25 The Domestic Cat, *Felis catus*, as a Model of Hereditary and Infectious Disease

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ABSTRACT

The domestic cat, currently the most frequent of companion animals, has enjoyed a medical surveillance, as a nonprimate species, second only to the dog. With over 200 hereditary disease pathologies reported in the cat, the clinical and physiological study of these feline hereditary diseases provides a strong comparative medicine opportunity for prevention, diagnostics, and treatment studies in a laboratory setting. Causal mutations have been characterized in 19 felid genes, with the largest representation from lysosomal storage enzyme disorders. Corrective therapeutic strategies for several disorders have been proposed and examined in the cat, including enzyme replacement, heterologous bone marrow transplantation, and substrate reduction therapy. Genomics tools developed in the cat, including the recent completion of the 2-fold whole genome sequence of the cat and genome browser, radiation hybrid map of 1793 integrated coding and microsatellite loci, a 5-cM genetic linkage map, arrayed BAC libraries, and flow sorted chromosomes, are providing resources that are being utilized in mapping and characterization of genes of interest. A recent report of the mapping and characterization of a novel causative gene for feline spinal muscular atrophy marked the first identification of a disease gene purely from positional reasoning. With the development of genomic resources in the cat and the application of complementary comparative tools developed in other species, the domestic cat is emerging as a promising resource of phenotypically defined genetic variation of biomedical significance. Additionally, the cat has provided several useful models for infectious disease. These include feline leukemia and feline sarcoma virus, feline coronavirus, and Type C retroviruses that interact with cellular oncogenes to induce leukemia, lymphoma, and sarcoma.

Key Words: Domestic cat, *Felis catus*, Gene therapy, Whole genome sequence, Radiation hybrid map, Knockout model, FIV, SARS.

INTRODUCTION

Mankind has held a centuries-long fascination with the cat. The earliest arch eological records that have been linked to the domestication of *Felis catus* date to approximately 9500 years ago from Cyprus,[1] with recent molecular genetic analyses in our laboratory suggesting a Middle Eastern origin for domestication (C. Driscoll *et al.*, unpublished observations). Currently the most numerous of companion animals, numbering close to 90 million in households across the United States (http://www.appma.org/press_industrytrends.asp), the cat enjoys a medical surveillance second only to the dog and humankind. In this chapter we review the promise of the cat as an important model for the advancement of human hereditary and infectious disease and the genomic tools that have been developed for the identification, and characterization of genes of interest.

For many years we have sought to characterize genetic organization in the domestic cat and to develop genomic resources that establish *F. catus* as a useful animal model for human hereditary disease analogues, neoplasia, genetic factors associated with host response to infectious disease, and mammalian genome evolution.[2,3] To identify genes associated with inherited pathologies that mirror inherited human conditions and interesting pheno-types in the domestic cat, we have produced genetic maps of sufficient density to allow linkage or association-based mapping exercises.[4–11]

The first genetic map of the cat, a physical map generated from a somatic-cell hybrid panel, demonstrated the cat's high level of conserved synteny with the human genome, which offered much promise for the future application of comparative genomic inference in felid mapping and association exercises.[12] Several radiation hybrid (RH) and genetic linkage (GL) maps have since been published.[4–9,11,13,14]

THE DOMESTIC CAT RADIATION HYBRID MAP

Although previous versions of the cat gene map, based on somatic cell hybrid and ZOO FISH analysis,[15,16] revealed considerable conservation of synteny with the human genome, these maps provided no knowledge of gene order or intrachromosomal genome rearrangement between the two species, information that is critical to applying comparative map inference to gene discovery in gene-poor model systems. Radiation hybrid (RH) mapping has emerged as a powerful tool for constructing moderate- to high-density gene maps in vertebrates by obviating the need to identify interspecific polymorphisms critical for the generation of genetic linkage maps.[7]

The most recent RH map of the cat[8] includes 1793 markers: 662 coding loci, 335 selected markers derived from the cat 2X whole genome sequence targeted at breakpoints in conserved synteny between human and cat, and 797 short tandem repeat (STR) loci. The strategy used in developing the current RH map was to target gaps in the feline–human comparative map, and to provide more definition in breakpoints in regions of conserved synteny between cat and human. The 1793 markers cover the

From: *Sourcebook of Models for Biomedical Research* (P. M. Conn, ed.),

length of the 18 feline autosomes and the X chromosome at an average spacing of one marker every 1.5 Mb (megabase), with fairly uniform marker density.[8] An enhanced comparative map demonstrates that the current map provides 86% and 85% comparative coverage of the human and canine genomes, respectively.[8] Ninety-six percent of the 1793 cat markers have identifiable orthologues in the canine and human genome sequences, providing a rich comparative tool, which is critical in linkage mapping exercises for the identification of genes controlling feline phenotypes. Figure 25–1 presents a graphic display of each cat chromosome and blocks of conserved syntenic order with the human and canine genomes.[8] One hundred and fifty-two cat–human and 134 cat–dog homologous synteny blocks were identified. Alignment of cat, dog, and human chromosomes demonstrated different patterns of chromosomal rearrangement with a marked increase in interchromosomal rearrangements relative to human in the canid lineage (89% of all rearrangements), as opposed to the more frequent intrachromosomal rearrangements in the felid lineage (95% of all rearrangements) since divergence from a common carnivore ancestor 55 My ago.

With an average spacing of 1 marker every 1.5 Mb in the feline euchromatic sequence, the map provided a solid framework for the chromosomal assignment of feline contigs and scaffolds during assembly of the cat genome assembly,[17] and served as a comparative tool to aid in the identification of genes controlling feline phenotypes.

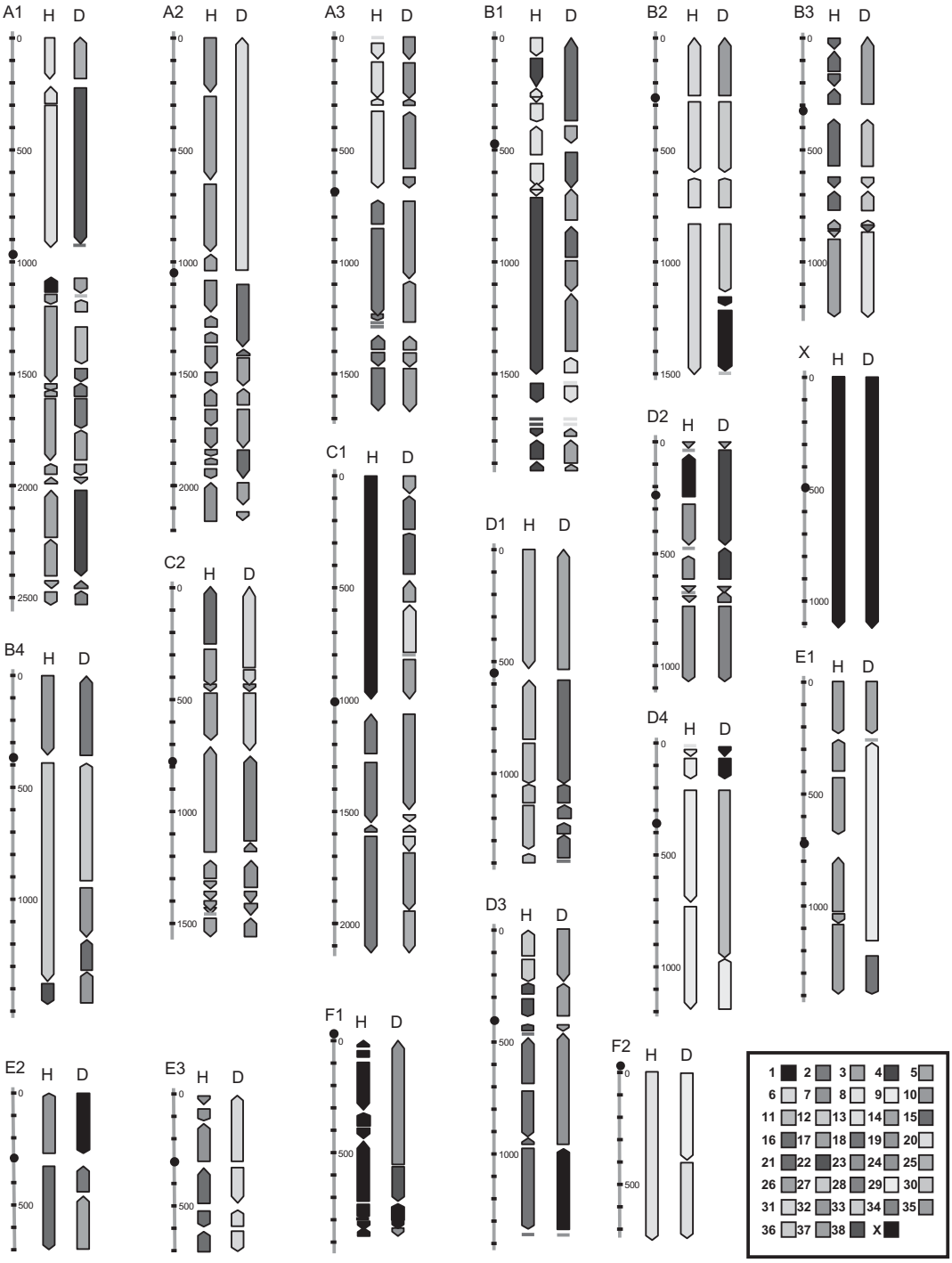

Figure 25–1. Feline chromosome maps (labeled at top) and homologous synteny blocks (HSBs) in the human (H) and dog (D) genomes. HSBs are shown to the right of each cat chromosome map (only the map scale is shown). The dark cross-marks on each cat chromosome correspond to 100-cR$_{5000}$ intervals. The inferred centromere positions are shown by dark circles. HSBs are color coded by human or dog chromosome, defined by the key in the bottom right corner. (Reprinted from Murphy *et al.*[7] Copyright 2007, with permission from Elsevier.) (See color insert.)

THE DOMESTIC CAT GENETIC LINKAGE MAP

As a complement to the RH map of the cat, a third generation linkage map of 625 STRs is currently nearing completion. The map has been generated in a large multigeneration domestic cat pedigree (n = 483 informative meioses).[18] Previous first- and second-generation linkage maps of the cat were generated in a multigeneration interspecies pedigree generated between the domestic cat and the Asian leopard cat, *Prionailurus bengalensis*,[7] to facilitate the mapping and integration of Type I (coding) and Type II (polymorphic STR) loci.[7] The current map, which spans all 18 autosomes with single linkage groups, has twice the STR density of previous maps, providing a 5-cM resolution. There is also greatly expanded coverage of the X chromosome, with some 75 STR loci. Marker order between the current generation RH and GL maps is highly concordant.[8]

Approximately 85% of the STRs are mapped in the most current RH map of the cat,[8] which provides reference and integration with Type I loci. Whereas the third-generation linkage map is composed entirely of STR loci, the sequence homology of extended genomic regions adjacent to the STR loci in the cat 2X whole genome sequence,[17] to the dog's homologous region,[19] has enabled us to obtain identifiable orthologues in the canine and human genome sequences for over 95% of the STRs. Thus, practically every STR acts as a "virtual" Type 1 locus, with both comparative anchoring and linkage map utility. Combined with the cat RH map, these genomic tools provide us with the comparative reference to other mammalian genomes critical for linkage and association mapping.

THE DOMESTIC CAT WHOLE GENOME (2X) SEQUENCE

The domestic cat is one of 26 mammalian species endorsed by the National Human Genome Research Institute (NHGRI) Human Genome Annotation committee for a "light" 2-fold whole genome sequence, largely to capture the pattern of genome variation and divergence that characterizes the mammalian radiations (http://www.hgsc.bcm.tmc.edu/projects/bovine/, http://www.broad.mit.edu/mammals/). Although light genome coverage provides limited sequence representation, (~80%),[20] one of the rationales for these light genome sequences included "enhancing opportunities for research on species providing human medical models." The 2-fold assembly of the domestic cat genome has recently been completed for a female Abyssinian cat, "Cinnamon," [17] and a 7X whole genome sequencing effort is planned in the near future.

A total of 9,161,674 reads were assembled to 817,956 contigs, covering 1.642 Gb with an N50 (i.e., half of the sequenced base pairs are in contigs <N50) contig length of 2378 bp. Assembled supercontigs (N = 217,790) had an N50 length of 117 kb[17] (http://hosted. abcc.ncifcrf.gov/cgi-gin/gbrowse/cat/). The estimated size of the genome was 2.7 Gb and the genome coverage was approximately 2-fold, predicting an average inclusion of 80–85% of the eukaryotic genome sequence.[21]

Feline coding genes were identified using a comparative approach based upon sequence homology and syntenic orthology of neighboring gene homologues in the genomes of six index mammal species (human, chimp, mouse, rat, cow, and dog). The results revealed nearly 21,080 feline genes plus 132,493 conserved sequence blocks (CSBs) used to build the gene map,[17] depending upon the framework RH map of 1794 ordered Type 1 markers.[8] The 2X feline genome sequence detects 83% of human genes, 89% of chimp or cattle genes, and 92% of dog genes based upon sequence identity to approximately 1000 bp of reciprocal base match[17] between the cat sequence and the genome sequence of the six index mammals.

A genome browser has been developed from the cat assembly, named Genome Annotation Region FIELD (GARFIELD), which provides a physical map of the 18 autosomes and the X chromosome, which can be inspected for sequence representation, including genes and the proportion of that gene available in the 2X cover, single nucleotide polymorphisms (SNPs), and STRs, which can be used in linkage and association mapping, and other genome features (http://ccr.cancer.gov/ labs/lab). Figure 25–2 illustrates a

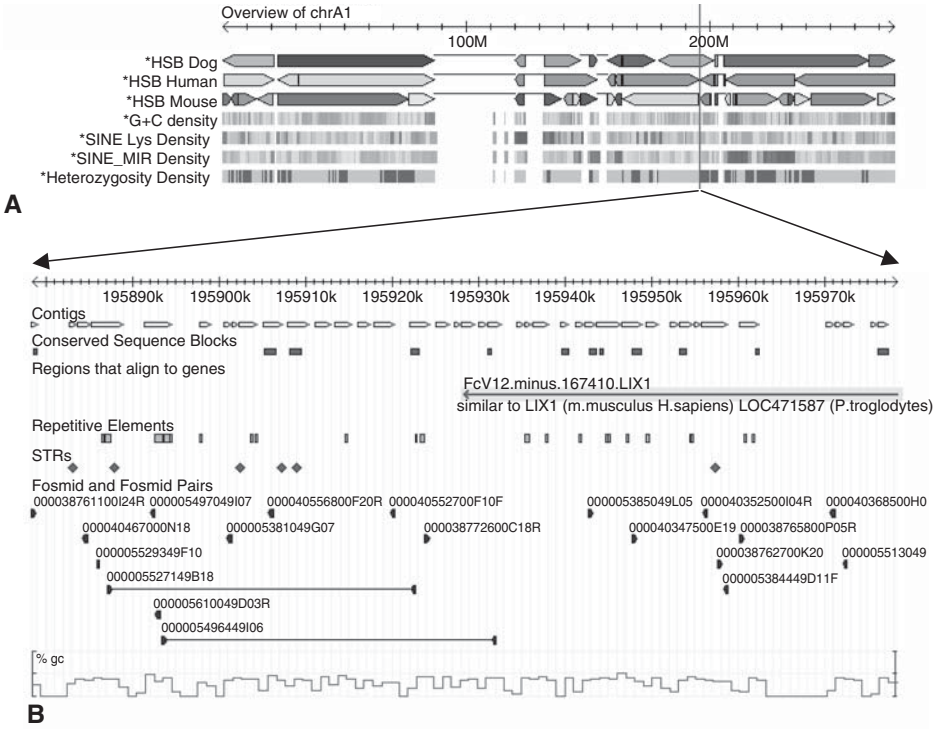

Figure 25–2. Gene annotation region fields (GARFIELD) displayed using Generic Genome Browser at two levels of resolution for the *LIX1* gene on chromosome A1. (A) Chromosome view showing homologous synteny blocks (HSB) for dog, human, and mouse, representation of G + C density, and of SINE, LINE, and SNPs. (B) A 200-kb view showing contigs of the region, conserved sequence blocks (CSB), regions that align to annotated genes in other mammalian genomes, regions masked by repeat masker, single tandem repeats, Fosmid reads with their partners, and a histogram of local GC content.

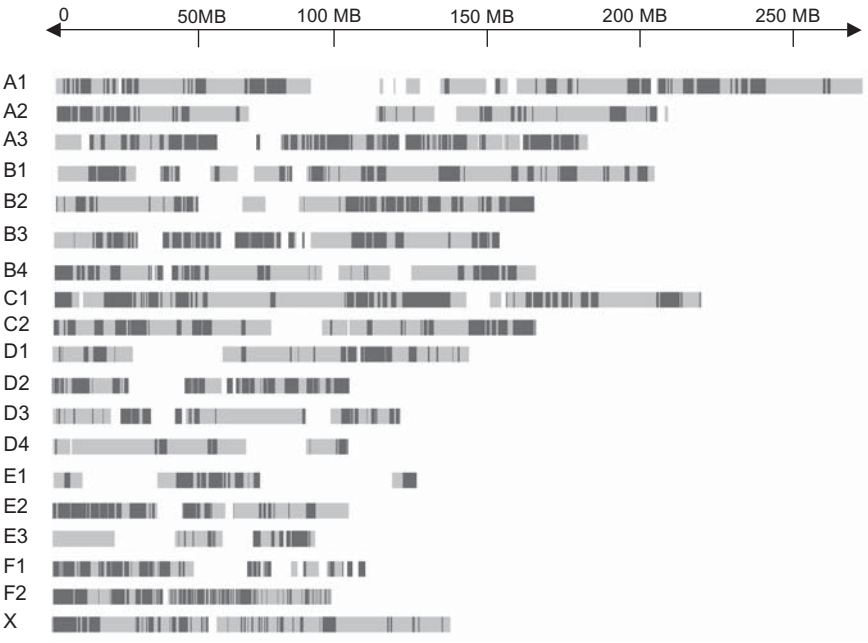

Figure 25–3. SNP profile across the 2X whole genome sequence of the domestic cat, Cinnamon. Heterozygosity across Cinnamon's chromosomes is represented in nonoverlapping windows of 100 kb. Black represents regions with more than two SNPs per 100 kb while gray represents the homozygous region (less than two SNP, 0/100 kb). White represents gaps in the chromosome assembly.

representative view of GARFIELD demonstrating features for the *LIX1* gene on chromosome A1.[17]

SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS DEMONSTRATES POTENTIAL OF LINKAGE DISEQUILIBRIUM MAPPING IN CAT BREEDS

A total of 421,000 SNP variants were identified in Cinnamon's sequence, representing an incidence of 1/600 bp.[17] Approximately 43% of Cinnamon's genome was heterozygous and 57% homozygous, which was not unexpected in a breed cat that is also the member of a highly inbred pedigree for retinal atrophy.[17,22] Long stretches of alternating homozygous and heterozygous segments were observed (Figure 25–3), which represent the consequences of close inbreeding during the domestication process, and the more recent generation of fancy breeds and inbred disease pedigree.[22] Similar patches of homozygous/heterozygous segments were observed in the recently released whole genome sequence of the dog.[19] The length of the segments is influenced by breed-specific history including effective population sizes, use of popular sires, and population bottlenecks.[23,24]

Linkage disequilibrium (LD) mapping has recently emerged as a powerful approach in humans for association mapping.[25] Long stretches of linkage disequilibrium in the target population greatly facilitate the success of the strategy and decrease the number of markers required for analysis.[25] The extended LDs observed in dog breeds, up to one hundred times the length observed in human populations,[26] is proving to be a powerful mapping strategy for identification of genes associated with breed-specific phenotypes,[27] including hereditary pathologies.[28,29]

The potential of this mapping approach in cat breeds was evaluated by examining breed-specific patterns of common segment homozygosity in 24 Cat Fancier Association (CFA) (http://www.cfainc.org) breeds.[17] The level of homozygosity reflected in a group of 665 SNPs was roughly half that seen in dogs,[26] likely reflecting a more extensive recent inbreeding within dog versus cat breeds.[30] This level of homozygosity was used to estimate[19] that some 45,000 equivalently spaced SNP variants would be required for a linkage disequilibrium/haplotype-based association genome search of a complex heritable disease within cat breeds.

Recently, tyrosinase-related protein 1 (*TYRP1*), one of the key enzymes in the melanogenic pathway, was linked to two coat color variants in the cat by association mapping in 38 cat breeds due to extensive LD.[31] Two DNA polymorphisms in *TYRP1*, an A3G substitution in the signal peptide and an in-frame insertion *TYRP1*–421ins17/18, were associated with the *chocolate* (b) allele. A premature UAG stop codon at position 100 of *TYRP1* was associated with a second allele of the B locus, *cinnamon* (bl).[32] SNP discovery is planned in the 7X whole genome sequencing of the cat through a resequencing strategy of selected genomic regions in several cat breeds as was recently performed in the 7X whole genome sequencing of the dog.[19]

THE MATURITY OF CURRENT FELID MAPPING RESOURCES DEMONSTRATED IN SUCCESSFUL WHOLE GENOME AND ASSOCIATION MAPPING EXERCISES

The majority of hereditary pathologies in the domestic cat for which the gene defect has been elucidated have resulted from the analysis of candidate genes (Table 25–1). However, with the availability of a detailed comparative map, and integration with developing GL and RH maps, and the cat 2X whole genome sequence, linkage and association-based mapping techniques have recently identified causative mutations for hereditary disease genes,[33,34] as well as several feline phenotypes (Table 25–1).[18,32,35–57]

Once a genomic region is implicated from association-based or linkage mapping exercises, fine mapping has been accomplished by development of new STRs or SNPs in the targeted region using the cat 2X whole genome sequence data accessed

Table 25–1
Feline genetic diseases/phenotypes characterized at a molecular level

Disease/phenotype	*Gene*	*Mutationa*	*Reference*
α-Mannosidosis	MAN2B1	1749_1752delCCAG leads to premature stop	38
Gangliosidosis G_{M1} (Sandhoff disease)	GLB1	R482P	39
Gangliosidosis G_{M2}	HEXB	39delC leads to premature stop or 1467_1491 inv; del exon12	40, 41
	GM2A	Del4bp in 3′ region leads to frame shift	42
Glycogenosis IV	GBE1	Gene rearrangement with insertion and large deletion in exon 12	43, 44
Hemophilia B	F8	R338X, C82Y	45
Hypertriglyceridemia (lipoprotein lipase deficiency)	LPL	G412R	46
Hypertrophic cardiomyopathy	MYBPC3	A31P	47
Mucolipidosis II (I-cell disease)	GNPTAB	C2655T	48
Mucopolysaccharoidosis Type I	IDUA	107_109 delCGA	49
Mucopolysaccharoidosis Type VI	ARSB	L476P (severe phenotype) D520N (mild phenotype)	50
Mucopolysaccharoidosis Type VII	GUSB	E351K	51
Muscular dystrophy, Duchenne type	DMD	Deletion in the dystrophin muscle promoter	52
Niemann–Pick disease, Type C	NPC1	G2864C	53
Oculocutaneous albinism (Type II)	TYR	R422Q	54
Polycystic kidney disease	PKD1	C3284X	33
Pyruvate kinase deficiency	PKLR	Splicing defect leads to 13 bp deletion in exon 6	55
Retinal degeneration in Abyssinian cats (rdAc)			
Spinal muscular atrophy	LIX1	~140 kb deletion	34
Albino	TYR	del975C leads to premature stop	36
Brown	TYRP1	A3G and 421_422 ins 18AA/19AA	32
Burmese	TYR	G227W	32, 37
Cinnamon	TYRP1	R100X	32
Dilute	MLPH	del83T leads to premature stop	35
Melanism	ASIP	123_124delCA leads to frame shift	18
Melanism (jaguar)	MC1R	301_315del	18
Melanism (jagaroundi)	MC1R	283_306del	18
Siamese	TYR	G301R	32, 37
Sweet taste receptor	TAS1R2	454_700del	56

[a]Mutation notation according to den Dunnen and Antonarakis.[53]

through the cat genome browser, GARFIELD.[17] A recent report of the mapping and characterization of a novel gene causative of feline spinal muscular atrophy[34] marked the first identification of a disease gene purely from positional reasoning.

Human spinal muscular atrophies (SMAs) are a genetically heterogeneous group of neuropathies that varies in clinical severity, from lethal in infancy to onset of mild weakness in adulthood, but all are characterized by neurogenic muscle atrophy due to degeneration of lower motor neurons of the spinal cord.[58] For approximately 97% of people affected with SMA, disease pathology is attributable to a mutation in the *SMN1* gene, on human chromosome 5q13, which is subject to a high frequency of deletions and gene conversion events with the divergent and only partially functional centromeric copy/copies of the duplicated *SMN2* locus.[59,60]

A domestic cat model of SMA has been described that is a model of autosomal recessive juvenile-onset SMA.[61] With the feline *SMN* gene excluded as the disease locus,[61] a full genome linkage scan was conducted in a pedigree segregating for SMA.[61] The disease phenotype was linked to chromosome A1q,[34] in a region of conserved synteny to human chromosome 5q15. Fine mapping was accomplished with development of new STRs and sequence tagged sites (STS), utilizing sequence information from the cat 2X whole genome sequencing effort, which ultimately identified an ~140 kb deletion and a novel gene candidate, *LIX1*[34] (Figure 25–4). Though the function of *LIX1* is unknown, the predicted secondary structure is compatible with a role in RNA metabolism. An exon sequence screen of 25 human SMA cases, not otherwise explicable by mutations at the *SMN1* locus, failed to identify comparable *LIX1* mutations.[34]

The *SMN1* gene product, SMN, is a ubiquitously expressed protein member of multiple ribonucleoprotein complexes with diverse roles in RNA metabolism, splicing, and transport in all cells.[62,63] A central focus of SMA research remains to discern the disease mechanism(s) and to understand why the primary disease pathology is localized to spinal lower motor neurons when all cells require SMN function. *LIX1* expression is largely restricted to the central nervous system (CNS), primarily in spinal motor neurons, thus offering an explanation of the tissue restriction of pathology in feline SMA. Determination of *LIX1* function may well provide fresh insight into the mechanisms of human SMA pathology, impetus for more targeted therapeutics, and answers to fundamental questions of motor neuron development, maintenance, and/or function.

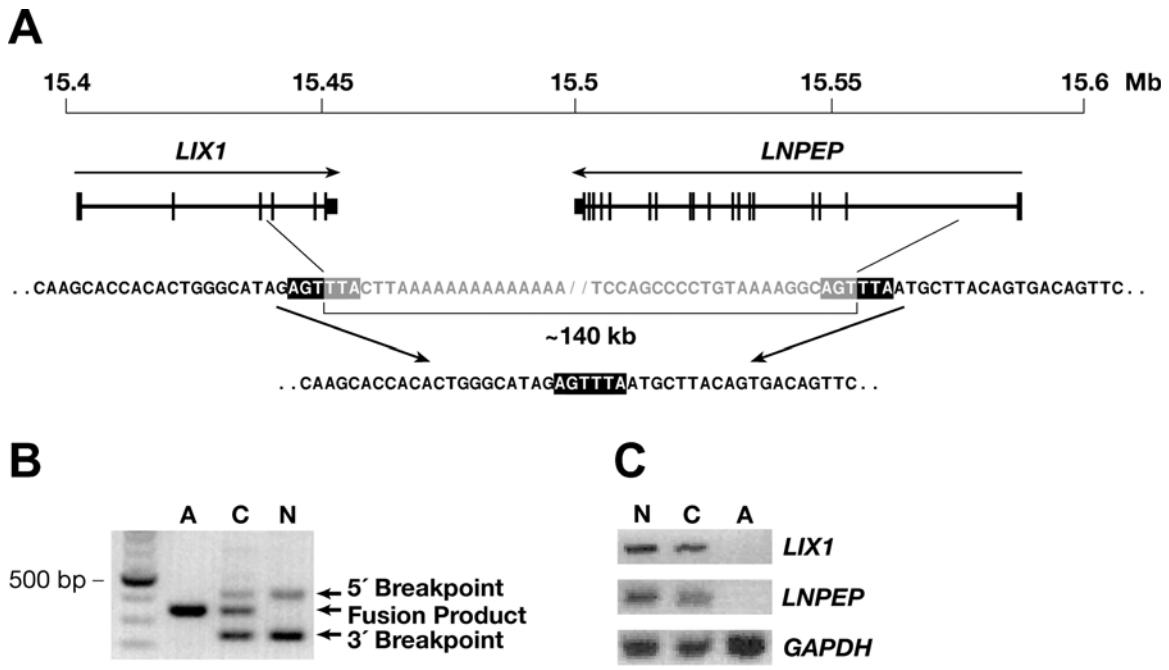

Figure 25–4. An ~140-kb deletion abrogates *LIX1* and *LNPEP* expression in SMA affected cats. (A) A schematic of the genomic organization of *LIX1* and *LNPEP* on FCA A1q. Arrows indicate the direction of transcription of each gene. The scale of chromosome coordinates is from the region of conserved synteny in the dog genome (CFA 3) (UCSC Genome Browser; July 2004 assembly). Sequences of the deleted and normal alleles are aligned showing the precise breakpoints *below* the schematic. The immediately flanking 6-bp sequence, AGTTTA, is in bold. (Note that the genome browser did not itself identify exon 1 of *LNPEP* by homology with RefSeq genes, but limited homology was identified by BLAT search of the dog genome with a cat sequence that was 82% identical over 300 bp of the human exon 1 and flanking 5′-UTR and intron 1 sequences.) (B) PCR products produced from genomic DNA in a multiplex reaction designed to amplify across both deletion breakpoints when the normal allele (lane N) was present. A third product corresponding to the affected cat sequence shown in (A) was amplified when the deleted allele was present, either in homozygous affected (lane A) or heterozygous carrier (lane C) cats. (C) RT-PCR products amplified from cervical spinal cord ventral horn RNA of a genetically normal cat (lane N), a clinically normal carrier (lane C), and an SMA affected cat (lane A). PCR primers for *LIX1* were from exons 1 and 4, *LNPEP* primers were from exons 14 and 15, and *GAPDH* primers were from exons 6 and 8. (Reprinted from Fyfe *et al.*[32] Copyright 2006, with permission from Cold Spring Harbor Laboratory Press.)

THE CAT AS ANIMAL MODEL FOR HUMAN HEREDITARY DISEASE

The world's veterinary schools produce thousands of practitioners each year, most of whom carefully document genetic and chronic diseases of our pets. The result is a comprehensive veterinary literature that has described over 200 feline hereditary pathologies[17] (http://www.angis.org.au/). The clinical and physiological study of these feline hereditary diseases provides a strong comparative medicine opportunity for prevention, diagnostics, and treatment studies in a laboratory setting. Additionally, large animal homologues are similar to humans in natural genetic diversity and offer the possibility of evaluating long-term effects of treatment.[64]

To date, causal mutations have been characterized in 19 felid genes that cause hereditary disease (Table 25–1). The largest representation comes from lysosomal storage enzyme disorders that arise from defects in genes playing a role in degradation of macromolecules targeted to the lysosomes. Many of the genes that cause these pathologies have been mapped in the cat.[65]

Corrective therapeutic strategies have been proposed and examined in the cat, including enzyme replacement, heterologous bone marrow transplantation, and substrate reduction therapy.[64,66] Limitations to these treatment strategies include high morbidity and mortality, limited positive outcomes, incomplete response to therapy, cost, and in some cases requirements for continuous lifelong therapy.[66] Gene therapy poses the most recent of intervention strategies. Feline models have been important in elucidating molecular pathogenesis and are now playing a critical role in evaluating and optimizing the range of therapeutic strategies prior to clinical trials in humans.

The mucopolysaccharidoses are disorders that result from the deficiency of lysosomal enzymes involved in the degradation of mucopolysaccharides. The cat offers homologous models for mucopolysaccharidosis Types I, VI, and VII.

Mucopolysaccharidosis Type I (MPS I), which results from a genetic deficient activity of the enzyme α-L-iduronidase (IDUA), can lead to mental retardation, growth abnormalities, and shortened life span in humans.[67] Naturally occurring models have been characterized in the cat[68,69] and dog.[70] Immune responses can nullify the effect of gene corrective therapy. It has been demonstrated that cats, but not dogs, mount a potent CTL response to canine IDUA after neonatal gene therapy, which can be prevented with transient CTLA4-Ig.[71] The efficacy of neonatal retroviral therapy has also been explored in the cat.[66] The cat model, additionally, provides an ideal system to study mechanisms of brain neurodegeneration and neural-directed strategies, especially given a large body of preexisting literature on cat neurology.

MPS VI or Marteaux–Lamy disease, deficient activity of arylsulfatase B (ARSB), is characterized in humans and cats with growth retardation, coarse facial features, corneal opacity, and skeletal deformities.[72–75] The feline model also exhibits abnormal lysosomal storage in occasional neurons and glia distributed throughout the cerebral cortex.[76] Fibroblast-mediated *in vitro* gene therapy has been examined in this cat model.[77] Recently, an adeno-associated vector containing feline *ARSB* has demonstrated gene therapy-based correction of corneal clouding in the MPS VI cat.[78]

MPS VII results from deficiency of β-glucuronidase (GUSB), which in humans manifests as cartilaginous and bony malformations, growth and mental retardation, abdominal organ enlargement, and corneal clouding.[79] Naturally occurring animal models have been described in mice,[80] dogs,[81] and the cat.[82] Enzymatic activity has been restored in fibroblasts and restored by retroviral gene transfer of rat GUSB cDNA. As GUSB is an essential housekeeping enzyme, this feline model is important for examination of exogenous genes and gene product delivery to a variety of tissue types, and could prove especially valuable due to extensive research conducted on the anatomy and physiology of the cat central nervous and visual systems. Three different serotypes of adeno-associated viral constructs of *GUSB* demonstrated the efficacy of this vector to achieve gene transfer in the normal cat brain, as a model for the efficacy of this construct in a large mammalian brain.[83]

Deficiency of lysosomal α-mannosidase leads to an accumulation of mannose-rich oligosaccharides,[84] which leads to mental retardation, recurrent infections, skeletal changes and hearing impairment.[85] This feline model was initially important in achieving bone marrow transplantation as corrective strategy for neuronal storage diseases of the CNS.[86,87] Recently, adeno-associated viral (AAV) constructs of feline α-mannosidase were used to demonstrate the efficacy of CNS gene therapy. Treated cats exhibited widespread improvement of neuropathology, showing the efficacy of this treatment in a large mammalian brain for CNS correction of human lysosomal enzyme deficiencies.[88]

Lipoprotein lipase (LPL) is a crucial enzyme involved in the regulation of lipoprotein and lipid metabolism ability to thrive.[89] Cats with LPL deficiency display a remarkably similar phenotype to humans, including severe pancreatitis, chylomicronemia, and failure to thrive.[46] There is currently no adequate treatment for this pathology in humans. Cats could prove to be the most valuable animal model of LPL deficiency, as of numerous animal model systems examined including the mouse, the cat most closely resembles the lipoprotein pattern and lipid transport system of humans. Recently, AAV-mediated transfer of a human LPL ^{S447X} variant into feline muscle cells demonstrated correction of the hypertriglyceridemia associated with feline pathology; this offers much promise for treatment of human LPL deficiency.[90]

A separate class of lysosomal storage disorder characterized in the cat is the gangliosidoses, G_{M1} and G_{M2}, which are heritable neurodegenerative diseases. A deficiency of lysosomal β-galactosidase results in the neuronal accumulation of the G_{M1} ganglioside, while the degradation of the G_{M2} ganglioside is initiated by coordinated action of at least three gene products, the α and β submits of β-*N*-acetylhexosaminidase and the G_{M2} activator (GM2A) protein.[42,91] Mutations in any of these enzymes result in an accumulation of gangliosides G_{M1} and G_{M2} in the lysosomes of affected neurons, resulting in progressive deterioration of the CNS. Feline models have been especially important in characterizing the pathobiology and molecular biology of these diseases. G_{M2}-gangliosidosis has been characterized in cat models deficient in the G_{M2} activator protein and HexB,[40,42,92,93] exhibiting remarkably similar pathology to human Sandhoff's disease.[91] Limited reduction in G_{M2} neuronal storage has been reported following bone marrow therapy.[86] Feline models will be important in the development of therapeutic strategies for these disorders.

Mucolipidosis II (I-cell disease) is caused by deficient activity of the enzyme *N*-acetylglucosamine-1-phosphotransferase, which leads to a failure to internalize enzymes into lysosomes. The cat is the only known animal model for this pathology.[94]

Congenital diseases of feline muscle and neuromuscular junction have been reviewed by Gashen *et al.*[95] Some pathologies have been observed in isolated breeds, including hypokalemic myopathy of Burmese cats,[96] glycogen storage disease type IV in Norwegian Forest cats,[44] and myopathy observed in the Devon Rex.[97] The cat is the only reported animal model for type IV glycogen storage disease. Myotonia congenital,[95,98] muscular dystrophy (dystrophin deficient),[52] and laminin α_2 deficiency[52,99] have also been reported in the cat.

X-linked muscular dystrophy in humans is characterized by progressive degeneration of skeletal and cardiac muscle. Mutations in humans lead to either an absence of or abnormality in the protein product dystrophin.[100,101] A deletion in the dystrophin muscle promoter characterized in the cat eliminates expression of muscle and Purkinje neuronal dystrophin isoforms.[52] Marked clinical heterogeneity is observed in these models, from severe disability exhibited in human and dog, to minor muscle fibrosis and an actual regenerative process leading to muscle hypertrophy in mouse and cat.[102–104] These different sequellae could be important in characterizing immediate and secondary consequences of the lack of dystrophin[105] and points out the importance of multiple animal models.

Hypertrophic cardiomyopathy is a clinically heterogeneous myocardial disease contributing to one of the most common causes of sudden cardiac death in young adults.[106] The cat represent the first spontaneous large animal model for this familial disease[47] and will prove to be valuable for examining pathophysiological processes and therapeutic interventions.

THE POTENTIAL FOR KNOCKOUT CATS

There is a continued demand for alternative mammalian models for studying human diseases. Compared to traditional murine models, the cat's more similar physiology, increased size, and longevity have made it ideal for testing the safety and efficacy of some therapeutic modalities in naturally occurring feline disease models.[107–110] Additionally, cats have a genetically heterogeneous background, similar to humans. A good example of a human disease in need of a better mammalian model is cystic fibrosis, as mice targeted with the most common human mutation (ΔF508) in the cystic fibrosis transmembrane receptor (*CFTR*) fail to spontaneously develop the same opportunistic lung infections that plague human patients with cystic fibrosis.[111,112] One group has been working to produce a ferret cystic fibrosis model through gene targeting of somatic cells for nuclear transfer.[113,114] While reproductive cloning has a consistently low success rate (1–4%) across mammalian species,[115] targeting genetic loci through homologous recombination in somatic cells is currently the only viable method for producing knockout models in all mammalian species other

than the mouse, for which targeted embryonic stem cells are routinely used. Nuclear transfer of targeted fibroblasts has been successfully used to produce viable α-1,3-galactosyltransferase knockout pig and cow models for xenotransplantation studies.[116,117] With the successful reproductive cloning of cats by several groups,[118–120] the development of gene-targeted cat models through nuclear transfer is now feasible.[121] The imminent release of the annotated feline genome project,[17] integration of recombination and radiation hybrid maps,[5–10,122] and availability of multiple PAC, BAC,[123] flow-sorted autosome, and Y-chromosome libraries[124,125] (J. Pecon-Slattery *et al.*, unpublished observations) at the Laboratory of Genomic Diversity will facilitate efforts by researchers to develop new cat models of specific human genetic diseases.

COAT COLOR GENES IN THE DOMESTIC CAT

The coat color loci influence the development, maturation, and migration of melanocytes as well as the synthesis of melanin and the formation, transport, and transfer of melanosomes. Genes involved in these processes often have pleiotropic effects, which impact other important biochemical pathways. Coat color loci in the mouse have been known to be part of diverse cellular, developmental, and physiological processes and in some cases to be implicated in pathologies such as anemia, sterility, and neurological disorders.[126–128] The cat is an excellent model system with which to study coat color phenotypes. At least nine different coat color loci have been identified in the cat,[129] and several are now characterized on a molecular genetic level including, *a* (nonagouti) responsible for melanism,[18] *b* (Brown), which changes black pigmentation to brown or variants of brown,[32] *c* (color), causing the darker pigmentation at extremities (i.e., ears, tail), observed in Siamese and Burmese cats[32,37] and albinism,[36] and *d* (dilute), which causes dilution of expected color (i.e., black pigmentation appears gray).[35] The cat is also unique in mammalian species in exhibiting a variation in coat pattern, demonstrating agouti (*A*) (nonpatterned coat) and variants of the *T* (tabby) locus, which affect striping and spotting patterns.

VIRAL PATHOGENS OF THE DOMESTIC CAT

The cat has provided several useful models for infectious disease. These include feline leukemia and feline sarcoma virus, feline coronavirus, and Type C retroviruses that interact with cellular oncogenes to induce leukemia, lymphoma, and sarcoma.[130–132] Historically, many of the human oncogenes that define signal transduction pathways were originally discovered in the context of feline leukemia virus interaction in cat models. The cat provides the only naturally occurring model for human AIDS pathogenesis, in its endemic fatal transmissible feline immunodeficiency virus (FIV).[133,134] Similar to its close phylogenetic relative HIV, FIV induces CD4-T lymphocyte depletion in affected cats, an immune system collapse, and susceptibility to adventitious microbial agents as a prelude to wasting disease and death.[133,135] Interestingly, over 10 wildcat species (including lions, leopards, cheetahs, ocelots, pumas and other big cats) are endemic with their own species-specific strain of FIV[136–141]; however, unlike strains in domestic cats, the wildcat FIV strains do not appear to cause acute immunodeficiency in the wildcat species, perhaps a consequence of historic natural selection of host genetic resistance to the fatal virus.[139,142] Lion and puma-specific FIV strains have recently been demonstrated to utilize novel, more promiscuous mechanisms for cell entry than FIV, suggesting a divergent tropism and biological properties of these viruses.[143]

The World Health Organization reported a new human respiratory illness outbreak (severe acute respiratory syndrome, SARS) that emerged in Guangdong Province, China in 2003.[144,145] Sequence analysis demonstrated that the infectious agent was a previously unrecognized coronavirus.[146,147] An animal model demonstrating clinical symptoms and pathology of SARS-infected patients has not been reported.[148] Of interest, the recent report of a highly virulent feline coronavirus epidemic in captive African cheetahs, a disease model for human SARS, illustrates the critical role of ancestral population genetic variation.[132] In addition, cats injected with the SARS virus developed clinical symptoms, an important insight in implicating the virus in the SARS epidemic.[149–152]

The feline panleukopenia (feline distemper) virus has revealed a natural history parable in its abrupt transformation of the cat virus to an epidemic, fatal canine parvovirus, that emerged in the world's puppy population in 1978.[153] In contrast, the canine distemper virus, which is normally restricted to canid species, precipitously adapted to and decimated East African lions in 1994, killing one-third of the lions in the Serengeti ecosystem within a 9-month outbreak.[154] A clear involvement of host defense mecha-

Table 25–2
Feline genome project resources (September, 2006)

1. Somatic cell hybrid panel framework physical map > 100 Type I genes	12, 152
2. Interspecies backcross (ISB) genetic linkage maps	6, 7
3. Intraspecies Nestlé Purina pedigree genetic linkage map	Unpublished
4. 5000-rad radiation hybrid panel and maps	5, 6, 8–10, 122
5. Flow sorted feline chromosome libraries: reciprocal chromosome paint map	15, 125, 153
6. Arrayed BAC and PAC libraries	123
7. Tissue/cell line DNA repository of >10,000 exotic and domestic feline specimens	154, 155
8. Domestic cat breed forensic database of 38 breeds, 11 multiplexed optimized STRs	31
9. Domestic cat Y chromosome cosmid library	Unpublished
10. Completed sequence	
a. Whole genome sequence (2-fold coverage)	17
b. mtDNA sequence	156
c. Major histocompatibility complex	157
11. Cat genome browser (GARFIELD)	17

nisms in these and other infectious disease episodes renders the cats and their pathogens an excellent candidate species for characterizing the interaction of microbial adaptation and host disease gene defenses. Given the critical importance of infectious disease in scores of chronic and acute human disease, there are powerful research opportunities in the cat family.[142,155]

Finally, recent concern over the emergence of avian flu H5N1 has shown a strong susceptibility of cats, both domestic and large cats, again raising possibilities for pathogenesis and therapy development.[156,157]

With the development of genomic resources in the cat (Table 25–2) and the application of complementary comparative tools developed in other species, the domestic cat is emerging as a promising resource of phenotypically defined genetic variation of biomedical significance. Exploration of similar resources in other species, particularly the dog and mouse, has provided important insight into otherwise unexplained biomedical disorders.

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