Dogs really are man's best friend — Canine genomics has applications in veterinary and human medicine!

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Abstract

In 2003, the US National Human Genome Research Institute (NHGRI) agreed to fund a project to sequence the entire genome of a boxer dog named Tasha. Although the USA is a country of dog lovers, with approximately 38 million households owning one or more dogs, why did one of the National Institutes of Health countenance the use of $30m for such a purpose? The answer is that the NHGRI recognised the value of the dog as an unrivalled model for the study of human disease. In this paper, the reasons why the dog is such a good model are examined. Examples of where the study of disease in dogs is increasing the understanding of the genetic basis of human disease, of the development of improved diagnostic assays and of the evaluation of clinical therapies are provided.

INTRODUCTION

The domestic dog, *Canis familiaris*, has more naturally arising inherited diseases than any other species, with the exception of man.[1–3] The relevance of this for human medicine is that at least half of the more than 400 hereditary canine diseases are known to have equivalent human diseases, including many common human diseases (Table 1). Such diseases have similar clinical characteristics and, indeed, are often ascribed the same name. Causative single-gene mutations have been identified for approximately 30 of the inherited canine diseases (reviewed by Patterson[1] and Switonski *et al.*[44]) enabling the development of diagnostic tests to identify carrier and affected animals (Table 2). Significantly, the majority of monogenic canine diseases for which a point mutation has been identified are caused by a genetic lesion in a gene that is orthologous to that containing the mutation responsible for the corresponding human disorder.[47,62–66]

The major reasons why the dog represents a suitable model for the study of human disease are not limited to similarities in physiology, disease presentation and clinical response. Many common inherited human diseases (eg asthma, diabetes, epilepsy and cancer) are the consequence of complex interactions between multiple genes and environmental factors. Consequently, when considering the selection of an authentic model for a multifactorial human disease, of particular relevance is that dogs share a common environment with man. For example, because of similarities between canine lymphoma and human non-Hodgkin's lymphoma, common environmental risk factors have been postulated.[67,68]

WHY ARE DOGS SO UNHEALTHY?

The dog population of the UK is estimated at around 6 million, and the increasing number of pet insurance policies purchased is testament to the spiralling level of expenditure devoted to improving veterinary treatments. A pertinent question is therefore: 'Why are dogs afflicted with so many diseases?' In the answer to this question lies the reason

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Table 1: Examples of human diseases shown to be inherited in dogs, or for which there is a distinct pedigree dog breed predisposition

Disease	Mode of inheritance	Affected dog breeds
Progressive retinal atrophies (PRA)	**Cf**: Autosomal dominant,[4] autosomal recessive,[5–9] or X-linked,[10] according to disease	Many breeds affected by one, or more than one, form of PRA
Mammary neoplasia	**Hs**: Familial form associated with two or more autosomal dominant susceptibility genes[11,12]	Boxer, Cocker Spaniel, Dachshund, English Springer Spaniel
Cardiomyopathies	**Hs**: Familial forms associated with autosomal dominant,[13–20] autosomal recessive[21] and X-linked[22] genes	Doberman Pinscher, English Cocker Spaniel, Great Dane, Irish Wolfhound, Saint Bernard
Deafness	**Cf**: Autosomal recessive for some breeds,[23,24] but for majority of breeds mode of inheritance and number of contributing genes unknown[25–28]	Many breeds, including Australian Cattle Dog, Bull Terrier, Dalmatian, English Cocker Spaniel, English Setter
Diabetes mellitus (Type I)	**Hs**: Multiple susceptibility genes[29–32]	Many breeds, including Beagle, Fox Terrier, German Shepherd, Keeshond, Miniature Schnauzer, Poodle, Pug, Samoyed, Siberian Husky
Epilepsy	**Hs**: Single-gene syndromes[33] and multiple-gene disorders[34–36]	Many breeds, including Belgian Tervuren
Muscular dystrophy	**Cf**: X-linked[37]	Belgian Shepherd, Golden Retriever, Irish Terrier, Miniature Schnauzer
Prostate cancer	**Hs**: Familial form likely associated with multiple susceptibility genes[38–42]	Bouvier des Flandres
Spinal muscular atrophy	**Cf**: Autosomal dominant[43]	Brittany Spaniel

The mode of inheritance is denoted for *Canis familiaris* (Cf) where known, otherwise for *Homo sapiens* (Hs).

Pedigree dog breeds have been created by man and essentially represent reproductively isolated populations

why purebred dogs are such suitable models for the study of the genetic basis of human diseases.

The purebred dog population was created by man and consists of partially inbred isolates called breeds, which are akin to geographically isolated human populations such as Ashkenazi Jews or Icelanders, as they are the products of closed genetic pools. The enormous phenotypic variation between dog breeds is a manifestation of the genetic differences between breeds. Many modern dog breeds have arisen from a small number of founder individuals considered by breeders to exhibit the best physical or behavioural traits required of the breed. For some breeds, genetic diversity has been further restricted by world events that have reduced effective breeding stocks to only a few dogs. The tenet of dog breeding is that through selective mating it is possible to raise dogs that are more likely to have a desired temperament, working ability, size or other physical features. To achieve these objectives, male dogs with particularly desirable physical characteristics have been employed to sire tens of litters, while other male dogs have been neutered. An economic incentive for avoiding inter-breed mating is the pedigree designation ascribed to a dog in recognition that it is purebred, with the prerequisite that both of a dog's parents are registered as members of the same breed. Pedigree dogs command many times the price of mongrels. In the UK, The Kennel Club[69] currently recognises 196 breeds and prescribes the breed standards against which dogs are judged at licensed dog shows. While pedigree dog breeding has selected for traits desirable to man, the by-product of years of selective inbreeding is that many breeds of dog are predisposed to inherited diseases such as cancer, heart disease, deafness, blindness and autoimmune diseases.

ADVANTAGES OF STUDYING DISEASES IN DOGS

Clinical studies

There are a variety of reasons why results derived from the clinical investigation of diseases in dogs are most likely to be extrapolative to human medicine. The most compelling argument for the dog being the most appropriate model organism for studying diseases that afflict

Table 2: Examples of diagnostic tests for hereditary canine diseases

Disease	Mode of inheritance/Gene mutation	Type of test	Affected breeds
Canine leukocyte adhesion deficiency	Autosomal recessive, missense mutation in beta-2 integrin[45]	Mutation detection	Irish Setter, Irish Red and White Setter
Cone degeneration	Autosomal recessive, deletion in *CNGB3*[a,8]	Mutation detection	German Short Haired Pointer
Congenital stationary night blindness	Autosomal recessive, deletion in RPE65[5]	Mutation detection	Briard
Copper toxicosis	Autosomal recessive, deletion in COMMD1[46]	Linkage detection	Bedlington Terrier
Cystinuria	Autosomal recessive, nonsense mutation in SLC3A1[47]	Mutation detection	Labrador Retriever, Newfoundland
Fucosidosis	Autosomal recessive, 14bp insertion in alpha-L-fucosidosis[48]	Mutation detection	English Springer Spaniel
Globoid cell leukodystrophy	Autosomal recessive, mutation in GALC[b,49]	Mutation detection	Cairn Terrier, West Highland White Terrier
GM1 gangliosidosis	Autosomal recessive, point mutation in beta-galactosidase[50]	Mutation detection	Portugese Water Dog
Mucopolysaccharidosis IIIb	Autosomal recessive, L1 retrotransposon-mediated insertion in Naglu[c,51]	Mutation detection	Schipperke
Mucopolysaccharidosis VI	Autosomal recessive, point mutation/deletion in 4S[d,52]	Mutation detection	Miniature Pinscher
Mucopolysaccharidosis VII	Autosomal recessive, missense mutation in beta-glucuronidase[53]	Mutation detection	German Shepherd Dog
Muscular dystrophy	X-linked, mutation in dystrophin[54]	Mutation detection	Golden Retriever
Myotonia congenita	Autosomal recessive, mutation CIC-1[e,55]	Mutation detection	Miniature Schnauzer
Narcolepsy	Autosomal recessive, mutation in *HCRTR2*[f,56]	Mutation detection	Dachshund, Doberman, Labrador Retriever
Muscle phosphofructokinase deficiency	Autosomal recessive, nonsense point mutation in PFK[g,57]	Mutation detection	American Cocker Spaniel, English Springer Spaniel
Progressive retinal atrophy (progressive rod-cone degeneration, prcd)	Autosomal recessive, point mutation in prcd-g[9]	Linkage detection	American Eskimo Dog, Australian Cattle Dog, Chesapeake Bay Retriever, English Cocker Spaniel, Entlebucher Mountain Dog, Labrador Retriever, Miniature and Toy Poodle, Nova Scotia Duck Tolling Retriever, Portugese Water Dog
Progressive retinal atrophy (rod-cone dysplasia-1, rcd-1)	Autosomal recessive, nonsense mutation in *PDE6B*[h,6]	Mutation detection	Irish Setter, Irish Red & White Setter
Progressive retinal atrophy (rod-cone dysplasia-1, rcd-1a)	Autosomal recessive, 8 bp insertion in *PDE6B*[h,58]	Mutation detection	Sloughi
Progressive retinal atrophy (rod-cone dysplasia-3, rcd-3)	Autosomal recessive, mutation in *PDE6A*[i,59]	Mutation detection	Cardigan Welsh Corgi
Progressive retinal atrophy (autosomal dominant, ADPRA)	Autosomal dominant, point mutation in RHO[i,4]	Mutation detection	English Mastiff, Bull Mastiff
Red blood cell pyruvate kinase deficiency	Autosomal recessive, 6bp insertion in R-PK[k,60]	Mutation detection	American Eskimo Dog, Basenji, Beagle, Cairn Terrier, Dachshund, West Highland White Terrier
Severe combined immunodeficiency	X-linked, point mutation in DNA-PK[l,61]	Mutation detection	Basset Hound, Welsh Corgi

[a] *CNGB3* = cyclic nucleotide-gated channel beta-subunit gene; [b] GALC = galactocerebrosidase; [c] Naglu = N-acetyl-α-D-glucosaminidase; [d] 4S = N-acetylgalactosamine-4-sulfatase; [e] CIC-1 = skeletal muscle voltage-dependent chloride channel; [f] *HCRTR2* = hypocretin (orexin) receptor-2 gene; [g] PFK = phosphofructokinase; [h] *PDE6B* = cyclic guanosine monophosphate phosphodiesterase beta-subunit gene; [i] *PDE6A* = cyclic guanosine monophosphate phosphodiesterase alpha subunit gene; [j] RHO = rhodopsin; [k] R-PK = erythrocyte pyruvate kinase; [l] DNA-PK = DNA-dependent protein kinase.

Diagnostic tests have been developed for many inherited canine diseases

both dogs and people is that in the dog (as in man) the conditions are naturally occurring and are similar biologically, histologically and in clinical course. The dog also exhibits a physiology more suited to gross comparison with the human than many traditional model organisms.

Evaluation of the efficacy of potential therapeutics is central to clinical studies of disease. Treating naturally occurring diseases of the dog does not attract the ethical dilemmas seen with experimentally induced disease and may provide a more robust model, as the complex and

Clinical and epidemiological studies of disease in dogs are often relevant to human medicine

sometimes unexpected tissue interactions can be studied. The physiology of the dog is such that it responds to and metabolises drugs in a comparable way to humans, consequently the dog is used routinely for pharmaceutical toxicological studies.[70] There is increasing interest in the value of comparative dog–human oncological studies, and this area of research arguably has the potential to deliver the greatest benefit to human medicine. Cancer is the most frequent canine disease. Dogs develop a number of spontaneous tumours that display similar biological behaviour and histopathological characteristics to tumours that occur in man (Figure 1).[71–73]

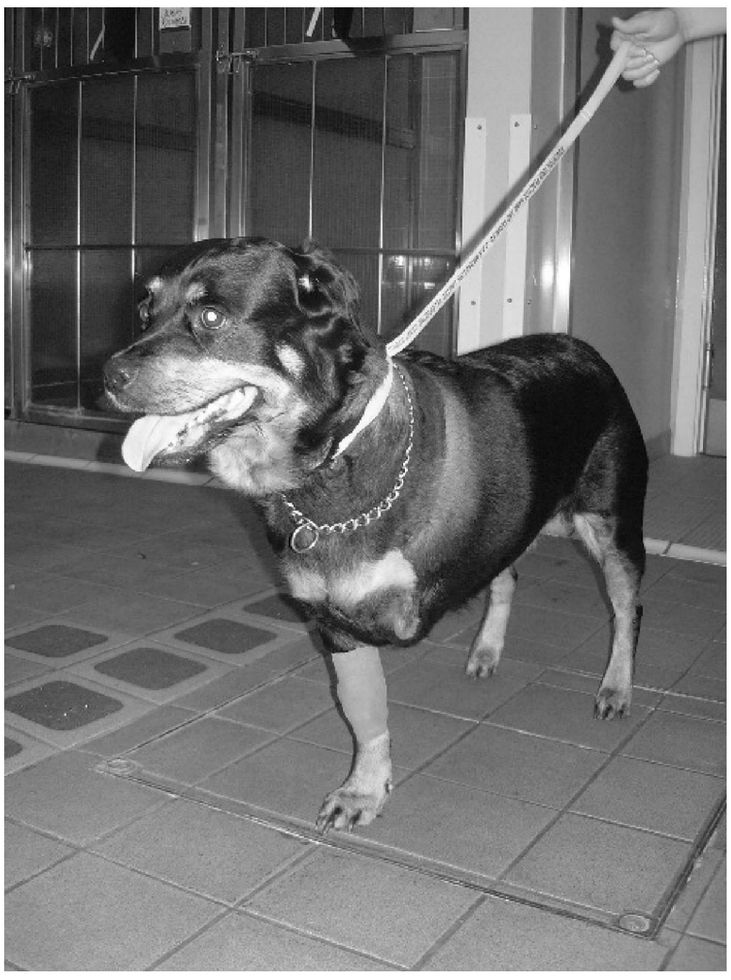

Figure 1: A Rottweiler following limb amputation to treat an osteosarcoma. Osteosarcoma is an example of a naturally occurring canine cancer that is similar in terms of clinical presentation and histopathology to a corresponding human cancer.

In addition, there is reason to believe that there may be more similarities between the mechanisms of tumour development in humans and dogs than between man and other model organisms. For example, in dogs investigations of the role that inhibition of telomeric diminution plays in cell immortalisation and proliferation are apposite, as they have telomeres that more closely resemble human telomeres than murine telomeres.[74] Among the practical benefits of clinical investigation of cancer in dogs is the fact that, as dogs have a shorter life span, clinical intervention can be studied over a condensed period of time. Survival rates for dog cancer cases are quoted over one year, rather than five, as in human oncology,[73] and so it is possible to achieve relatively rapid results when performing clinical trials and monitoring disease progression.

The importance of autopsy of disease cases to confirm diagnosis, characterise the structural, and perhaps functional, changes associated with disease and possibly confirm the appropriateness of medical care cannot be underestimated. However, in the aftermath of the events at Alder Hey Hospital (Liverpool, UK),[75,76] however, it is arguable that, in the UK, dog owners are more likely to allow a full post-mortem examination than families of affected human beings.

Epidemiological study of the risk factors associated with complex human diseases may also be approachable via the study of canine cohorts.

As dogs share a common environment with man, it is possible that the aetiology of canine diseases is similar to those of their human counterparts. Although sharing an environment, dogs, however, do not smoke (except passively) nor drink alcohol and mostly have a non-varied diet. Hence, the effects of passive smoking and exposure to pesticides, asbestos, radon and lead have all been studied without the confounding influences of direct tobacco

or alcohol use.[77–80] There is also an increased awareness of dogs as sentinels for vector-associated disease (eg Lyme disease[81]) and diseases that have an environmental component. This is particularly pertinent, as dogs often develop clinical signs of disease more quickly than humans.[82]

Genetic mapping

Mapping of disease genes is easier in the dog than in man because of the natural history and structure of breeds and the availability of detailed genealogical records.

Genetic mapping of disease genes is easier in the dog than in man

The prevalence of inherited diseases in dogs was the motivation in the mid-1970s for the inception of dog registries, in which entries detail ownership, often include a pedigree and record medical conditions. Prior even to the development of genetic tests for single-gene disorders, the objective was to attempt to minimise the inheritance of disease genes in any mating by providing breeders with details of disease-affected and disease-carrying individuals in a dog's family.[83] For family-based linkage analysis of monogenic disorders, the availability of pedigrees with associated phenotypic data allows the geneticist to predict mode of inheritance and identify individuals required for genotyping. In man, high-risk families characterised by multiple generations of affected individuals are typically small, and key elderly family members may be deceased. By contrast, the large sizes of canine families serve to increase the statistical power of linkage analyses, while the condensed life span of dogs means that it is possible to collect DNA samples from three generations, a prerequisite for unambiguous identification of recombination between disease and marker alleles. In the absence of suitable families, it is also possible to set up directed matings to enable examination of disease segregation.[84] Selected mating of dogs from different breeds with apparently similar diseases can also be used to establish whether diseases are allelic.[85]

Many common hereditary diseases do not exhibit Mendelian modes of inheritance but are the product of many alleles, each with a low to moderate effect. Mapping of such genes in outbred human populations is intractable by linkage-based studies, due in part to the fact that different genes may be responsible for disease clustering in different families. In theory, the mapping of complex diseases will be somewhat less complicated in dog breeds. It appears that at least 46 per cent of inherited dog diseases are breed specific.[1] This suggests that breeds are enriched for a small number of disease alleles, which are relatively rare in the overall dog population, as they are derived from one or a small number of common ancestors. The implications of this are that alleles associated with disease susceptibility may be mapped using extended dog families and modest numbers of samples.

In order to side-step the requirement for multigenerational sampling demanded by linkage analysis, association studies[86] are increasingly being deployed for genetic mapping of common polygenic human diseases. With the development of high-density, whole-genome single nucleotide polymorphism (SNP) maps and technological advances that will reduce the cost of genotyping, it is likely that genome-wide association studies will succeed current candidate gene-based investigations in order to increase the probability of detecting associations between markers and disease. As it is not feasible to genotype all possible alleles of all genes to detect an association, however, it is necessary to identify a minimum set of alleles capable of reporting on all other alleles. The number of markers in a so-called 'tagging set' is dependent upon the strength of the linkage disequilibrium (LD) between each marker and other markers in close proximity. Although the extent of LD is highly variable in different chromosome regions and in different human populations,[87–89] it is estimated that it will be necessary to genotype in the order of 200,000 to

500,000 SNPs to report adequately on all SNPs.[90]

Recent analysis of sequence variation at five genetic loci in each of 20 unrelated dogs from each of five breeds of distinct origin[91] identified LD that was 20–100 times more extensive than in humans, suggesting that a proportionally smaller number of markers could be used for whole-genome association studies in dogs than in humans. In addition, the study[91] identified the sharing of haplotypes between the different dog breeds, suggesting that a single tagging set of SNP markers could be constructed for genome-wide mapping of traits in a large number of dog breeds.

Whole-genome associated studies of polygenic diseases in different dog breeds may be possible using a single set of SNP markers

CREATION OF REAGENTS FOR THE ANALYSIS OF INHERITED CANINE DISEASES

Genetic linkage maps

The first canine linkage maps were constructed in 1997[92,93] by genotyping microsatellite markers on large pedigrees of dogs and using meiotic linkage analysis to determine the order and spacing of markers along the chromosomes. In the first map, 94 microsatellites were analysed and 43 of them were placed into 16 linkage groups, whereas the superior mapping power of the pedigrees used in the second map enabled 139 to be linked to at least one other marker, identifying 30 linkage groups. At the time, it was not possible to differentiate between the 38 pairs of acrocentric canine chromosomes, and so linkage groups could not be associated with specific chromosomes.

Creation of maps of the canine genome

The second-generation linkage map for the dog[94] expanded the number of mapped markers to 276 and identified 39 linkage groups. The size of the canine genome was suggested to be about 27 Morgans. A third-generation linkage map[95] comprised 341 markers; the average distance between the markers was 9.0 centimorgans and the map was estimated to cover over 95 per cent of the genome. Fourteen of the 37 autosomal linkage groups contained either gene-associated or anonymous markers localised to cosmids that had been assigned to specific canine chromosomes by fluorescence *in situ* hybridisation (FISH).

Radiation hybrid maps

The first whole-genome radiation hybrid (RH) map of the canine genome was published in 1998 and comprised 400 markers, including 218 genes.[96] Limitations in the cytogenetic resolution of dog chromosomes meant that only 14 of the 57 RH groups could be assigned to specific chromosomes.

Chromosome identification

In 1999, canine whole-chromosome-specific FISH probes (chromosome paints) were developed to unequivocally identify each chromosome in a metaphase spread.[97–99] The ability to identify each canine chromosome, and also to perform cross-species chromosome painting, enabled meaningful genome-wide comparative studies that examined the levels of synteny between the canine and other mammalian genomes (Figure 2).[99–101]

Work is currently ongoing to develop a procedure in which a panel of publicly available canine chromosome-specific bacterial artificial chromosome (BAC) clones can be employed to identify simultaneously all canine chromosomes in a single hybridisation,[102] such as following comparative genomic hybridisation analysis[103] of tumour DNA.

Integrated maps

By 2000, map integration was achieved with linkage and radiation hybrid groups being linked to specific canine chromosomes.[104] A subsequent development was a 1 megabase radiation map[105] comprising 3,270 markers, including 1,596 microsatellites, 900 cloned gene sequences and expressed sequence tags (ESTs), 668 canine-specific BAC ends and 106 sequence-tagged sites. A minimal screening set of 325 highly

informative, well-spaced markers was defined for use in genome-wide screens. The map also identified 85 fragments conserved between the dog and human genomes, enabling initial linkage findings resulting from genome-wide screens to be followed up by candidate gene studies. The most recent RH map[106] encompasses 4,249 markers at a density of one marker every 900 kilobases, and contains 1,760 BAC clones localised to 1,423 unique positions, 851 of which have also been FISH mapped. For 2,233 markers, the orthologous human genes have been established, allowing the identification of 79 segments conserved between the dog and human genomes (Figure 2).

EXAMPLES OF BENEFITS OF CANINE GENOMICS RESEARCH TO HUMAN MEDICINE

Similarities between the clinical features and molecular bases of analogous canine and human diseases enable effective testing of new treatments for human diseases in dogs

Over a number of years, it has become clear that investigations into a variety of sporadic and inherited canine diseases are able to inform investigations into similar or analogous human diseases. Many of the clinical and molecular features of these canine diseases are very similar to analogous diseases of humans, making them closer clinical models than equivalent mutant or transgenic rodents. This, coupled with their 'large' size and a physiology that is comparable to humans, also makes dogs excellent candidates for testing of novel clinical therapies.

Inherited retinal diseases

There are approximately 100 canine breeds that exhibit forms of inherited degenerative retinal disease. Using a candidate gene approach, informed by studies into inherited retinal degenerations in humans, numerous distinct mutations have been identified in a variety of canine breeds (Table 2). By identifying affected dogs by clinical examination and, more recently, by a diagnostic assay that identifies both affected and carrier dogs, many of these degenerative retinal diseases are being eliminated from the breeds.[107,108]

Positional cloning studies have initiated gene therapy experiments that target the gene products that are specifically affected in some of these dog breeds.[109,110] This highlights one of the distinct advantages of working with dogs, rather than transgenic rodents. The size of the eye in dogs, particularly in medium to large breeds, is comparable to humans. Consequently, recent gene therapy trials in dogs[111] are potentially more relevant than similar studies performed in transgenic mice, since they are more likely to be predictive of the effects of such treatments in people. In such experiments, it is likely that significant volumes of vehicle/gene product need to be injected. By using a large animal as a model system, the effects of both the introduced gene product and the injection technique can be evaluated.

Inherited neurological diseases

A canine model of human epilepsy has recently been identified and characterised at a molecular level.[66] This study demonstrated that a dodecamer repeat in the *Epm2b* (*Nhlrc1*) gene is responsible for a significant number of 'idiopathic' epilepsy cases. Because of the relatively high frequency of 'idiopathic' epilepsy in the canine population, the identification of mutations within specific loci may enable both improved treatment of the canine patients and evaluation of new therapeutic regimens.

Of further interest is narcolepsy, an uncommon but serious condition in humans, which has been identified as an inherited disease in both Dobermans and Labrador Retrievers.[112] Prior to the identification of the gene mutations involved, pharmacological and physiological studies in people had been facilitated by the use of the canine model.[113] However the identification of causative mutations in the hypocretin/orexin-receptor-2 gene,[114] will promote increased understanding of the molecular basis of the disease, paving the way for the development of new treatment strategies.

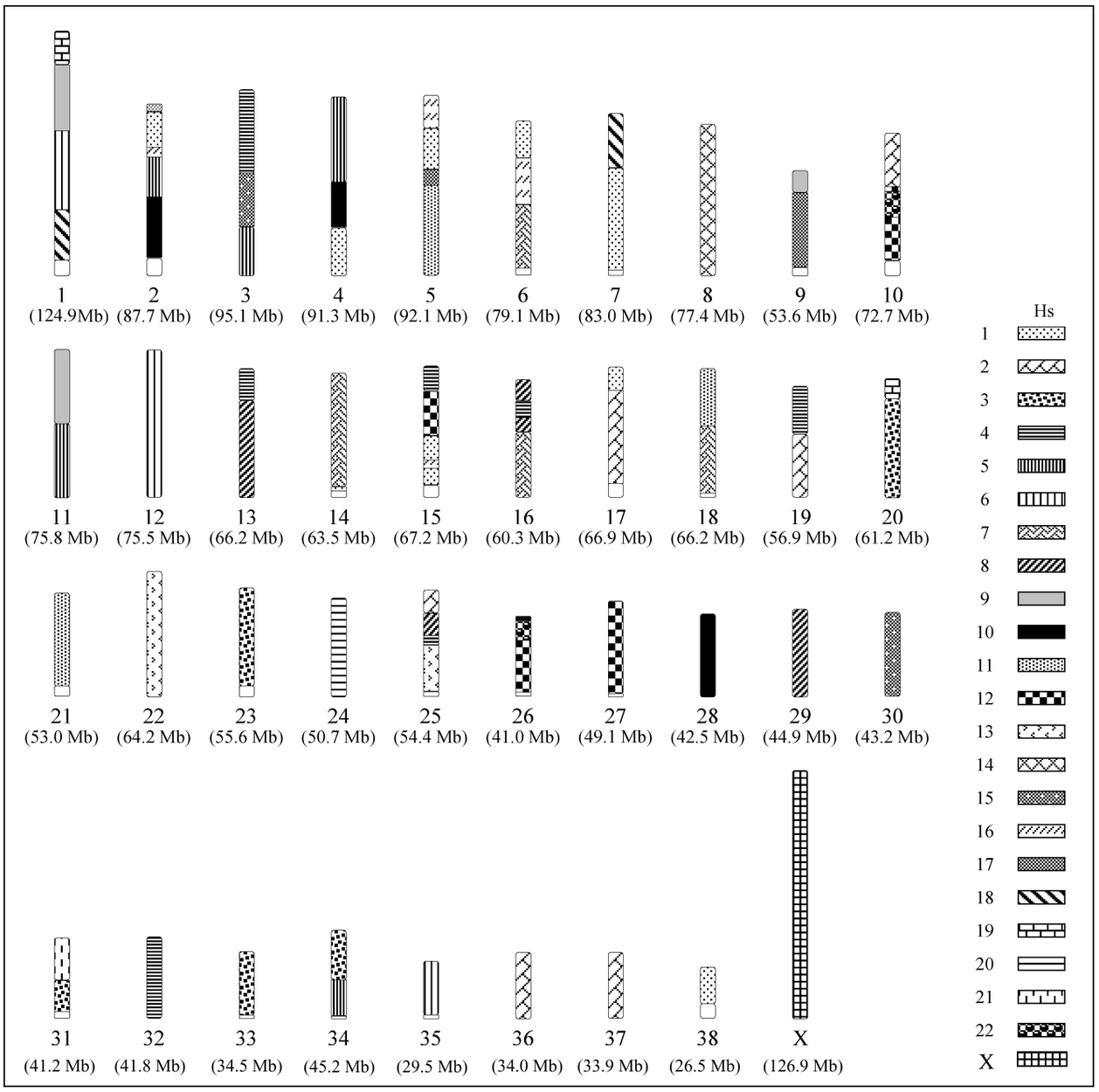

Figure 2: Representation of the sequence conservation between the dog and human genomes. Canine chromosomes (of length predicted in the first Ensembl annotated release of the assembled dog genome sequence[138]) are overlaid with segments of *Homo sapiens* (Hs) chromosomes with which they share significant sequence similarity. Abstracted with permission from Kirkness, E. F., Bafna, V., Halpern, A.L. *et al.* (2003), 'The dog genome: Survey sequencing and comparative analysis', *Science*, Vol. 301, pp. 1898–1903. Copyright 2003 AAAS.

Substantial sequence similarity exists between the canine and human genomes

Inherited storage diseases

Inherited lysosomal storage diseases are rare diseases, which have been reported in humans and a wide range of animal species (as reviewed by Jolly and Walkley;[115] see Table 2). Due to the restricted gene pools of pedigree dog breeds, it is conceivable that a relatively high proportion of the breeding population of certain breeds (in particular, rarer breeds such as the Portuguese Water Dog) may be carriers of recessive causative mutations. These affected dogs are not only informative in respect of the basic biology of the disease process, but additionally they can be used as 'large

animal' models to test a variety of therapeutic modalities.

Gene therapy trials in dogs

Gene therapy trials have been attempted in dogs with mucopolysaccharidosis I[116,117] and VII,[118,119] and enzyme replacement therapy attempted for other storage diseases such as fucocidosis.[120] Transfer of gene therapy techniques from transgenic mice to humans has been somewhat hampered because low levels of gene expression can often apparently fully correct the enzyme deficiency in knockout transgenic mice; however, similar levels of enzyme expression are associated with clinical disease in human and most large animal models of these diseases. Therefore, it is anticipated that to explore the potential of gene therapy for treating human lysosomal storage diseases most effectively, it will be necessary to use a large animal model, such as the dog, which may carry the same, or similar, mutations in the affected genes.

A very well characterised copper storage disease has been described in Bedlington Terriers[121] and is used as a model for Wilson's disease — an analogous inherited copper storage disease of humans.[122] Of much interest is that the two most likely candidate genes, the copper transporting ATPase *ATP7B*,[123] defective in Wilson's disease, and the copper chaperone *ATOX1*,[124] have both been excluded as causes of copper toxicosis in Bedlington Terriers. Screening of genes localised on canine chromosome 10q26 revealed mutations in the *MURR1* gene.[125] Exon 2 of this gene is deleted in both alleles of all affected Bedlington Terriers and in single alleles in obligate carriers. The function of the *MURR1* gene is unknown,[125] but does not appear to be affected in other non-Wilsonian hepatic copper storage diseases in humans.[126] The genetic studies of copper toxicosis in the dog provide an apt illustration of how genetic defects identified in a canine model of a human disease highlight potential genetic mutations in the human counterpart.

Comparative human-dog studies of inherited and sporadic cancers are of increasing interest

Comparative oncology

There is increasing interest in using canine, breed-specific predispositions to certain tumour types to help to identify genes that may be involved in a variety of carcinogenesis pathways. An example is identification of the *RCND* mutation, which is associated with renal cystadenocarcinoma and dermatofibrosis in German Shepherd dogs.[129] This mutation occurs in exon 7 of the Birt–Hogg–Dube locus. At this stage, it remains to be seen whether the orthologous gene is affected in humans afflicted with the same conditions.

The canine tumour that has been most investigated at a molecular level is the mast cell tumour (MCT). This is the most common cutaneous tumour of dogs, and several breeds — such as boxers — are highly predisposed to developing them. Approximately 30–50 per cent of these tumours contain internal tandem duplications within exons encoding the transmembrane domain of c-KIT.[128] The same gene is frequently mutated in gastrointestinal stromal tumours (GISTs) of humans.[129] Although MCTs are extremely rare in people, the canine MCT represents a potential molecular and therapeutic model for investigation of both human MCT and GIST. This is particularly pertinent for GIST, since the canine disease is much more accessible, being localised in the skin as opposed to in the walls of the intestine.

Novel immunological therapies have been tested in rodent models of human cancers with varying degrees of success (reviewed by Srinivasan and Wolchok[130]). Significantly, however, a Phase I preclinical trial of a xenogeneic melanoma vaccine has been successfully performed in dogs[131] exhibiting oral melanoma, concurrently with similar xenogeneic trials of the same type in humans.[130] Comparative parallel studies of this type are likely to become increasingly common as canine models of human sporadic and inherited diseases become better characterised.

SEQUENCING THE CANINE GENOME

In 2001, a proprietary 1 × sequence of the genome of a male Standard Poodle, Shadow, was generated by Celera Genomics. The case for a dog genome sequence available in the public domain was presented in the 2002 White Paper.[132] A publicly available canine genome sequence was required to provide a framework for, and to expedite, the comprehensive sequence analyses that would have to be performed by disparate research groups in order to identify the new and informative polymorphisms (eg SNPs) that would enable high-resolution mapping of candidate disease regions. Validation and annotation of a draft canine genome sequence would be assisted by a number of canine EST projects.[133–135]

The sequence of the genome of the female Boxer, Tasha

In June 2003, the National Human Genome Research Institute (NHGRI)-funded sequencing of the dog genome was initiated at the Broad Institute[136] and Agencourt Biosciences Corporation.[137] SNP typing of 60 candidate breeds identified a breed of comparatively low sequence heterozygosity; the genome of a female Boxer dog, Tasha, was therefore selected for sequencing by a whole-genome shotgun sequencing strategy. The assembled sequence[138–140] is constructed from a 7.6-fold sequence coverage and is estimated to cover 96–98 per cent of the canine genome, assuming a haploid genome of 2.4×10^9 base pairs. Initial analysis of the assembled sequence annotated 20,439 genes encoding 32,548 transcripts. Sequence similarity searching of the dog genome with annotated human gene sequences, however, suggests that the Ensembl analysis and annotation pipeline[141] has erroneously failed to annotate the orthologues of around 700 human genes (Michael Schuster, personal communication).

In parallel with the boxer genome sequencing project, 100,000 reads were generated from across the genomes of nine other breeds, to identify, by comparison with the assembled Boxer genome sequence, a large set of SNPs[142] that could ultimately be used as markers for high-resolution genetic mapping of disease traits in any breed. The validation rate for the SNPs varies between 90–98 per cent (Kerstin Lindblad-Toh, personal communication), according to the number of reads representing a given allele required and the nature of the base-specific quality scores[143] employed by the SNP discovery algorithm SSAHA-SNP.[144]

In the meanwhile, The Institute for Genomic Research (TIGR) extended analysis of the Standard Poodle, generating (from a 1.5 × sequence coverage) a publicly available assembly[145] estimated to cover about 78 per cent of the genome, and identifying 974,400 putative SNPs.[146] The length of unique human genome sequence that could be aligned to the 1.5× dog sequence (Figure 2) was twice that which could be aligned with the 8 × mouse genome sequence[146], suggesting a higher degree of nucleotide sequence conservation between the genomes of dog and human than between human and mouse. Among the 650×10^6 base pairs of dog sequence that aligned uniquely to the human genome were fragments of putative orthologues for 18,473 of 24,567 annotated human genes.

THE FUTURE

Sequencing of the canine genome will have profound implications for both veterinary and human medical research. There is already evidence that the availability of the genome sequence has enabled high-resolution mapping of candidate disease regions implicated by initial demonstration of linkage in canine families.[147] The construction of such maps will facilitate the successful conclusion of positional cloning endeavours. Of even greater significance is the feasibility of whole genome association studies (featuring 5,000 to 30,000 SNP markers) in dog breeds given the identification of extensive LD.[91] The high degree of haplotype conservation in different breeds[91] may

enable the development of a universal whole-genome SNP marker for the association studies in different breeds that will be required to identify the alleles conferring susceptibility to many complex diseases that are common to dogs and humans.

The identification of genetic markers will form the basis of tests for presymptomatic diagnosis and the identification of dogs carrying inherited disease alleles, and has a clear benefit for promoting canine health. What may be less apparent, however, is that the associated benefits to man, of breeders being guided to make informed decisions concerning breed management, go beyond pedigree dog owners having healthier dogs that live longer. Ultimately, the identification of markers for complex traits, such as temperament, will be extremely valuable to guide- and service dog organisations that have breeding colonies.

Comparative veterinary-human studies are the way forward

There are numerous examples of situations in which human medical researchers have adopted the dog as the model by which to study their disease of interest. The value of projects conducted jointly by human and veterinary researchers, however, has recently been aptly exemplified by the identification of a gene associated with an inherited form of canine epilepsy similar to Lafora disease, the most severe teenage-onset human epilepsy.[66] The most productive way ahead is likely to be through collaborative comparative veterinary–human studies that serve to accelerate the rate of discovery and the extent to which human medicine accrues benefits from veterinary research. If proof were needed, acknowledgment of the importance of veterinary research to human medicine has been provided by the establishment of the Comparative Oncology Program within the US National Cancer Institute's Center for Cancer Research and the appointment of a senior researcher, whose laboratory has led the field of canine genetics research for the past decade, to a pivotal position in the Cancer Genetics Branch of the NHGRI.

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