

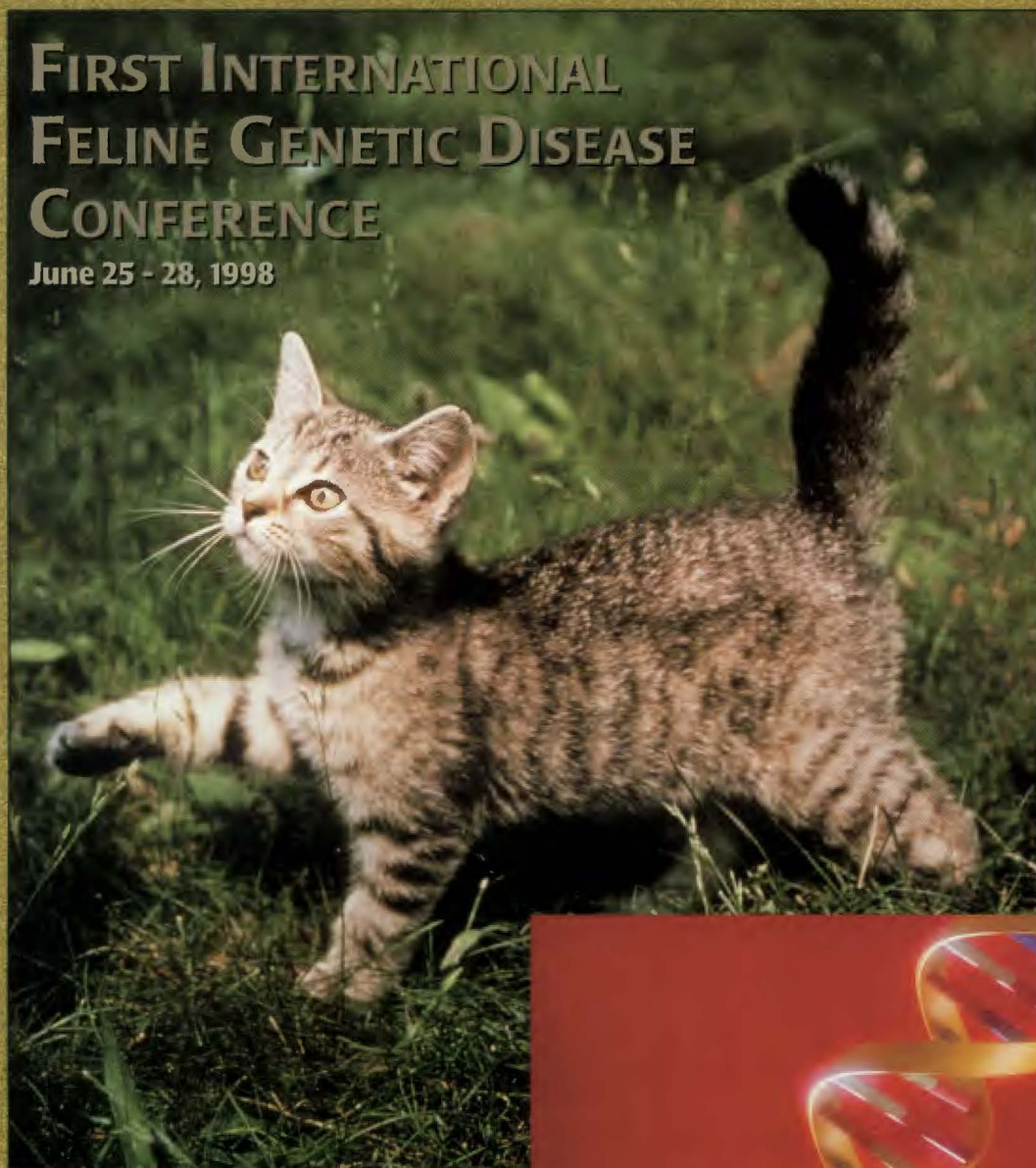
# Feline Practice<sup>®</sup>

FELINE SPECIAL SUPPLEMENT 1999

*The Journal of Feline Medicine  
and Surgery for the Practitioner*

## FIRST INTERNATIONAL FELINE GENETIC DISEASE CONFERENCE

June 25 - 28, 1998



School of Veterinary Medicine  
University of Pennsylvania  
Veterinary Hospital  
3850 Spruce Street  
Philadelphia, Pennsylvania



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*Ralston Purina, the leader in molecular nutrition™*





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School of Veterinary Medicine, University of Pennsylvania, Veterinary Hospital, 3850 Spruce Street, Philadelphia, PA  
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## From the Sponsor

June 3, 1998

On behalf of Ralston Purina Company, I'd like to welcome you to the First International Feline Genetic Disease Conference and thank you for your interest in and support of these efforts which we believe will improve the health and happiness of cats everywhere.

Ralston Purina is proud to sponsor this event and participate with the University of Pennsylvania's School of Veterinary Medicine and Winn Feline Foundation. Purina has over 70 years of experience in improving the health of cats through nutrition research conducted at the Purina Pet Care Center and through collaboration with our colleagues in veterinary colleges across the country. Our long-standing commitment to research has provided a steady stream of new knowledge which we incorporate into all of our products.

Ralston Purina and Winn Feline Foundation share a common interest in helping to continually improve the lives of cats. Sponsoring this conference indicates our dedication toward that interest. The potential of what any cat "can be" is the product of genetics, nutrition and environment. We are particularly interested in participation with you in discovering the secrets of feline genetics, the very blueprint of a cat's biological being, so as to provide optimal feline nutrition which will lead to the best possible life for cats.

Again, we would like to thank you for your attendance at this important conference and hope your experience will benefit you. Purina is pleased you are here and we look forward to a continuing partnership in improving the lives of cats.

Sincerely,

Dr. David M. Bebiak  
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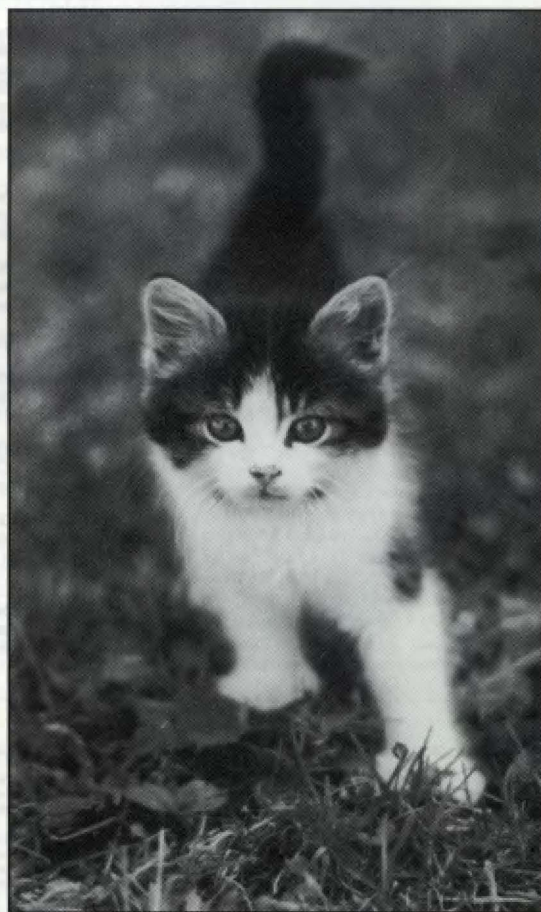
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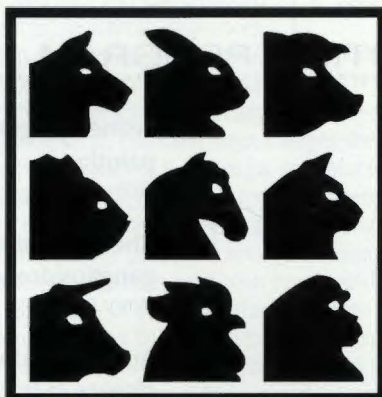
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June 3, 1998

Dear Conference Participants:

We would like to take this opportunity to welcome you to the First International *Feline Genetic Disease Conference* at the School of Veterinary Medicine, University of Pennsylvania. This is the first international conference dedicated to hereditary disorders in cats and the feline gene map focusing on the future of feline health. Currently, over 150 genetic diseases have been reported in domestic and purebred cats and several additional disorders are being discovered every year. During this decade the molecular defects for nearly 10 hereditary disorders have been identified. Based upon major advancements in molecular genetics and the human genome project, a first feline gene map has also recently been developed.

We believe that this conference will provide a state-of-the-art program on the emerging field of feline genetics: a two-day conference for scientists and clinical investigators is followed by a one-day conference for cat breeders, pet owners, and feline practitioners. This format of the conference, with over 70 presentations, should provide new insight into feline genetics for everyone, offer novel approaches for investigators, and generate new collaborative studies between scientists, clinicians, and breeders. Such efforts will likely help to eliminate many hereditary disorders and reduce the frequency of known genetic diseases in future generations.

The School of Veterinary Medicine at the University of Pennsylvania has a long-standing history of contributing to clinical and basic genetics and is very proud to host the First International *Feline Genetic Disease Conference*. We are grateful to the Ralston Purina Company and the Winn Feline Foundation for their involvement in improving the well-being of cats and for their co-sponsoring this event; this generous support is greatly appreciated.

Sincerely,

Alan M. Kelly, BVSc, MRCVS, PhD

The Gilbert S. Kahn Dean of Veterinary Medicine

Urs Giger, PhD, Dr.med.vet, MS

The Charlotte Newton Sheppard Professor of Medicine and Medical Genetics

School of Veterinary Medicine

University of Pennsylvania

3800 Spruce Street

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# FIRST INTERNATIONAL FELINE GENETIC DISEASE CONFERENCE

School of Veterinary Medicine, University of Pennsylvania, Veterinary Hospital, 3850 Spruce Street, Philadelphia, PA  
June 25 - 28, 1998

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*Erik Gruys*
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*Donald Patterson*



The classification of the species of the family Felidae has been controversial for 200 years. Classic taxonomies range from clumping cats into two major lineages to liberals splitting cats into as many as twenty lineages. Currently, at least four different classification schemes are common for cats. Our laboratories have employed high and low G-banded karyotypes, albumin immunological distances, retroviral sequences, DNA/DNA hybridizations, isozymes, 2-D protein electrophoresis, minisatellites and both mitochondrial RFLPs and sequences to tackle the ancient relationships of the saber-toothed tiger to present divisions of sub-species. Early work with albumin immunological distances separated cats into three major lineages; the ocelot lineage of South American cats split nearly 12 million years ago, followed by the domestic cat branch of 8 - 10 m.y.a. and most recently the big cat lineage of *Panthera*, about 4-6 m.y.a. This most recent split also showed smaller branches, including a recent split between modern great cats and lynxes only 2 m.y.a. Low resolution G-banded karyotypes supported this early work. Fifteen of the 19 chromosome pairs are invariant in all cat species. Most of the karyotypic variation occurs in the early phase of the pantherine lineage. Retroviruses, RD-114 and FeLV, liquid hybridizations and isozyme data recapitulated these results and different techniques have been investigated to more finely resolve the major feline lineages. Mitochondrial RFLP studies have been able to refine some of the relationships within the major lineages; grouping lions and leopards and tigers and snow leopards within *Panthera*, splitting the ocelot lineage into three sister taxa, and dividing the domestic cat lineage into several smaller lineages. Sequence analyses of mitochondrial DNA have further resolved the cats into eight major clusters, including: the ocelot lineage, the domestic cat lineage, the *Panthera* genus, the puma group, the *Lynx* genus, the Asian leopard cat group, the caracal group and the bay cat group. Our lab will continue to employ these standard and more novel techniques to clarify the relationships of our feline friends and to provide insight to a broad range of applications, including the study of endogenous retroviruses, gene evolution, population bottlenecks, infectious disease transmission, and species conservation.

## Evolution of the Family Felidae

Stephen J. O'Brien<sup>1</sup>, Glen E. Collier<sup>1</sup>, William G. Nash<sup>2</sup>, William S. Modi<sup>1</sup>, Raoul E. Benveniste<sup>1</sup>, Dianne N. Janczewski<sup>1</sup>, Jill Pecon-Slattery<sup>1</sup>, Warren E. Johnson<sup>1</sup>, Peter A. Dratch<sup>1</sup>, Eduardo Eizirik<sup>1</sup>, Leslie A. Lyons<sup>1</sup>

<sup>1</sup>Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD;  
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Lysosomes and their "housekeeping" enzymes function in degrading large complex substrates which have been taken into a cell by endocytosis. In normal cells, lysosomal enzymes are translocated into the lumen of the endoplasmic reticulum where high mannose oligosaccharides are added. Further post-translational modification results when enzymes add a mannose-6-phosphate (Man-6-P) moiety, recognized by membrane glycoprotein receptors, permitting transfer of the enzymes to lysosomes. A proportion of the Man-6-P-enzymes may also leave the cell via secretory granules. Secreted enzyme can ultimately reach the lysosome because Man-6-P receptors are present in the plasma membrane of many cells.

Degradation of most substrates occurs by the activity of a series of hydrolases in a stepwise fashion, each step requiring the action of the previous enzyme to modify the substrate, thereby permitting catabolism to proceed to the next enzyme step in the pathway. If one step in the process fails, further degradation ceases. The lysosomal storage diseases are a group of genetic disorders of this cellular catabolism.

Most lysosomal storage diseases are inherited as autosomal recessive traits and result from mutations in the coding sequence of one of the acid hydrolases located in lysosomes. The reduction in enzyme activity results in the accumulation within lysosomes of the substrate of that enzyme, hence the name lysosomal storage disease. The first definitive discovery of a lysosomal storage disease in a feline was  $G_{M1}$  gangliosidosis in a Siamese cat by Baker et al. in 1971. Additional diseases have been described: globoid cell leukodystrophy,  $G_{M2}$  gangliosidosis, glycogen storage disease II, alpha-mannosidosis, and mucopolysaccharidosis I, VI, and VII. Mucopolipidosis II is an exception to the usual pathogenesis of a lysosomal storage disease. This disorder results from a failure in a post-translational phosphotransferase, producing many lysosomal enzymes which lack the Man-6-P signal responsible for efficiently directing the hydrolases to lysosomes. Thus, little of many enzymes reach lysosomes, and large amounts are secreted into the extracellular fluid and can be detected in the serum.

As substrates accumulate, the lysosomes swell and occupy more and more of the cytoplasm. This increase in the number and size of lysosomes may obscure the other cellular organelles, and may deform the nuclear outline. As the process continues, the affected cells swell leading to organomegaly. With the exception of the central nervous system, and cartilage and bone, the pathophysiology of these disorders is related to the increase in the cell, tissue, or organ size. The predominant clinical signs are related to the CNS, skeleton, eye, cardiovascular system, and organomegaly. A final diagnosis for lysosomal storage diseases requires the demonstration of a particular enzyme deficiency. Enzyme assays can usually be performed on serum, white blood cells, cultured fibroblasts, or liver. Generally, there is a profound deficiency in activity of the enzyme. Many of these disorders in cats have been described at the molecular level, including the gangliosidoses, alpha-mannosidosis, and mucopolysaccharidoses making the detection of heterozygotes more straightforward than by enzyme activity determination.

Therapy: The secretion of lysosomal enzymes by cells, and uptake via the plasma membrane Man-6-P receptor system, forms the basis for current approaches to therapy. Providing normal enzyme to abnormal cells permits the enzyme to be taken up by the plasma membrane receptor, resulting in delivery of the normal enzyme to lysosomes where it can catabolize stored substrate. Current approaches to deliver normal enzymes include 1) parenteral injection of purified recombinant normal enzyme, 2) heterologous bone marrow transplantation, which provides a continuous source of normal enzymes from bone marrow-derived cells, and 3) gene therapy whereby a viral vector is used to transfer a copy of the normal enzyme cDNA to a patient's own cells, which then act as a source of normal enzyme.

Supported by NIH grants RR 02512, DK 25795, EY 07705, and DK 54481.

Haskins, M. E. and Giger, U. (1997) Lysosomal Storage Diseases in Clinical Biochemistry of Domestic Animals, 5th edition, Kaneko, J.J., Harvey, J.W., and Bruss, M.L. (eds.), Academic Press, New York, pp 741-761.

## Lysosomal Storage Diseases in Cats: An Overview

Mark E. Haskins VMD, PhD

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA



## Feline Hereditary Coagulopathies

Marjory B. Brooks

Comparative Coagulation Section, Diagnostic Laboratory, Cornell University, Ithaca, NY

Coagulation disorders in cats are caused by deficiency or dysfunction of clotting factors. Clotting factors are plasma proteins that interact to generate fibrin clots at sites of blood vessel injury. Heritable coagulation disorders are caused by mutations in the various genes coding for specific clotting factors. New or spontaneous mutations can arise in any purebred or DSH/DLH cat. Once a mutation occurs, defects are most often propagated when asymptomatic carriers are bred.

### I. Clinical Signs of Coagulopathies

The most common signs of clotting factor deficiencies include spontaneous bleeding into joints, muscles or body cavities (chest, abdomen), and development of hematoma (swelling filled with blood) beneath the skin. Prolonged or excessive bleeding occurs from sites of surgery or trauma, and may occur from the gums in association with tooth eruption. Clinical severity of the bleeding tendency varies for deficiencies of different factors, and may also vary between defects appearing in unrelated individuals.

### II. Diagnosis of Coagulopathies

Coagulation screening tests (abbreviated aPTT, PT, TCT) and fibrinogen assays detect functional defects in groups of factors within the coagulation cascade. These assays are useful for initial evaluation of cats suspected of having inherited or acquired factor deficiencies. It is important that testing laboratories specifically validate their assays for feline patients. The results of coagulation screening tests provide the basis for determining whether and which individual factor assays should be performed. Specific factor assays establish definitive diagnosis of an inherited factor deficiency and offer predictions of clinical severity.

### III. Specific Heritable Coagulopathies

The most common inherited feline coagulation disorders include dysfibrinogenemia (factor I deficiency), hemophilia (factors VIII or IX deficiency) and factor XII deficiency.

Dysfibrinogenemia is a deficiency or dysfunction of fibrinogen, seen most frequently in DSH cats. It is an autosomal trait; both males and females are affected. Spontaneous bleeding episodes are uncommon, but prolonged bleeding is typically seen from venipuncture and after surgery or trauma.

Hemophilia is the most common severe factor deficiency in cats. There are 2 forms; hemophilia A caused by factor VIII deficiency and hemophilia B caused by factor IX deficiency. Both forms are X-linked, recessive traits. Hemophilic males inherit an abnormal gene from their dams and express a bleeding tendency. Female carriers have one normal and one abnormal gene, and are clinically normal. Hemophilia has been identified in DSH/DLH, British shorthair, Havana Brown, Himalayan, Persian, and Siamese cats.

Factor XII deficiency is common in DSH/DLH cats. It is an autosomal trait, both males and females are affected. This defect is unusual because deficiency of factor XII does not cause a bleeding tendency, although coagulation screening tests are prolonged. In human beings, factor XII deficiency may be associated with thrombotic tendencies.

## The Feline Genome Project: Development of the Type I Linkage Map of the Cat Using Feline Interspecies Hybrids

Leslie A. Lyons, Stephen J. O'Brien

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A genetic recombination map is being developed for the domestic cat. Both coding genes, Type I markers, and microsatellites, Type II markers, are being mapped in a feline interspecies hybrid backcross between the domestic cat, *Felis catus*, and the Asian leopard cat, *Prionailurus bengalensis*. The feline hybrids were produced by both natural and assisted reproduction techniques. There are over 70 backcross cats in the hybrid cross, which will ensure a genetic map that can determine recombination distances between 4-28 cM with LOD scores >3.0 for fully informative markers. Over 350 Type I markers and 250 Type II markers have been developed for the mapping project. The Type I markers are represented by the comparative anchor tagged sequences, CATS, which are primer sets that amplify intronic regions of coding genes. The CATS were selected

by combining information from the human physical and murine linkage maps, ensuring adequate genome coverage and even spacing. The parental cats of the interspecific hybrid diverged approximately 5-8 million years ago, thus a majority of the Type I markers are fully informative in this reference family. The integration of the Type I and Type II markers on the same pedigree will facilitate gene mapping efforts and population genetic studies in the domestic cat and establish the cat as a strong representative for the carnivora in genomic evolution studies. We will present the Type I linkage data for markers found on five different human chromosomes. This data will be combined with the feline chromosome painting results and compared to published data from other species. The feline genetic map will facilitate the identification of disease genes such as; polycystic kidney disease, progressive retinal atrophies, spasticity, and congenital head defects, and phenotypic homologs, such as dwarfism, coat colors and fur types, found in other mammals.

## Niemann-Pick Disease Type C

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Niemann-Pick disease includes two metabolically distinct disorders, both of which have been reported in cats. Niemann-Pick types A and B are acid sphingomyelinase deficiencies resulting from mutations in the gene coding for lysosomal sphingomyelinase. Niemann-Pick type C (NPC) is an autosomal recessive disorder characterized by abnormalities of intracellular transport of exogenous cholesterol with sequestration of unesterified cholesterol in lysosomes and storage of glycolipids,

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sphingomyelin and gangliosides in central nervous system. Feline NPC has been clinically, biochemically, and morphologically characterized, and a breeding colony has been established for further characterization and therapeutic trials, including bone marrow transplantation.

At least two separate genes induce identical biochemical phenotypes of human NPC. A gene (NPC1) with insertion, deletion and missense mutations has been identified in the more common form of human NPC; this gene has been mapped to chromosome 18q11. The NPC1 protein function is not yet known, but has sequence homology to several known proteins involved in cholesterol homeostasis. The genetic defect in two murine models has been independently localized to mouse chromosome 18 in a region syntenic to human NPC1 locus, and the two mouse loci belong to the same complementation group as human NPC1.

Cell fusions were performed to assess whether multinucleated cells that are formed between human NPC and feline NPC fibroblasts showed a reversal of the NPC phenotype. Reversal of the disease phenotype would indicate that the cells each harbor a mutation of distinct (unrelated) genes. Conversely, the remaining presence of the NPC phenotype in multinucleated heterokaryons would suggest that each of the parent cells (human and feline) harbors a mutation of orthologous genes (NPC1). Cultured fibroblasts from NPC affected humans and NPC affected cats were hybridized and then analyzed for complementation by challenging the cells with LDL and subsequently staining with filipin to visualize any abnormal accumulation of unesterified cholesterol. Persistence of positive filipin staining in heterokaryons indicated an absence of complementation, suggesting that the underlying defect in the human chromosome 18 variant and the feline model involve orthologous genes. Studies are currently being conducted to identify the feline ortholog of NPC1 and to determine if this gene is disrupted in the feline NPC model.

Following the identification of a proband, a colony of Maine coon cats with a heritable form of hypertrophic cardiomyopathy (HCM) was developed. Selective breedings and periodic echocardiographic examinations were performed to study the phenotypic expression, natural history, and heritable characteristics of the disease. Phenotypically, the disease is characterized primarily by marked papillary muscle hypertrophy, progressive left atrial enlargement in cats with severe disease, and systolic anterior motion of the mitral valve. Approximately half (12/22) the offspring produced by mating Maine coon cats from the colony with HCM (affected) to Maine coon cats from the colony without HCM (unaffected) developed HCM. Breeding affected to affected colony cats produced unaffected cats ( $n=2$ ), affected cats ( $n=4$ ), and stillborn kittens ( $n=3$ ). One unaffected to unaffected breeding produced two unaffected cats. Equal numbers of males and females were affected. Whenever, an affected cat was bred to another cat, at least one affected cat was produced. Males and females both transmitted the disease. Multiple instances of male to male transmission were identified. This pattern is compatible with an autosomal dominant trait with 100% penetrance in which the stillborn kittens represent lethal homozygotes. In cats from affected to unaffected matings, HCM was not evident before one year of age, usually became apparent by 2 years of age, and usually progressed to severe disease by young adulthood (2 to 4 years of age). Males in this group developed more severe disease. In cats from affected to affected matings, HCM became apparent as early as 3 months of age and progressed to severe disease between 6 and 18 months of age. Males and females in this group both developed severe disease. Cats with severe disease died suddenly ( $n=4$ ) or of heart failure ( $n=3$ ). Five of the seven cats that died were males. At postmortem examination, each severely affected cat had moderate to marked myocardial fiber disarray, intramural coronary arteriosclerosis, and interstitial fibrosis. We conclude that HCM in Maine Coon cats closely mimics human familial HCM in which sarcomeric gene mutations are responsible for the disease. It most closely mimics the disease observed in human families that have light chain mutations.

### Heritable Characteristics, Phenotypic Expression, and Natural History of Hypertrophic Cardiomyopathy in Maine Coon Cats

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Familial hypertrophic cardiomyopathy (FHCM) in humans is a primary disease of the cardiac muscle characterized by concentric hypertrophy of the left ventricular walls, dynamic outflow obstruction, and diastolic dysfunction. It is frequently caused by a point mutation in the  $\beta$ -myosin heavy chain ( $\beta$ MHC) gene. All but one of the reported mutations in this gene are found in the region that codes for the head of the molecule. Clinical and pathological features of hypertrophic cardiomyopathy (HCM) in the domestic cat closely resemble those of FHCM. Feline HCM has been demonstrated to be a familial defect inherited as an autosomal dominant trait in some feline families. DNA from cats with familial feline HCM was evaluated for the  $\beta$ MHC mutations observed in humans with FHCM. Polymerase chain reaction-based sequencing identified nine base pair changes within exons 3-23, the head region of the molecule. The base pair changes did not affect the amino acid produced and were observed with equal frequency in the normal cat population. Thus, the identified base pair changes in this gene reflect normal feline polymorphisms rather than disease specific mutations. Although a causative mutation was not found in this study, we can not rule out the importance of this gene in some cases of feline hypertrophic cardiomyopathy, since the disease has significant genetic heterogeneity between families in humans. Additional studies should also be performed to examine the other candidate genes for this feline disease.

### Identification of Nine Polymorphisms Within the Head Region of the Feline Beta Myosin Heavy Chain Gene

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## Erythrocyte Pyruvate Kinase Deficiency in Cats

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**R**-type pyruvate kinase (R-PK) deficiency is the most common erythroenzymopathy in humans. R-PK-deficient dogs represent the only animal model described thus far, but they develop a unique M<sub>2</sub>-PK expression and terminal osteosclerosis not typically seen in affected humans. We now describe the clinicopathologic and biochemical abnormalities as well as the molecular basis of R-PK deficiency in cats, a close homologue of the human disease.

Nine 1 to 10-year-old male and female Abyssinian and Somali cats were identified with signs of chronic intermittent hemolytic anemia and mild splenomegaly. The anemia was macrocytic-hypochromic and regenerative, and no poikilocytes were seen. The osmotic fragility of erythrocytes was normal or slightly increased. Glycolytic metabolites proximal of the PK step were markedly increased in affected erythrocytes when

compared to controls. Erythrocytic R-PK enzyme activity of affected cats was low but variable with only 6-20% of that in normal cats. Parents and other relatives of R-PK-deficient cats had intermediate PK activity, supporting an autosomal recessive mode of inheritance. There was a complete absence of R-PK by immunoblotting of erythrocyte homogenates from affected cats.

The normal R-PK cDNA was sequenced from feline liver cDNA clones and RT-PCR products from normal cats (except for first exon), and was found to be 87% and 88% homologous to the human and canine R-PK cDNA sequence, respectively. Furthermore, on Northern blots the R-PK message was markedly reduced in reticulocytes from affected cats versus healthy control cats. Gel electrophoresis and sequence analysis of RT-PCR products exon 6 of affected cats revealed the existence of a cDNA length polymorphism in this region (13 bp deletion). Subsequent sequence analysis of genomic DNA spanning the same region led to the identification of an intronic G → A transition of affected cats. This transition may be involved in altering the donor splice site selection of intron 6 and hence lead to the use of cryptic splice sites located nearby, resulting in a frameshift and premature stop codon. These studies permit the accurate diagnosis and genetic control of PK deficiency in Abyssinian and Somali cats and allow the development and evaluation of gene transfer for this common erythroenzymopathy.

## Feline MPS VI as a Model to Study Pathology and Evaluate Efficacy of Therapy for Maroteaux-Lamy Syndrome Patients

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**M**ucopolysaccharidoses (MPS) are a group of ten inherited lysosomal storage disorders characterised by an inability to degrade specific mucopolysaccharides. Mucopolysaccharidosis type VI (MPS VI) results from a deficiency in N-acetylgalactosamine-4-sulfatase (4S) leading to lysosomal storage of the mucopolysaccharide dermatan sulfate in connective tissue cells and dermatan sulfate-uria. Children with MPS VI display a range of phenotypes from extremely severe, with multitissue involvement, to attenuated clinical forms. The skeletal system is a major site of pathology in MPS VI children. This pathology includes dwarfism, facial dysmorphism and dysostosis multiplex. Widespread soft tissue pathology includes heart valve thickening, corneal clouding, joint stiffness and enlarged liver and spleen. Severely affected patients usually die before twenty years from cardiac or respiratory complications. MPS VI has also

been described in cats with the clinical and tissue pathology closely paralleling that observed in humans. These cats provide a valuable model to investigate the pathophysiology of MPS VI and to develop and evaluate the efficacy of new therapies for use in humans.

We have identified two mutations in our feline MPS VI colony originally obtained from Siamese cats first described in Philadelphia. Homozygous L476P alleles cause a severe clinical presentation with osteopenia, whereas L476P/D520N cats have normal growth with a high incidence of degenerative joint disease. L476P/L476P MPS VI cats, treated with weekly injections of recombinant human 4S from birth for six months, showed a dose responsive effect of enzyme replacement with a complete reduction of lysosomal storage vacuoles in all soft tissues except cartilage and cornea. Treated cats were heavier, more flexible, without spinal cord compression, and had an improvement in most pathologies associated with MPS VI except degenerative joint disease. Treatment with recombinant feline 4S gave a significantly improved dose response over the human 4S equivalent. We anticipate that the benefits observed in these studies will also be observed in human MPS VI patients who undergo enzyme replacement therapy.

## Construction and Analysis of Bacterial P1 Artificial Chromosome Libraries from the Domestic Cat

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**T**he domestic cat serves as a model for over 30 heritable genetic defects which are analogous to human genetic diseases, and the domestic cat is subject to epidemics of two viruses, FeLV and FIV, that cause immunodeficiencies and neoplasms, providing a useful animal model for the study of virus and host genome interactions. Artificial chromosome libraries have become essential research tools for positional cloning of disease genes. Although yeast artificial chromosome libraries have been a method of choice because of their large insert size, the advantages of bacterial chromosome vectors for genomic cloning in terms of lower frequency of chimerism, higher transformation efficiency and ease of purification from host genome has made these vectors

increasingly important. Bacterial artificial chromosome vectors consist of a single copy replicon for stable propagation of large insert clones derived either from fertility factor (BAC) or bacteriophage P1 (PAC), an antibiotic resistance gene for positive selection of vector containing clones and a SacBII domain for positive selection for recombinants.

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We have constructed a domestic cat genomic DNA library composed of 91,900 independent P1 artificial chromosomes (PACs) with an average insert size of 80,000 bp and a size range of 50-150 kb as determined by pulsed field gel electrophoresis of 35 random clones. The theoretical number of genome-equivalents represented was estimated at 2.5 and the probability of finding any given gene sequence as 91%. Screening of the feline PAC library with 52 Comparative Anchor Tagged Sequences (CATS) using the polymerase chain reaction (Lyons, et al., *Nature Genetics* 15:47,1997) and comparison with PCR products obtained using cat genomic DNA by agarose gel electrophoresis indicated that the actual probability of finding any given gene is ~83%. This cat genomic PAC library has been successfully screened for feline MHC class I and class II genes and the clones characterized using high-throughput mini preparations in a 96-well plate format for rapid isolation of BAC/PAC/plasmid DNAs, clone-based physical mapping, and nucleotide sequence analysis. A feline BAC library consisting of 150,000 - 200,000 colonies with 150 kb inserts for an 8 - 10 fold redundancy is currently under construction which will be arrayed into 384-well microtiter plates and made available to the feline genetic disease community.

It has been reported that cats have 2 major types of hemoglobin (Hb) named HbA ( $\alpha_2\beta^A_2$ ) and HbB ( $\alpha_2\beta^B_2$ ). HbA and HbB have identical  $\alpha$ -globin chains, but  $\beta^A$  differs from  $\beta^B$  by four amino acids, and only  $\beta^B$  is acetylated at the amino terminus. The HbA:HbB ratio appears to vary between different cats from 45:55 to 95:5. Alleles for high and low expression of  $\beta^B$  were thought to determine the Hb ratio.

In order to further characterize the feline Hb system, we analyzed feline Hbs by isoelectric focusing electrophoresis, by reversed-phase high performance liquid chromatography (RP-HPLC) and by Southern blotting, cloning and sequencing of the  $\beta$ -globin gene region. Hemolysates from 84 cats of different breeds, gender, and age were analyzed. Twelve families were included into the study.

Consistent with previous reports using other techniques a single  $\alpha$ -globin was found by RP-HPLC. However, by RP-HPLC analysis a total of 6  $\beta$ -globins (I to VI) was identified, with each cat having 1 to 4  $\beta$ -globin chains. From analysis of the number of  $\beta$ -globins, their relative amounts, and Hb mixing studies, 17  $\beta$ -globin patterns were observed. The  $\beta$ -globin pattern in healthy and anemic cats was identical. Family studies documented an autosomal codominant mode of inheritance of the adult  $\beta$ -globins and a linkage of 2  $\beta$ -chains forming haplotypes. Analysis of the  $\beta$ -globin gene region by Southern blotting using a human  $\beta$ -globin DNA probe revealed multiple DNA restriction fragment length patterns providing further evidence for the complex  $\beta$ -globin system.

In conclusion, evidence for the greatest polymorphism of adult  $\beta$ -globins thus far described in any species is provided. A feline  $\beta$ -globin gene region with 2 linked  $\beta$ -globin gene loci and 2 and 5 alleles is proposed.

### Hemoglobin Polymorphism

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Members of a family of domestic shorthaired cats showed fasting chylomicronemia with hypertriglyceridemia and hypercholesterolemia. Affected cats showed fasting hyperlipemia, lipemia retinalis and xanthomas in skin and other tissues. Peripheral neuropathies due to compression of nerves by xanthomas was a significant clinical sign. Reduced body mass, slowed growth rates and increased still birth rates were observed in kittens homozygous for the mutation and reduced oral fat tolerance in homozygotes and heterozygotes. Homozygotes show reduced LDL-C and elevated (male) HDL-C. The fat content of milk of homozygotes contains 25% of that of normal cats with enrichment in shorter chain fatty acids (C14:0, 16:0). Biochemical analyses confirmed that the cats had defective lipoprotein lipase (LPL) catalytic activity in the presence of a high level of LPL mass. The cats produce an abnormal LPL protein which is inactive and fails to bind to the vascular endothelium. Cloning the normal and affected cat LPL cDNA has shown that the affected cat has a nucleotide change resulting in a substitution of arginine for glycine at residue 412 in exon 8. In vitro mutagenesis and expression studies, in addition to segregation analysis, have confirmed that the DNA change is the cause of LPL deficiency. There is an autosomal recessive mode of inheritance with heterozygotes having intermediate LPL enzymatic activity. These cats are a model of human LPL deficiency and may be useful for the new approaches for treatment of LPL deficiency, including gene therapy.

### Chylomicronemia & Inherited Lipoprotein Lipase Deficiency in Domestic Cats

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Hypertrophic feline muscular dystrophy (HFMD) is due to an inherited X-linked recessive dystrophin deficiency and is a homologous animal model for the human disease Duchenne (DMD) muscular dystrophy, a crippling condition leading to muscle atrophy and fibrosis and premature death of affected young men. Dystrophin, a rod shaped protein, is present in the subsarcolemmal space. It may exert several effects on permeability, mechanical protection or calcium regulation, although its exact function remains unclear.

Remarkably, the phenotype of dystrophin deficiency in cats is characterized by pronounced and potentially lethal appendicular and axial muscle hypertrophy, with involvement of the tongue and diaphragmatic muscles. We studied the clinical course of the dis-

### Dystrophin-Deficient Hypertrophic Feline Muscular Dystrophy in the Cat

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ease in our colony of HFMD cats. Affected kittens first show an increase in serum CK levels at age 5-6 weeks. Hypertrophy of axial and appendicular muscles becomes obvious around 3 months of age. Muscle hypertrophy is durable. Serum concentrations of muscle enzymes such as CK, AST but also ALT are consistently increased. Hypertrophic cardiomyopathy develops with ultrasonographic myocardial changes indicative of multifocal mineralization. However, these cats usually do not show clinical signs of heart failure. Typical EMG changes consist of bizarre high frequency discharges. Some of the cats are very sensitive to various stresses including volatile anesthetic agents or simple restraint, and develop stress-related lethal peracute rhabdomyolysis. Histologically, the disease is characterized by multiple foci of muscle fiber degeneration and regeneration without significant inflammatory infiltrate, and myofiber hypertrophy. Dystrophin immunoblots show muscle dystrophin concentration reduced to less than 10 percent of normal in skeletal muscle, and no dystrophin in heart muscle. In spite of its different phenotype, HFMD is an interesting and original model particularly for the early stages of human dystrophinopathies.

### **Glycogen Storage Disease Type IV in Norwegian Forest Cats: Molecular Detection of Carriers**

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**G**lycogen storage disease type IV (GSD IV) is an autosomal recessive disorder due to glycogen branching enzyme (GBE) deficiency in humans and Norwegian forest cats. In cats, phenotypic variation included fatal cardiopulmonary collapse at birth or progressive neuromuscular degeneration beginning at 5 months and causing complete debilitation by 10 months of age. Sudden, fatal cardiac decompensation was observed in a 1-year-old cat. Though not in stillborn kittens, deposition of abnormal glycogen in skeletal and cardiac muscle and in neurons coincided with tissue degeneration in juveniles. Liver and smooth muscle cells showed no evidence of pathology other than accumulation of abnormal glycogen.

Feline GBE cDNA was cloned, and molecular characterization of feline GSD IV demonstrated lack of immunoprecipitable GBE in liver, undetectable GBE RNA on northern blots, variant GBE splicing products detected by RT-PCR, and restriction fragment length differences evident on Southern blots (in preparation).

These data suggested deletion of an exon of GBE on the GSD IV allele. To examine this hypothesis and to develop convenient GSD IV carrier detection in clinically normal Norwegian forest cats, we PCR-amplified genomic DNA from normal and affected cats across the putative deletion. Sequence of the products demonstrated a complex rearrangement that created a 230 bp insertion immediately 5' of a 6 kb deletion, eliminating a 172 bp exon. A common forward primer and allele-specific reverse primers were designed to PCR-amplify 315 and 254 bp products from normal and rearranged GSD IV alleles, respectively. In a family study, all affected cats were homozygous for the GSD IV allele, obligate carriers exhibited both normal and GSD IV alleles, and 50 unrelated normal cats were homozygous for normal alleles. The PCR based, allele-specific carrier test of blood samples submitted by Norwegian forest cat breeders detected carriers in three such catteries. Examination of pedigrees did not reveal a common ancestor for all detected carriers, suggesting either misidentified parentage or dispersion of the GSD IV allele in the breed prior to importation to the USA. This test is available to breeders of Norwegian forest cats to eliminate the GSD IV allele from breeding stock.

### **Feline Cytogenetics: Karyotypes, FISH and Painting**

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**T**he domestic cat has 38 chromosomes, 18 autosomal pairs and the X and Y. G-banding of the domestic cat and its feline relatives have shown the karyotype to be highly conserved across all felids. Thirty-three of 37 felid species have been G-banded by several investigators and 13 chromosomal species groups can be established based on eight polymorphic autosomes. Approximately two-thirds of the felid species can be combined into three large groups. Most of the large cats have the same G-banded pattern and include the panthera and lynx genera. Most small cats of the Felis genus are in a second group and a

third group accounts for several large and small cats including the leopard cat, cougar and jaguarundi. Early G-banded and mapping data showed that at least human chromosomes 1p, 2, 12, and X were extremely conserved to cats and a majority of the felid chromosomes have been suggested to represent the ancestral carnivoran chromosomes. The shared homology of genetic structure is also conserved at the sequence level between cats and humans. Dozens of human and cat specific coding gene loci and microsatellites have been hybridized to feline metaphase spreads, further demonstrating the conservation in gene sequence and positions between the two species. Most recently, the feline chromosomes have been flow sorted and individual chromosome paints have been established. Feline chromosomes have been hybridized to the human karyotype and human chromosomes have been hybridized to cats. These data show that as few as ten rearrangements of the cat karyotype can transform the genome to a human karyotype. The cytogenetic data have supported the gene mapping data of the strong conservation between humans and cats and these techniques are continuing to resolve the feline genetic map using both feline radiation hybrids and interspecies hybrids.



**T**he gangliosidoses are fatal inherited diseases affecting Siamese, Korat and non-purebred cats. Although the first report of a feline gangliosidosis was published in 1971, their molecular bases have been discovered only recently, providing an unprecedented opportunity to apply molecular diagnoses for carrier detection. GM1 and GM2 gangliosidosis result from mutations in the respective genes (GALB, HEXA or HEXB) encoding one of the lysosomal enzymes ( $\beta$ -galactosidase, N-acetylhexosaminidase A or B) responsible for degrading either GM1 or GM2 ganglioside. Feline GM1 gangliosidosis affecting Siamese cats results from a G to C substitution (CGT  $\rightarrow$  CCT) at base 1486 resulting in a change of an amino acid from Arg to Pro and loss of hydrolytic activity. Recently, we have shown that the same mutation is responsible for GM1 gangliosidosis in both Siamese and Korats. Both feline GM2 gangliosidoses described to date result from different mutations of the HEXB gene and loss of all hexosaminidase activity measured by hydrolysis of synthetic substrates. The Korat mutation (fHEXBkorat) results from a deletion of cytosine at base 39 resulting in a premature stop codon and very early termination. In contrast, the second mutation observed in non-purebred cats (fHEXBbaker) results from a 25 base inversion at the extreme 3' end of the open reading frame, causing cessation of translation only 8 amino acids premature of the full length protein. Immunologically cross reactive protein is present, but hydrolytic activity is lost. Remarkably, both GM1 and GM2 gangliosidoses occur in the Korat breed, complicating clinical and pathological diagnoses of similar diseases caused by entirely different mutations. Although these diseases can be diagnosed by enzyme assays, there is sufficient overlap between carrier and normal biochemical phenotypes that indeterminate results are frequent, complicating use of the enzyme results for breeder selection. Therefore, unambiguous molecular testing is critically important to detect carriers and encourage their elimination from breeding stock. International screening of Korats for the gangliosidosis mutations is currently in progress.

### The Molecular Bases of Feline GM1 and GM2 Gangliosidoses

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**S**pontaneous ADPKD in Persian cats closely resembles the human disease. In cats with ADPKD, renal function gradually deteriorates leading to end-stage renal disease by 3-10 years of age. Diagnosis was based on ultrasonography, gross and microscopic renal pathology, or both. Results of Chi square analysis were compatible with autosomal dominant inheritance. Renal cysts were lined by cuboidal or squamous epithelium and chronic tubulointerstitial nephritis was present in most affected cats. Biliary hyperplasia and fibrosis were observed in the livers of many affected cats. Na<sup>+</sup>-K<sup>+</sup> ATPase immunohistochemistry showed strong basilar staining of thick ascending limbs and distal tubules and weak basilar staining of proximal tubules. Less than half of the cysts stained for Na<sup>+</sup>-K<sup>+</sup> ATPase. Staining with peanut agglutinin suggested that cysts were derived from both proximal and distal nephron segments.

Primers designed from exons 36 and 37 of human PKD1 were amplified in domestic cat DNA generating a 200 bp product. Coding regions are conserved bearing 86% homology between cat and human sequences. The intronic region is 66% conserved between the two species. A sequence polymorphism between domestic and leopard cat, identified in the intronic region, was genotyped in a 110 member interspecies pedigree using single stranded conformational polymorphism. Approximately 250 polymorphic microsatellites have been genotyped and are currently in linkage analysis. One dinucleotide repeat locus, FCA476 was identified which is linked to feline PKD1 at 10cm with a LOD of 4.0. The locus is moderately heterozygous exhibiting an average heterozygosity in a sample of outbred domestic cats of 0.64. FCA476 will be genotyped in the Ohio State Persian cat pedigree and examined for linkage to a putative disease locus. The pedigree also will be genotyped and examined for linkage to a putative disease locus with an additional 25 microsatellites mapped to cat B1 which has conserved synteny with human chromosome 4, the location of PKD2.

### Autosomal Dominant Polycystic Kidney Disease in Persian Cats

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**A** genetic linkage map of the domestic cat has been constructed of 253 microsatellite loci including 246 autosomal and 7 X-linked loci. Two hundred thirty five dinucleotide (dC.dA)<sub>n</sub> (dG.dT)<sub>n</sub> and 18 tetranucleotide repeat loci were genotyped in a 3-family, 107 member multi-generation interspecies pedigree between the domestic cat (*Felis catus*) and the Asian leopard cat (*Prionailurus bengalensis*). Two hundred twenty nine loci were linked to at least one other marker with a LOD score, 3.0, identifying 34 linkage groups. Linkage groups were physically mapped using a somatic cell hybrid panel to 17 of the 19 cat chromosomes. An average intermarker distance of 11.3 cM was observed and the estimated length of the sex-averaged map is approximately 3400 cM.

### A Genetic Linkage Map of Polymorphic Microsatellites in the Domestic Cat (*Felis catus*)

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## Assisted Reproductive Technology for Facilitating Management of Feline Hereditary Disease Models

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technology has the potential to become a cost-effective, efficient means for sustaining these various models. In studies conducted at the University of Pennsylvania, Colorado State University, and Wake Forest University, semen was collected via electroejaculation from cats ( $n=36$  males, 104 ejaculates) representing nine disease models: mucopolysaccharidosis (MPS-I, MPS-VI),  $\alpha$ -mannosidosis, Gm<sub>1</sub> gangliosidosis, Ehlers-Danlos syndrome, porphyria, spasticity syndrome, Chediak-Higashi syndrome, and Niemann-Pick (Type C). Semen evaluation revealed that all males produced spermic ejaculates with adequate sperm motility (50-80%), but with variable sperm concentration (20-650 million/ml) and percentage of normal sperm (30-70%) per ejaculate. Recovered sperm were either used fresh for AI or extended with cryoprotectant and frozen by pelleting on dry ice. Intrauterine AI of queens stimulated with exogenous gonadotropins has proven useful for generating viable offspring in non-model domestic cats. In studies conducted at the University of Pennsylvania and Colorado State University, females representing several hereditary diseases (MPS-I, MPS-VI, porphyria, spasticity syndrome) were subjected to intrauterine AI via laparoscopy or laparotomy. Insemination of MPS models ( $n=32$ ) with fresh spermatozoa collected from both MPS-affected and MPS-carrier males resulted in 13 pregnancies (~41%) whereas AI of MPS model females ( $n=6$ ) with frozen-thawed sperm produced 2 pregnancies (~33%). Insemination of porphyria ( $n=1$ ) and spasticity syndrome ( $n=1$ ) models with freshly-collected sperm also produced pregnancies. An alternative method for model propagation involves the use of IVF and embryo cryopreservation combined with ET. One advantage of this technology is that IVF may allow generation of homozygous embryos that can be transferred to non-model recipients to produce affected kittens exclusively. In addition, using embryo cryopreservation, disease models may be maintained entirely with genome resource banks until needed for research purposes. Our ongoing studies with MPS populations are demonstrating the feasibility of this approach. In a recent trial, MPS-I and MPS-VI affected females ( $n=4$ ) and non-MPS females ( $n=2$ ) were treated with exogenous gonadotropins and oocytes were recovered from ovarian follicles via laparoscopy. Oocytes (mean  $\pm$  SEM,  $11.8 \pm 1.5$  oocytes/queen) were inseminated in vitro with sperm collected from MPS-affected ( $n=2$ ) or non-MPS ( $n=2$ ) males, and resulting embryos were cultured for 6 days. Fertilization percentage was similar for MPS-homozygous (67%) and heterozygous (41%) combinations, and embryos from each demonstrated comparable development ( $14.5 \pm 2.8$  and  $16.6 \pm 2.3$  cells/embryo, respectively) after 6 days of culture. In follow-up studies, IVF embryos derived from MPS-affected cats will be cryopreserved, transported to the Cincinnati Zoo and transferred into non-MPS recipients to produce offspring. Findings from earlier embryo transfer trials with non-MPS cats suggests that, if post-thaw embryo viability is adequate, we may expect pregnancy success at least equivalent to that observed following AI. In conclusion, assisted reproductive technology, when fully developed, should facilitate efficient management of cat research colonies and be applicable to a variety of feline hereditary disease models.

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## The Heritability of Susceptibility to Infectious Disease in Domestic Cats

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of susceptibility to infectious disease for two reasons. Infectious disease susceptibility may appear in obscure patterns on pedigrees in part due to reduced penetrance because of uneven exposure among cats to the infectious agents. Secondly, selection experiments to determine the heritability to infection in cats have not been performed. Whether or not the heritability is precisely known, it would be possible to reduce the incidence of infectious diseases in cats by artificial selection. Artificial selection is commonly applied to characters of conformation in cats, but rarely for resistance to infection. The current presentation discusses parallel studies in mice and other animal models and the limited data available for feline susceptibility to infectious disease. The feline enteric coronavirus model system is described in detail as an example of the approach to the study of feline genetics and infectious disease. Approaches are suggested that might enable us to estimate the heritability of susceptibility to infectious disease and evaluate the success of selection regimes for increasing resistance.

Assisted reproductive technology, including electroejaculation, artificial insemination (AI), in vitro fertilization (IVF), embryo transfer (ET), and sperm/embryo cryopreservation, could be useful for assisting in the propagation and management of valuable feline hereditary disease models. For many disease models, affected cats have skeletal deformities or other pathological defects that preclude natural reproductive processes such as copulation, conception, gestation and/or parturition. In addition, maintenance of viable research colonies for each hereditary model is extremely expensive, especially for autosomal recessive conditions that require large heterozygous populations for continued propagation. Recent research advances suggest that assisted reproductive

Domestic cats are commonly affected by bacterial, viral, protozoal, and other infectious agents, particularly in multiple-cat settings such as shelters, catteries, and feral cat colonies. To the extent that susceptibility has a genetic component, the problem has been exacerbated in catteries where cat breeding populations may be small, mating may be strictly regulated, and artificial selection may be intense, leading to small effective population sizes ( $N_e$ ). In cats it is difficult to evaluate the heritability



In comparison to the dog species, cats are affected by relatively few inherited eye diseases. However, several specific problems of the anterior and posterior structures of the eye have been described especially in purebred cats. Below is a short description of the most common defects diagnosed in cats in which a hereditary background is suspected or has been proven.

- Eyelid agenesis is a developmental defect of cats which nearly exclusively affects the upper, lateral eyelids. Iris, lens and optic nerve colobomas (notches) may be present concurrently. The defect is most often seen in Birman and in Burmese cats but may affect any breed.
- Epibulbar dermoids are also congenital malformations, which result in displacement of skin elements, often in the lateral angle of the eye. Again Birman and Burmese cats are often affected although the defect can occur in other breeds as well.
- Entropion is an inward turning of the lower eyelid which causes hairs from the surrounding skin to rub on the cornea. Only part of the eyelid may be affected or the entire lid. Persian cats are most often affected.
- Corneal sequestrum (black body) is a condition of unknown cause that is unique to the cat. There is a breed disposition in that the condition, which often is bilateral, is seen in Colorpoint, Persian, Siamese, Himalayan, Birman and Burmese cats. Mixed breed cats may also be affected with a unilateral condition, which indicates that other factors such as chronic irritation or tear film deficiencies may be involved in the disease mechanism.
- Cataract is an opacity of the lens or its capsule which is infrequently seen in the cat species. Congenital cataract has been reported in the Persian and British shorthaired breeds and presumed to have an autosomal recessive inheritance. Cataracts secondary to inherited progressive retinal atrophy (PRA) are not seen in cats.
- Retinal dysplasia (RD) is an abnormal development of the neural retina and can involve more or less widespread parts of the retina. In cats mainly the focal type of RD is seen which usually does not cause any visual problems. Cases of RD have been found sporadically and especially in the Abyssinian.
- Inherited enzyme deficiencies or retina as well as other body systems. The recessively inherited diseases include alpha-mannosidosis, manifested in the central part of the fundus, GM1-gangliosidosis, associated with focal fundus lesions and gyrate atrophy (ornithine deficiency), resulting in PRA.
- Chediak-Higashi syndrome (CHS) is an autosomal recessive disease of cats and other species. Eyes of CHS affected kittens are hypopigmented and there are changes in retinal structures. A membrane defect has been reported in CHS, involving an increase in unsaturated fatty acid content.
- PRA has been reported in only a few breeds of the feline species. Retinal degeneration with a suspected hereditary basis has been reported in Siamese, Persian and in mixed-breed domestic cats. Only in the Abyssinian cat has the mode of inheritance for the defect been proven. PRA in Abyssinian cats can be inherited either in an autosomal recessive mode or as an autosomal dominant trait. The former type of disease appears to be more common in the general population of Abyssinian cats, especially in northern Europe. The disease in the recessive form is a slowly progressive disorder clinically affecting cats at the age of 1.5 to 2 years of age but leading to blindness at 3-5 years of age. In the latter type of disorder affected cats are congenitally visually impaired, some have nystagmus and severe visual impairment or blindness at the age of 12-16 weeks.

## Hereditary Eye Diseases

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In order to develop a genetic and physical map of the domestic cat MHC, we have constructed a PAC (P1 artificial chromosome) library from a male domestic cat genomic DNA. This library consists approximately 80 Kb average insert size and 2.5 times genome equivalent complexity. Utilizing this library and domestic cat MHC cDNA and PCR clones as probes, we have isolated 250 Kb class II and 330 Kb class I feline MHC region. Restriction mapping using end labeling method and high-throughput shotgun sequencing in 96 well plate format revealed that the isolated class I PAC clones have 4 contigs, while class II PAC clones contain three contigs. These class II contigs included DPA, DNA, RING3 and DMA genes; LMP2, TAP1, LMP7, TAP2 and DOB genes; DRB and DRA genes in DR subregions. Comparison of gene organization in human, mouse, and cat MHC class II regions showed that mouse and cat class II regions are much more compact than human class II region, approximately 50 % size of human HLA for mouse H2 and 60 % for cat MHC. One feline PAC clone (75.6 Kb) contained a single DRB and a single DRA genes with 17 Kb interval sequence and also 5' region of this DRB gene had a high sequence homology specific to human DRB1 functional gene, suggesting that this cat MHC DR subregion has very simple gene organization.

## Characterization of Genomic Organization in Feline MHC Class I and II Region Using Feline PAC Genomic Library

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## Feline $\alpha$ -Mannosidosis: From Molecular Genetics to Therapy

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The lysosomal storage disease,  $\alpha$ -mannosidosis, was first described in humans in 1967. Caused by a genetic deficiency of lysosomal  $\alpha$ -mannosidase, the disorder is characterized by psychomotor retardation, deafness, hepatomegaly, increased susceptibility to infections, and partial dysostosis multiplex. Feline  $\alpha$ -mannosidosis was first recognized independently by Blakemore and by Vandevelde in the early 1980's and several breeding colonies have been established. The gene for feline lysosomal  $\alpha$ -mannosidase has been identified and the defect in our colony of cats with  $\alpha$ -mannosidosis determined. The enzyme is synthesized as a single chain precursor with a 50 aa signal peptide followed by a polypeptide chain of 957 aa, with the latter being cleaved into three polypeptides in the mature state. In our mutant kittens, a 4 base pair deletion was found to result in a frame shift and premature termination, and the production of a truncated protein with little or no hydrolytic activity. Absence of lysosomal  $\alpha$ -mannosidase results in storage of a variety of mannose-rich compounds in essentially all cells of brain and other tissues. There is also an unexplained accumulation of the glycosphingolipid, GM2 ganglioside, in many, but not all, CNS neurons in diseased cats. In addition to lysosomal storage, GM2 ganglioside-laden pyramidal neurons of the cerebral cortex sprout synapse-bearing ectopic dendrites identical to those documented in primary ganglioside storage diseases. We have also found that GABAergic neurons of cerebellum and other brain regions exhibit massive neuroaxonal dystrophy in a manner that closely correlates with clinical neurological dysfunction. Our attempt at developing therapy for this disease has involved use of bone marrow transplantation (BMT) with sibling kittens serving as donors. Affected kittens transplanted at 8-12 weeks of age revealed reversal of mild tremors present at the time of transplant, and failure to develop additional clinical neurological disease over long periods of time (currently 7 years). Our studies showed that BMT successfully delivered enzyme to CNS neurons and eliminated storage. This likely occurred via monocyte migration to brain and secretion of stable enzyme that was internalized by diseased brain cells. Feline  $\alpha$ -mannosidosis represents the most successful example of correction of a lysosomal storage disease with brain involvement and has led the way in development of potential therapies for other genetic brain diseases.

## Development Strategies for DNA-Based Tests for Cat Parentage Verification

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The genetic heritage of a registered cat is described by its pedigree. Owners highly value the ancestry records to provide information about their cats' expected potential. Parentage testing programs are a tool to help breeders attain their breeding goals by assuring pedigree accuracy, by providing highly effective solutions to paternity questions and by sorting out switched kitten scenarios.

Cat fancy associations and owners interested in parentage testing may wish to take advantage of the new generation of genetic tests

based on assays of inherited DNA sequence differences between individuals. A testing system that meets the needs of cat breeders and their registration authorities is likely to be similar to ones successfully used for DNA-based parentage tests of horses, cattle and other domestic animals. Characteristics of these genetic tests include:

- the genetic variants (alleles) detected show simple Mendelian inheritance, with a large amount of genetic variation (polymorphism) within breeds and a low frequency of undetectable (null) alleles;
- the typing procedures are fast, relatively inexpensive and can be automated;
- the detection systems provide highly reproducible and accurate results and do not use radioactive (isotopic) labeling;
- the tests require small amounts of DNA and are not limited to fresh blood;
- the alleles are characterized by a low mutation rate;
- the systems are genetically independent (unlinked);
- the alleles detected can be simply encoded for computer data storage and easy retrieval.

Currently only microsatellites have proven to have the spectrum of genetic properties needed for large-scale animal parentage verification programs. A microsatellite is a DNA segment characterized by simple repeat motifs. Based on the effective tests in other domestic animal species, the cat DNA parentage test will likely consist of 10-15 microsatellites. The efficacy of this test to detect incorrect parentage should be very high-calculated to be 99.99% or greater. Such an array of markers should be effective even in circumstances involving inbred pedigrees, a consistent feature of domestic animal breeds. With such a test, it is highly unlikely that any two cats except identical twins (if they exist) will have the same type. The test will be able to be performed using a variety of biological samples, but the routine cat test will probably use mouth swab samples. These samples can be taken by the owners, without the need for veterinary assistance, and sent to the laboratory by first class mail with no risks of breakage or of spoilage due to extremes of heat or cold.

With the availability of a parentage test, cat breeders may more effectively attain their breeding goals through verified pedigrees, and will have access to highly effective tools to sort out paternity questions and switched kitten scenarios.



**F**eline amyloidosis is a rare finding. In Abyssinian/Somali and Siamese/Oriental breeds, however, it is more common while being of the reactive type (AA-amyloidosis). The amyloid-A protein is derived from an acute phase protein of hepatic origin, serum amyloid A (SAA). For this protein in mammals different isotypes have been found. The exact pathogenesis of AA-amyloid deposition from the acute phase SAA is unknown. For the Abyssinian breed renal amyloidosis has been mentioned as major clinical problem in several papers; the Siamese amyloidosis has not received broad attention so far. The present report regards the amyloid findings as recorded in our Department of Pathology's diagnostic center from 1983-1997, and as found in the Felissana Foundation's pedigree database on Abyssinian and Siamese breeds. Amyloid was seen in 70 of 25498 referrals (0.2%). AA-amyloid was found in 38 and AIAPP-amyloid in 27 cats. Two cats had an amyloid containing calcifying epithelial odontogenic tumor, two a subcutaneous amyloid tumor of the AL-type and another cat had amyloid in a mammary carcinoma. In 1.5% of 710 Siamese and 3.1% of 258 Abyssinian cat referrals AA-amyloidosis was diagnosed. Cats with AA amyloid (all breeds) were of significantly younger age than animals with the other types of amyloid ( $P < 0.0001$ ). The Siamese patients appeared to be familiarly interrelated and to descend from three different males. Cases from England (mentioned by Godfrey, Vet Rec 1996;139:352) and Sweden (sent in) appeared to belong to the same cat families as the Dutch patients. Major gross lesions were liver hemorrhages, enlarged spleen and dilated small intestine. Most Abyssinian cases had renal medullary involvement and showed significantly more inbreeding ( $P < 0.002$ , ANOVA) than the Siamese cats (mean ICs calculated for eleven at random chosen cats per category with seven known generations of ancestors were for AbyApos = .11 and AbyAneq = .08; and for SlamApos = .04 and SlamAneq = .03). For both breeds the differences between the mean ICs for amyloid positive versus amyloid negative cats calculated, were not significant. AA-protein amino acid sequences found, revealed slight differences. In comparison to the Abyssinian AA-sequence which was similar to those published by others, two non-directly related Siamese ones both had amino acid transpositions at pos 46: Q  $\rightarrow$  R, and pos 52: A  $\rightarrow$  V. A domestic shorthair AA-protein (Johnson et al., Comp Biochem Physiol 1989; 94B:765-768) did at pos 30: I  $\rightarrow$  K, pos 43: D  $\rightarrow$  E and pos 49: P  $\rightarrow$  R. The identical amino acid sequences found in both Siamese cats indicate a possible more close genetical relationship than could be deduced from their pedigree data so far. The finding of amino acid substitutions in the cat indicates the occurrence of different genes for amyloidogenic feline SAAs. An explanation for the higher prevalence of amyloidosis in some breeds might be more amyloidogenic SAAs, although other options exist as well. In 25% of the Abyssinian cats with amyloid, 36% of the Siamese cases and 63% of other breed cats with AA-amyloid inflammatory processes such as rhinitis or feline infectious peritonitis which might be considered primary to their reactive amyloid, were encountered. Recent avian *in situ* hybridization findings concerning mRNA for SAA in amyloid-containing joint capsules (Ovelgonne et al., to be published) were included in the discussion on amyloid and its site of deposition.

## Feline Amyloidosis

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**I**n my first attempts to conceive and deliver lectures in medical genetics for veterinary students over thirty years ago, I learned early the necessity of using disease examples in relevant species and the importance of pictures. In those days examples in domestic species were few, but thanks to cat breeders and some astute cat lovers and scientists who were genetically-inclined, a good deal was known about coat color inheritance and the population genetics of cats. There were even a few chromosome defects and monogenic diseases to talk about. I was grateful. Since that time, knowledge of the feline genome has increased greatly and the evolving field of veterinary medical genetics has revealed a myriad of feline genetic disorders. Their ever-increasing number is currently of major concern to cat owners and breeders. While veterinarians have been responsible for identifying and describing the clinical features of most of the genetic disorders presently known in cats and dogs, veterinary medicine as a profession has so far not taken a major role in reducing the frequency of genetic diseases in cat and dog populations. The chief reason for this deficiency has been the lack of education of veterinarians in medical genetics, and the lack of substantial financial support for the research needed to provide basic tools for accurate genetic diagnosis, carrier testing, and genetic counseling in clinical practice. This is the challenge of the present and the future - a challenge that applies to all of the animal species in the realm of veterinary medicine. The challenge can be met by veterinary schools willing to modernize their curricula, and by agencies that provide funding for education and research in animal health. It is encouraging that this conference, one of the first to deal primarily with genetic diseases of the cat, has been so generously supported by Ralston Purina and the Winn Foundation. My objective in the remaining portion of this brief presentation is to discuss some intriguing aspects of cat genetics and genetic diseases not dealt with by other speakers at this conference. Included will be a number of unpublished and published personal observations, drawing in part on the many collaborations I have enjoyed with fellow scientists who also are fascinated by the physical and behavioral beauties of the cat and by the things cats can teach us about genetics and genetic diseases.

## Medical Genetics and the Domestic Cat - A Potpourry of Thoughts, Experiences, and Some Genetic Disorders Encountered During 30 Years in the Field

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# FIRST INTERNATIONAL FELINE GENETIC DISEASE CONFERENCE

School of Veterinary Medicine, University of Pennsylvania, Veterinary Hospital, 3850 Spruce Street, Philadelphia, PA  
June 25 - 28, 1998

## BREEDER/PRACTITIONER SESSION

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### Feline Hereditary Diseases: An Overview

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With the control of most infectious diseases, nutritional imbalances, and intoxications, hereditary disorders have surfaced as a major problem in feline medicine. Currently, over 150 hereditary disorders have been documented in purebred and domestic shorthair/longhair cats; many of them are identical to the ones described in dogs and humans. They represent a large and growing, heterogeneous group of disorders which may affect any organ system and include congenital malformations, inborn errors of metabolism, specific organ dysfunctions, and susceptibilities to infectious, immune-mediated processes, and cancer. Data bases covering all feline hereditary disorders are being developed and many diseases are being discussed during this conference.

Most reported disorders in cats represent single gene defects and are inherited by an autosomal recessive trait. Diseased cats have two mutant (abnormal) genes (alleles) and are generally the offspring of clinically asymptomatic parents who carry a mutant and a normal allele known as carriers. Affected cats may have littermates that are also affected, are carriers, or are clear of a mutant allele. Certain inbreeding practices have led to an increased frequency of specific genetic defects in various breeds. Furthermore, examples of x-linked recessive traits are hemophilias and muscular dystrophy; polydactyly and polycystic kidney disease are autosomal dominant traits. Hip dysplasia, patellar luxation, and congenital heart defects may be complex or polygenic disorders.

Much progress has been made in the characterization of the underlying genetic defect, such as enzyme deficiencies and alterations in many other protein functions or expressions (receptors, transporters, and plasma proteins). The major advancement of the human genome project led recently to the first establishment of a feline gene map. This feline gene map will enable the discovery of mutations in novel genes responsible for other genetic diseases.

Clinical manifestations are extremely variable ranging from benign to debilitating, but are usually chronic and progressive. Beside routine clinical investigations, special biochemical or molecular screening tests are now available to reach a specific diagnosis of many inherited disorders. Some of these tests are not only useful to identify affected animals, but also to detect carriers (asymptomatic). Molecular genetic tests have simple sample submission requirements and are most accurate to determine affecteds, carriers, and normals. Thus, these tests together with appropriate genetic counseling provide avenues to eliminate mutant genes from the breeding pool, while preserving desirable traits.



**T**he fading kitten syndrome is characterized by anorexia, lethargy, and emaciation and is part of the kitten mortality complex which refers to a high incidence of neonatal mortality, birth defects, and reproductive failure in cats. Kittens may be stillborn, or born small, weak, and unable to nurse, resulting in dehydration, hypothermia, hypoglycemia, and death within the first few days of life. Other kittens appear healthy during the first weeks of life, become weak and anorectic, and die of starvation and dehydration. Underlying causes of neonatal kitten deaths include poor management, malnutrition, congenital and genetic defects, infections, and neonatal isoerythrolysis. Prewearing mortality rates between 14 and 23% have been found with peak deaths occurring at birth and at 6-7 weeks of age. Often the cause of death cannot be determined, but inbreeding may result in homozygosity of deleterious genes leading to lethal structural and metabolic defects.

Neonatal isoerythrolysis is caused by an incompatibility of the kitten's blood type with the queen's colostrum. Cats with blood group A have antibodies in their blood and colostrum against blood group B, but the levels of these antibodies are relatively low. However, cats with blood group B have very high levels of antibodies against type A in their blood, and most importantly in the colostrum. When kittens with blood group A are born to a queen with blood group B, they ingest milk containing antibodies against their own blood group. This leads to destruction of the kitten's red blood cells. The signs of disease are variable and include jaundice and death within the first two days of life, no signs at all (which is rare) or the tail tip may become necrotic and fall off at 10 to 14 days of life.

## The Fading Kitten Syndrome and Neonatal Isoerythrolysis

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**R**ecent advances in mammalian genetics offer us the opportunity to develop the "next generation" of genetics tests. These tests, which are often referred to as DNA-based genetic tests, are particularly important for genetic diseases that are inherited in an autosomal recessive fashion. Diseases showing this mode of inheritance are particularly problematic because animals that only carry one copy of a defective gene do not show signs of the disease, yet they pass their defective gene to 1/2 of their offspring, on average. Genetic tests that are based on DNA analysis can discriminate between normal appearing animals that carry one copy of the mutant gene (carriers) and normal animals that don't carry the defective gene (normal, both copies of the gene are normal and functional).

DNA-based genetic tests detect differences in the DNA sequence. DNA is composed of four different types of bases, or building blocks, abbreviated as A, C, G, and T, that are arranged like beads on a string. These bases encode all of the proteins in an animal's body, and each cell contains a complete copy of the animal's DNA, often referred to as the genome. The genome of any mammal is thought to contain 6 trillion base-pairs of DNA. If one thinks of the DNA of a single cell as four colors of glass seed beads on a string, it would take two strands of beads over 3000 miles long to represent an entire mammalian genome. Even more remarkable is that fact that the substitution, deletion, or addition of a single bead or base-pair can cause a lethal disease. Current technologies allow us to detect these minute, but sometimes life-threatening differences.

How are these tests done? The most time- and cost-consuming part of a DNA-based genetic test involves isolating a particular piece of DNA, such as a gene, that is associated with the genetic disease. This must be done from both normal and disease-affected animals, so that the DNA difference or mutation associated with the disease can be identified. Depending on what is known about a particular genetic disease in other species, in particular, in people, this process can take as little as a few months, to decades. The intense effort to isolate and sequence the human genome will be enormously helpful in the study of animal genetic diseases. The relatively small number of currently available genetic tests in cats and dogs attests to the effort necessary to develop these tests.

Once developed, DNA-based genetic tests can be performed shortly after birth using DNA isolated from various tissue sources. A drop of blood, hair follicles, or cheek swabs are all common sources of DNA and are obtained by relatively non-invasive techniques. The polymerase chain reaction (PCR), is invariably used to amplify selected DNA segments up to billion-folds so they can be examined. The DNA fragments are then examined in various ways, including DNA sequencing and various electrophoretic techniques, that detect the DNA change that causes or is associated with the particular genetic disease. These data are the diagnostic information that indicate if an animal is normal, a carrier, or is affected with the genetic disease. Once identified, carrier animals can still be bred, but only to normal animals, thus avoiding the production of any animals that will be affected with the disease. Furthermore, if the breeding of carrier animals is minimized, the disease-causing version of the gene eventually can be eliminated from a population. Clearly, an important component of genetic testing is the analysis of the test results in the context of the entire pedigree and the particular breed in general.

DNA-based genetic tests are currently available only for diseases caused by defects in single genes. As the day progresses, you will hear about DNA-based tests for specific genetic diseases and how the feline genome project will allow scientists to develop tests for those genetic diseases for which we don't yet know which gene is affected and for diseases caused by defects in multiple genes.

## Molecular Genetic Tests: How Do These Tests Work?

Paula Henthorn

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**H**eritable coagulation disorders in cats are caused by deficiency or dysfunction of specific clotting factors. Clotting factors are proteins that function as enzymes, coenzymes, or substrate in the process of coagulation that culminates in fibrin clot formation. Deficiency of any single clotting factor causes prolongation of in vitro clotting time, and in vivo bleeding diatheses of variable severity. Specific disease phenotypes are diagnosed on the basis of in vitro functional tests (coagulant activity assays). In general, in vitro factor activity is predictive of clinical severity. Unfortunately, delay in diagnosis is common, and many cats experience repeated episodes of bleeding before definitive diagnosis.

Many of the heritable factor defects found in human beings have been described in cats. The most common of these include hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency), factor XII deficiency and dysfibrinogenemia. These defects have been identified in different breeds including Siamese, Persian, British shorthair, Havana Brown, and in many unrelated DSH/DLH families. Factor XII deficiency is unusual because prolongation of clotting time is an in vitro phe-

## Feline Hereditary Coagulopathies

Marjory B. Brooks

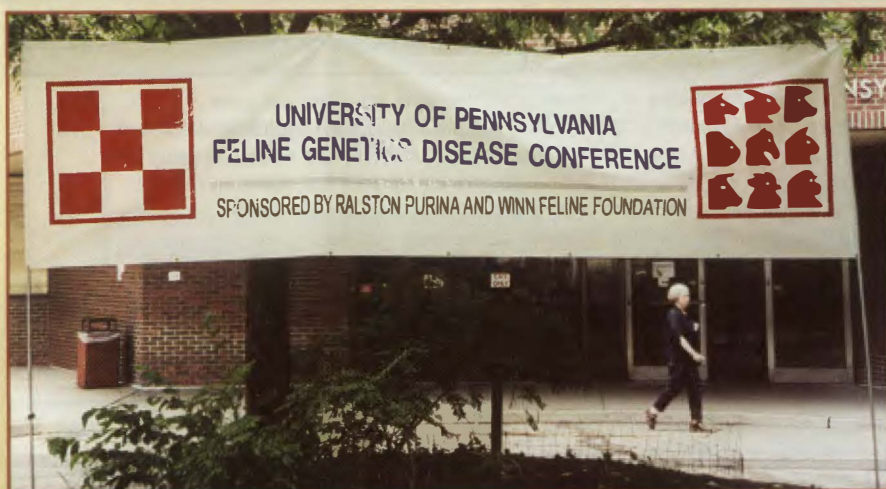
Comparative Coagulation Section, Diagnostic Laboratory, Cornell University, Ithaca, NY

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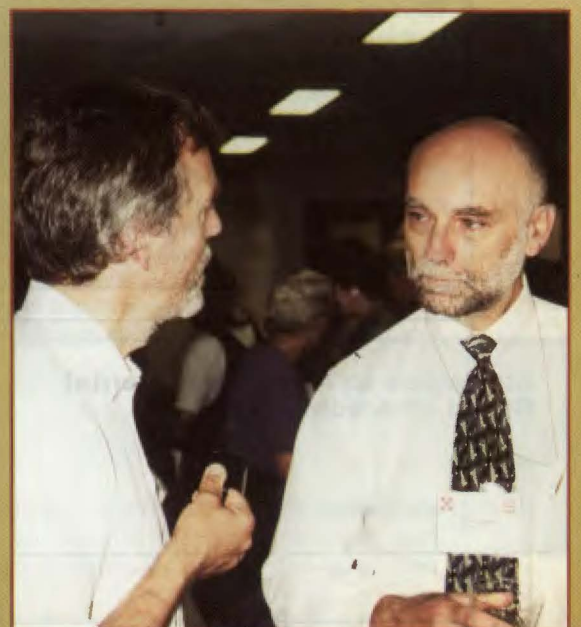


# FIRST INTERNATIONAL FELINE GENETIC DISEASE CONFERENCE

School of Veterinary Medicine, University of Pennsylvania, Veterinary Hospital, 3850 Spruce Street, Philadelphia, PA  
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nomenon, with no associated clinical bleeding disorder. Uncommon defects, identified in only a single breed or line, include factor VII deficiency and factor X deficiency in DSH cats, and deficiency of vitamin K-dependent factors in Devon Rex.

Hemophilia A and B are X-linked recessive traits, whereas the expression pattern of other factor deficiencies appears to be autosomal recessive. The common hereditary coagulopathies seem to arise from de novo mutations and are usually propagated when asymptomatic carriers are bred. The molecular genetic defects responsible for various disease phenotypes have not yet been identified in cats. In human beings, hemophilia A and B, and dysfibrinogenemia are molecularly heterogeneous disorders, with many different mutations identified in different unrelated families. Molecular genetic analyses of feline coagulopathies and clotting factor genes will prove whether similar mutation mechanisms occur in the homologous feline genes. Characterization of the feline genes may also prove useful in identifying marker loci for gene mapping studies and for developing clinical assays to enhance detection of heterozygous disease carriers.

### The Feline Genome Project: The Role of the Genetic Map of the Cat in Fancy Cat Breeding

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A genetic linkage map is being developed for the domestic cat. Over 500 markers of different types are being mapped in a feline cross-species hybrid between the domestic cat, *Felis catus*, and the Asian leopard cat, *Prionailurus bengalensis*. The feline hybrids are known as Bengals and they were produced by both natural and assisted reproduction techniques. A majority of the cats used in this family were supplied by Bengal cat breeders in the United States. There are over 70 second generation Bengals in the pedigree, which will ensure a genetic map that can determine marker associations at far genetic distances with high confidence. Over 350 of the markers used in the project are also being mapped in most every other species' genome project. This will help transfer genetic information about diseases across different

species, transferring information from mouse to humans to cats to dogs or any other species. The remaining markers will be useful for population studies, such as determining gene pool diversity, individual identification, parentage testing and forensic analyses. The genetic map identifies which markers are independent, which is necessary for their effective use in population studies. The feline genetic map will facilitate the identification of disease genes such as; polycystic kidney disease, progressive retinal atrophies, spasticity, and congenital head defects, and phenotypic homologs, such as dwarfism, coat colors and fur types, found in cats and other mammals. We will review how the genetic map facilitates gene mapping and population studies in the cat, how breeders can assist with the development of the map and disease gene hunting projects in their cats of interest and how to facilitate interactions between breeders, veterinarians and scientists.

### Heritable Characteristics and Natural History of Hypertrophic Cardiomyopathy in Maine Coon Cats

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Following the identification of a proband, a colony of Maine coon cats with a heritable form of hypertrophic cardiomyopathy (HCM) was developed. Selective breedings and periodic echocardiographic examinations were performed to study the phenotypic expression, natural history, and heritable characteristics of the disease. Phenotypically, the disease is characterized primarily by marked papillary muscle hypertrophy, progressive left atrial enlargement in cats with severe disease, and systolic anterior motion of the mitral valve. Approximately half (12/22) the offspring produced by mating Maine coon cats from the colony with HCM (affected) to Maine coon cats from the colony without HCM (unaffected) developed HCM. Breeding affected to affected colony cats produced unaffected cats (n=2), affected cats (n=4), and stillborn kittens (n=3). One unaffected to unaffected breeding produced two unaffected cats. Equal numbers of males and females were affected.

Whenever, an affected cat was bred to another cat, at least one affected cat was produced. Males and females both transmitted the disease. Multiple instances of male to male transmission were identified. This pattern is compatible with an autosomal dominant trait with 100% penetrance in which the stillborn kittens represent lethal homozygotes. In cats from affected to unaffected matings, HCM was not evident before one year of age, usually became apparent by two years of age, and usually progressed to severe disease by young adulthood (2 to 4 years of age). Males in this group developed more severe disease. In cats from affected to affected matings, HCM became apparent as early as 3 months of age and progressed to severe disease between 6 and 18 months of age. Males and females in this group both developed severe disease. Cats with severe disease died suddenly (n=4) or of heart failure (n=3). Five of the seven cats that died were males. At postmortem examination, each severely affected cat had moderate to marked myocardial fiber disarray, intramural coronary arteriosclerosis, and interstitial fibrosis. We conclude that HCM in Maine coon cats closely mimics human familial HCM in which sarcomeric gene mutations are responsible for the disease. It most closely mimics the disease observed in human families that have light chain mutations.

### Strategies to Avoid Detrimental Breed Characteristics

Joan Miller

The Winn Feline Foundation

A cat breed is a distinctive group of cats that is genetically isolated in some way. The very concept of breed development, improvement, refinement or preservation involves controlling inherited traits through reproductive selection. Yet when strictly considering sound health it is generally believed that animals with random genetic diversity will be superior. This contradiction is an interesting challenge for breeders and their veterinarians. Isolated populations of domestic cats, whether confined to islands or farm colonies, do seem to thrive in spite of a high degree of natural inbreeding. The intervention of cat breeders in reproductive selection is also not necessarily detrimental when there is awareness and planning. The 'cat

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fancy" exists to enhance and preserve natural or established historical appearance, desirable genetic mutations and unique breed personality characteristics. We need to develop better strategies to achieve these goals while avoiding heritable disorders and loss of general vigor. Some of the healthiest breeds have extremely small gene pools. Many of the breeds have a relatively static genetic makeup that has existed for over a century without serious problems. In the majority of breeds it is now impossible to find two cats unrelated to each other because every pedigree is based on a few early founding cats. Methods used to achieve consistent conformation are inbreeding, linebreeding, backcrossing to a superior prepotent male and outcrossing within or outside the breed. These same techniques can have both advantages and risks when planning a breeding program emphasizing sound health. As an example, breeders will sometimes start with cats of diverse background, then inbreed (siblings or father-daughter) to fix certain qualities and determine undesirable recessive genes, both for conformation or health. Careful linebreeding (cousin matings or moderate inbreeding) will allow slow development of features without causing general loss of vigor. Backcrossing to a superior prepotent male cat can greatly enhance appearance. If the male is a known producer of sound long-lived cats he can also benefit the health of a breed.

Unlike the cattle business keeping progeny records including detailed health/death information is not yet practiced in the cat fancy beyond informal word of mouth exchange of wisdom and experience. Knowledge of heritable diseases/disorders in cats is dramatically increasing but the sharing of information and taking action to deal with problems is slow. When a specific disorder is recognized in a breed strategy must differ. In the Scottish Fold the potential for joint disease/skeletal lesions requires avoidance through the use of outcross breeds approved by CFA. The Manx breed, with its mutant tailless feature that must have originated centuries ago, continues to be viable in spite of the in utero loss of homozygous kittens. According to CFA registration records Manx litter size is comparable to most other breeds. This is due to selection for reproductive superiority in females. Abnormalities thought to be related to the tailless gene have been avoided by breeders who are rigorous in their selection of sound cats. A disease such as familial renal amyloidosis in the Abyssinian and related breeds is a special challenge. The mode of inheritance is not entirely known, early diagnosis is not possible without a reliable test and affected cats often die at an older age after producing litters. Pedigree analysis has become second nature to experienced breeders along with the increased use of 10 year and older stud cats and cats with minimal inflammatory problems. In the case of polycystic kidney disease in Persian cats testing clinics are being established to help identify affected cats prior to breeding. The CFA Health Committee, The Winn Feline Foundation, a mentoring program for new breeders, breed standards that allow for conformation variability and stricter penalties for unsound features, show rules that prohibit surgical correction, are examples of the ways in which the Cat Fanciers' Association is helping to improve the genetic health of pedigreed cats.

**T**he Abyssinian cat has been shown to be affected by a hereditary retinal degeneration that shares many similarities with human retinitis pigmentosa (RP). The disease was characterized by clinical and laboratory investigations in groups of normal cats and Abyssinian cats affected by ophthalmoscopically obvious disease. In short, the fundus appeared normal until the animals reached young adulthood, at the age of 1.5-2 years, at which time slowly progressive changes began, that led to generalized retinal atrophy by the time the cats were middle-aged. DC-electroretinography (ERG) showed that there was a primary effect by the disease on the function of rod photoreceptors with a later involvement also of the cone system. The function of the retinal pigment epithelium (RPE) was not affected, however, until at a late stage of the degenerative disease process. These changes were verified by ultrastructural studies which showed changes primarily in solitary rod outer segments or in several rods in patches, while cones and other retinal cells were normal appearing. The morphological changes were more severe in midperipheral and peripheral areas of the retina compared to the central parts, which seemed to be spared until late in the disease, when there was a generalized drop-out of both rods and cone photoreceptors. Through retrospective studies of breeding pairs and their off-spring a simple recessive mode of inheritance for the defect was found.

During the last decade this rod cone degenerative disease has been further studied in order to more thoroughly characterize the disorder as to early functional and morphological changes, including the use of immunohistochemical and immunochemical methods. Biochemical studies of blood and affected tissue have also been performed as well as, more recently, specific physiologic and molecular genetic studies. Candidate gene analysis of genes encoding for proteins and enzyme systems mainly of the photoreceptor transduction cascade has so far not led to the elucidation of the genetic defect. During recent years also treatment trials have been initiated, utilizing the technique of neuro-retinal transplantation.

It is clear that this Abyssinian cat mutant now is a well characterized animal model for RP, which offers the advantage of a large eye with several similarities to the human eye. Routine ophthalmic examinations can be performed in cat as well as the utilization of medical and surgical techniques regularly in use for human patients. Furthermore, the cat has since long been an established species in the field of basic and applied Neuroscience, which so far has rendered a great amount of basic scientific knowledge especially in the fields of Neurology and Ophthalmology. It is therefore of great importance that research into the Abyssinian cat degeneration be further pursued. Investigations of priority include:

- To elucidate the gene defect in rdAc
- To pursue the issue of retinal transplantation with focus on structure and function.
- To investigate the cone mechanisms in hereditary rod-cone degeneration
- To study the physiology of ocular circulation in retinal heredo-degeneration.

Still another hereditary retinal disease exists in the Abyssinian cat. In England a dominantly inherited rod cone dysplasia has been described. Affected cats are congenitally visually impaired, some have nystagmus and blindness is obvious at the age of 12-16 weeks. Morphologically the rods and cones never develop normally in this Abyssinian cat mutant. The genetic defect has not been found.

### **Progressive Retinal Atrophy in Abyssinians**

**Kristina Narfström**

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## Lysosomal Storage Diseases in Cats: An Overview

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age) within lysosomes of the substrate of that enzyme, hence the name lysosomal storage disease. The name of each disease usually refers to the substrate which is stored. The first definitive discovery of a lysosomal storage disease in a cat was GM1 gangliosidosis in a Siamese cat by Baker et al. in 1971. Nine additional diseases have now been described in cats: GM2 gangliosidosis, glycogen storage disease II, alpha mannosidosis, mucopolysaccharidosis I, VI, and VII, globoid cell leukodystrophy, Niemann Pick disease type C, and mucopolipidosis II.

Cats with lysosomal storage diseases frequently have central nervous system problems with head tremors and abnormalities in gait. Some also have cloudy comeas, and abnormalities in bone development with short legs, small ears, and a flat face. Many of the diseases result in severe disability and death within the first year of life, while others are less severe with life spans extending up to 8 years.

There are no uniformly successful approaches to treat lysosomal storage diseases. Preventing mating of cats which carry a mutation can result in the eradication of a lysosomal storage disease from an individual family of cats, while retaining the desirable characteristics of the line. The diagnosis of carriers for these diseases is becoming increasingly more accurate with the availability of molecular techniques which have been developed after the gene for the enzyme has been cloned. Enzyme assays on blood or cells grown from skin biopsies are helpful in those diseases or families where the mutation has not been identified.

Current research to develop approaches to therapy include 1) injection of normal enzyme, 2) bone marrow transplantation, usually from a sibling, and 3) gene therapy, where a copy of the normal gene is put into an animal's own cells.

## Molecular Screening for the Feline Gangliosidoses in Korats

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The gangliosidoses are inherited diseases affecting several cat breeds, most notably Korats. Although neurological diseases resembling the gangliosidoses have been observed in Korats since the 1960s, the first documented report of GM2 gangliosidosis in Korats was published in 1985, and this year Castagnaro et al described the first confirmed case of GM1 gangliosidosis in Korats. The occurrence of two entirely different mutations in a single breed, which result in diseases with very similar clinical and pathological characteristics, is remarkable and seriously complicates diagnoses and epidemiological studies. Because the gangliosidoses are inherited as autosomal recessive traits, the periodic appearance of these diseases over four decades may represent an ominous presence of many carriers of these mutations in the breed. A breed wide screening program is the only valid approach to accurately estimate the incidence of these mutations and eliminate carriers from breeding stock. Although these diseases can be diagnosed by enzyme assays, there is sufficient overlap between carrier and normal biochemical phenotypes that indeterminate results are frequent, complicating use of these enzyme results for breeder selection. Therefore, unambiguous molecular testing is critically important to detect carriers and encourage breeder participation. Muldoon, et al described the mutation responsible for Korat GM2 gangliosidosis in 1994. Recently, we described the sequence of the normal feline  $\beta$ -galactosidase gene, discovered the mutation responsible for GM1 gangliosidosis in Siamese and discovered the mutation responsible for GM2 gangliosidosis in nonpurebred cats. Our studies demonstrate that the GM2 mutations of Korat and nonpurebred cats are different and we have shown that the same mutation is responsible for GM1 gangliosidosis in both Siamese and Korats. These discoveries provide an unprecedented opportunity to apply molecular diagnoses for carrier detection. An international program to screen Korats for the gangliosidosis mutations has been organized and testing is currently in progress. To date, 69 samples from Korats located in 6 countries have been received for testing. Carriers for both GM1 and GM2 gangliosidoses have been detected in these submissions.

## Coat Genetics in Domestic Cats

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Artificial selection by humans has resulted in preservation and proliferation of a large variety of coat colors, length, and texture, in the domestic cat. Currently approximately 15 genes, each with two or more alleles, cause easily observable differences. In addition, there are polygenic factors that affect coat color.

Color and coat genes encompass a wide variety of modes of inheritance. These include the familiar sex-linked orange/red color which produces textbook mosaicism in heterozygous females, as well as simple dominant/recessive alleles such as the dilute gene which affects pigment deposition in the cells of the hair shaft, easily visible under low power magnification of individual hairs. Other color genes are pleiotropic, causing a number of effects besides color. The temperature sensitive allele of the albino gene causing pointed color in many cats including Siamese, affects the optic nerve pathways. Dominant white is pleiotropic, epistatic, and has variable expression. White color apparently results from altered development, particularly programmed cell death, and occasionally causes deafness through hair cell degeneration in the inner ear after birth. The rex genes cause similar phenotypes of curly coats through different genes. Tabby patterns were thought to be created by several alleles of one gene. Data collected from the cat fancy indicates that tabby patterns are probably caused by at least three separate genes with multiple alleles.

When the genes that are involved in coat color are located on the feline chromosomes they will provide genetic markers whose linkage to other genes should be easy to observe and thus they should contribute markedly to the feline genome project.



**C**anine hip dysplasia (HD) and patellar luxation (PL) are common, well-recognized orthopaedic conditions. They are considered separate disease entities and are thought to occur infrequently in the same dog. Hip dysplasia is particularly prevalent in large and giant breed dogs, whereas PL is commonly found in toy and miniature breed dogs. In contrast, HD in cats presents clinically less frequently and it is not common to test cats radiographically or clinically for HD. Therefore, feline HD is less well characterized. Patellar luxation has rarely been reported in cats and isolated cases have been thought to be traumatic or congenital with little genetic component. A few limited surveys have found a higher prevalence of medial patellar luxation in Abyssinian and Devon Rex cats suggesting a hereditary basis. The objectives of our study were to examine the occurrence of hip dysplasia (HD) and patellar luxation (PL) in cats, to determine whether an association exists between these 2 orthopaedic conditions, and to relate degenerative joint disease and laxity in a group of cats by the use of NA, DI and OFA scoring.

A non-random population of 78 cats were examined for the existence of PL and HD. Complete history was obtained, physical examination was performed and radiographs of the stifles and hips were made under sedation. The hip-extended radiographs were evaluated by a board-certified radiologist using the standard 7-point scoring scheme established for dogs by the Orthopedic Foundation for Animals (OFA). Evidence of DJD was recorded. Joint laxity was quantitated from the hip-extended radiograph using the Norberg Angle method and from the distraction radiograph using the distraction index (DI). The relationship of hip DJD to objective hip score was examined. There were 43 males and 35 females with a mean age of 2.5 years. Eleven of the 78 cats had clinical signs of pelvic limb disease. Hyperlaxity of the patella-subluxation sub-grade 1- was seen in 31 of 33 cats otherwise diagnosed as normal in the stifle joints. Medial patellar luxation (MPL) was found in 45/78 (58%) of the cats. Seventy-one percent (32/45) of the cats with MPL had bilateral involvement. Cats mainly had Grade 1 MPL. Of the breeds represented Abyssinians and Devon Rex cats were affected with MPL more frequently and more severely. Concurrent MPL and HD was seen in 19/78 cats (24%), and an association existed between MPL and HD ( $\phi=0.25$ ;  $p=0.045$ ). Cats with MPL were 3 times more likely to have HD than those without.

Hip dysplasia was diagnosed radiographically in 25/78 (32%) using the OFA scoring system. Nineteen cats had mild, 4 moderate, 2 severe HD. Eighteen of the 25 cats with HD had bilateral disease. Fifteen of the 25 cats had DJD. Mean NA in the 78 cats was 92.4° (range 56-105°). The mean NA in cats with DJD was significantly lower (84°) than in cats without DJD (95°). No cat had a NA greater than 105°. The mean DI for all cats was 0.51 (range 0.2-0.84). The mean DI for cats with DJD was significantly higher (0.6) than without DJD (0.49). No cat with a DI less than 0.4 had DJD. The chance of having DJD increased with increasing DI and decreasing NA and with a worse OFA score.

Patellar luxation and HD may occur in cats more frequently than reported. The two conditions were observed to occur simultaneously in several cats (24%) and a weak association was found between PL and HD. Clinical signs were generally mild, and none were observed with grade 1 medial PL or mild hip dysplasia. Cats in this small sample had hip laxity comparable to that observed in breeds of dogs having high incidence of HD, such as Golden Retrievers and Rottweilers. As in the dog, there was a positive relationship in the cat between hip laxity and the expression of DJD.

## Patellar Luxation & Hip Dysplasia in a Group of Cats

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**D**omestic cats are commonly affected by bacterial, viral, protozoal, and other infectious agents, particularly in multiple-cat settings such as shelters, catteries, and feral cat colonies. To the extent that susceptibility has a genetic component, the problem has been exacerbated in catteries where cat breeding populations may be small, mating may be strictly regulated, and artificial selection may be intense, leading to small effective population sizes ( $N_e$ ). In cats it is difficult to evaluate the heritability of susceptibility to infectious disease for two reasons. Infectious disease susceptibility may appear in obscure patterns on pedigrees in part due to reduced penetrance because of uneven exposure among cats to the infectious agents. Secondly, selection experiments to determine the heritability to infection in cats have not been performed.

Whether or not the heritability is precisely known, it would be possible to reduce the incidence of infectious diseases in cats by artificial selection. Artificial selection is commonly applied to characters of conformation in cats, but rarely for resistance to infection. The current presentation discusses parallel studies in mice and other animal models and the limited data available for feline susceptibility to infectious disease. The feline enteric coronavirus model system is described in detail as an example of the approach to the study of feline genetics and infectious disease. Approaches are suggested that might enable us to estimate the heritability of susceptibility to infectious disease and evaluate the success of selection regimes for increasing resistance.

## The Heritability of Susceptibility to Infectious Disease in Domestic Cats

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## Autosomal Dominant Polycystic Kidney Disease in Persian Cats

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Spontaneous ADPKD in Persian cats closely resembles the human disease. In cats with ADPKD, renal function gradually deteriorates leading to endstage renal disease by 3-10 years of age. Diagnosis was based on ultrasonography, gross and microscopic renal pathology, or both. Results of Chi square analysis were compatible with autosomal dominant inheritance. Renal cysts were lined by cuboidal or squamous epithelium and chronic tubulointerstitial nephritis was present in most affected cats. Biliary hyperplasia and fibrosis were observed in the livers of many affected cats. Na<sup>+</sup>-K<sup>+</sup> ATPase immunohistochemistry showed strong basilar staining of thick ascending limbs and distal tubules and weak basilar staining of proximal tubules. Less than half of the cysts

stained for Na<sup>+</sup>-K<sup>+</sup> ATPase. Staining with peanut agglutinin suggested that cysts were derived from both proximal and distal nephron segments.

Primers designed from exons 36 and 37 of human PKD1 were amplified in domestic cat DNA generating a 200 bp product. Coding regions are conserved bearing 86% homology between cat and human sequences. The intronic region is 66% conserved between the two species. A sequence polymorphism between domestic and leopard cat, identified in the intronic region, was genotyped in a 110 member interspecies pedigree using single stranded conformational polymorphism. Approximately 250 polymorphic microsatellites have been genotyped and are currently in linkage analysis. One dinucleotide repeat locus, FCA476 was identified which is linked to feline PKD1 at 10cm with a LOD of 4.0. The locus is moderately heterozygous exhibiting an average heterozygosity in a sample of outbred domestic cats of 0.64. FCA476 will be genotyped in the Ohio State Persian cat pedigree and examined for linkage to a putative disease locus. The pedigree also will be genotyped and examined for linkage to a putative disease locus with an additional 25 microsatellites mapped to cat B1 which has conserved synteny with human chromosome 4, the location of PKD2.

## Development Strategies for DNA-Based Tests for Cat Parentage Verification

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A parentage testing system that meets the needs of cat breeders and their registration authorities is likely to be similar to ones successfully used for DNA-based parentage tests of horses and other domestic animals. Among the variety of available DNA technologies, currently only short tandem repeats (microsatellites) have proven to have the spectrum of genetic properties needed for large-scale animal parentage verification programs. A microsatellite is a DNA segment characterized by simple repeat motifs. The efficacy of a microsatellite-based test to detect incorrect parentage should be very high-calculated to achieve efficacies of 99.99% or greater. The test can be performed using a variety of biological samples including blood or hair, but the routine cat

test will probably use mouth swab samples. With the availability of a parentage test, cat breeders may more effectively attain their breeding goals through verified pedigrees, and will have access to highly effective tools to sort out paternity questions and switched kitten scenarios. The same DNA-based procedures for verification of pedigrees can be used as genetic identification tools to provide forensic evidence in legal proceedings.

## A Forensic Case Involving a Cat

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A PCR-based method of genetic individualization of domestic cats has been developed which has been applied to DNA isolated from a single hair of a domestic cat found on a jacket left at a homicide scene and the blood of the suspect's pet cat. Ten feline STR loci were selected from a subset of 400 dinucleotide (dC.dA)<sub>n</sub> . (dG.dT)<sub>n</sub> repeat loci isolated from genomic DNA of *Felis catus* which had been genotyped in a 70 member pedigree to generate a genetic recombination map. The STR loci were unlinked, demonstrated Mendelian inheritance, exhibited high heterozygosity and amplified

with as little as one nanogram of DNA generating strong, clean electrophoretogram profiles. A "match window" was empirically established for each STR locus based on determining the measurement precision of alleles known to be unambiguously identical by descent in the three generation pedigree. A genotype survey of two small population groups was performed which demonstrated that the ten loci exhibit expectations consistent with Hardy-Weinberg equilibrium.



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June 25 - 28, 1998

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## **Geneva: A Case-Based Learning Tool for a Lysosomal Storage Disease in a Family of Cats**

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**A** Macintosh-based interactive program is available to teach the basics of a lysosomal storage disease present in a family of cats. The program was developed using Macromedia Authorware which permits combining text, pictures, movement, and sound.

The learning process requires further reading to come to a definitive diagnosis which must be entered before the program will permit the student to progress. This further reading requires the student to be able to search databases, and have a library available which contains at least one

of a series of veterinary or human textbooks or journals.

The program presents the history and signalment, and asks for decisions about tests to be performed, giving the results of those tests selected, and illustrating abnormalities by contrasting them to normal values or pictures. The program includes clinical information, pathology, genetics, and a small section on therapy.

Geneva was used in the first year course in Medical Genetics at the University of Pennsylvania in 1998 and received good reviews from the students.

## **Feline Genetic Disease Information System**

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**T**hus far, over 150 hereditary disorders have been reported in domesticated cats and several new genetic diseases are being discovered every year. They occur in many different breeds as well as domestic shorthair/longhair cats, and represent a very heterogeneous group involving every organ system. They include congenital malformations, inborn errors of metabolism, and susceptibilities to immune disorders, infections, and cancer. Although several review articles summarize various inherited diseases, a comprehensive information source does not yet exist. We are presently developing a computer-based feline genetic

ic disease information system which contains on every disease a summary, clinical signs and laboratory test abnormalities, pathologic findings, diagnostic tests for affecteds and carriers, mode of inheritance, and recommendations for control of the disease. A first version of the computer program has been developed and a few hereditary diseases have been entered. Additional information and illustrations on hereditary diseases in cats are solicited.

## **Genetic Counseling Recommendations Should Account for the Demographics of the Population and the Epidemiology of the Defective Genes**

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**G**enetic counseling recommendations are often either, "Do not breed any affected or carrier cats," or "Breed affected and carrier cats, but only to genetically normal cats." A recommendation to eliminate carriers of a defective gene may result in a significant loss of breed genetic diversity. This could also concentrate previously unknown defective genes through a genetic bottleneck. A recommendation to breed carriers keeps the gene pool diverse, but does not provide selective pressure to decrease the frequency of the defective gene.

Genetic counseling recommendations should account for the characteristics of the genetic disease being controlled, and the dynamics of the breeding population. The character of a genetic disease includes its severity, typical age of onset, mode of inheritance, and genetic spread in the population. Genetic diseases that cause death or significant discomfort, should have a high priority. A late age of onset allows genetically affected cats to be bred before becoming clinically affected. PCR-based tests measure the genotype, and can be run at any age, before the age of onset. Polygenic disorders and recessive disorders without carrier tests are difficult to control, because carriers of defective genes cannot be identified. With these disorders, knowledge of the carrier or affected status of the littermates, the parents, and the littermates of parents (breadth of pedigree) provides important selection information on the possible genotype of the individual.

Widespread defective genes are a concern for all breeders. Conversely, a recent mutation may involve only a small portion of the breeding population. In this case, genetic disease control should be more stringent, to prevent the defective gene(s) from spreading further in the gene pool. Due to the non-random nature of cat breeding, the genetic spread of defective genes does not necessarily relate to the

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frequency of a gene in the population. Most defective genes in a purebred population originated from a single ancestral source. A defective gene that is rare in the population, but present in two distantly related cats, indicates an older and dispersed gene. While infrequent, a wide range of the population will need genetic testing and counseling.

An effective genetic disease control program requires set goals, based on; the breed, the disease, and the genetic tests available. An immediate goal is to decrease the incidence of affected cats being born. With an established testing program, the breed can monitor the frequency of the defective gene in the population, and work to decrease the percentage of carriers. With reliable carrier tests, carriers with desirable traits can be bred to genetically normal cats. Desirable, normal testing offspring should be selected for future breeding stock. By not selecting against carrier breeders, you are selecting for a carrier frequency of 50 percent; much higher than most breed averages. The proper use of genetic tests is not one that continually multiplies carriers in a breeding program. It should be geared toward producing quality, genetically normal cats. As more breeders work away from the defective gene(s), the problem for the breed as a whole diminishes.

**T**he development of whole-genome radiation hybrid mapping has revolutionized the field of gene mapping. Construction and characterization of human RH panels composed of less than 100 clones has allowed high-resolution physical mapping intermediate to that of genetic and YAC-based contig maps. The main benefit of RH mapping is its ability to integrate both genetic and EST-based maps due to the ability to score any non-polymorphic marker that differs between the donor cell line and recipient rodent cell line. This method holds great promise for the field of comparative genomics and the subsequent development of domestic species as model organisms. Construction of RH panels has been completed or are in progress in almost a dozen phylogenetically diverse vertebrate species, from mice to zebrafish. We describe the development of a 5,000 rad radiation hybrid panel for the domestic cat, *Felis catus*. The impetus for constructing a cat RH panel is twofold. First, the resolution presently available with the domestic cat/leopard cat interspecies cross is 5-30 centimorgans. Thus, finer resolution will require a method such as RH mapping. In addition, the RH panel will be essential for ordering loci on parts or all of cat chromosomes D2, F1, and Y, which cannot be accomplished using the interspecies cross. The utility of this panel is demonstrated by ordering 30-40 Type I and Type II loci from feline chromosome B4. The specific implications of sex chromosome comparative mapping will also be discussed.

### **Development of a Whole Genome Radiation Hybrid Panel for Feline Gene Mapping**

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Laboratory of Genomic Diversity, National Cancer Institute, FCRDC, Frederick, MD

**S**hort tandem Repeat (STR) loci have proven to be useful for parentage and identity verification in humans and animals. We have isolated and characterized 13 tri- and tetranucleotide repeat loci within the genome of the domestic cat (*Felis catus*), suitable for use in automated identity and parentage testing. Over 100 mixed breed, non-related individuals and 50 to 75 non-related purebred Manx, Abyssinian, and Persians have been genotyped. All genotyping was performed using 4-color fluorescent technology on an ABI Prism 373 Sequencer. PCR products are fluorescently labeled during PCR using a primer with a dye attached to the 5' end. Markers that separate electrophoretically in a gel lane by size and color can be co-loaded in a single lane. Use of the in-lane size standard (350 Rox), in a fourth color, allows for precise size calling and minimizes gel to gel variation. Genotypes can be automatically determined using Genotyper 2.0 software. PCR products from the 13 loci studied can be loaded in two lanes, one lane containing products from 8 loci (F27, F42, F37, F41, F155, F36, F163, F85), one lane containing products from 5 loci (F164, F75, F62, F95, F115). Allele frequencies, PIC, heterozygosity, and power of exclusion values were calculated for each marker, and Mendelian segregation was assessed for all loci tested.

### **Automated Genotype Analysis in Felines Using Tri- and Tetranucleotide Repeat Sequences**

M. K. Hutton, M. M. Johal

PE AgGen, Davis, California



## **Brainstem Auditory-Evoked Responses (BAER) for Diagnosis of Congenital Deafness in White Cats**

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**W**hite cats are presented to the University of Munich in increasing numbers for evaluation of hearing with brainstem auditory evoked responses (BAER) because the guidelines of the largest association of feline breeders in Germany (1. Deutscher Edelkatzenzuchtverband) specify according to current German law that (1) there is no permission to breed deaf cats, (2) hearing needs to be evaluated by BAER in every white cat prior to breeding, (3) white cats may not be bred to each other.

BAER were recorded from 36 normal cats to describe the configuration of the feline BAER and to establish reference ranges for peak latencies. Cats were anesthetized with propofol. Technical parameters were identical to a protocol used for dogs.<sup>1</sup> BAERs were recorded with platinum needle electrodes from the "vertex-mastoid position". The feline BAER consisted of a series of 6 to 7 vertex-positive peaks identified as P1a, P1b, P2, P3, P4, P5 and P6. Peak latencies at suprathreshold stimulation intensities (90 dB SPL) were  $0.92 \pm 0.03$  (P1a),  $1.26 \pm 0.05$  (P1b),  $1.72 \pm 0.08$  (P2),  $2.45 \pm 0.11$  (P3),  $3.26 \pm 0.14$  (P4),  $4.34 \pm 0.23$  ms (P5) and  $5.18 \pm 0.04$  (P6). Peaks P1b and P5 were present in 96 % and 84 % of the recordings, respectively, and were the first ones to disappear with decreasing stimulus intensities. 64 % of the recordings showed an additional peak P6 at stimulation intensity levels  $\geq 90$  dB SPL.

BAER were then recorded from 27 cats with white haircoat presented for evaluation of hearing either at the owner's own request or because required prior to breeding. The otoscopic and neurologic examinations were normal in all cats. BAER were bilaterally absent in 8 cats and unilaterally absent in 3 cats. Affected breeds were British Shorthair (2), Persian (3), and Maine Coon (6).

These results demonstrate that BAER testing is a useful diagnostic technique for the diagnosis of congenital deafness in cats similar to the situation in dogs. There is broad acceptance by the breeders for this type of examination. In cats, congenital deafness may also occur unilaterally as has been shown in dogs. Further studies are required to determine the true incidence of bilateral and unilateral deafness in white cats and to establish the pattern of inheritance.

## **Preliminary Studies Into the Anatomy and Biology of the Feline Olfactory Epithelium**

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**S**tudies of olfactory neurophysiology have progressed rapidly over the last decade in a wide variety of species, ranging from invertebrates such as lobster and drosophila to vertebrates including amphibians, fish, rodents and humans. Many basic elements of olfactory neurophysiology have been found to be remarkably conserved across diverse species, but species differences have also been found that suggest evolutionary changes have occurred to optimize function. Remarkably, virtually nothing is known of olfactory physiology in any obligate carnivore species such as the cat. In addition to being of interest in

order to fill-in this gap in our knowledge, the olfactory neuron is of interest as a tool to help understand neuronal processes occurring elsewhere in the body. Our laboratory has developed a technique to study individual olfactory neurons obtained from biopsies from living human subjects. As this tissue is capable of regeneration, biopsies can be obtained without permanent damage to the epithelium. If a similar biopsy technique can be developed for use with cats, we could study the impact of a variety of diseases on neuronal function at the cellular level from live animals. We have therefore begun structural and functional studies of the olfactory epithelium in young and adult cats obtained immediately after sacrifice. Preliminary results include anatomical studies to localize the olfactory epithelium within the nasal cavity and characterize the cell types present; molecular biological studies to begin to identify feline olfactory-specific genes, and studies using calcium imaging techniques to characterize olfactory neuron function.



**E**ighteen cases of a syndrome of episodic muscle weakness were identified in Burmese kittens. Clinical signs first became apparent between 2-6 months of age. Ventral neck flexion and limb weakness were the most consistent signs. Low potassium levels ( $<3.5$  mmol/L) were documented in 16 kittens and CPK levels ranged from 454 to over 100,000 iu/L. EMG recordings and muscle biopsies were normal. There was no evidence of inadequate intake or excessive loss of potassium and the syndrome bears similarities to hypokalaemic periodic paralysis in man which is related to a calcium channel disorder. Signs resolved in half the kittens but recurred occasionally associated with persistent hypokalaemia in others. A familial relationship between affected cats was identified.

### **Hypokalemic Episodic Weakness in Burmese Kittens**

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**I**ncidence of mediastinal lymphosarcoma in young FeLV-negative pedigreed Oriental Shorthair cats has been observed since the early 1980's. Affected cats are typically between 3 months and 2 years of age, and present initially with dyspnea or difficulty in eating. Characteristically further examination reveals a large mediastinal mass with metastases to surrounding lymph nodes. Tumors grow rapidly and are quickly terminal unless treated. Tumors regress rapidly in response to chemotherapy and there are currently a number of affected cats that have survived for a year or more with treatment.

Pedigree analysis reveals that the vast majority of affected Oriental Shorthair's are descendants, on both their maternal and paternal sides, of a single male who died of lymphosarcoma in the early 1980's. A few affected cats have this line of descent from only one parent. In spite of those individuals who are not obviously inbred on the propositus, this lymphosarcoma has many attributes of a recessive genetic disease: most affected cats trace back to the propositus on both sides, many generations of unaffected cats can be the ancestors of an affected litter, usually only approximately 1/4 of the kittens in a litter are affected, and when a cat that later develops the disease is bred to a relative, much larger percentages of kittens in the litter are affected.

### **Hereditary Lymphosarcoma in Oriental Shorthair Cats**

Heather E. Lorimer

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**T**his study is based on a retrospective analysis of 29,545 surgical pathology cases submitted to the Laboratory of Pathology, University of Pennsylvania, School of Veterinary Medicine over a 10-year period between 1986 and 1995. The tumors were classified using the International Histologic Classification of Skin Tumors, Goldschmidt, MH. et. al. (In press) and Soft Tissue Tumors, Hendrick, MJ. et. al. (In press). Odds ratios with 95% confidence limits were calculated for all feline breeds at risk for developing skin tumors. Statistical significance ( $p < 0.05$ ) was determined by the Fisher's exact test.

Tumors found to have an increased or decreased breed risk include basal cell tumor, squamous cell carcinoma, sebaceous adenoma, apocrine carcinoma, anal sac gland carcinoma, ceruminous gland carcinoma, melanocytoma, fibrosarcoma, lipoma, hemangiosarcoma, and mast cell tumors.

### **Skin and Soft Tissue Tumors of the Cat: A Retrospective Study and Analysis of Breed Risk**

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## **Congenital Portosystemic Vascular Shunts in Cats: 98 Cases**

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A retrospective study using the Veterinary Medical Data Program was undertaken to identify cats with portosystemic vascular shunts. Congenital shunts were confirmed in 98 cats at 17 institutions. There were slightly more males (56%) than females. Mixed breed cats accounted for 73% of the cases, and 2 related breeds (Persians and Himalayans) comprised another 16% of cases. Clinical signs attributed to hepatic encephalopathy were reported by the owners by 6 months of age in 80% of

cats, although diagnosis was often delayed. Heart murmurs were ausculted in 13% of cats, and 18% of males were cryptorchid. The most common clinical signs were salivation (85%), stunted growth (83%), seizures (67%), ataxia (60%), vision deficits (54%), tremors or twitching (48%), and mydriasis (38%).

RBC microcytosis was present in 36% of cats tested. High ALP (54%) and ALT (24%) and low BUN (46%) and creatinine (35%) were the most common biochemical abnormalities. Urine specific gravity was below 1.025 in 32% of cats, and 12 cats had a history of ammonium biurate urolithiasis. Liver function testing indicated increased BSP retention (83%), fasting hyperammonemia (97%), fasting bile acids (87%), and post-prandial bile acids (100%).

The most common type of shunt was the single extrahepatic portocaval anomaly. Surgery performed in 83 cases resulted in 31 complete ligations, 40 partial ligations, and 14 perioperative deaths or euthanasias. Overall, clinical condition improved following shunt correction surgery, particularly if a complete ligation was achieved.

## **Inherited Degenerative Joint Disease in Cats due to Mild Mucopolysaccharidosis Type VI**

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H uman mucopolysaccharidosis type VI (MPS VI) is a genetically inherited lysosomal storage disease caused by a deficiency of the lysosomal hydrolase N-acetylgalactosamine-4-sulfatase (4S), and leads to lysosomal accumulation of its undegraded substrate dermatan sulphate (DS). This causes pathology in connective tissues, with severe skeletal deformity and death usually in late childhood. Variability in disease severity is observed in human patients due to different mutations in the 4S gene. A naturally occurring

feline model of MPS VI was first described in Siamese cats in Philadelphia (Jezyk et al., Science 198:834-836, 1977), and has since been described in Longhaired Siamese and domestic cats in many states in the USA and in Italy (Haskins et al., JAVMA 182:983-985, 1983; Di Natale et al., JIMD 15:17-24, 1992). Disease in MPS VI cats is similar to the human disease. A colony derived from the original cats has been established in Adelaide, Australia, to develop therapies for MPS VI patients. DNA analysis identified two disease causing 4S mutations within this colony. Cats homozygous for an amino acid substitution (L476P) exhibit severe clinical disease, with dwarfism, corneal clouding, degenerative joint disease, very low leukocyte 4S/ $\beta$ -hexosaminidase ratios, DSuria, and lysosomal inclusions in leukocytes and in most connective tissues. Cats homozygous for an amino acid substitution (D520N) and compound heterozygote cats (L476P/D520N) have similar biochemical features but have normal growth, lack corneal clouding, and only have inclusions in leukocytes and some chondrocytes. L476P/D520N cats also have a high incidence of degenerative joint disease. We conclude that L476P/D520N cats have a very mild MPS VI phenotype distinct from the L476P/L476P phenotype. The study of L476P/D520N and D520N/D520N genotypes will improve understanding of genotype to phenotype correlations and the pathogenesis of skeletal dysplasia and joint disease in MPS VI.



**L**ysosomes contain many acid hydrolases that are involved in the stepwise catabolism of several large compounds including glycosaminoglycans (GAGs), also known as mucopolysaccharides (MPS), other polysaccharides, sphingolipids, glycolipids, and glycoproteins. Deficiency in activity of any one of these hydrolases causes substrate accumulation in lysosomes. Most of these lysosomal storage diseases (LSD) are inherited as autosomal recessive traits, and are associated with progressive neurologic, cardiovascular, musculoskeletal, hepatic, and/or ophthalmic signs. Thus far, 10 different LSDs have been reported in cats. The metabolic screening laboratory at the School of Veterinary Medicine, University of Pennsylvania is an integral part of the NIH-supported Referral Center for Animal Models of Human Genetic Disease and has established reliable methods to study many lysosomal enzyme activities, essential tools to specifically diagnose cats with LSD. Here we present data on 14 lysosomal enzyme activities in leukocytes and plasma from 30 healthy cats: acid  $\alpha$ -galactosidase, acid  $\alpha$ -glucosidase, acid sphingomyelinase, arylsulfatase A, arylsulfatase B,  $\alpha$ -L-fucosidase, galactosylceramidase,  $\beta$ -galactosidase,  $\beta$ -glucosidase,  $\beta$ -glucuronidase,  $\beta$ -hexosaminidase,  $\alpha$ -L-iduronidase,  $\alpha$ -mannosidase, and  $\beta$ -mannosidase. These data are compared to those in cats with specific enzyme deficiencies studied in this laboratory. The availability of these assays not only allows the diagnosis of the specific LSD, but in many cases also offers an opportunity to test for carriers, preventing the further spread of these diseases. Finally, these enzyme assays are helpful to assess the effects of gene transfer experiments.

### Lysosomal Enzyme Activities in Feline Leukocytes and Plasma

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**F**eline hemoglobin is particularly susceptible to oxidative damage and, thereby, to the formation of methemoglobin (Met-Hb) and Heinz body in erythrocytes. NADH-methemoglobin reductase (EC1.6.2.2; cytochrome  $b_5$  reductase; NADH diaphorase) is important in the reduction of Met-Hb to oxy-hemoglobin (Hb). We describe here a family of domestic shorthair cats with severe methemoglobinemia.

One male and one female domestic shorthair kitten were found to have dark brown mucous membranes and tongue on physical examination. They experienced no apparent exercise intolerance, but these cats were kept indoor. Neutering under general anesthesia was uneventful except for the dark brown/blue discoloration of genital and other internal organs. At 10 months of age both cats had a moderate polycythemia (packed cell volume 54; normal 30-45), mild reticulocytosis, and severe methemoglobinemia (Met-Hb 50 and 52%, normal <1%). Erythrocyte Met-Hb reductase activity, measured using ferricyanide as substrate, was completely deficient in both cats (control 9-13 IU/g Hb at 30 degrees C). Following acetylcysteine administration for 2 days, the Met-Hb concentrations were 38 and 39%, respectively; and ascorbic acid treatment lowered the Met-Hb level to 32% in one treated cat. Although the parents were not available for study, a male littermate to the probands had pink mucous membranes, normal Met-Hb levels (0.2%) and hematocrit (38%), but the Met-Hb reductase activity was only 3.8 IU/g Hb (40% of control).

In conclusion, a severe methemoglobinemia due to a complete Met-Hb reductase deficiency was characterized in a family of domestic shorthair cats. The intermediate Met-Hb reductase activity in a littermate suggests an autosomal recessive mode of inheritance. Affected cats are at increased risk because feline Hb is highly susceptible to oxidative damage. Treatment with reducing agents appears helpful in lowering the Met-Hb concentration.

### Familial Methemoglobin Reductase Deficiency in Domestic Shorthair Cats

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**C**ystine urolithiasis, a disorder associated with a transport defect for cystine and other basic amino acids, has been extensively studied in humans and dogs. In contrast, there is a paucity of information about cystine urolithiasis in cats.

In our series, 17 of 18 feline uroliths were composed of 100% cystine and 1 cystine calculi also had a small quantity of struvite in the outer layer. All uroliths were radiodense and were obtained from the lower urinary tract (calculi from 11 cats came from the

### Feline Cystine Urolithiasis - 18 Cases

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bladder, while samples from one cat came from the bladder and urethra, samples from one cat came from the urethra, and 5 samples were voided). Large radiodense bilateral renoliths subsequently developed in one cat that initially had only urocystoliths. Small nephroliths appeared in another cat. The initial clinical signs of affected cats were characteristic of feline lower urinary tract disease (hematuria, dysuria, pollakiuria, and/or urethral obstruction). Feline cystine uroliths occurred in males (8 male castrated) and females (7 spayed; 2 intact) with equal frequency (the gender of one cat was unknown). Mean age of cats at the time of diagnosis of cystine urolithiasis was 3.6 years (range = 4 months to 12.2 years). Most cats were of the domestic short hair breed ( $n = 12$ ), but 3 Siamese, one Maine Coon, Korat, and domestic longhair cat were also affected. The mode of inheritance has not been determined. Cystine crystalluria was a characteristic finding in urine samples collected from cats with cystine urocystoliths. Some cats also had struvite and calcium oxalate crystalluria. Hematuria was also a consistent finding. The urine pH of affected cats was variable, ranging from 6.0 to 8.0. Bacterial urinary tract infections were not observed.

Evaluation of urine amino acid profiles of four affected cats revealed increased levels of arginine, lysine, and ornithine in addition to cystine. In conclusion, cystine urolithiasis may occur more commonly than previously reported, and is an important differential diagnosis of feline lower urinary tract disease.

### **The Role of Basic Fibroblast Growth Factor and Mast Cells in the Pathogenesis of Feline Muscular Dystrophy**

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Identical cats ( $n=13$ ) were compared to normal control cats ( $n=15$ ) and carriers of the gene defect ( $n=13$ ) at the age of 3 and 6-9 months. We examined the localization of bFGF in the muscle tissue by immunohistochemistry with a monoclonal antibody against human bFGF. With several markers of muscle regeneration like desmin, vimentin and utrophin on consecutive sections, we determined the relationship of bFGF to different stages of regeneration. 3 subtypes of mast cells could be distinguished by an immunohistochemical and histochemical double-labelling method for the proteases chymase and tryptase. bFGF was increased in HFMD cats, mainly in the fiber cytoplasm, around nuclei or satellite cells, and in areas of fiber necrosis and mononuclear infiltration. Fibers containing bFGF in their cytoplasm were almost exclusively regenerating fibers. An accumulation of mast cells of the tryptase subtype was observed in HFMD animals. The mast cells were concentrated in the endomysium in areas of necrosis and early regeneration. These results indicate an important role of bFGF and mast cells in the pathogenesis of dystrophin deficiency, possibly through enhancement of tissue repair, vascularization and satellite cell proliferation.

### **Hereditary Thrombopathias Causing Bleeding in Two Domestic Shorthair Cats**

Mary Beth Callan,<sup>1</sup> Monika Griot-Wenk,<sup>1</sup> Susan Hackner,<sup>2</sup> Urs Giger<sup>1</sup>

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**H**ypertrophic Feline Muscular Dystrophy (HFMD) is a homologous animal model for Duchenne Muscular Dystrophy (DMD), an X-linked recessive disorder in humans. Contrary to DMD patients, HFMD cats do not develop muscle atrophy and fibrosis, but show a pronounced and durable muscle hypertrophy. Factors that could influence this difference in regenerating capacity are growth factors and inflammatory cells, especially mast cells. Mast cells are able to activate growth factor such as bFGF, an enhancer of myoblast proliferation, and may also play a role in the development of fibrosis. In order to investigate the role of bFGF and mast cells, we examined muscle biopsies of a HFMD-breeding colony at the University of Bern. Dystrophin-deficient

**H**ereditary platelet function disorders clinically characterized by hemorrhage, prolonged bleeding time, but normal platelet count and coagulation tests are uncommon in cats. A platelet  $\delta$ -storage pool deficiency ( $\delta$ -SPD) has been identified as part of the Chediak-Higashi syndrome in blue smoke Persian cats, but there are no previous reports of an isolated intrinsic platelet function defect in the cat. Here we describe inherited thrombopathias in two young unrelated domestic shorthair cats. A 1.5-year-old neutered male cat was presented for evaluation of recurrent bilateral epistaxis, petechiation of the pinnae, and gingival bleeding, and a 7-month-old female cat was presented with a histo-

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ry of intermittent gingival bleeding, severe enough to result in a blood loss anemia (PCV 12%) and need for a blood transfusion. Platelet counts and measurements of plasma coagulant function and von Willebrand factor were normal in both cats. However, buccal mucosal bleeding times were prolonged in both cats (male, >8 minutes; female 6.5 minutes; normal <2 minutes). In repeated aggregation studies, platelets from both cats underwent a shape change and minimal aggregation (<5%) in response to ADP ( $\leq 100 \mu\text{M}$ ); there was no aggregation in response to low concentration of collagen (2  $\mu\text{g/ml}$ ) and a diminished response (<40%) to a high concentration of collagen (50-100  $\mu\text{g/ml}$ ). The platelet ATP:ADP ratio was increased in the female cat (5.8; normal <3.6) but normal in the male cat (1.9). Platelet morphology assessed by light microscopy was normal in both cats, and by electron microscopy was normal in the one cat (male) tested. The increase in the platelet ATP:ADP ratio in the female cat, together with impaired platelet aggregation, suggests that a platelet  $\delta$ -SPD may be the cause of bleeding in this cat. In light of a normal platelet ATP:ADP ratio in the male cat, his platelet aggregation abnormalities are suggestive of a secretion/signal transduction disorder, possibly due to a defect in arachidonic acid metabolism. Hereditary thrombopathias may be an important.

**F**our related domestic shorthaired kittens were investigated after detection of abnormalities in their gait and difficulty eating and chewing. They walked with a stiff, stilted gait although the apparent stiffness reduced during exercise.

Clinical examination confirmed difficulty opening their mouths, skeletal muscle hypertrophy and facial and skeletal muscle spasms with hyperextension of the limbs and falling into lateral recumbency when they received a sudden fright. A diagnosis of *Myotonia congenita* was made based on the clinical signs and presence of spontaneous waxing and waning, high frequency discharges on electromyography and the "dive bomber" sound on audioprojection. Histopathologically there was moderate myofibre hypertrophy; occasional fibres had central nuclei and rounded sarcoplasm. No histochemical abnormalities were detected.

The findings in these cats resemble those of *M. congenita* of dogs goats and humans. As all kittens were related, being from litters from related queens, the disease was thought to be inherited as an autosomal recessive mode of inheritance. The sire was an unknown stray. This is the first report of this disease in this species and the pathogenesis is thought to be associated with decreased chloride conductance of muscle cell membranes as it is in those species.

The molecular basis of human *M. congenita* is established as being caused by a mutation of the gene encoding for the muscle chloride channel. Nine point mutations in exons, a base change affecting a splice consensus site, and two deletions have been described in the human chloride channel gene. Similar investigations are planned for the myotonic cats.

### Congenital Myotonia in Related Cats

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**N**ine new-born Birman kittens were referred to the University of Zurich, School of Veterinary Medicine showing hairless of practically the entire body. At the breeders request, they were euthanized to determine the cause of disease. At necropsy, no thymus could be found; in its usual location were loose connective tissue and fat. On histologic examination, hair follicles, sebaceous glands, and sweat glands were hypoplastic and reduced in number. Histologic examination of the normal anatomical site of the thymus revealed an absence of lymphocytes and Hassall's corpuscles. The lymph nodes, spleen, and Peyer's patches showed a reduction in germinal follicles and the paracortical regions contained few lymphocytes.

The pedigree revealed nine affected kittens, five males and four females, and nine normal kittens born in five litters to five normal par-

### Congenital Hypotrichosis With Thymic Aplasia in Birman Kittens

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ents with one common ancestor. Pedigree analysis suggested an autosomal recessive mode of inheritance.

Hairless Birman kittens have been previously reported in Great Britain. Of the affected kittens which were not killed shortly after birth, none survived to 13 weeks of age. It appeared that all succumbed to infections which are normally not lethal in immunologically intact cats. Unfortunately necropsies were not performed, but this finding suggests that these kittens might have also been athymic.

Congenital hypotrichosis with thymic aplasia has been described in mice, rats, guinea pigs, and two calves. The clinical appearance and the histologic findings in these species were very similar to those found in our kittens, but further studies are needed to characterize the genetics of this defect and the immunologic status of the affected kittens.

## Coat Color Genes in Domestic and Wild Cats

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**D**iverse patterns of coat color variation are observed in the domestic cat, which can be related in several possible ways to color phenotypes present in other felid species. Since the beginning of this century, interesting genetic and evolutionary questions have been raised regarding the mechanisms underlying coat color determination and its evolution in the cat family. However the genes involved in these traits have not yet

been characterized in these organisms, precluding further advance in this area. The present study aims to (1) map the chromosomal locations of the major genes involved in coat color determination in the domestic cat, and assess homology with other mammalian species; (2) characterize at the molecular level some of these genes in the domestic cat and other felid species, and identify mutations producing different phenotypes; (3) address evolutionary issues in the Felidae pertaining to these loci. We have ascertained a large domestic cat pedigree in which several coat color traits are segregating, and which will be used for the mapping approach along with the Laboratory of Genomic Diversity interspecific backcross pedigree and other pedigrees which may become available. We are currently characterizing at the molecular level the *agouti* locus, believed to be involved in melanistic phenotypes, through conserved primer design, PCR amplification and direct sequencing in different felid species, characterization of clones from a domestic cat PAC genomic library, and analysis of mRNA obtained from skin biopsies in two different species. We are starting to investigate using similar approaches the extension locus, possibly also involved in melanistic phenotypes in some species, and other traits such as brown, albino (siamese/tyrosinase), silver (inhibitor of melanin), piebald spotting, dominant white, orange, and tabby.

## The AB Blood Group System in Wild Felids

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**B**lood type A and B erythrocyte antigens represent the only blood group system recognized in domestic cats (*Felis catus*) and are determined by glycolyl and acetyl neuraminic acids on membrane glycolipids and -proteins. The A allele is dominant over the B allele, and type AB is inherited as a third allele. Blood type A is the most common type. The frequency of type A and B varies between geographic areas worldwide among domestic short-hair cats and also between breeds of cats. Domestic cats possess naturally-occurring alloantibodies that are responsible for neonatal isoerythrolysis and hemolytic transfusion reactions. In this study, we surveyed the blood type of non-domesticated felids.

A total of 131 wild cats belonging to 26 felid species (1-16 members per species) from zoos and parks were studied. Based upon a tube hemagglutination assay established for domestic cats, 80% of felids had type-A and 18% type-B blood. Felids in the Puma group and African and Asian golden cats had blood type B, whereas all other species were found to have blood type A. In addition, two cheetahs and one bobcat had type-AB blood (2%). Red cell glycolipids analyzed by high performance thin layer chromatography revealed a similar ganglioside pattern in wild cats as previously reported in domestic cats. Sera from only 8% of wild felids agglutinated type A and/or type B cells from domestic cats independent of the AB blood group system. Incompatible blood crossmatch reactions were only detected between species and more likely between members of different phylogenetic groups.

In conclusion, wild felids display AB-erythrocyte antigens as domestic cats, and the same blood typing procedures can be applied. The majority of felid species had type A blood. The blood typing data followed the proposed phylogenetic classification (Johnson and O'Brien 1997). Blood incompatibility reactions were observed among different felid species. Therefore, it appears advisable to blood type and crossmatch wild cats to ensure blood compatibility when transfusing and breeding them.



Carrier detection remains the single most effective means of controlling the spread of recessively inherited disease. Unfortunately, many biochemical assays are equivocal when used to examine animals who are carriers of inherited disease. Accurate results may be obtained from a variety of molecular genetic tests, however these are often complicated, time consuming, expensive, and subject to contamination, requiring extensive quality control. Novel methods must be developed to allow rapid, accurate, and inexpensive carrier detection. Currently, three methods are being evaluated to detect the mutation of feline beta-galactosidase responsible for GM1 gangliosidosis in the Korat and Siamese breeds. These methods include automated direct sequencing of genomic PCR fragments, which represents the current method employed by the laboratory for genetic diagnosis. A 5'-3' exonuclease fluorogenic release assay has been developed using two oligonucleotide probes, one corresponding to the sequence of each allele. The amount of fluorescence released is directly proportional to the number of copies of each allele, allowing specific alleles to be counted. A final assay under development utilizes genetic bit analysis, where oligonucleotides are used as primers to extend across the mutation site with labeled dideoxy nucleotides. DNA sequencing remains the "gold standard" as it allows for directly reading the sequence of the DNA involved. However, the expense incurred, the need for multiple post PCR manipulations and interpretation of the data make this the least desirable of the three techniques for clinical implementation. The 5' fluorogenic release assay presents an attractive alternative to sequencing as it completely eliminates all post PCR processing, and could be totally automated. However, the need for specialized equipment to perform the assay means that its use may be confined to large commercial or academic laboratories. Genetic bit analysis presents an appealing compromise between sequencing and the 5' fluorogenic release assay as it allow the sequence at one particular base to be determined and only requires one post PCR manipulation. Additionally, results may be determined on the bench top, without specialized equipment. With the advent of smaller and less expensive thermocyclers, this assay has the potential of being offered in small clinical laboratories and perhaps even in the veterinarian's office.

### **Novel Molecular Methods for Carrier Detection of Inherited Feline Diseases**

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A PCR-based method of genetic individualization of domestic cats has been developed which has been applied to DNA isolated from a single hair of a domestic cat found on a jacket left at a homicide scene and the blood of the suspect's pet cat. Ten feline STR loci were selected from a subset of 400 dinucleotide (dC.dA)<sub>n</sub> . (dG.dT)<sub>n</sub> repeat loci isolated from genomic DNA of *Felis catus* which had been genotyped in a 70 member pedigree to generate a genetic recombination map. The STR loci were unlinked, demonstrated Mendelian inheritance, exhibited high heterozygosity and amplified with as little as one nanogram of DNA generating strong, clean electrophoretogram profiles. A "match window" was empirically established for each STR locus based on determining the measurement precision of alleles known to be unambiguously identical by descent in the three generation pedigree. A genotype survey of two small population groups was performed which demonstrated that the ten loci exhibit expectations consistent with Hardy-Weinberg equilibrium.

### **Genetic Individualization of Domestic Cats Using Feline STR Loci for Forensic Applications**

**Marylin Menotti-Raymond, Victor A. David, J. Claiborne Stephens, Leslie A. Lyons, Stephen J. O'Brien**

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## Microsatellite Analyses of Two Re-Introduced Eurasian Lynx (*Lynx lynx*) Populations in Switzerland Using Heterologous Tools

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have commenced a pilot study in order to determine the genetic diversity of the two Swiss lynx populations. The genetic analysis has been performed using microsatellites which were developed in cats. Genomic DNA was isolated from whole blood samples from the collection of the KORA. PCR amplification of 10 feline fluorescently-labelled microsatellites (FCA 026,043, 058, 080, 088, 090, 096, 126, 132, 149) has been carried out in 10 unrelated, age and sex matched individuals per group. Furthermore, we tested 4-5 offspring of 2-3 females per population, in order to study the mode of inheritance of the newly defined alleles. The allele length was determined in polyacrylamide gels using an ABI 373 sequencer with ABI Genescan and Genotyper software. Statistical analysis was performed using the GENEPOP program. All feline microsatellites amplified well in lynx. The observed products were inherited in a Mendelian fashion. The number of alleles detected per locus ranged from 2 to 7. The allele size ranges differed slightly from those detected for the cat. The average heterozygosity over all loci was 0.48 (0.21-0.70). When testing for Hardy-Weinberg, 9 out of 10 loci were in equilibrium. Population-specific alleles could only be found in a very low frequency (e.g. alleles no. 4, fca 149, 10%). A statistically significant ( $p < 0.0001$ ) difference of the allele frequencies for the two populations could be seen for two loci (FCA 090 and 096). Our preliminary results represent the first microsatellite analysis performed in the lynx and confirm the usefulness of *Felis catus* specific markers for this purpose. With the completion of the genetic map of the domestic cat, additional loci will be at our disposal to answer the questions of interest for the lynx species.

## Using Canine Recombinant Granulocyte Colony Stimulating Factor (CG-CSF) for Treatment of Ringworm in Cats

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Our question is, does canine recombinant granulocyte colony stimulating factor (cG-CSF) assist in the treatment of cats with intractable dermatophytosis and or with dermatophytosis and immune suppression? The rationale for this study is that a colony of Persian cats with mild dermatitis and one cat with intractable dermatophytosis and immune suppression were successfully treated with G-CSF and both human recombinant and cG-CSF have been successfully administered to cats. We have recently initiated a study involving the treatment of pet cats that present with a variety of diseases in which the use of cG-CSF is warranted. The results we have obtained to date will be discussed.

Lynx were extinct in most of Central and Western Europe at the end of the 19th century. In the 1970s, re-introduction programs started in the Alps and in adjacent mountain ranges of Switzerland, Slovenia, Italy, Austria, and France. In Switzerland, 6 lynx (4M/2F) were officially released in the Alps and 4 animals (2M/2F) in the Jura Mountains. Due to additional clandestine releases the number of founder individuals probably was 10-12 lynx for the Alps, and 8-10 for the Jura Mountains, respectively. All known animals originated from the wild population of the Carpathian Mountains of Slovakia. The spread of the Swiss populations has slowed down or halted in the mid-1980s. Today, the two populations are estimated to consist of about 50 reproducing lynx each. From this situation, several questions arise: (1) Do the two geographically separated lynx populations of Switzerland differ genetically? (2) Do the two populations have a reduced genetic variability compared to the Slovakian source population? (3) Did the founder individuals all originate from the autochthonous population of the Carpathian Mountains? To address the first question, we

Dermatophytosis is a superficial infection of the keratinized layers of the skin, hair, and nails by zoonotic fungi. Three dermatophytes commonly cause infection in cats: *Microsporum canis*, *M. gypseum*, and *Trichophyton mentagrophytes* and *T. rubrum* (the common human athlete's foot fungus) is occasionally isolated from cats. *Microsporum canis* is the most common isolate. While topical fungicidal agents are effective, these generally have no effect on spores and hyphae that may be present elsewhere on the animal. The use of systemic agents are also effective, but their side effects, specifically bone marrow suppression, will occasionally preclude their use in immune suppressed patients.