

Leading the way: canine models of genomics and disease

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In recent years *Canis familiaris*, the domestic dog, has drawn considerable attention as a system in which to investigate the genetics of disease susceptibility, morphology and behavior. Because dogs show remarkable intrabreed homogeneity, coupled with striking interbreed heterogeneity, the dog offers unique opportunities to understand the genetic underpinnings of natural variation in mammals, a portion of which is disease susceptibility. In this review, we highlight the unique features of the dog, such as population diversity and breed structure, that make it particularly amenable to genetic studies. We highlight recent advances in understanding the architecture of the dog genome, which propel the system to the forefront of consideration when selecting a system for disease gene studies. The most notable benefit of using the dog for genetic studies is that dogs get many of the same diseases as humans, with a similar frequency, and the same genetic factors are often involved. We discuss two approaches for localizing disease genes in the dog and provide examples of ongoing studies.

Introduction

The domestic dog, *Canis familiaris*, reportedly bears over 450 diseases; approximately 360 of which are analogous to human diseases (Patterson, 2000; Parker and Ostrander, 2005; Wayne and Ostrander, 2007). As a spontaneous model for many heritable human diseases, the dog provides an excellent system for the identification and study of disease loci (Parker and Ostrander, 2005; Wayne and Ostrander, 2007; Karlsson and Lindblad-Toh, 2008), particularly cancer loci (Cadieu and Ostrander, 2007). Specific advantages of the dog system include the fact that the dog has a unique population structure, with each breed arising from a limited number of founders (American, 1998). This fact, combined with the frequent use of popular sires, means that each domestic breed is a closed population, with limited locus and disease heterogeneity (Ostrander and Kruglyak, 2000; Ostrander and Wayne, 2005; Parker and Ostrander, 2005; Karlsson and Lindblad-Toh, 2008). As a result, genetic studies in dogs are theoretically simpler and more straightforward than those conducted in complex populations, offering many of the statistical

advantages of studies performed in isolated human populations, such as those carried out in Finland or Iceland (Ostrander and Kruglyak, 2000). Additional advantages are offered by the architecture of the dog genome itself (Lindblad-Toh et al., 2005); the dog is known to have long stretches of linkage disequilibrium (LD), reducing the overall number of markers needed to investigate the genome (Sutter et al., 2004; Lindblad-Toh et al., 2005). In addition, analysis of the whole-genome sequence assembly of the dog at both a 2× and 7.8× coverage shows that it is, as expected, more homologous in sequence conservation to humans than mice (Kirkness et al., 2003; Lindblad-Toh et al., 2005). Finally, with humans and dogs sharing a common environment, food, immunologic profile (Storb and Thomas, 1985) and carcinogenic load (Glickman et al., 2004), it is entirely predictable that the domestic dog would emerge as a viable model for cancer genetics.

Breed structure and disease predisposition of dogs

The domestic dog population is divided into over 350 discrete breeds worldwide, with

approximately 160 breeds recognized in the USA by the American Kennel Club (AKC) (<http://www.akc.org/>) (American, 1998). Each breed exhibits a distinct phenotype created by selective breeding practices that took place largely during the Victorian era (Clark and Brace, 1995; Wilcox and Walkowicz, 1995). Today, kennel clubs in both the USA and Europe impose strict restrictions on dog registration; to be an official member of a breed the ancestors of each dog must be registered members as well. Thus, each dog breed represents a closed breeding population of individuals with high levels of phenotypic homogeneity. Not surprisingly, there is reduced genetic diversity within breeds and greater genetic divergence between breeds. Indeed, 27% of the total genetic variance observed in dogs is between breeds, compared with the 5-10% that exists between distinct human populations (Parker et al., 2004).

The strong selection that breeders have imposed in order to produce a homogenous population of individuals with common morphological and behavioral traits has led to an excess of inherited diseases in domestic dogs. Although this is unfortunate for the companion animal community, the fact that many breeds display an excess of disease offers a unique opportunity to identify genes that have been difficult to localize through the study of human families and populations, with cancer providing an excellent example (Cadieu and Ostrander, 2007).

The frequency of occurrence and response to treatment of canine tumors often parallels human neoplasms (Dorn, 1976; Cadieu and Ostrander, 2007; Breen and Modiano, 2008) much better than, for instance, rodent tumors. Similar to in humans, canine tumors usually appear spontaneously, whereas rodent tumors are frequently induced. In terms of the epidemiology of cancer, it is at least as common in dogs as in humans (Table 1). In a necropsy series of 2000 dogs, 23% of all dogs, and 45% of dogs that were over 10 years old, died of cancer (Bronson, 1982;

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Table 1. Tumor incidence rates (all sites) estimated in pet dogs by country and tumor characteristics

Country	Years	Population location	Number of dogs estimated to be at risk	Tumors	Incidence rate*
UK	1997-1998	UK insured dogs†	130,684	Cancers‡	747.9
				All neoplasia-related claims	1948
California, USA	1963-1966	Alameda County	1031	Cancers	381
				Nonmalignant tumors	1130
Ontario, Canada	1999	Veterinary clinics	63,500	Cancers	850
				All tumors	3970
Italy	1985-1994	Genoa County	127,600	Cancers	310
				Nonmalignant tumors	760
Italy	2001	Local health unit	9812	Cancers	958.4

All data obtained from Merlo et al. (Merlo et al., 2008).

*Crude rate defined as the number of cases per 100,000 dogs/year.

†All dogs insured with a single UK pet insurance company.

‡Claims for veterinary treatment identified as being related to neoplasia.

Vail and MacEwen, 2000). The challenge has been to make use of this information to identify genes that are important in both human and companion animal disease; to do this, two general approaches have been used.

Mapping disease genes in dogs

Finding genes that predispose any disease begins with the identification of a susceptible population. For family-based studies, DNA samples are collected from both affected and unaffected individuals in families segregating an excess of disease. Phenotypes are carefully established and a

genome-wide linkage scan is performed using published approaches (Fig. 1) (Acland et al., 1998; Lingaas et al., 1998; Acland et al., 1999). In the past, these scans relied on the use of microsatellite repeat-based markers (Ostrander et al., 2000; Ostrander and Wayne, 2005), and were used to identify the gene for a canine form of a kidney cancer, known as Birt-Hogg-Dubé (BHD) disease, and the genes for several vision disorders (Acland et al., 1998; Acland et al., 1999; Jónasdóttir et al., 2000; Lingaas et al., 2003). Evidence of a locus is indicated by calculation of a Lod (log of the odds) score (Ott, 1976), which indicates the likelihood that a

given region of the genome is segregating with the disease state because it is genetically linked, versus the likelihood that the segregation occurs by chance.

With the completion of a 7.8× draft assembly of the dog genome sequence (Lindblad-Toh et al., 2005), a canine single nucleotide polymorphism (SNP) chip has been developed, producing between 40,000 and 50,000 data points for each individual tested (Karlsson et al., 2007). Not only can the SNP chip be used for linkage-based studies, but this resource has also proven useful for performing genome-wide association studies (GWAS) in which a popula-

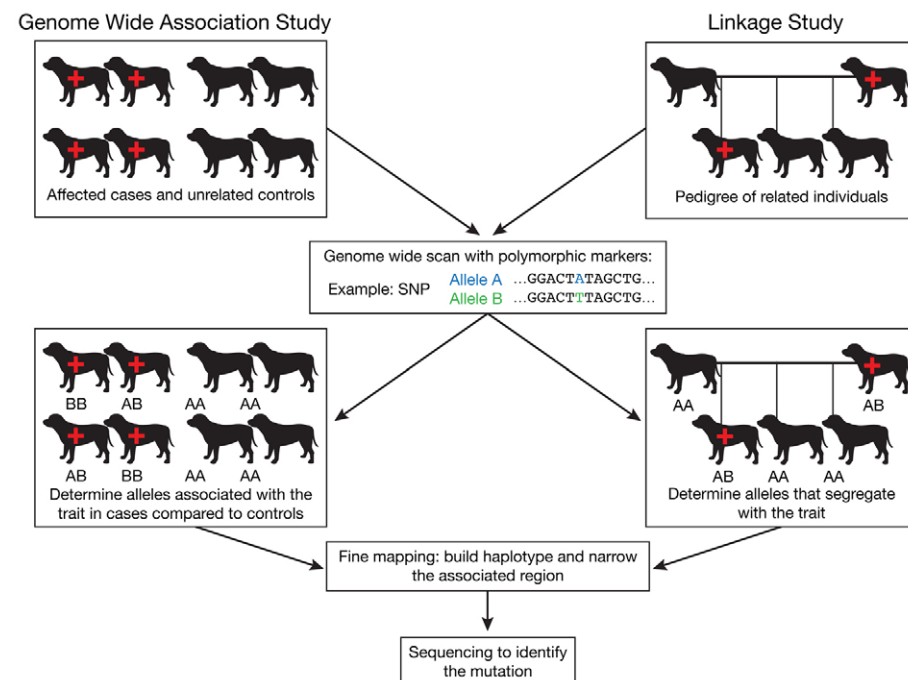


Fig. 1. Genome-wide association study (GWAS) versus linkage-based study.

A GWAS study compares a dense set of genotypes from animals that have a particular trait (cases) with unrelated controls, in order to ascertain alleles that are associated with the trait. Specific corrections need to be applied to account for factors such as population stratification. By comparison, a linkage study analyzes a genome-wide distribution of markers through multiple generations of a pedigree. Statistical methods are used to find a marker whose alleles segregate with disease status more often than would be expected by chance. Both types of approaches can be successful in identifying a disease locus. Fine mapping with more markers is used to identify a shared pattern of alleles from adjacent markers (haplotype). Sequencing is used to ultimately identify the disease mutation. The red cross symbol indicates an individual that is affected with the disease. The polymorphic markers that are used most frequently for linkage-based studies include SNPs and microsatellites, whereas GWAS studies use SNPs exclusively.

tion of dogs with well-documented disease is compared with a population of healthy controls of the same breed (Fig. 1). This approach is very powerful in dogs, particularly if multiple breeds are used and, for example, has resulted in the mapping of both morphologic (Salmon Hillbertz et al., 2007; Drogemuller et al., 2008; Cadieu et al., 2009; Parker et al., 2009) and disease-associated traits (Wiik, 2008; Awano, 2009). Of note, samples are relatively easy to collect for GWAS, because one can avoid the tedious process of tracking specific family members and any diseased individual who meets a pre-specified set of criteria is usually accepted into the study.

Two key factors have led to the rapid and successful growth of GWAS studies in dogs. The first is that far fewer markers are needed to perform a GWAS study in dogs than, for instance, in humans. This is largely because of the extensive LD that is observed in dogs (Sutter et al., 2004; Lindblad-Toh et al., 2005). Whereas LD in humans rarely extends for more than 10-20 Kb, it is not uncommon for LD in dogs to be in the order of megabases (Sutter et al., 2004; Lindblad-Toh et al., 2005). Thus, whereas human studies require in the order of 500,000 to a million markers, both Sutter et al. and Lindblad-Toh et al. (Sutter et al., 2004; Lindblad-Toh et al., 2005) predicted, correctly, that 30,000-50,000 biallelic markers would more than suffice for GWAS studies in dogs. This has since been proven by the localization of genes for several canine traits (Salmon Hillbertz et al., 2007; Drogemuller et al., 2008; Wiik, 2008; Awano, 2009; Cadieu et al., 2009; Parker et al., 2009).

The second advantage to performing genetic mapping studies in the dog is embedded in the breed structure itself. Many dog breeds can be grouped into genetic clusters (Parker et al., 2004; Parker et al., 2007). Genetic loci conferring shared phenotypes within breeds that are members of each cluster can be identified much more readily than when studies are confined to a single breed. In addition, examination of LD in regions of association for multiple, related dog breeds can be used to narrow a crucial region of linkage or association, thus facilitating the task of moving from a linked or associated marker to a gene. This was aptly demonstrated by Parker et al. who used a combination of 20 dog breeds to find an expressed retrogene that is responsible for the chondrodysplasia phenotype in several

dog breeds including the Corgi, Basset Hound, Dachshund, etc (Parker et al., 2009). Cadieu et al. also demonstrated the same principle in the search for genes controlling hair type, combining data from dozens of breeds to identify genes controlling fur length, curl and texture (Cadieu et al., 2009).

Cancer

Canine malignancies have been established as strong comparative models for many types of human cancers including transitional cell carcinoma (TCC) of the bladder (Knapp et al., 2000), non-Hodgkin's lymphoma (Leifer and Matus, 1986; Valli et al., 2006), leukemias, osteosarcoma (Mueller et al., 2007), melanoma (particularly oral melanoma) (MacEwen, 1990; Bergman, 2007) and soft tissue sarcomas (Onions, 1984; Moore and Rosin, 1986; Affolter and Moore, 2000). Mammary (Taylor et al., 1976; Gilbertson et al., 1983) and squamous cell carcinomas (O'Brien, 1992; Henry et al., 2005) have also been reported in the dog. Many of these cancers exhibit an increased prevalence in particular breeds of dogs (Table 2), indicating a genetic predisposition (Cadieu and Ostrander, 2007). Some examples are discussed below.

Renal cystadenocarcinoma and nodular dermatofibrosis in the German shepherd dog

One of the most compelling examples of the use of genome-wide linkage scans to identify canine cancer loci comes from the study of renal cystadenocarcinoma and nodular dermatofibrosis (RCND), a hereditary kidney cancer of the German shepherd dog (GSD) that is characterized by bilateral, multifocal renal tumors, skin nodules and, in females, uterine leiomyomas (Jónasdóttir et al., 2000; Moe et al., 2000). The disease is metastatic in about 50% of cases. In 2000, following a genome-wide linkage scan, the disease locus was mapped to canine chromosome 5 (CFA5) using a large family of several sibships that originated from a single affected popular sire (Fig. 2) (Jónasdóttir et al., 2000). A mutation was found to segregate with affected dogs in exon seven of the folliculin (*FLCN*) gene (Lingaas et al., 2003). Mutations in folliculin cause a similar disorder in humans, also characterized by renal neoplasias, called BHD syndrome (Schmidt et al., 2001; Nickerson et al.,

2002). In dogs, however, tumors develop at a much earlier age and the disease progresses at an accelerated rate, providing an excellent model for the study of this unique cancer. Although this is a relatively rare disease, this study aptly demonstrates the power of popular sires and large mapping families in establishing canine disease models.

Transitional cell carcinoma

Another disorder that lends itself to genetic studies is TCC of the bladder, the most common malignancy of the urinary tract in dogs. Epidemiological studies reveal a number of risk factors, including breed and female gender, as well as environmental factors, such as insecticide exposure (Glickman et al., 1989; Glickman et al., 2004). The tumor is difficult to remove surgically and responds poorly to chemotherapy. The human and canine forms of the disease share many similarities, including similar clinical signs and metastases in 50% of cases, and both express cyclooxygenase-2 in the majority of cases (Glickman et al., 2004).

Only about five major breeds of dog are affected by TCC, with the Scottish terrier having a 20-fold increased risk compared with other dog breeds (Hayes, 1976; Glickman et al., 2004). Other at-risk breeds include the West Highland white terrier, the Shetland sheepdog and the Beagle, each of which are reported to have an increased risk of between threefold and fivefold (Table 2) (Knapp et al., 2000). The West Highland white terrier and Scottish terrier have been shown to share a common phylogenetic lineage (Parker et al., 2007), suggesting that the consideration of these two breeds together is likely to increase the power in a GWAS. With much remaining to be learnt about the genetic factors that predispose to human TCC, studies of the canine disorder are likely to be revealing.

In addition to the value of canine TCC as a genetic model, the disease also serves as a therapeutic model. The cyclooxygenase inhibitor piroxicam has been studied in canine drug trials for the treatment of TCC. One study showed a complete remission in two dogs and a partial remission in four out of 34 dogs (Knapp et al., 1994). Another trial undertaken by Mohammed and colleagues (Mohammed et al., 2002) demonstrated a reduction in tumor volume in 12 out of 18 dogs, suggesting the need for a parallel human trial.

Table 2. Breed predisposition for a variety of malignancies and associated relative risks

Cancer	Breeds predisposed	Relative risk	Reference
Gastric carcinoma	Chow Chow	10-20	Paoloni and Khanna, 2007; Stomach cancer study in dogs [†]
	Belgian shepherd dog (familial)		Head, 2002
Hemangiosarcoma of the soft tissues	Golden retriever	12.4*	Goldschmidt and Hendrick, 2002 Tamburini et al., 2009
	German shepherd dog Boxer		
Lymphoma	Boxer	4.5 [†]	Tamburini et al., 2009
	Golden retriever	2.2 [†]	
Malignant histiocytosis/histiocytic sarcoma	Bernese mountain dog		Goldschmidt and Hendrick, 2002; Pool and Thompson, 2002
	Flat-coated retriever Rottweiler		
	Golden retriever		
Mammary carcinoma	Doberman pinscher	3.2	Egenvall et al., 2005
	English springer spaniel Dachshund	2.7	
	Pointer		Misdorp, 2002
Mast cell tumor	Boxer	16.7	Goldschmidt and Hendrick, 2002; Welle et al., 2008
	Boston terrier	8.0	
	Several other breeds		
Melanoma	Chow Chow	7.9	Ramos-Vara et al., 2000; Paoloni and Khanna, 2007 Goldschmidt and Hendrick, 2002
	Scottish terrier	3.8	
	Standard schnauzer	3.5	
	Miniature schnauzer	3.5	
	Irish setter	2.8	
	Golden retriever	2.1	
	Doberman pinscher	2.1	
Subungual malignant melanoma	Scottish terrier	12.1	Goldschmidt and Hendrick, 2002
	Standard schnauzer	7.4	
	Irish setter	4.2	
	Miniature schnauzer	4.2	
	Rottweiler	3.1	
	Golden retriever	1.9	
Osteosarcoma	Great Dane	Giant breeds: 60.9 Large breeds: 7.9	Thompson and Pool, 2002
	Saint Bernard		
	German shepherd dog		
	Irish setter		
	Rottweiler		
	Boxer		
	Greyhound		
	Scottish deerhound		
Pancreatic carcinoma	Airedale	16.9	Priester, 1974
	Boxer	3.5	
Squamous cell carcinoma	Keeshond	3.6	Goldschmidt and Hendrick, 2002
	Standard schnauzer	2.5	
	Basset hound	2.2	
	Collie	1.9	
Subungual squamous cell carcinoma	Giant schnauzer	15.0	Goldschmidt and Hendrick, 2002
	Gordon setter	13.3	
	Standard poodle	5.9	
	Standard schnauzer	4.9	
	Scottish terrier	3.7	
	Several other breeds		
Transitional cell carcinoma	Scottish terrier	18.1	Knapp et al., 2000
	West Highland white terrier	3.0	
	Shetland sheepdog	4.5	
	Beagle	4.2	

The overall cancer rate in dogs was calculated to be 23% (Cadiou and Ostrander, 2007).

*7% of all canine cancers are hemangiosarcomas (Modiano et al., 2005), and one in five Golden retrievers get hemangiosarcomas (Tamburini et al., 2009).

[†]24% of all canine cancers are a lymphoma (www.akcchf.org/pdfs/2009FundingRequest.pdf, Breen abstract), and one in four Boxers (www.americanboxerclub.org) and one in eight Golden retrievers (Tamburini et al., 2009) get lymphomas.

[†]Stomach cancer study in dogs (<http://cvm.msu.edu/research/research-centers/center-for-comparative-oncology/comparative-oncology-research-studies/stomach-cancer-study-in-dogs>).

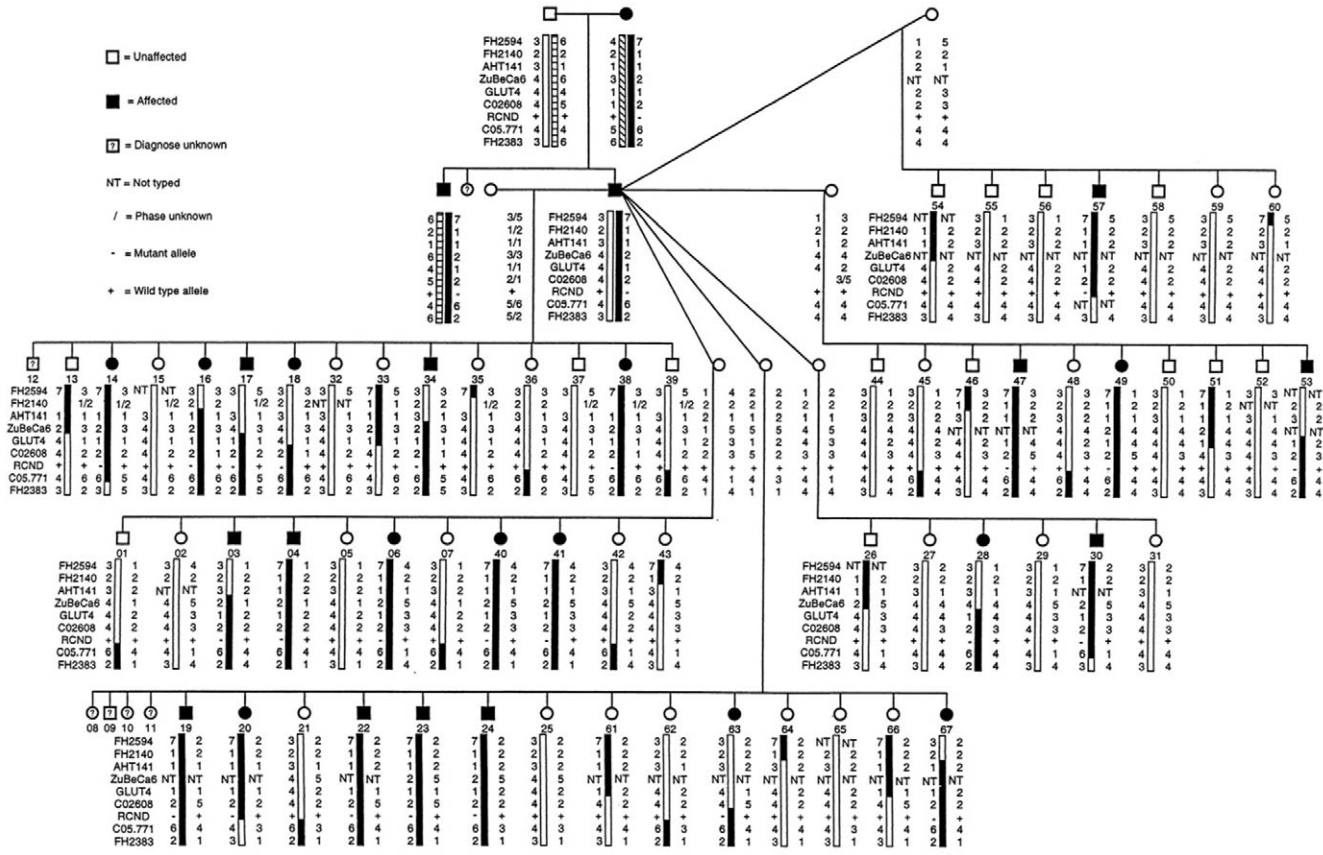


Fig. 2. Example of a linkage pedigree. The GSD pedigrees segregating RCND; this is an example of a powerful canine linkage study that was used to identify a cancer locus (Jónasdóttir et al., 2000). Several highly linked microsatellite markers established CFA5 as the location (Jónasdóttir et al., 2000), which was subsequently identified as a mutation in exon 7 of the folliculin gene (Lingaa et al., 2003). Figure reproduced from Jónasdóttir et al. (Jónasdóttir et al., 2000). Copyright (2000) National Academy of Sciences, USA.

Malignant histiocytosis in the Bernese mountain dog

Although TCC and RCND display the strong comparative power of the canine model, there is also enormous value in the study of canine diseases that do not directly mimic a human disease. For example, malignant histiocytosis (MH), also referred to as disseminated histiocytic sarcoma, is a highly aggressive cancer of the dendritic cells of the soft tissues that occurs in a small number of dog breeds such as the Bernese mountain dog (BMD), with 25% of BMDs succumbing to the disease (Abadie et al., 2009). However, malignant histiocytic neoplasms are extremely rare in the human population (Favara et al., 1997), resulting in little opportunity to study the behavior of aberrant dendritic cells in humans. MH in the BMD thus provides an opportunity to learn about genes that are essential for dendritic cell function, as well as to better understand the

utility of dendritic cells in the human immune system.

Appendicular osteosarcoma

For some canine diseases, a human counterpart occurs in only a subset of the population. Osteosarcoma (OSA) occurs most frequently in children and adolescents, with 400 cases per year in the population of the USA that is less than 20 years old; 85% of OSA cases occur in the extremities (Hawkins and Arndt, 2003). However, in dogs, OSA occurs about ten times more frequently (Khanna et al., 2006). Canine OSA tumors, as well as human OSA tumors, display chaotic and heterogeneous karyotypes with hypodiploidy, hyperploidy, and structural and numerical chromosomal aberrations. The dog breeds with an increased incidence include the Rottweiler, GSD, Great Dane, Scottish deerhound and Greyhound (Thomas et al., 2009). The

latter three are interesting because they are all long-limbed hounds, suggesting that there is a particular physiology in dogs that is associated with disease incidence.

One approach for unraveling the relationship between the disease and its underlying genetics is to look for copy number alterations in tumors (Wang et al., 2002; Rueda and Diaz-Uriarte, 2007). When comparing Rottweiler tumors with Golden retriever tumors, Thomas et al. demonstrated breed-specific differences in copy number aberrations in OSA cells (Thomas et al., 2009). Thomas et al. hypothesized that differences in the genetic backgrounds of each breed could yield hints about the life cycle of the tumor. Indeed, several loci with known tumor suppressor or oncogenic functions, including *WT1*, *TP53* and *CDKN2A*, displayed either genomic amplifications or deletions that were significantly associated with the specific dog breed.

Advantages of *Canis familiaris* as a model for genetic susceptibility to disease

- The canine genome exhibits greater homology to the human genome than other mammalian models for disease
- The dog population is divided into breeds that were generated by small founder populations and propagated by a closed breeding pool, thereby maintaining a high level of genetic homogeneity within most breeds
- The breed structure reduces disease heterogeneity and is an attribute for identifying disease susceptibility loci in a single breed or related breeds
- Dogs are susceptible to many of the same diseases as humans, particularly complex diseases like cancer
- Cancer occurs spontaneously in dogs, at a rate that is at least equal to human cancer, and often closely mimics characteristics of human tumors
- Humans and dogs share many of the same environmental factors that may influence disease onset or progression

Chronic myelogenous leukemia

In some cases, human and canine cancers share the same genetic hallmarks. For instance, Breen and Modiano recently reported that both human and canine chronic myelogenous leukemia (CML) results from the production of a breakpoint cluster region (BCR)-Abl fusion gene product, which is a tyrosine kinase that is involved in cell division and apoptosis (Breen and Modiano, 2008). In humans this so-called 'Philadelphia chromosome' involves a translocation event between chromosomes 9 and 22, t(9;22)(q34;q11). HSA 9q34 and 22q11 correspond to CFA 9q25-26.1 and 26q23-24, respectively, in the dog, and the translocation of these loci is denoted as t(9;26)(25-26.1;23-24). Although the numbers are small, studies of tumors from five dogs affected with CML demonstrated that 11-34% of cells carry the same translocation. No published studies exist that evaluate the use of the highly

touted drug imatinib, which was developed for human CML and inhibits the breakpoint cluster kinase; however, these data suggest that the dog may be a useful system in which to study appropriate targeted therapies.

Conclusions and long-term issues

In the last 15 years, the domestic dog has emerged as a powerful genetic tool for the study of heritable human diseases. Human disorders associated with immunodeficiency, narcolepsy, metabolic disease, cancer, autoimmune function, vision and epilepsy have all been studied in the dog (Jezyk et al., 1989; Lingaas et al., 1998; Lin et al., 1999; Jónasdóttir et al., 2000; Hungs et al., 2001; Sidjanin et al., 2002; Chase et al., 2005; Clark et al., 2005; Lohi et al., 2005; Zangerl et al., 2006). Advances in several disorders, particularly those associated with vision, sleep and immune function, have informed us about how to better understand the comparable human disease (Acland et al., 1998; Acland et al., 2001; Chabas et al., 2003; Lingaas et al., 2003; Chase et al., 2006). The recent introduction of cancer into the portfolio is exciting and suggests that new and relevant discoveries are around the corner (Khanna et al., 2006).

As more canine cancers are localized at a genomic level, the next steps will be the isolation of specific causative mutations. As we further define the molecular mechanisms responsible for various cancers, the assessment of those that are most similar in humans and canines will aid in targeting drug development efforts. Here, again, the dog will be of use, because it is vital to the development of new treatment modalities (Paoloni and Khanna, 2007). For example, the dog may prove useful in developing new treatments for CML patients who have developed resistance to imatinib, as we now know that the genetic translocations are the same in both humans and canines (Breen and Modiano, 2008). The advantages of the comparative disease approach are at the forefront of the minds of the collective genomics community as we now recognize that a clearer understanding of canine diseases is certain to lead to improved treatment options for both man and his best friend.

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COMPETING INTERESTS

The authors declare no competing financial interests.

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