

# Feature

## INHERITED DISEASE

# DNA testing Man's best friend

Since the complete canine genome was sequenced in 2004, the number of DNA tests available to detect different mutations in a dog's genetic makeup has increased rapidly. But, what is DNA testing, why do it and what do the results mean? Cathryn Mellersh of the Animal Health Trust explains.

IN December 2004, a US \$30 million project funded by the National Human Genome Research Institute (NHGRI) in the USA to sequence the entire dog genome was completed and the results were made publicly available. The NHGRI made the decision to fund such an expensive project because it recognised the dog as an unrivalled model organism with which to study the genetics of inherited disease.

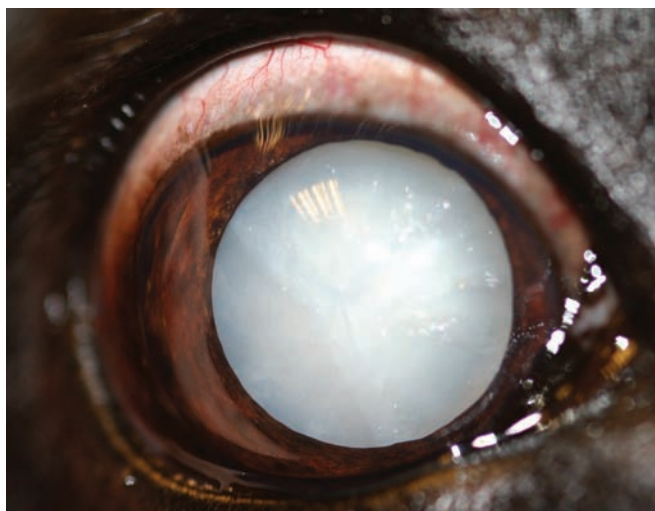
Although the NHGRI's motives for sequencing all the DNA in the dog were primarily human-centric, the findings that emerged six years ago have had profound implications for both veterinary and human medical research. Most importantly, the pace at which genetic mutations responsible for inherited canine diseases have been discovered has increased dramatically and will continue to do so, as the tools available to dissect the genetic basis of canine inherited traits become increasingly sophisticated.

Currently, DNA tests are available for close to 100 different canine mutations, and over 120 breeds are able to take advantage of at least one DNA test. As pressure to improve the health of purebred dogs continues to intensify, dog breeders will come under increasing pressure to make full use of all the DNA tests available to them, and many will turn to their vets for advice and information regarding the benefits as well as the limitations of specific DNA tests.

### DNA tests

The development of a DNA test for any inherited disease always starts as a research project which, very simply, compares DNA from affected and unaffected dogs to locate and identify the mutation responsible for the condition under investigation. Once a mutation has been identified, a DNA test can be developed and offered to the public.

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Inherited cataract in a labrador

Worldwide, there are now many facilities offering canine DNA tests. The process of DNA testing involves the submission of a sample of a dog's DNA to an appropriate testing laboratory. The DNA can often be submitted as a simple cheek swab that an owner can take themselves, although some tests/laboratories may require a blood sample. The testing laboratory analyses the DNA for the presence or absence of the relevant mutation and will report back, usually within a few weeks, with the result (the dog's 'genotype'). The results will inform the owner whether the dog being tested has zero, one or two copies of the mutation being tested for.

### What do DNA test results mean?

It is worth considering what the results of a DNA test mean to the owner. Clinically similar conditions can be caused by different mutations. For example, forms of progressive retinal atrophy (PRA) are known to affect many different breeds; currently around a dozen different mutations have been identified that cause PRA in specific breeds, whereas the causal mutation for many more breeds has yet to be identified.

Although clinically affected dogs of the same breed will usually share the same causal mutation, it is also possible for genetically

distinct forms of the same disease to exist within the same breed. It is important for owners and vets to appreciate that a DNA test only assays for a single, specific mutation, and not for any other mutations that cause clinically similar conditions. For example, mutations in the gene *HSF4* have been associated with hereditary cataract (HC) in the Staffordshire bull terrier, the French bulldog, the Boston terrier and the Australian shepherd dog, and DNA tests are currently offered to these four breeds ([www.aht.org.uk](http://www.aht.org.uk)) (Mellersh and others 2006, 2007, 2009). HC is known to affect many more breeds but *HSF4* involvement has been excluded in most, meaning different mutations, as yet unidentified, are responsible for the condition in these other breeds.

A clear DNA test result is not, therefore, an absolute guarantee that a dog will never develop a clinically similar disease to that being tested for, although dogs that are clear of specific mutations can be considered at very low risk of developing disease. Because many clinically similar inherited ocular conditions are known to have different genetic causes, a DNA test cannot, and should not, replace a clinical eye examination that has the ability to diagnose multiple defects and also detect newly emerging conditions.

Most of the DNA tests currently available are for mutations responsible for 'simple' or single gene diseases. This means that the disease is a result of a single mutation; no other genes or environmental factors are involved. For these diseases, the results of DNA tests are easy to interpret and an individual dog's risk of developing the condition can be estimated with a very high level of certainty from the DNA test results.

Many simple inherited conditions have a recessive mode of inheritance. Recessive diseases are the result of mutations that cause the loss of function of a biologically important gene, as opposed to dominant conditions, which usually result from mutations that cause an inappropriate gain of function of a gene. Every dog has two copies of each gene, one inherited from the dam and one from the sire, and carriers that have inherited a single copy of the normal gene from one parent and a single copy of a mutant gene from the other parent usually have sufficient functional protein to remain clinically healthy. It is only when a dog inherits a faulty gene from both parents that it becomes clinically affected.

Consequently, if a mutation is recessive, then dogs with no or one copy of the mutation will remain clinically free of the disease, although heterozygous carriers will pass the mutation onto half of their offspring. Dogs with two copies of the mutation (homozygotes) will almost certainly develop the disease during their lifetime, although they might be clinically clear at the time of testing.

If the mutation is dominant, dogs with one or two copies of the mutation will develop the condition, unless there is evidence of incomplete penetrance (meaning that not all the dogs with the mutation will develop the disease), whereas dogs that are clear of the mutation will remain healthy.

Examples of single gene DNA tests that are available include those for L-2-hydroxyglutaric aciduria and HC in the Staffordshire bull terrier, canine leukocyte adhesion deficiency in the Irish and Irish red

and white setters, and progressive rod-cone degeneration in multiple breeds.

Some diseases are more complex, and result from mutations in multiple genes or the interaction between genes and the environment. Individual mutations might increase a dog's risk of developing the associated condition, but cannot predict with certainty whether a dog will become

*'If used wisely, DNA tests can be very powerful tools with which to control and eliminate inherited disease and also to aid disease diagnosis'*

clinically affected. One such example is the DNA test for a mutation associated with hyperuricosuria (HUU) in the Russian black terrier, the bulldog, the large Munsterlander and the dalmatian. The mutation increases an individual dog's risk of developing urinary calculi (stones), which may then require surgery, although some dogs that carry two copies of the mutation remain clinically free of the condition. It is suspected that additional mutations and/or environmental factors exist that modify the effects of the HUU mutation and explain why some dogs remain healthy (Bannasch and others 2008).

### Why DNA test?

DNA tests can play a critically important role in the control and eventual elimination of inherited diseases. Recessive diseases are notoriously difficult for the dog breeder to eliminate, because of the existence of clinically healthy carriers within the population that can only be detected retrospectively, once they have produced affected offspring or one of their parents has been diagnosed as affected. The problem is confounded for late-onset conditions where affected animals may be innocently bred with before they are themselves diagnosed, and this problem is applicable to dominant as well as recessive diseases.

The availability of a DNA test is often the only way in which a recessive condition or a late-onset dominant condition can be reliably eliminated from a breed. Breeders should have their breeding stock tested before mating and make sensible breeding choices, based on the genotype of their dog, that minimise the risk of producing affected offspring.

Disease mutations can be very common within specific breeds

and once a DNA test becomes available, the instinct of many breeders is to breed only from clear dogs. This practice will obviously eliminate the disease mutation from the breed very rapidly, but may do so at the expense of genetic diversity if large numbers of dogs are instantly removed from the gene pool. High levels of inbreeding and loss of genetic variation are well documented to have detrimental effects on the health and fertility of animals. For common recessive mutations it is therefore advisable for breeders to continue breeding with carriers, at least for the first generation following DNA test development. Provided all carriers are paired with DNA-tested, clear mates, only clear and carrier puppies will be born; no clinically affected dogs will be produced and breeders can select a clear dog to breed on from the resulting litters. Table 1 details the outcomes of mating dogs with different genotypes with respect to a recessive mutation, and whether they can result in clinically affected offspring.

For dominant mutations the situation is different. All offspring that inherit a disease-associated dominant mutation will develop clinical signs at some stage during their lives so breeding with animals that carry such mutations is harder to justify.

In addition to being very important tools with which to control and eliminate inherited diseases, DNA tests can also help the clinician. As discussed above, clinically similar conditions can be caused by different genetic mutations and conditions with different aetiologies can have clinical signs in common. DNA tests can thus aid differential diagnosis in such cases or help to distinguish between inherited and non-inherited forms of a condition, such as cataracts.

### Non-disease-associated DNA tests

Although DNA tests for disease mutations will be of primary interest to the clinician, there is a steadily growing list of DNA tests available for 'cosmetic' traits such as coat colour and length. Although of limited health benefit, such tests might be usefully used to avoid the production of 'undesirable' pups that unscrupulous breeders might euthanase on cosmetic grounds. In addition, the bobtail DNA test can be used to prove that dogs of particular breeds have a naturally occurring, rather than docked, short tail.

There is also an increasing number of DNA tests available that attempt to determine the breed 'composition' of mixed-breed dogs. Owners should be aware that these tests will rarely determine a dog's precise ancestry; rather, they will identify breeds that have made 'major', 'intermediate' and 'minor' contributions to their pet's genetic makeup. Companies that market this type of test suggest the benefits

TABLE 1: Outcome of mating dogs of different genotypes with respect to a recessive mutation

Combination of dogs	Outcome	Possibility of clinically affected offspring?
Clear x clear	All puppies will be clear	No
Clear x carrier	50 per cent of puppies will be clear 50 per cent of puppies will be carriers	No
Clear x affected	All puppies will be carriers	No
Carrier x carrier	25 per cent of puppies will be clear 25 per cent of puppies will be affected 50 per cent of puppies will be carriers	Yes
Carrier x affected	50 per cent of puppies will be affected 50 per cent of puppies will be carriers	Yes
Affected x affected	All puppies will be affected	Yes

of knowing a mixed-breed dog's makeup include the ability to provide suitable behavioural training and meet breed-specific nutritional needs.

### How accurate are DNA tests?

All laboratories offering DNA tests should provide clear and detailed information about the test. They should make it clear whether the DNA test is a mutation-based or a linkage test, and if it is a linkage test what the estimated error rate is. As described above, mutation-based tests examine a dog's DNA for the presence or absence of the precise mutation that causes a particular disease. The vast majority of current DNA tests are mutation-based tests and, within the limits of human error, a well-designed mutation-based test is 100 per cent accurate. Linkage-based tests, in contrast, do not detect the disease-causing mutation itself, but instead analyse DNA markers that are known to be located very close to the mutation. Linkage-based tests can be inaccurate in a small percentage of dogs tested, because of the potential for genetic recombination to occur between the mutation and the markers being analysed.

The DNA testing facility should also explain whether the mutation being tested for is recessive or dominant, and the associated risks to dogs with different genotypes of developing clinical disease. If the mutation is incompletely penetrant, then that should be documented. The laboratory should also give details of any genetically distinct forms of the same disease that are known to exist within the breed so owners can appreciate their dog's risk of developing a disease even if it receives a clear DNA test result. It is also useful if the service provider can communicate the frequency of a specific mutation within a breed as a whole or at least from a specific geographic location, so that owners and breeders can use their judgement regarding the need to test their dogs. One way in which the validity of a DNA test can be judged is its publication in a peer-reviewed journal and it would seem prudent for owners and vets to remain sceptical about tests that remain unpublished 12 months after their launch dates.

### Powerful tools

Rapid advances in current technology mean that the number of DNA tests available for

use in the dog will steadily increase in the coming years. If used wisely, DNA tests can be very powerful tools with which to control and eliminate inherited disease and also to aid disease diagnosis. As the media continues to focus attention on the health of purebred dogs, it should be the responsibility of anyone who breeds, cares for or treats dogs to understand the benefits and uses, as well as the limitations, of DNA tests.

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