PROSPECTS FOR COMPARATIVE GENOME ANALYSES AMONG MAMMALS

Leslie A. Lyons and Stephen J. O'Brien

Laboratory of Viral Carcinogenesis
Frederick Cancer Research and Development Center
National Cancer Institute
Frederick, MD 21702-1201

INTRODUCTION

The discovery of hypervariable microsatellite markers and the technical advances of marker typing has facilitated efforts towards the construction of genetic linkage maps in several diverse vertebrate species. These mapping projects have advanced the exploration of each organism's genome and its biology. But less effort has been focused on the mapping of coding genes, genetic markers which allow comparisons of genome organization across species. The establishment of universal Type I (coding gene) genetic markers, which can be mapped in all species, will allow true comparisons of genome organization across species and will provide the framework for comparative genetic research (O'Brien, 1991). Comparative genetics allows the gene poor species to consider the information of gene rich species, exponentially increasing the perspective available for disease and genetic trait analyses. In developing the genetic map of the cat (O'Brien and Nash; Nash and O'Brien; Gilbert et al., 1988; O'Brien et al., 1988), we have focused on the mapping of Type I markers in order to capture the information locked in the genome of other species and to assist the use of the feline genome in evolutionary and inherited disease research (O'Brien et al., 1993).

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THE FELINE GENOME AND COMPARATIVE GENETICS

The past decade has witnessed a gradual development of the genetic map of the cat. The karyotypes of felids are highly conserved, 2n=19 for most felids, and the chromosome pairs are easily distinguishable by standard G-banding*See*SeeSee methods (Wurster-Hill and Centerwall, 1982; Modi and O'Brien 1988). The diverse size and morphology of the feline chromosomes facilitated the characterization and usage of a Chinese hamster x cat and a mouse x cat somatic cell hybrid panel. These hybrid panels have permitted the syntenic mapping of nearly 100 isozymes, oncogenes, and other coding genes on the feline chromosomes (Marshall et al., 1995). Comparisons of the syntenic maps of cats and cows have suggested that the cat and cow genomes are 3-4 times less rearranged than mice, when compared to the structure of the human genome (O'Brien et al., 1988). This difference laid the ground work and enthusiasm for comparative genetic studies across several diverse species. These crude syntenic maps and karyotypic investigations quickly led to evolutionary considerations of genome organization and the role of genomic rearrangements in species development, as well as the possible cross-species inferences of biological processes.

The investigation of inherited disease is an important area of comparative genetics that would benefit from a cat gene map. The location for the genes causing disease should be predictable in one species when they are mapped to a chromosomal segment of homology in another. Homologous disease gene locations are not always certain in mouse-human comparisons due rearrangement of the two genomes into over 120 chromosomal segments of conserved homology (Nadeau et al., 1991; Copeland et al., 1993). Homologous disease gene locations are often found to be near rearrangement breakpoints between mouse and human genomes, while the fewer rearrangements in the feline make chromosomal predictions more reliable. For example, Figure 1 depicts the location for polycystic kidney disease type 2 locus on human chromosome 4. This human chromosomal segment is in a region homologous to two mouse chromosomes, 5 and 3. The same region is conserved as a larger block of homology in the cat genome on cat chromosome B1; thus mapping efforts and gene localization techniques could concentrate on that single chromosome in the cat. The cat also provides a comparative model for cardiomyopathies, retinal atrophies, viral immunodeficiencies, and other inherited and acquired diseases (Barnett and Curtis 1985; Hardy et al., 1980; Pedersen and Floyd 1985; Pedersen 1987). The diverse morphology of the domesticated feline breeds is a potential resource for the elucidation of polygenic interactions of developmental and quantitative traits. Therefore, the development of additional mammalian species as disease models will offer valuable and unique insight into gene interaction and organismal biology.

The discovery of feline-human genome conservation from the rudimentary syntenic map of the cat has fueled the investigation of

the cat genome. The feline genome project's major objectives are to provide a carnivore representative for genetic evolutionary analyses and to aid in the advancement of human and feline health issues. These objectives have reached beyond syntenic relationships and justify the construction of a genetic linkage map for the cat.

THE FELINE GENOME PROJECT

The Laboratory of Viral Carcinogenesis has committed to an organized effort to develop the genetic map of the cat. The project is designed to utilize the biology of the cat in several research areas

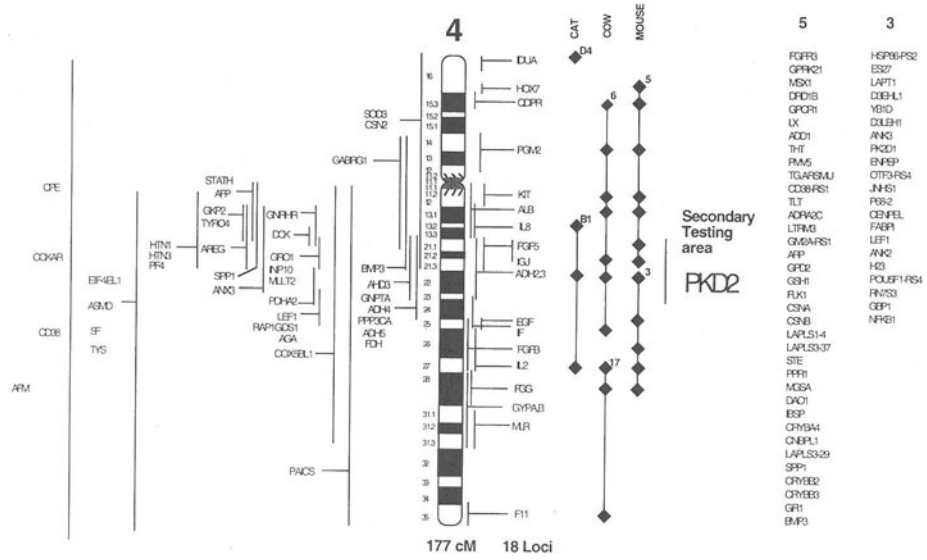

Figure 1. Comparative Mapping: Gene Rich to Gene Poor. (Left) Human genes mapping to 4q21-q23 from HGM11. (Right) Mouse genes mapping to mouse chromosomes 5 and 3 in regions homologous to human 4q21-q23 from Mouse Genome Database. Diamonds denote genes mapped in corresponding species with species chromosome number indicated. Loci to right of idiogram represent comparative anchor loci.

that are described, but which so far have little genetic information. The cat is an excellent model for host-virus interactions. Feline leukemia virus is an excellent model for viral carcinogenesis (Hardy et al., 1980). Feline immunodeficiency virus (FIV) causes a disease

progression similar to human immunodeficiency virus (HIV) in a percentage of infected domestic cats, but is apparently non-pathogenic in larger felids (O'Brien 1986). The recent radiation and the popularity of the 37 extant felids makes cats ideal for evolutionary and population studies (O'Brien 1986; Wayne et al., 1989). Domestic cats are common household pets and non-domestic feline species are attractive zoo exhibits. The human fascination with the feline has assisted the investigation of the cat's biology by a growing field of scientists, veterinarians, breeders, and reproductive-physiologists. The charismatic nature of the cat has led to the establishment of over thirty fancy cat breeds throughout the world. Fancy cat breeding has led to the detection of inherited disease models and has facilitated the establishment of cat colonies segregating for a variety of genetic diseases. Over two dozen inherited diseases which have analogous phenotypes in humans have been described in cats (Nicholas 1996) (Table 1). Our laboratory has had an inherent interest in viral and

Table 1. Hereditary Human Diseases with Feline Models.

Disease	Affected Protein	Gene symbol	Human Locus	Feline Locus
Albinism*	Tyrosinase	TYR	11q14-q12	D1
Amyloidosis*	Cystatin	CST†	20p	
Cardiomyopathy				
Chediak-Higashi Syndrome*	(unknown)	CHS	1q43	
Corneal Dystrophy	(unknown)	CDGG1	5q22-q33 3	
Diabetes Mellitus, insulin dependent*	Insulin	IDDM	6p21.3	
Diabetes Mellitus, non-insulin depend*	Islet amyloid polypeptide	NIDDM	12	B4
Ehlers-Danlos Syndrome	Collagen			
Ehlers-Danlos Type VII	Procollagen protease			
Endocardial Fibroelastosis	(unknown)	EFE	Xq28	
Fabry Disease	Alpha-galactosidase	GLA	Xq22	X
Factor XII	Factor XII	F12	5q33-qter	
Fucosidosis	Alpha-L-fucosidase	FUCA1	1p34	C1
Globoid cell leukodystrophy*	Galactosylceraminidase	GALC	14q24 3-q32	
Glycogen Storage Disease Type II	Alpha-1,4-glucosidase	GAA	17q23	D1
Glycogen Storage Disease Type IV	Glycogen branching enzyme	GBE1	3p12	
GM1 Gangliosidosis	Beta-galactosidase	GLB1	3p21.33	B3
GM2 Gangliosidosis (Sandhoff's)	Beta-hexosminidase A	HEXB	5q13	(B3)§
Hemophilia A	Factor VIII	F8C	Xq28	
Hemophilia B	Factor IX	F9	Xq27.1-q27 2	
Hyperlipoproteinaemia*	Cholesterol metabolism			
Mannosidosis	Alpha-mannosidase	MANB	19cen-q12	(B3)§
Mucopolysaccharidosis Type I	Alpha-L-iduronidase	IDUA	4p16.3	D4
Mucopolysaccharidosis Type VI	Arylsulfatase B	ARSB	5q11-q13	
Neuronal ceroid lipofuscinosis*	(unknown)	CLN†		
Niemann-Pick Type A	Sphingomyelin phosphodiesterase-1	SMPD1	11p15.4-p15	
Niemann-Pick Type C*	(unknown)	NPC	18q11-q12	
Ornithine aminotransferase deficiency	Ornithine aminotransferase	OAT	10q26	
Polycystic Kidney Disease	(unknown)	PKD1	16p13 31-p13	
Progressive Retinal Atrophy				
Testicular Feminization*	Androgen receptor	AR	Xq11-q12	X
Von Willebrand Disease*	Coagulation factor VIII	F8VWF	12p13 3	

*diseases with murine models
† several
§ HEXA-B3, MANA-B3
Current collaborations in bold.

evolutionary felid studies, and with the further development of the genetic map of the cat, we have initiated collaborations to investigate the genetic basis of many feline diseases.

The reference family for the cat genome linkage project is a feline interspecific backcross pedigree. This pedigree has been constructed by mating female domestic cats to male Asian leopard cats, *Prionailurus bengalenesis.* The leopard cat is within the size range of domestic cats, but has an intractable temperament. Leopard cats will occasionally naturally mate with domestic cats, if reared together from kittenhood, but mature leopard cats require assisted reproduction methods for the production of hybrid cats (Lyons et al., 1996). Our pedigree, which is currently 50 backcross offspring, has been developed by both natural and assisted reproduction. Approximately one-third of the backcross offspring have been produced by artificial insemination techniques, whereas the remaining two-thirds of the pedigree samples have been collected from cat breeders. Hybrids between the leopard cat and the domestic have been common in the United States for over 20 years. A fancy cat breed has been developed from these hybrids and is commonly known as the Bengal. Fourth generation Bengals qualify for championship competition and represent one of the few breeds which are bred for their spotted coats.

The F1 males of the interspecific feline cross follow Haldane's rule and are sterile (Haldane 1922). Female F1's can be mated to either male domestic or leopard cats. Backcross males, from either cross, are also sterile. This sterility of the F1 males prohibits the production of F2 offspring and reciprocal backcrosses, although male fertility has been noted in subsequent generations. We have investigated the physiological basis of sterility in F1 males. Histological and morphological examination of the F1 male testis reveals Sertoli cell degeneration and the complete lack of spermatogenesis. These hybrid cats exhibit an earlier arrest in spermatogenesis than the F1 males of the interspecies mouse cross, *Mus spretus* x *mus domesticus* (Bonhomme 1978). Mouse hybrids, as well as horse x donkey hybrids (Chandley et al., 1974), display arrested spermatogenesis after meiosis I. Subsequent generations of the interspecies cat hybrids are fertile, thus the correlation of fertility/sterility with leopard/domestic cat gene inheritance should reveal the segregation of specific genes involved with reproduction. Reproductive isolation is one of the mechanisms leading to speciation. The identification of the genes leading to reproductive isolation in the cat could help elucidate the process of species development in the Felidae.

It is recognized that the hybridization of less divergent species of cats would likely provide a more prolific cross, but sterility studies, interesting viral studies, and polymorphism information content may be compromised with other such crosses. The availability of pure African and/or European wildcats is also more limited than access to leopard cats. Genetic differences between the leopard cat and the domestic cat may provide valuable insight to viral diseases and other inherited traits. Leopard cats are resistant to FeLV (Hardy et al., 1980) and do not have the endogenous retrovirus, RD-114 (Reeves and

O'Brien 1984). The cross between the domestic and the leopard cat allows the investigation of these unique feline viruses. As non-responders, the leopard cats also facilitate the mapping of the dominant gene controlling the response to catnip.

The 8-10 million years of evolutionary divergence between the domestic and leopard cat (Collier and O'Brien 1985) permit the development of an extremely efficient pedigree for genetic linkage studies. Over 95% of domestic cat specific microsatellites primers amplify in both cat species (Menotii-Raymond and O'Brien 1995). Southern blot analyses of coding genes reveal unique band differences between the two cat species with screening only 3-4 restriction enzymes. Thus, this completely phase-known and completely heterozygous pedigree provides an extremely efficient family for the mapping of both Type I, coding gene markers, and Type II, microsatellite markers. As few as 61 offspring can effectively produce a 5 cM genetic map (Lyons et al., 1994). Our present pedigree of 50 backcross offspring would be sufficient to establish a 10 cM map for the cat. Thus, although not highly prolific, the domestic x leopard cat cross is most robust and versatile for genetic studies of felines.

Marker Development

The advancement of microsatellite typing techniques has greatly improved the feasibility of rapidly and efficiently developing a genetic linkage map of Type II markers for any species. Yet microsatellite markers are not very effective for across species comparisons, even within species of the same order. Type I markers are efficacious for such comparisons, but inefficient and time consuming typing methods have led to the paucity of coding gene markers on all genome maps, with the exception of the mouse (Coopland et al., 1993). Without the advantage of a diverse genetic cross for a reference pedigree, the tedious detection of polymorphism and the low heterozygosity of coding genes has impeded Type I marker mapping for all species.

Our laboratory has focused on the advancement of Type I marker usage for mammalian genome projects. In collaboration with members of the comparative mapping community, 321 genes were selected as anchor loci (O'Brien et al., 1993). The genes selected as anchor loci had been previously mapped in more than one species, were publically available as DNA clones, and were evenly spaced by using human cytogenetic and mouse linkage data. Preference was given to genes shown to be involved in disease and reproduction and genes which flanked known blocks of homology when compared to the human genome. Thus, a set of markers that could be mapped by all species mapping efforts, has been established to provide a framework for genome structure comparisons across mammalian orders.

Even though a common set of comparative anchor markers has been established, time consuming typing methods and low heterozygosity levels remain major drawbacks for mapping efforts. We have explored techniques for establishing polymerase chain

reaction (PCR) based typing of universal Type I markers in any mammal species, in a manner which will also increase the likelihood of polymorphism detection for these highly conserved markers. Portions of the following procedure have been automated to enable the rapid construction of a set of primers for a given Type I gene which amplify the corresponding gene in diverse species (Lyons et al., 1996).

The initial step in our procedure is to identify all the published sequences for a particular human gene. We start by searching Genbank for the gene accession numbers, searching with human gene symbols and gene names. After verifing that the accession numbers are the correct gene, we then attempt to build a contiguous single gene sequence from identified sequences, by splicing together exons and 5' or 3' sequences into one larger gene sequence. This "contig" sequence is then used to search the vertebrate DNA sequence database to identify all non-human species sequenced for the same gene. For each species, the sequences which are the longest and have the greatest homology to the human gene are selected for alignment. The gene sequences from the various species are aligned and any known intron/exon boundary information is noted. The consensus sequence in downloaded into a primer designing program for the selection of primers. Primer parameters are held constant for all the genes. The gene primers are selected from the middle of adjacent exons, in the areas with the highest conservation amongst the aligned species. Primers are designed to amplify the intron region between the two exons. This placement ensures primers to be well conserved across species, but amplifies intron regions which have little selection pressures and should accumulate mutations and DNA polymorphism more rapidly than exon regions of the same gene.

Our goal is to develop universal PCR primers for a group of comparative mapping anchor loci which can be assayed by PCR methods in any mammal species and will have 5-10 cM speciation across the murine and human genomes, respectively. Over 400 gene specific primers have been tested for gene recognition in selected mammalian species (dog, seal, horse, deer, sheep, pig, cow, bat, echidna, Tammar wallaby, mouse, hamster, rabbit and humans). Preliminary results reveal that the primer design is robust and that these species can be amplified with over half of the primers using PCR conditions that were optimized empirically for cat genomic DNA. A concerted attempt to place these Type I loci in species from several mammalian orders offers the prospect of deriving comparative inference from across the mammalian radiations.

Comparative Map Usage

Effective comparative genetics depends on availability of both a Type I and a Type II genetic map (O'Brien 1991). Since the microsatellites developed from domestic cats can be mapped in the leopard cat (Menotti-Raymond and O'Brien 1995), the feline interspecific pedigree will produce an integrated Type I and Type II

marker map. Both marker categories are required for effective disease gene homology determinations. Once a disease phenotype has been identified in two different species, gene homology must be determined. Verification of the homologous gene as the locus responsible for the disease phenotype in different species is necessary for development of clinical diagnostics and therapies. Effective treatments developed in the cat could be used in humans for the genetically homologous disease. For example, the gene for polycystic kidney disease (PKD1) has been recent identified and mapped to human chromosome 16p (Intern. Polycycstic Kidney Disease Consortium 1995) (Figure 2). An autosomal dominant form of PKD has been described in cats (Biller et al., 1995) but its map location has not been determined. The comparative mapping anchor loci for human 16p are *HMBA1*, *PRM1*, *IL4R*, and *PRKCB1*. The feline interspecific backcross pedigree will have these 4 genes mapped as well as linked microsatellites. Once it is shown that this region is conserved as a large block between humans and cats, the cat microsatellites linked to the human 16p markers can be screened in the feline PKD pedigree for linkage to the PKD disease phenotype. Linkage would strongly suggest disease gene homology (Figure 2). True homology cannot be determined until the exact causative mutations are discovered in each species.

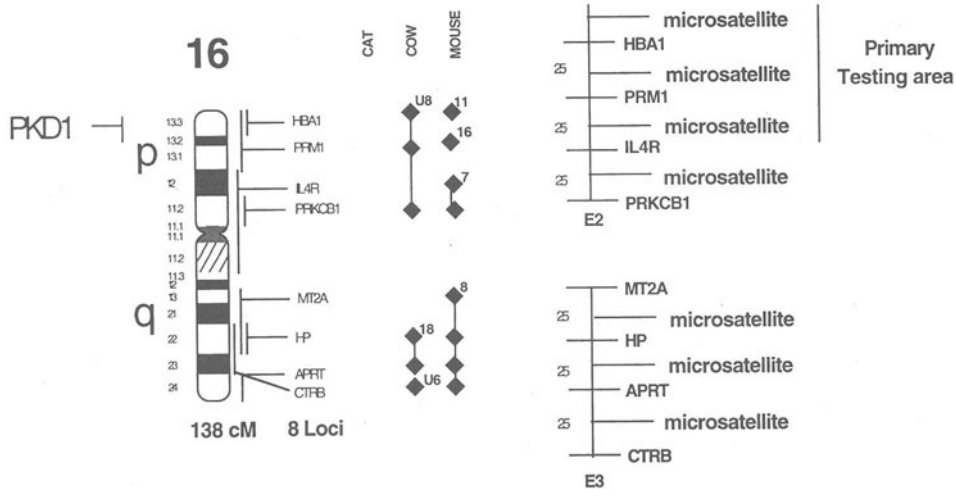

Figure 2. Comparative Mapping of Polycystic Kidney Disease. known felinehuman homology blocks allow rapid disease homology determination. Microsatellites linked to the Type I markers in the region where PKD1 is mapped can be analyzed in feline PKD families to determine homology.

Comparative genetics allows the exploitation of the gene rich species, mice and humans, by the gene poor species. Once comparative mapping anchor loci have been mapped in different species, then all the genetic information identified for those species is interpretable for the other species. As presented in Figure 2, if the feline PKD is not homologous to human PKD1, the region homologous to human chromosome 4 should then be searched since a second locus for polycystic kidney disease has been mapped to this region in humans, although gene has not yet been identified. Human 4q21-q23 is maintained as a single conserved unit in the cat. If PKD maps to the homologous segment on cat chromosome B1, then the genes located in the region 4q21-q23 of human and in homologous segment of cat chromosome B1 become possible candidates.

Certain linages of Abyssinian cats have an inherited form of progressive retinal atrophy (PRA) (Barnett and Curtis 1985). Humans also have progressive retinal atrophies but neither specific genes nor the homologous disease between humans and cats have been identified. The cat disease pedigree can be screened with Type II markers for linkage to the feline PRA. Once linkage has been established, the neighboring Type I markers can be identified. These markers now become candidate genes. These linked Type I markers allow the identification of homologous chromosome segments in the human and mouse. The plethora of genes mapped in these two species within the homologous gene regions provides a wide selection of

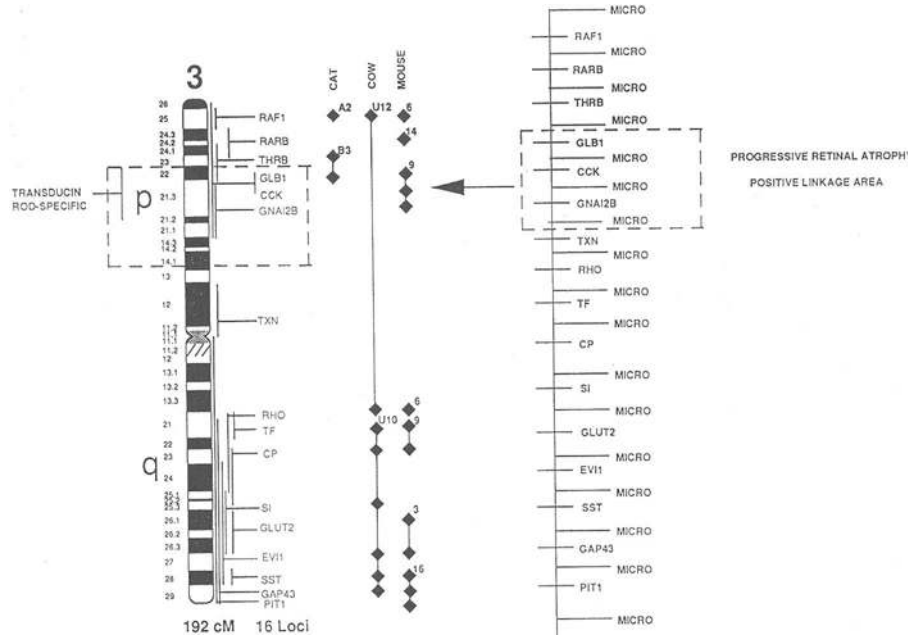

Figure 3. Reverse Comparative Genetics. Hypothetical model for the mapping of a progressive retinal atrophy in the cat. Type II markers showing linkage to the disease phenotype can be used to find the homologous regions in a gene rich species. Type I markers linked to the Type II markers allow human and/or mouse regions to be identified for candidate gene selection.

candidate gene possibilities (Figure 3). Every gene does not have to be mapped in all species, just enough loci to delineate the blocks of conservation. This reverse comparative approach may identify a retinal specific gene, mapped in either mouse or humans, which now would become the prime candidate for the feline and human PRA.

SUMMARY

Comparative genomics has progressed beyond the role of mapping homologous genes. As the genomes of more diverse species become mapped with both Type I and Type II markers, the mechanisms of biological interactions will begin to unravel. Type II markers have helped identify genes involved with economically important traits, such as meat and milk quality and quantity in agriculturally important species. The genetic map of the mouse is rich in both Type I and Type II markers, making the mouse the principle animal model in diverse fields of biological research. As Type I marker maps become more developed in diverse species such as cows, cats, deer, and wallabies, comparative mappers will begin to complement the comparative inference begun by human and murine gene mapping. Second and third models for human inherited disease will be developed in these additional species, as well as models for genes controlling development and complex traits. Eventually, comparative mapping may become a genomic "web" of information, allowing each species to be as rich as the other, exponentially more so than when each stands alone.

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