J Vet Intern Med 2003;17:495–498

Prevalence of Mucopolysaccharidosis Type VI Mutations in Siamese Cats

A.C. Crawley, F.H. Muntz, M.E. Haskins, B.R. Jones, and J.J. Hopwood

Mucopolysaccharidosis type VI (MPS VI), a lysosomal storage disease, is one of the more prevalent inherited diseases in cats and is commonly found in cats with Siamese ancestry. The prevalence of 2 known MPS VI mutations in cats was investigated in 101 clinically normal Siamese cats, in 2 cats with clinical signs of MPS VI, and in 202 cats from 4 research colonies. The mutation L476P, which causes a severe clinical phenotype, was present on both alleles in the known MPS VI cats from Italy and North America and was present in all research colonies that originated from North America. However, L476P was not detected in the Siamese population screened. In contrast, the mutation D520N, which causes a mild clinical phenotype, was identified in 23 of 202 (11.4%) alleles tested in Siamese cats from 3 continents, 2 of which were homozygous for D520N. Thus, the D520N mutation was widespread, and it is likely that cats inheriting both mutations (L476P/D520N compound heterozygotes) would be in the general Siamese population, particularly in North America. Practitioners should note the high incidence of degenerative joint disease in these animals.

Key words: Animal models; Genetic disease; Inherited joint disease; Lysosomal storage diseases.

Lysosomal storage diseases are inherited disorders of cellular metabolism. More than 40 lysosomal storage diseases have been described in humans,[1] a number of which have also been described in domestic animals.[2] Affected animals are normal at birth, but they then have progressive growth abnormalities and generally exhibit neurologic abnormalities, skeletal abnormalities, or both. Affected animals are usually purebred or inbred.

Mucopolysaccharidosis type VI (MPS VI) is an autosomal recessive lysosomal storage disease caused by deficient activity of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase (4-sulfatase). This enzyme is essential for the degradation of the glycosaminoglycan dermatan sulfate, which is found in various connective tissues, particularly cartilage and bone.[3] Skeletal disease is the predominant abnormality associated with this disorder.

MPS VI was 1st described in a family of Siamese cats[4] and has since been reported in at least 7 families of Siamese cats in North America.[5] Other cases have been reported in a Siamese cat in Quebec, Canada,[6] in a long-haired Siamese cat in Naples, Italy,[7] and in a domestic long-haired kitten in Maine.[8] Several separate breeding colonies have been established for research purposes from 3 of the early Siamese cat families from New York City, northern New Jersey, and the western United States. These colonies have been used to investigate the pathophysiology of the disease and to evaluate the efficacy of bone marrow transplantation, enzyme replacement, and gene therapies for future application to human MPS VI patients.

Clinical features in MPS VI cats are 1st evident at 6–8 weeks of age and include a broad face and shortened nose, small ears, stunted growth, and reduced flexibility of the cervical spine. Severe widespread skeletal disease is apparent radiographically, including a generalized osteopenia, with a coarse trabecular pattern and severe epiphyseal dysplasia of the vertebrae. Animals have progressive difficulty walking, with hindlimb paresis or paralysis developing in some animals due to the bony compression of the spinal cord. A progressive degenerative joint disease also occurs, which is evidenced radiographically by an irregular subchondral bone outline and osteophyte development. A range in skeletal disease severity has been observed between littermates.[5,7,9,10]

In an MPS VI cat colony derived from one family (family 3) of Siamese cats,[9] the feline 4-sulfatase complementary DNA (cDNA) from MPS VI–affected cats and littermates was screened for disease mutations to assist with other research objectives. Two separate mutations were identified. Cats homozygous for a leucine to proline substitution at codon 476 (L476P/L476P) exhibited classic MPS VI, with severe skeletal disease.[11,12] In contrast, cats homozygous for an aspartic acid to asparagine substitution at codon 520 (D520N/D520N) had no evidence of clinical disease; however, they had an enzyme deficiency characteristic of MPS VI.[12,13] Because both mutations were found in the same colony, some cats had inherited both mutations as separate alleles (L476P/D520N compound heterozygotes). A high incidence of degenerative joint disease with normal skeletal growth was observed in these animals as well as the enzyme deficiency characteristic of MPS VI.[12] The leukocytes from cats that were L476P and D520N homozygotes and L476P/D520N compound heterozygotes had 4-sulfatase activity values of 0.3, 1.9, and 1.0%, respectively, compared to those of normal cats. Obvious radiographic changes were evident in some L476P/D520N compound heterozygotes as early as 1 year of age; however, other animals with the same genotype were radiographically normal at 3–4 years of age. Some animals were inactive, had

From the Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, Adelaide, South Australia (Crawley, Hopwood); the Department of Veterinary Pathology, University of Glasgow Veterinary School, Glasgow, Scotland, UK (Muntz); the Department of Pathobiology and Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA (Haskins); and the Department of Small Animal Clinical Studies, Faculty of Veterinary Medicine, University College Dublin, Dublin, Ireland (Jones).

Reprint requests: Allison Crawley, PhD, Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, 72 King William Road, North Adelaide, 5006 South Australia; e-mail: allison.crawley@adelaide.edu.au.

Submitted September 9, 2002; Revised November 13, 2002; Accepted January 14, 2003.

0891-6640/03/1704-0004/$3.00/0

a stiff gait, and were reluctant to jump; however, this was also quite variable among different animals.

Mutation analysis of purebred Siamese cats, cats known to have MPS VI, and cats from MPS VI research colonies was undertaken to determine the prevalence of both of these mutations in the wider Siamese cat population. This would also enable an assessment of the likely occurrence of cats with mild MPS VI (L476P/D520N compound heterozygotes), which may have previously been diagnosed with nonspecific degenerative joint disease. In addition, the analysis of MPS VI animals in the different research colonies would establish whether they shared the same genotypes and hence whether research observations were directly comparable between cats from different research colonies.

Materials and Methods

Known MPS VI Cats

Purified genomic DNA was obtained from 1 long-haired Siamese cat from Italy known to have MPS VI.[7] DNA from 1 other known MPS VI domestic short-haired cat of unknown ancestry from Pennsylvania was also sampled. These known MPS VI cats were diagnosed on clinical grounds and then by urinalysis and enzymology (as described by Di Natale et al[7]).

MPS VI Research Colonies

Whole blood spotted onto filter paper (''Guthrie'' cards) that had been dried and stored at room temperature was obtained from 19 cats from 3 MPS VI research colonies in North America. These research colonies were established from 3 distinct and apparently unrelated families of Siamese cats from within the United States, each originating from a cat known to have MPS VI (families 2 and 3 as described by Haskins et al[9]). No interbreeding occurred between these colonies. Family 3 was the source of heterozygote cats used to establish a 4th research colony in Australia, which was also sampled ($n > 200$ cats). All research animals were cared for according to the principles outlined in either the National Institutes of Health Guide for the Care and Use of Laboratory Animals or the National Health and Medical Research Council Australian code of practice for the care and use of animals for scientific purposes.

General Siamese Cat Population

DNA samples were obtained from Siamese cats in 6 countries (England [$n = 5$], Ireland [$n = 12$], Argentina [$n = 2$], the United States [$n = 12$], the Netherlands [$n = 4$], and Australia [$n = 66$]). Most samples were collected as whole-blood Guthrie samples from clinically normal cats by veterinary practitioners. All Australian samples were from a commercial veterinary diagnostic laboratory where whole blood collected into EDTA for diagnostic purposes was retrieved from cats designated Siamese on submission forms. EDTA blood samples were frozen at −20°C until use. DNA was crudely prepared from the EDTA blood samples by alkali digestion of white cell pellets. Polymerase chain reaction (PCR) was performed on the supernatants.[11,13]

The genotype of animals was established from PCR-based analysis of dried blood spots or from purified genomic DNA.[11,13] Restriction analysis of PCR products encoding a region of the feline 4-sulfatase cDNA was performed with the *Hae*III restriction enzyme to identify a leucine to proline change at codon 476[11] and with the *Bsl*I or *Ava*II restriction enzyme to identify an aspartic acid to asparagine change at codon 520.[13]

Results

Both cats known to have MPS VI from Italy and Pennsylvania were L476P homozygotes (4 of 4 alleles tested); one was a long-haired Siamese (as described by Di Natale et al[7]), and the other was a domestic shorthair of unknown ancestry (Table 1). All MPS VI cats within established research colonies were also L476P homozygotes. The 3 original research colonies were established from apparently unrelated MPS VI clinical cases in North America (families 2 and 3 as described by Haskins et al[9]). The 4th research colony in Australia was derived directly from family 3 heterozygote cats imported from the United States. The identification of the D520N mutation in 2 of the family 3 Pennsylvania cats (Table 1) as well as in the Australian colony confirmed that the D520N mutation 1st described within the Australian colony originated from the American research colony.[13]

Table 1. Detection of feline 4-sulfatase mutations in established MPA VI colonies or known MPS VI clinical cases.

Origins	No. Cats Genotyped	Mutations Present
Pennsylvania family 2[a] & 3[b] colonies	3	L476P/L476P
	2	L476P/D520N
	8	L476P/+[c]
Colorado colony[d]	1	L476P/L476P
	5	L476P/+
Australia colony[e]	>50	L476P/l476P
	>50	L476P/D520N
	>50	L476P/+
	>50	D520N/+
Italy—known MPS VI cat	1	L476P/L476P
Pennsylvania—know MPS VI cat	1	L476P/L476P

MPS VI, mucopolysaccharidosis type VI.

[a] Originally from New York City and maintained as an isolated breeding line.

[b] Originally from New Jersey and maintained as an isolated breeding line.

[c] + denotes wild-type allele.

[d] Originally from the western United States and maintained as an isolated breeding line.

[e] Established from Pennsylvania family 3 cats.

The D520N mutation was detected in 23 of 202 alleles (11.4%, $n = 21$ of 101 cats) tested from the general Siamese cat population from several countries, namely Ireland, Argentina, the Netherlands, Australia, and the East Coast of the United States (Table 2). A similar prevalence of 11% ($n = 13$ of 66 cats) for the D520N mutation was observed in the largest sample group, from Melbourne, Australia. Two animals were homozygous for the D520N mutation—1 in Australia and 1 in Pennsylvania. The L476P mutation was not detected in any samples from the general Siamese cat population.

Discussion

Variable degrees of inbreeding are inevitable in the less common breeds of domestic pets, increasing the chances of encountering recessively inherited genetic diseases. MPS VI, an autosomal recessive disorder, has been most commonly reported in Siamese cats and is due to absent or defective activity of intralysosomal 4-sulfatase. Animals homozygous for the feline 4-sulfatase mutation L476P ex-

Table 2. Prevalence of feline 4-sulfatase mutations in Siamese cats from the general Siamese population.

Origins	No. Alleles Tested	L476P	D520N	(%)
Bristol, England	10	0/10	0/10	
Dublin, Ireland	24	0/24	3/24[a]	(12.5%)
Argentina	4	0/4	2/4[b]	(50%)
The Netherlands	8	0/8	1/8	(12.5%)
Melbourne, Australia	132	0/132	14/132[c]	(10.6%)
Pennsylvania	24	0/24	3/24[c]	(12.5%)
Total	202	0	23	(11.4)

[a] Two animals possibly related.
[b] Possibly littermates.
[c] One cat was a D520N homozygote.

hibit classic MPS VI, with extensive and progressive skeletal disease that usually presents as stunted growth and facial dysmorphia as early as 6 weeks of age.[12] The detection of the L476P mutation in all 3 North American research colonies (1 in Colorado and 2 in Pennsylvania) and in both the Italian and Pennsylvanian MPS VI clinical cases indicates that there are pockets of higher frequency of the L476P mutation, particularly in North America. It can be speculated that the published reports of at least 10 apparently unrelated MPS VI cats exhibiting a severe phenotype, which were found predominantly in North America, are also due to the L476P mutation.[5–8] However, the absence of this mutation in the general Siamese cat population suggests that its frequency is either relatively low or that it may have a higher prevalence within certain breeding lines that were excluded in the sampling process.

Conversely, the high prevalence (11.4%) of the D520N feline 4-sulfatase mutation in the general Siamese cat population in 5 of the 6 countries sampled implies an extensive presence of carriers, to the extent that 2 D520N homozygotes were detected. Of 18 D520N homozygotes up to 5 years of age evaluated radiographically, only 1 exhibited an atypical joint disease in the shoulder as seen in the L476P/D520N compound heterozygotes (see below).[12] Therefore, in the clinical situation, these animals would most likely be indistinguishable from normal Siamese cats, although it is unknown whether more clinically observable joint lesions may develop in older animals than in those previously studied. It is possible that some animals sampled in the current study were closely related; however, the carrier frequencies from a number of centers were comparable, which suggests that results were not skewed.

The relatively high frequency and wide distribution of the D520N mutation and the presence of the L476P mutation in several centers suggest that L476P/D520N compound heterozygotes must be present in the Siamese cat population, particularly in North America. These animals have normal skeletal growth but a high incidence of degenerative joint disease.[12] In a radiographic study of cats that were 25 L476P/D520N compound heterozygotes between 0.9 and 6.7 years of age, 64% had mild-to-severe remodeling of the caudal aspect of the proximal humeral epiphysis, and 20% had generally less severe bilateral degenerative changes in the femorotibial joints. Joint disease was quite variable in severity and did not necessarily correlate with age.[12] Therefore, mild MPS VI should be discussed as a differential diagnosis in Siamese cats presenting with joint stiffness or radiographic evidence of joint disease, which, in turn, may alter disease management and the use of animals in pedigree breeding. Both D520N homozygotes and L476P/D520N compound heterozygotes have characteristically mild basophilic cytoplasmic granulation in neutrophils on routinely stained blood films.[12] More extensive morphologic changes are present in leukocytes from L476P homozygotes. Therefore, a blood film examination may be a useful initial screening test.

A diagnosis of MPS VI cannot be made on clinical grounds alone, because other lysosomal storage diseases with skeletal manifestations have been detected in cats, such as MPS I and VII and mucolipidosis.[14–16] Accurate diagnoses of MPS VI must ultimately be made by specialized laboratories that measure urinary glycosaminoglycans and 4-sulfatase activity in leukocytes or fibroblasts. The PCR-based mutation analysis of DNA samples as described in this paper can identify the L476P and D520N mutations in feline 4-sulfatase; however, other mutations potentially present in populations of cats would remain undetected by these methods.

In summary, the presence of both L476P and D520N mutations in the feline 4-sulfatase gene in Siamese cats from North America compared with the absence of the L476P mutation in the United Kingdom and Australia should alert North American practitioners to the potential for the presence of both a severe and mild MPS VI disease syndrome in Siamese cats. Hence, when generalized skeletal disease or atypical degenerative joint disease is observed in Siamese cats, classic severe MPS VI or mild MPS VI should be discussed as a diagnosis.

Acknowledgments

The authors gratefully acknowledge the collection of dried blood spots or genomic DNA from the following people: Margaret Weil from the University of Pennsylvania, Philadelphia, PA; Dr Mary Anna Thrall from Colorado State University, Fort Collins, CO; Dr Tim Gruffydd-Jones from the University of Bristol, UK; Dr Paola Di Natale from the University of Naples Federico II, Naples, Italy; and Dr Bernard van Oost from the University of Utrecht, Utrecht, the Netherlands. We also greatly appreciated the cooperation of the staff at the Central Veterinary Diagnostic Laboratory, Melbourne, Australia, for the retrieval of Siamese blood samples from general diagnostic samples. This work was supported by an NHMRC program grant (ACC, JJH) and by NIH grants DK25759 and RR02512 (MEH).

References

1. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA 1999;281:249–254.

2. Jolly RD, Walkley SU. Lysosomal storage diseases of animals: An essay in comparative pathology. Vet Pathol 1997;34:527–548.

3. Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D, ed. The Metabolic and Molecular Bases of Inherited Disease, 8th ed. New York, NY: McGraw-Hill; 2001:3421–3452.

4. Jezyk PF, Haskins ME, Patterson DF, et al. Mucopolysacchari-

dosis in a cat with arylsulfatase B deficiency: A model of Maroteaux-Lamy syndrome. Science 1977;198:834–836.

5. Haskins ME, Bingel SA, Northington JW, et al. Spinal cord compression and hindlimb paresis in cats with mucopolysaccharidosis VI. J Am Vet Med Assoc 1983;182:983–985.

6. Breton L, Guerin P, Morin M. A case of mucopolysaccharidosis VI in a cat. J Am Anim Hosp Assoc 1983;19:891–896.

7. Di Natale P, Annella T, Daniele A, et al. Animal models for lysosomal storage diseases: A new case of feline mucopolysaccharidosis VI. J Inherited Metab Dis 1992;15:17–24.

8. Beekman GK. Mucopolysaccharidosis VI in a kitten: A case report and discussion of feline Maroteaux-Lamy syndrome. Feline Pract 1993;21:7–11.

9. Haskins ME, Jezyk PF, Patterson DF. Mucopolysaccharide storage disease in 3 families of cats with arylsulfatase B deficiency: Leukocyte studies and carrier identification. Pediatr Res 1979;13:1203–1210.

10. Konde LJ, Thrall MA, Gasper P, et al. Radiographically visualized skeletal changes associated with mucopolysaccharidosis VI in cats. Vet Radiol 1987;28:223–228.

11. Yogalingam G, Litjens T, Bielicki J, et al. Feline mucopolysaccharidosis type VI: Characterization of recombinant N-acetylgalactosamine 4-sulfatase and identification of a mutation causing the disease. J Biol Chem 1996;271:27259–27265.

12. Crawley AC, Yogalingam G, Muller VJ, Hopwood JJ. Two mutations within a feline mucopolysaccharidosis type VI colony cause three different clinical phenotypes. J Clin Invest 1998;101:109–119.

13. Yogalingam G, Hopwood JJ, Crawley A, Anson DS. Mild feline mucopolysaccharidosis type VI: Identification of an N-acetylgalactosamine-4-sulfatase mutation causing instability and increased specific activity. J Biol Chem 1998;273:13421–13429.

14. Haskins ME, Jezyk PF, Desnick RJ, et al. Mucopolysaccharidosis in a domestic short-haired cat—A disease distinct from that seen in the Siamese cat. J Am Vet Med Assoc 1979;175:384–387.

15. Gitzelmann R, Bosshard NU, Superti-Furga A, et al. Feline mucopolysaccharidosis VII due to beta-glucuronidase deficiency. Vet Pathol 1994;31:435–443.

16. Bosshard NU, Hubler M, Arnold S, et al. Spontaneous mucolipidosis in a cat: An animal model of human I-cell disease. Vet Pathol 1996;33:1–13.