al. 1973). The breeding studies reported here of the male half sibling of the original domestic shorthair cat with ML II clearly demonstrated an autosomal recessive mode of inheritance. Approximately one-quarter of all kittens were affected, with an equal number of both genders born to phenotypically normal related parents, but no affected kittens were born from outcrossings to unrelated cats. Parents of affected kittens had moderately increased serum activity of several lysosomal enzymes. Similarly parents of one child with ML II were studied and demonstrated a twofold increase in serum lysosomal enzyme activities (Weismann et al. 1971). The increase in these enzymes in obligate carriers suggests that the partial deficiency of GlcNAc-phosphotransferase activity leads to enzyme leakage into plasma, which allowed the identification of heterozygotes in this family of cats. However, this partial defect did not result in any clinical abnormalities in heterozygotes.

As in human patients with ML II (Beck et al. 1995; Sprigz et al. 1978), clinical signs in cats can be recognized within the neonatal period and there is some clinical variability between individuals. The facial and skeletal morphologic changes are characteristic of MPS, but are generally not specific for a particular type of MPS. Radiologic features of ML II in children includes claw hands, pes valgus, short metacarpals, coarse trabecular pattern of the long and short bones, coxofemoral and shoulder dislocation, lumbar scoliosis, inferior vertebral body beaking, dysostosis multiplex, diffuse mineralization, osteopenia with periosteal diaphyseal new bone formation, and pathologic fractures (Patriquin et al. 1977; Sprigz et al. 1978; Whelan et al. 1983). These changes occur earlier in life than with other mucolipidoses (Taber et al. 1973). The kittens affected with ML II demonstrated very similar radiologic signs, which were present in the first month of life and progressed rapidly. It appears likely that the skeletal abnormalities are caused by deficient activity of lysosomal enzymes relative to substrate load in bones and cartilage, resulting in severe storage, as demonstrated in chondrocytes (Bosshard et al. 1996).

The psychomotor development in affected children does not proceed beyond 6–9 months of age and death occurs in the first decade of life (Kornfeld and Sly 2000; Okada et al. 1985; Taber et al. 1973). Similarly the cats with ML II appeared behaviorally dull and failed to thrive. To the best of our knowledge, lesions have not been documented in the brain of affected human patients, although inclusions in the cerebral cortical neurons were reported in the original ML II affected cat (Bosshard et al. 1996).

Corneal storage has not generally been appreciated in human patients (Okada et al. 1985; Sprigz et al. 1978; Taber et al. 1973; Whelan et al. 1983) or cats with ML II, except for a couple of human patients (Libert et al. 1977). However, the

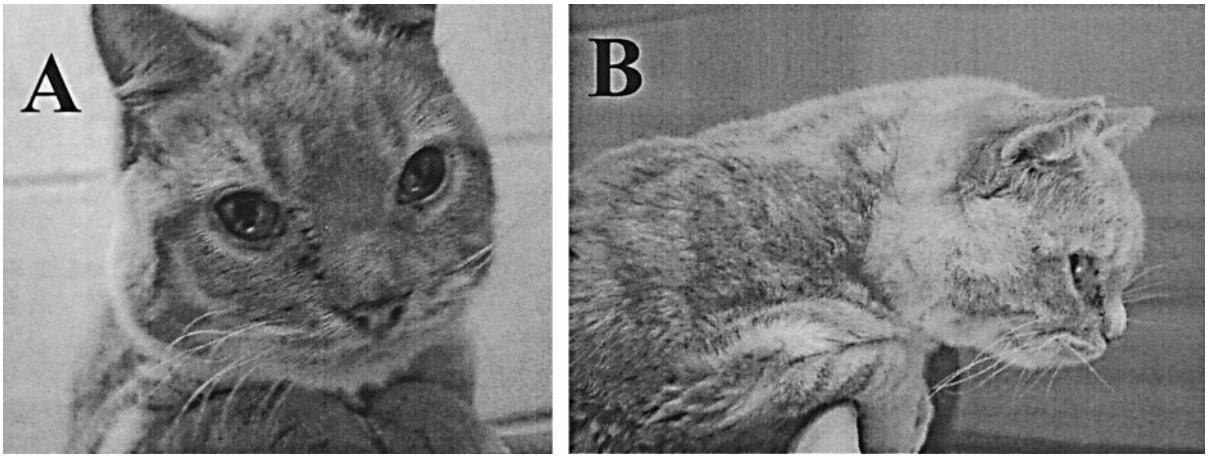

Figure 5. Facial abnormalities of kittens with mucolipidosis II were present early, including **(A)** thickened eyelids, wide-spaced eyes (hypertelorism), and a flat, broad face, as well as **(B)** frontal bossing, depressed nasal bridge, and low-set ears.

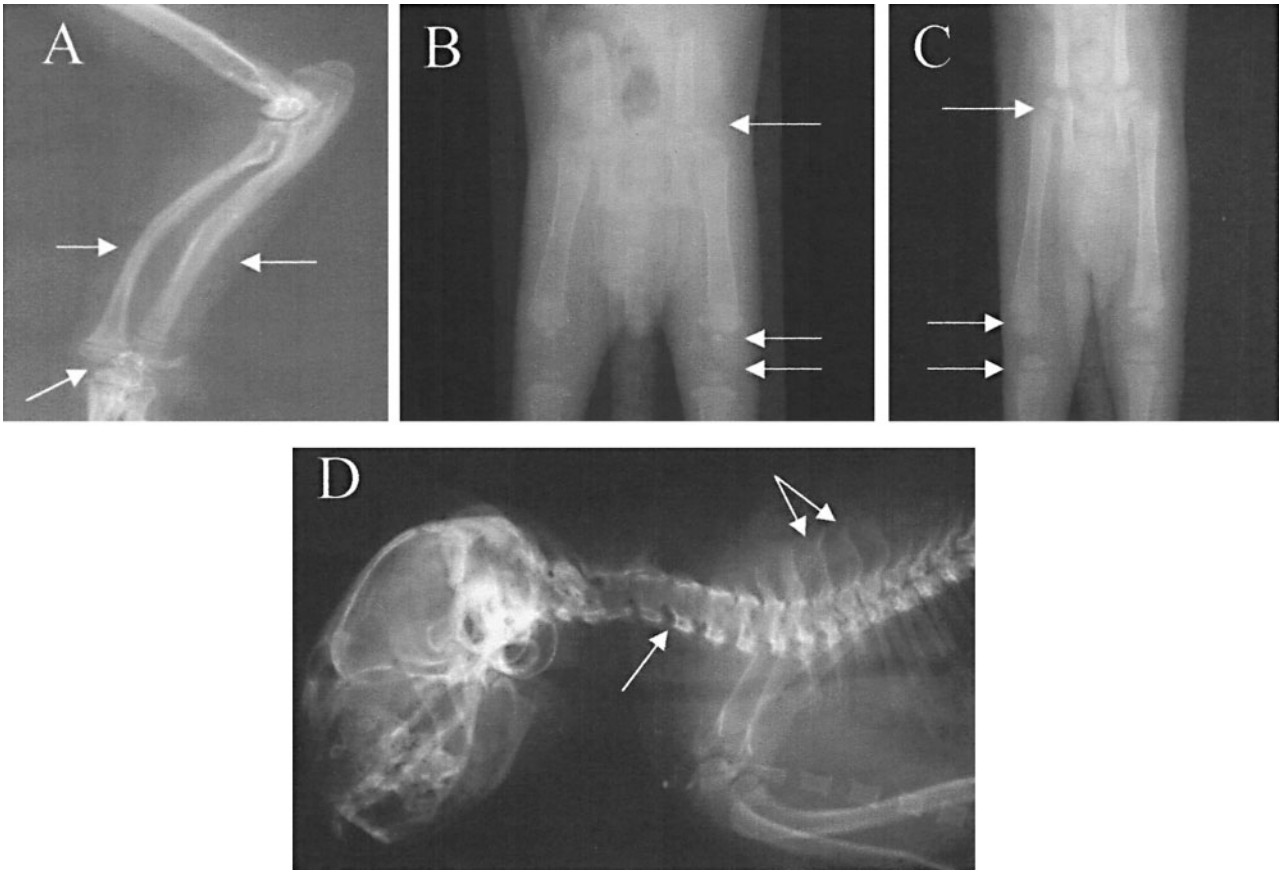

Figure 6. Radiographic findings of kittens with mucolipidosis II. **(A)** Radial and ulnar bowing and luxation of the antebrachial-carpal joint in a 6-month-old affected cat. **(B)** Delayed mineralization of the femoral head, distal femur, and proximal tibia in an affected cat compared to **(C)** his control littermate, both at 1 month of age. **(D)** Cervical vertebrae of a 6-month-old affected cat. The vertebral bodies are shortened and the dorsal spinal processes are misshapen. (Arrows point to all abnormalities.)

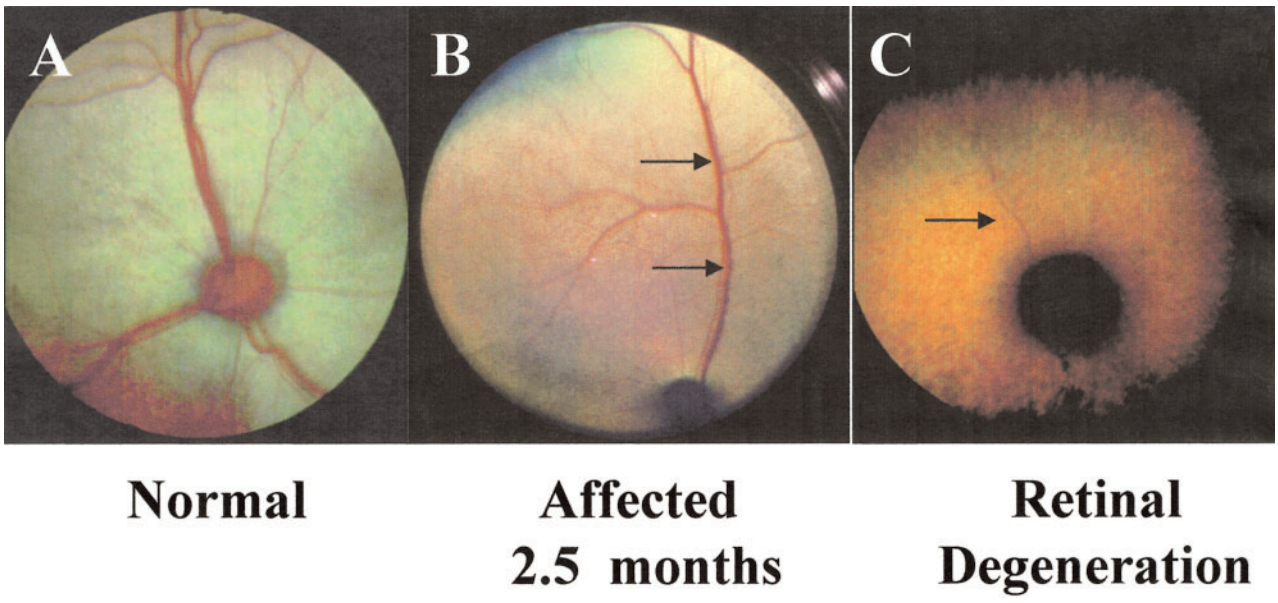

Figure 7. Retinal changes of kittens with mucolipidosis II. **(A)** A normal control retina compared to **(B)** the retina of an affected cat at 2.5 months of age, with notable thinning of retinal venules (arrows), and **(C)** the retina of an affected cat at 5 months of age with signs of diffuse retinal degeneration, including tapetal hyperreflectivity and vascular attenuation (arrows). The optic disc is dark due to a photographic effect.

affected kittens studied developed progressive retinal degeneration resulting in blindness by a few months of age, which does not appear to be a typical feature of the human disease. Only one human patient with retinal degeneration has been reported (Okada et al. 1985); however, ophthalmologic studies of human patients are limited. The cause of the photoreceptor degeneration is unclear. Because of the trafficking defect, essential enzymes, such as proteases, may be functionally absent in the lysosomes, resulting in rhodopsin accumulation, which would lead to retinal degeneration. Of interest is that humans with ML III (Trabousi and Maumenee 1986) and some MPS disorders develop retinal abnormalities.

As in affected kittens, ML II in human patients is rapidly progressive. The causes of death in the first decade of life have not always been determined, but some children die due to heart failure exacerbated by pneumonia (Kornfeld and Sly 2000; Okada et al. 1985). Likewise, some kittens affected with ML II developed upper respiratory infections and cardiac failure; others died suddenly without an identifiable cause. Systolic murmurs (Okada et al. 1985; Sprigz et al. 1978), cardiomegaly, in particular left ventricular enlargement on chest radiographs (Satoh et al. 1983; Sprigz et al. 1978), cardiomyopathy (Muller et al. 2000), and congenital cardiac anomalies such as dextrocardia and mitral valve prolapse accompanied by a prolong QT interval (Satoh et al. 1983) have been described in human patients. Pathology reports in ML II-affected children have revealed heart failure due to aortic regurgitation (Tang et al. 1995) and storage of foam cells in the myocytes with thickened heart valves (Satoh et al. 1983). The presence of cardiomegaly, thoracic transudates, and pansystolic heart murmurs in affected kittens was consistent with heart failure.

Therapy for most LSDs involves providing the enzyme deficient in activity by intravenous injection of purified enzyme, heterologous tissue transplantation (which acts to make and secrete normal enzyme), or correcting autologous cells by gene therapy. In ML II, the enzyme that is deficient in activity is in the Golgi apparatus and would require gene therapy of many cell types to correct the primary defect. However, the clinical signs of ML II are the result of trafficking failures of several distinct lysosomal enzymes. As different enzymes may be responsible for the clinical signs due to storage of various substrates in different tissues, the most promising therapeutic approach would be heterologous tissue transplantation (such as bone marrow) that would secrete a variety of normal lysosomal enzymes for uptake by different tissues. A recent report of bone marrow transplantation in three children younger than 1.5 years appeared to transiently slow disease progression (Peters et al. 2002). Thus far this approach in two 1-month-old ML II kittens does not appear to be helpful (Haskins et al., unpublished data).

In conclusion, this colony of cats is currently the only animal model of human ML II. As in children with ML II, affected kittens had early onset and progressive disease, with similarly characteristic clinical signs, a short life span, abnormal biochemical findings, and an autosomal recessive mode of inheritance. Future investigation will define the histopathologic lesions and the molecular basis of ML II in cats. The close homology of the disease in these two species indicates that cats with ML II will be a suitable model for the investigation of the pathophysiology of ML II and the

development and assessment of novel therapeutic strategies for this unique lysosomal storage disease.

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