



Genetic screening in dogs



Matthew Binns, BSc (Hons), PhD

The Royal Veterinary College, London, UK

Dr. Binns is Professor of Genetics at the Royal Veterinary College, London. He took this position in Autumn, 2004, after 14 years working at the Animal Health Trust (AHT). His research focuses on genetic diseases in horses and dogs, with the aim of improving their health and welfare through the development of DNA-based screening tests. Dr. Binns has chaired the horse and dog gene mapping committees for the International Society for Animal Genetics and published more than 150 scientific papers.

■ Introduction

The dog was probably the first species domesticated by man and has maintained a special place in human affections ever since. The enormous range of phenotypic diversity exhibited by different dog breeds led Darwin to conclude that they could not all have originated from a single recent ancestral species. In fact, recent molecular data demonstrates that all dog breeds are derived from the grey wolf (1). Whilst man's initial selection of dogs

KEY POINTS

- ◆ The recent sequencing of the dog genome has accelerated the rate at which the mutations underlying genetic diseases in the dog are being identified
- ◆ Increasing numbers of genetic screening tests are commercially available which can be used to selectively breed healthier dogs through the reduction and elimination of deleterious mutations
- ◆ Genetic tools are now available that can be used to identify breed specific "fingerprints" for the majority of pure dog breeds. These same tools can be used to determine the breed composition of non-pure breed individuals, presenting opportunities to genetically characterize an enormous range of morphological, behavioral and disease traits
- ◆ The scientific progress made in characterizing dog diseases at the molecular level has increased interest in their use as biomedical models for equivalent human conditions, and it is anticipated that future results from mapping genetic diseases in dogs will have both veterinary and human clinical importance

concentrated on the development of functional characteristics to benefit the lives of man, such as hunting, herding, and guarding livestock, the development of dog shows, breed societies and stud books by the Victorians led to the emergence of new breeds based largely on appearance. These new breeds were often formed by inter-breeding existing types, and selecting for individuals with particular desirable structural or behavioral characteristics.

This interesting history of pure dog breeds is evident in the genetic signatures of their chromosomes, revealed following the sequencing of the dog genome, which show evidence of two genetic bottleneck events. The first bottleneck is thought to be associated with the original domestication events, where a restricted amount of genetic variation was captured from the wolf population. This probably happened more than 10,000 years ago and the architecture of the ancestral chromosomes involved has over time broken into small fragments by the process of recombination, which shuffles the genetic card deck at each generation. The second bottleneck is comparatively recent and is thought to reflect the formation of pure breeds, in which genetic variation in the domestic dog population was partitioned into the different breeds.

Most breeds were formed using relatively small numbers of founder dogs. The formation of closed stud books, which precluded the introduction of new genetic variants into the breed, essentially fixed the genetic options for each breed. Genetic variation was then lost, initially through the process of genetic drift which affects small populations in which the chromosomes of certain founders become common, whilst others are lost by chance. Dog breeders also strived to produce uniform individuals that matched breed standards as closely as possible. This was often achieved by the close inbreeding of individuals with the desired characteristics, and through the use of popular sires, who were often show champions. Together, genetic drift and inbreeding led to a reduction in the extent of DNA variation within most breeds. In fact, for those genes that control the characteristics that define the breed standard, it is likely that little or no genetic variation is left, *i.e.* the breeds are essentially fixed for those characteristics. These unusual features of the dog population are advantageous for the study of complex inherited diseases, such that the dog is increasingly recognized as a model species within which it may be possible to discover the genes underlying many of the most common canine and human diseases (2).

The molecular genetics of the dog have come a long way in a relatively short period of time. The dog has a difficult chromosome composition for genetic research, as it possesses a large number of

chromosomes ($2N=78$) which are hard to differentiate from one another. It was surprising and fortuitous that the technique of chromosome flow-sorting was able to separate the majority of chromosomes, such that tools to individually identify each dog chromosome could be produced (3). The first DNA genetic markers were developed in the early 1990's, and rudimentary genetic maps constructed shortly afterwards (4). These maps were an essential component in identifying the gene mutations that underlie the inherited diseases, morphological and behavioral traits of interest. Subsequent genetic linkage mapping, together with a candidate gene approach (see **Table 1**), led to the identification of several disease-causing mutations in the dog and the development of the first genetic screening tests for pure breed dogs. The disease tests available and the ways in which they can be used by breeders to selectively breed healthier dogs are described in more detail below.

A major turning point for progress in the study of inherited diseases in the dog came when scientists at the Broad Institute in Boston secured funding to sequence the entire dog genome. The genome of a female Boxer dog, Tasha, was sequenced, whilst partial sequences from nine other pure breed dogs, together with four grey wolves and one coyote were generated to identify large numbers of single nucleotide polymorphism (SNP) markers that can be used to rapidly map inherited diseases in dogs (5).

■ Inherited diseases

Approximately 500 genetic diseases have been identified in pure dog breeds. Information on these diseases has been collated on the "Inherited Diseases in Dogs" (IDID) website (www.vet.cam.ac.uk/idid). More than half of these disorders are inherited in a simple Mendelian autosomal recessive manner. This in part reflects the difficulty that breeders have in removing recessive mutations from their breed as they cannot differentiate between genetically normal and carrier individuals. In contrast, for simple Mendelian dominant conditions, where a single copy of the mutation is sufficient to produce disease, it is relatively simple for breeders to remove affected individuals from their breeding population. The majority of dominant diseases remaining in the dog

population are ones in which there is a late onset of disease signs, such that affected dogs have already been bred from before the disease becomes apparent.

Mutations can occur in any gene, such that any organ or tissue can be affected with hereditary disease. This is reflected in the wide range of diseases documented in the IDID database.

The same disease mutation can occur in several different breeds and this is likely to reflect an old mutation present in dogs before the breeds became established. An example of this is the mutation responsible for Type I von Willebrand's disease that was first identified in Doberman Pinschers. The same splice site mutation has subsequently been identified in at least eight other breeds, including the Bernese Mountain Dog, Drentsche Patrijshond, German Pinscher, Kerry Blue Terrier, Manchester Terrier, Papillon, Pembroke Welsh Corgi and Poodle. At the same time there are different mutations responsible for Type I von Willebrand's disease in other breeds including Irish Red & White Setters.

It can be difficult to obtain accurate estimates for the frequency of the mutations within breeds, even when a genetic test is available. There is a problem of ascertainment bias in the frequencies reported from laboratories carrying out testing, as they are far more likely to be contacted by breeders who have had a clinical problem in their lines than by others who have not had a clinical problem. The frequency of the same mutation in different breeds can also vary widely, presumably due to founder effects and the subsequent population history. For example, the frequency of Type I von Willebrand's disease was reported to be 28% in Doberman Pinschers whilst it was only about 1% in Bernese Mountain Dogs and Poodles.

For simple Mendelian diseases the future looks encouraging for pure breed dog breeders. They are increasingly able to test for the presence of a disease causing mutation and implement selective breeding plans to minimize the impact of the mutation on their dogs. The situation is not so encouraging for the most widespread diseases such as hip dysplasia, epilepsy, auto-immunity and heart conditions, where the genetics behind the disease is more complex and likely to involve large

numbers of genes. The identification of mutations increasing disease risk and their implementation in selective breeding schemes is going to be complicated. It is likely, though, that with the new high density genotyping tools becoming available, that mutations contributing to these widespread diseases will be identified over the next few years, which will be useful in reducing the severity of disease in affected breeds.

■ Genetic screening tests

DNA-based genetic screening tests have several advantages over other clinical diagnostic techniques. Once the mutation responsible for a disease has been identified it is generally straightforward to set up tests that are rapid, sensitive, cheap and definitive. In general, DNA tests use blood or buccal samples, and the first stage in most tests involves amplifying copies of DNA using a technique called the polymerase chain reaction (PCR). The DNA produced in the PCR can then be analyzed by a variety of methods.

The number of genetic tests available for inherited diseases in dogs is increasing steadily, and is likely to accelerate with the availability of the dog genome sequence. A list of currently available commercial genetic screening tests for inherited diseases in pure dog breeds is given in **Table 1**, which also includes the URLs for the organizations offering the tests. Genetic testing for inherited diseases in many species, including dogs, is sometimes subject to patent protection which limits which organizations can test for certain diseases.

The majority of the diseases in **Table 1** are inherited as autosomal recessive conditions, and it is the ability to identify carrier dogs that is a major advantage of DNA testing. Carrier dogs are clinically normal and it is not usually possible to distinguish between carriers and genetically clear dogs by veterinary investigation. The carriers act as "reservoirs" of disease for future generations and affected dogs are usually produced from matings between two carrier parents, where on average 25% of their litter will be affected with the disease.

DNA-based tests for recessive diseases present dog breeders with several options to improve the health and welfare of their dogs, and these choices are illustrated in **Figure 1**.

Table 1.
Genetic screening tests for inherited diseases in pure dog breeds

Disease	Breed	Company
Canine leukocyte adhesion deficiency	Irish Setter	AHT, Healthgene, Optigen
	Irish Red & White Setter	AHT, Optigen
Ceroid lipofuscinosis	Border Collie	AHT, Optigen
	Bulldog	GT
Collie eye anomaly/Choroidal hypoplasia	Australian Shepherd	Optigen
	Border Collie	Optigen
	Lancashire Heeler	Optigen
	Nova Scotia Duck Tolling Retriever	Optigen
	Rough Collie	Optigen
	Shetland Sheepdog	Optigen
	Smooth Collies	Optigen
Whippet	Optigen	
Cone degeneration	German short-haired Pointer	Optigen
Congenital hypothyroidism with goiter	Toy Fox Terrier	Healthgene
Congenital stationary night blindness	Briard	AHT, Healthgene, Optigen
Copper toxicosis	Bedlington Terrier	AHT, Vetgen
Cyclic neutropenia	Rough Collie	Healthgene
	Smooth Collie	Healthgene
Cystinuria	Labrador Retriever	PennGen
	Newfoundland	DDC, Healthgene, Optigen, PennGen, Vetgen
Factor VII deficiency	Alaskan Klee Kai	PennGen
	Beagles	PennGen
	Scottish Deerhound	PennGen
Factor IX deficiency (Hemophilia B)	Bull Terrier	Healthgene
	Lhasa Apso	Healthgene
	Labrador Retriever	Healthgene
Factor XI deficiency	Kerry Blue Terrier	PennGen
Familial nephropathy	English Cocker Spaniel	Optigen
Follicular dysplasia	Large Munsterlander	Healthgene
Fucosidosis	English Springer Spaniel	AHT, Finnzymes, PennGen
Globoid cell leukodystrophy	Cairn Terrier	Healthgene
	West Highland White Terrier	Healthgene
GM1 gangliosidosis	Portuguese Water Dog	Healthgene
Hereditary cataracts	Boston Terrier	AHT
	Staffordshire Bull Terrier	AHT
Ivermectin toxicity (MDR1)	Australian Shepherd	WSUCVM
	Collie	WSUCVM
	German Shepherd	WSUCVM
	Old English Sheepdog	WSUCVM
	Silken Windhound	WSUCVM
	Whippet	WSUCVM
L-2-hydroxy glutaric aciduria	Staffordshire Bull Terrier	AHT
Mucopolysaccharidosis IIIB	Schipperke	PennGen
Mucopolysaccharidosis VI	Miniature Pinscher	PennGen
Mucopolysaccharidosis VII	German Shepherd	PennGen
Muscular dystrophy	Golden Retriever	Healthgene
Myotonia congenita	Miniature Schnauzer	Healthgene, PennGen
Narcolepsy	Dachshund	Optigen
	Doberman Pinscher	Healthgene, Optigen
	Labrador Retriever	Healthgene, Optigen
Phosphofructokinase deficiency	American Cocker Spaniel	DDC, Healthgene, Optigen, PennGen, Vetgen
	English Springer Spaniel	AHT, DDC, Healthgene, Optigen, PennGen, Vetgen
Progressive retinal atrophy	American Eskimo Dog	Optigen
	Australian Cattle Dog	Optigen
	Australian Stumpy Tail Cattle Dog	Optigen
	Bullmastiff	Optigen
	Cardigan Welsh Corgi	CUVS, Healthgene, Optigen
	Chesapeake Bay Retriever	Optigen
	Chinese Crested	Optigen

>

Progressive retinal atrophy continued	English Cocker Spaniel	Optigen
	Entlebucher Mountain Dog	Optigen
	Finnish Lapphund	Optigen
	Irish Setter	AHT, Healthgene, Optigen, Vetgen
	Irish Red & White Setter	Optigen
	Kuvasz	Optigen
	Labrador Retriever	Optigen
	Lancashire Heeler	Optigen
	Lapponian Herder	Optigen
	Mastiffs	Optigen
	Miniature long-haired Dachshund	AHT
	Miniature Poodles	Optigen
	Miniature Schnauzer	Optigen
	Nova Scotia Duck Tolling Retriever	Optigen
	Portuguese Water Dog	Optigen
	Samoyed	Optigen
	Siberian Husky	Optigen
Sloughi	AHT, Optigen	
Swedish Lapphund	Optigen	
Toy Poodle	Optigen	
Pyruvate dehydrogenase phosphatase deficiency	Clumber Spaniel	GT
	Sussex Spaniel	GT
Pyruvate kinase deficiency	Basenji	Healthgene, Optigen, PennGen, Vetgen
	Beagle	PennGen
	Cairn Terrier	PennGen
	Chihuahua	PennGen
	Eskimo	PennGen
	Dachshund	PennGen
	West Highland White Terrier	AHT, DDC, Healthgene, PennGen
Severe combined immunodeficiency	Basset Hound	PennGen
	Welsh Corgi	PennGen
Von Willebrand's disease	Bernese Mountain Dog	Finnzymes, Vetgen
	Drentsche Patrijshond	Vetgen
	Doberman Pinscher	Finnzymes, Vetgen
	German Pinscher	Vetgen
	Irish Red & White Setter	AHT
	Kerry Blue Terrier	Finnzymes, Vetgen
	Manchester Terrier	Finnzymes, Vetgen
	Pembroke Welsh Corgi	Finnzymes, Vetgen
	Papillon	Finnzymes, Vetgen
	Poodles (all varieties)	Finnzymes, Vetgen
Shetland Sheepdog	Vetgen	
Scottish Terrier	Vetgen	

Website URLs for details of tests:

AHT: <http://www.aht.org.uk/>

DDC: <http://www.vetdnacenter.com/canine-disease-test.html>

Finnzymes: http://diagnostics.finnzymes.fi/index.php?lang=en&page=canine_inherited_disease

GT: <http://www.gtg.com.au/AnimalDNATesting/index.asp?menuid=080.150.010>

Healthgene: http://www.healthgene.com/canine/genetic_dna_testing.asp

Optigen: <http://www.optigen.com/>

PennGen: <http://w3.vet.upenn.edu/research/centers/penngen/services/alldiseases.html>

Vetgen: <http://www.vetgen.com/canine-services.html>

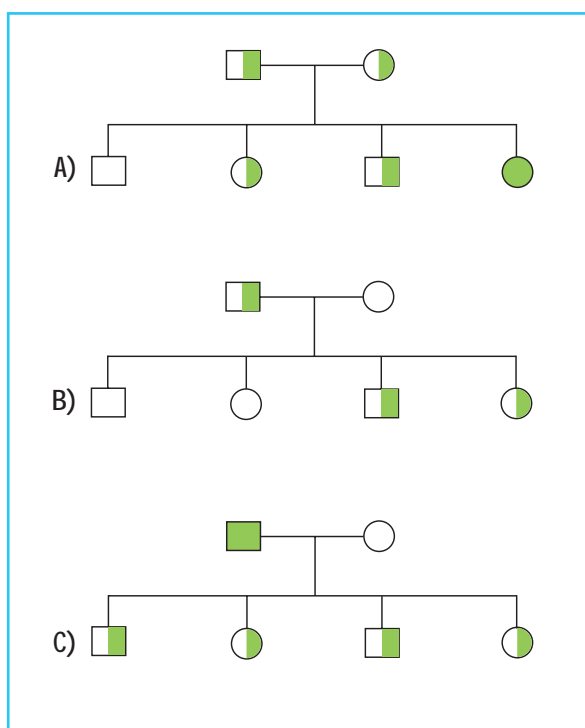
WSUCVM: <http://www.vetmed.wsu.edu/depts-VCPL/>

The main goal is to avoid mating two carrier individuals together, as this will produce affected pups (**Figure 1A**). If a breeder has a carrier that they would like to breed from, they should select a tested clear individual to mate with, as this will produce on average 50% carriers and 50% genetically clear pups (**Figure 1B**). Importantly, no affected pups are produced, and by testing the litter the breeder can select dogs matching their selection criteria that are now free of the disease. If the litter does not contain such individuals,

another round of carrier to tested clear matings can be undertaken. For some conditions it might even be realistic to breed from an affected dog, by again selecting a mate which has been tested clear. In this case all the offspring will be carriers (**Figure 1C**). These carrier pups can then be treated in the same way as the carriers above.

One option is to remove all carriers from the breeding population, and when the frequency of carriers in the breed is low (below < 5%) this may be

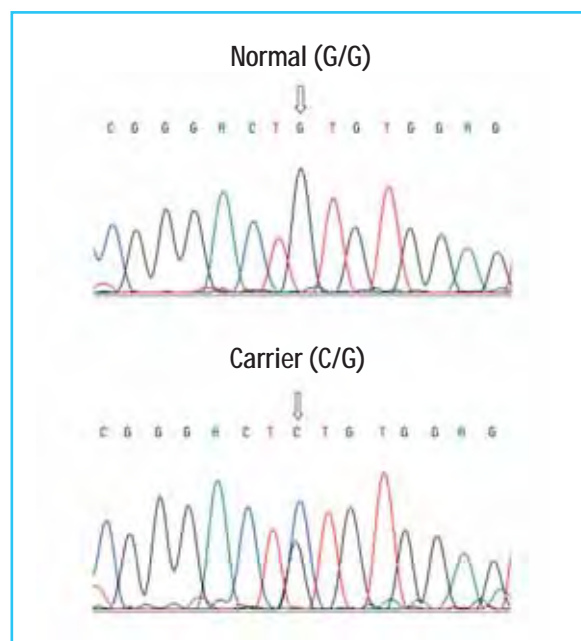
Figure 1. Selective breeding using the results of genetic testing for a recessive disease. **A** shows the situation when two carriers (half filled circles) are mated. On average half their offspring will be carriers, a quarter genetically clear (open circle) and a quarter will be affected (filled circle). In **B** a carrier has been mated to a tested clear individual. In this case, no affected dogs are produced, with half the litter being carriers and half the litter being genetically clear. Litter testing will identify which pups are genetically clear and thus potential breeding stock. **C** represents the situation when an affected dog has been mated to a tested clear individual. Again, no affected dogs are produced, with the entire litter being carriers for the disease mutation. These carriers can then be mated to a tested clear individual as in **B**.



the best option. However, in situations where there are a significant proportion of carriers in the breed, the overall health situation may be exacerbated by removing all the carriers from the breeding population as it would constrict the gene pool for the future. In effect, it might eradicate the disease being tested for, but increase the proportion of the breed carrying other deleterious mutations. When the initial carrier frequency is high, the slow removal of the disease mutation over many generations will maintain more genetic diversity in the breed.

An example of a successful genetic screening programme in the UK is that in Irish Setters for the autosomal recessive disease, canine leukocyte deficiency (CLAD). CLAD causes a failure of the dog's immune system that within months is fatal. Following the discovery of the mutation responsible for the disease (6) (see **Figure 2** for an example of a

Figure 2. DNA sequence based test for CLAD in Irish Setters. The arrows highlight the single nucleotide change causing this lethal disease. In normal individuals, both copies of their CD18 gene contain a G (black peak) nucleotide, whilst in carrier individuals, both C (blue) and G (black) nucleotides are present. The assay provides a clear identification of carrier individuals.



test result), the UK Kennel Club and the Irish Setter Breed Associations established a protocol whereby a five year period during which carrier animals could continue to be bred and registered was established. That period ended in July 2005, such that the only dogs that can be registered are tested clear of CLAD, or are the progeny of CLAD-clear tested parents. In this way, Irish Setter breeders have eliminated this lethal inherited condition from their breed, whilst at the same time maintaining genetic diversity. One precondition of such a scheme is that an open register of test results is maintained and available to breeders. In this case, in addition to maintaining a web-based database of CLAD test results, the UK Kennel Club added CLAD results to the dog's registration record and published them in the breed record supplement. Results are also placed on the registration certificates of their progeny to indicate that they are hereditarily clear of CLAD.

It is likely that many more such schemes will be introduced in the future leading to major improvements in the health and welfare of pure and mixed breed dogs through the elimination of harmful genetic diseases, by selective breeding.

■ Identifying the component breeds of mixed breed dogs

The formation of pure dog breeds from small numbers of founders, followed by intense inbreeding, has created a situation where there is enormous phenotypic diversity *between* breeds, but high levels of genetic similarity *within* breeds. Using the SNPs initially identified during the sequencing of the dog genome, scientists from the WALTHAM Centre for Pet Nutrition selected about 1500 SNPs spanning all 38 autosomes with which to test whether they could identify breed-specific genetic signatures for 120 of the most popular dog breeds (Paul Jones, personal communication). Samples were taken in both the UK and the USA and interestingly the results showed that some breeds, such as Irish Terriers and Gordon Setters, were genetically distinct based on their country of origin. In contrast, for other breeds such as German Shepherds and Bearded Collies, there was little difference between UK and USA derived samples. The results showed that there were clear patterns of particular SNPs within different pure breeds

that could be used to assign unknown samples to a breed with high reliability. The scientists then went on to look at a group of mixed breed dogs to see if they could identify which breeds they were derived from. In the majority of cases this was possible, and a sophisticated commercial test, Wisdom MX Panel, (using a further refined set of SNPs) that can do this will be available from Mars Veterinary later this year (<http://www.marsveterinary.com/>). The Wisdom MX test will enable owners of mixed breed dogs to learn more about the origins of their pets. In the test, pure breeds contributing more than 12.5% to the genetic constitution of the mixed breed dog are profiled in the results. These results will help owner's understanding of their dogs' physical attributes and provide insight about breed-driven dietary, exercise and training needs. Importantly, the analysis of mixed breed dogs also provides a powerful research strategy to map traits and diseases through the identification of SNPs shared by mixed breed individuals with the similar physical characteristics or disease.

REFERENCES

- Wayne RK. Molecular evolution of the dog family. *Trends in Genetics* 1993; **9**: 218-224.
- Neff MW, Rine J. A fetching model organism. *Cell* 2006; **124**: 229-231.
- Langford CF, Fischer PE, Binns MM, et al. Chromosome-specific paints from a high-resolution flow karyotype of the dog. *Chromosome Research* 1996; **4**: 115-123.
- Neff MW, Broman KW, Mellersh CS, et al. A second-generation genetic linkage map of the domestic dog, *Canis familiaris*. *Genetics* 1999; **151**: 803-820.
- Lindblad-Toh K, Wade CM, Mikkelsen TS, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 2005; **438**: 803-819.
- Kijas J, Bauer Jr TR, Gavvert S, et al. A missense mutation in the β -2 integrin gene (ITGB2) causes Canine Leukocyte Adhesion Deficiency. *Genomics* 1999; **61**: 101-107.