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Personal View

DNA testing man's best friend: Roles and responsibilities

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Background

Dogs and humans share a unique relationship. Over the last 20,000 years or so man has, literally, shaped the evolution of the domestic dog that now enriches the lives of millions of people the world over. I believe we owe the dog special responsibilities, not only to meet its material and behavioural needs but also to ensure the genetic health of the diverse range of breeds we have created. In June 2015 I was very honoured to be the co-winner of the Kennel Club Charitable Trust's (KCCT) International Canine Health Award. I received the KCCT award for the work my research team and I have done to develop DNA tests for a variety of inherited canine disorders. This article is my personal view on the role DNA testing has to play in safeguarding the genetic health of our purebred dogs and also the responsibilities various stakeholders have, both now and into the future, and as technology changes the way in which DNA tests are developed.

It is 26 years since the mutation responsible for haemophilia B was identified in a research colony of dogs, providing the first example of a canine Mendelian disorder to be characterised at the DNA level (Evans et al., 1989). Since that landmark publication the key mutations responsible for close to 200 Mendelian (single-gene) canine disorders have been characterised[1] and many of these mutations form the basis of DNA tests that are offered commercially to dog breeders and veterinarians by a small but steadily growing number of laboratories around the world.

The role of the DNA test

DNA testing has an undisputed role to play in the control and elimination of recessive inherited disorders in the dog. Many recessive diseases are debilitating and are very difficult to treat effectively. They are also extremely difficult to eliminate completely from a breed in the absence of a DNA test because clinically healthy carriers, which can only be detected retrospectively after they have produced affected offspring, act as a reservoir for the disease mutation in the population. Inbreeding and the extensive use of popular sires can result in specific disease mutations becoming very common within a breed. However, once the disease-mutation has been identified, widespread DNA testing used in parallel with a sensible breeding strategy can enable breeders to reduce the frequency of the mutation while preserving the genetic diversity of the breed. Providing advice to breeders once a DNA test is available is generally very straightforward; essentially all dogs can be safely bred, regardless of their genotype, provided both the sire and the dam have been tested and carriers and genetically affected dogs are only mated to dogs that are clear of the mutation.

An additional (although sometimes overlooked) role of the DNA test is also to assist the veterinarian with the differential diagnosis of disorders that share clinical signs (Mellersh, 2013), meaning that DNA tests can facilitate the appropriate treatment of affected dogs as well as play an important role in improving the genetic health of future generations of dogs.

DNA test development: Past, present and future

Ten years ago, the process of identifying the causal mutation responsible for an inherited disorder in a particular breed of dog was

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[1] See: http://omia.angis.org.au/home (accessed 25 August 2015).

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a lengthy and expensive one. DNA had to be collected from affected and unaffected dogs and then, typically, candidate genes were investigated, one by one, until the culprit was identified. The sequencing of the canine genome in 2004, followed by the development of SNP (single nucleotide polymorphisms) chips, which enabled genome-wide investigations, expedited the process considerably and the evolution of DNA sequencing techniques over the last 3 or 4 years has continued to reduce both the time and the cost associated with mutation identification and DNA test development. The collection of DNA from affected animals is still an absolute requirement for mutation identification, but the number of samples required has also reduced considerably, with nucleic acid (DNA or RNA) from a single affected animal occasionally being all that is required to find a disease mutation using whole-genome or whole-transcriptome sequencing (Forman et al., 2012; Gilliam et al., 2014).

These advances in technology, and the parallel reduction in the time and cost required to develop a DNA test for a breed-specific inherited disorder, are changing the way in which DNA tests are developed. When it was necessary to collect DNA from dozens of affected animals to enable the hunt for a disease-mutation to begin, and tens of thousands of pounds to pay for the research, success stories made the headlines and were rarely achieved without the extensive involvement of the relevant breed club(s) that typically helped with both the recruitment of DNA samples from affected dogs and the fundraising. It was not unusual for the development of a DNA test to take 10 years, or even longer, by which time the mutation and the disease may have become widespread within the breed.

A prime example is the development of the DNA test for primary lens luxation (PLL), a potentially blinding eye disorder that has been recognised as a familial trait in multiple breeds for over 80 years (Gray, 1932). Identification of the causal mutation, which was finally reported in the scientific literature in 2010, marked the culmination of over a decade of work, undertaken by research teams from both sides of the Atlantic, that involved the collection and analysis of DNA from hundreds of affected animals at an estimated cost of £100,000[2] (Sargan et al., 2007; Farias et al., 2010; Gould et al., 2011). By the time a DNA test was made available the mutation had become extremely common in some breeds, such as the Miniature Bull terrier where over 40% of dogs were carriers (N. Holmes, personal communication). The development of a DNA test for PLL was widely anticipated and embraced enthusiastically by the relevant breed stakeholders, almost certainly as a result of the high prevalence of the disease and because such large numbers of breeders and owners had personally witnessed the effects of this particular inherited disorder, which all too frequently leads to dogs needing to have their eyes removed on welfare grounds.

In the 12 months following the launch of the DNA test for PLL, the Animal Health Trust (AHT) alone commercially tested 6935 dogs, a number that has risen to 13,350 in the 6 years that the test has been available. Of these dogs, 381 are homozygous for the mutation and will invariably develop PLL during their lives. However, knowing their dogs will develop the condition gives owners the opportunity to monitor their dog's eyes and deliver prophylactic treatment at the earliest sign of the condition. In addition to the affected dogs, 4462 have been identified as carriers. Knowing the genotype of these dogs means they can all still be safely bred providing they are mated to dogs that are clear of the mutation, thus potentially saving the birth of many dogs that would have been destined to develop PLL had the DNA test not been available.

Advances in technology are, however, changing the landscape of DNA test development. Massively parallel (also called next-generation) sequencing technologies are enabling scientists to sequence DNA far quicker and far more cheaply than ever before, with the sequencing of entire genomes becoming readily available to even modestly sized laboratories at a cost of a few thousand pounds per dog. The result is that mutations for recessive inherited diseases can now be identified relatively cheaply, using DNA from a very small number of affected dogs. This, in turn, means that DNA tests are being developed for inherited conditions much earlier in the emergence process of the disease; in other words, much sooner after the disease mutation arose, and before the mutation (and thus the disease) has had a chance to become very widespread. To the casual observer this might sound like progress, but it means that breeders and even veterinarians are being given the opportunity to test their dogs/patients for diseases of which they are not even aware.

Two such examples are the DNA tests that the AHT has recently made available for macular corneal dystrophy (MCD) in Labrador Retrievers and primary open angle glaucoma (POAG) in Basset Hounds. Both these mutations were identified as the result of research carried out by ophthalmologists at the AHT, using samples collected with owner permission from cases they examined themselves. The respective research projects aimed at identifying the underlying mutations were funded by the AHT; both were completed successfully and very quickly and resulted in the development of DNA tests for both conditions. Both MCD and POAG are emerging conditions in their respective breeds and so are both, thankfully, relatively rare at the current time.

The availability of the DNA tests means that these conditions, both of which cause significant visual impairment and discomfort, can be stopped in their tracks, before any more dogs inherit the mutations and develop disease. It is perplexing, therefore, that only a handful of breeders have elected to test their dogs for either of these mutations, despite widespread publicity surrounding the new tests. The poor uptake is presumably because breeders are generally not aware of the respective diseases, probably have not heard personally of any affected dogs, and do not therefore consider the tests necessary or value for money.

The availability of DNA tests for very rare mutations, albeit ones that cause very debilitating conditions, raises important questions about how far breeders should be expected to travel along the health testing route to safeguard the genetic health of the dogs they breed. Are the scientists guilty of presenting breeders with an ever-increasing list of DNA tests that they are put under increasing pressure to use even though the associated diseases are very rare? Or should dog breeders be encouraged to use every available DNA test to minimise their risk of producing dogs that might develop an inherited disorder? DNA tests are all about prevention and we all incorporate risk-prevention into our own lives and the lives of our pets, even when there is a financial cost.

Most of us choose to insure our houses, cars, pets and even our washing machines. We also pay for our pets to be vaccinated. We are encouraged to adopt healthy lifestyles for both ourselves and our pets, to reduce the risk of developing certain health conditions, and we wear our seat belts when we drive to protect against injury in the event of an accident. So risk-aversion is not an alien concept by any means. So why do some dog breeders sometimes seem reluctant to pay for a DNA test when doing so would ensure the puppies they breed would be free from inherited disease? Most DNA tests cost little more than the cost of a bag of quality dog food, and they only need to be carried out once during the lifetime of the dog. In my opinion the responsibility for appropriate uptake of DNA testing lies with several stakeholders.

I believe the scientists who discover the mutations and develop the DNA tests have a responsibility to liaise and communicate with relevant breed clubs following the development of a new DNA test and also to work with the breed to estimate the true frequency of the mutation within the breed and provide customised breeding

[2] £1 = approx. €1.36, US$1.56 at 25 August 2015.

advice. I also believe veterinarians have a responsibility to familiarise themselves with the DNA testing process and to be able to offer accurate and informed breeding advice based on DNA testing results. And finally I believe dog breeders have a responsibility to engage with DNA testing, be willing to co-operate with scientists to help estimate the true frequency of the mutation within the breed and to disseminate information about new DNA tests to exhibitors and owners in an objective manner.

DNA tests for complex diseases

All of the discussion above refers to DNA tests for Mendelian (single-gene) diseases for which a single mutation accounts for virtually all disease risk. Genetically complex conditions that result from mutations in multiple genes or the interaction between genes and the environment represent a vastly different scenario from genetically simple traits. DNA sequence variants are now being identified that increase an individual dog's risk of developing an associated condition but do not predict with absolute certainty whether the dog will become clinically affected. These variants are known as risk factors.

Unravelling associations between specific genes and complex conditions is obviously important, will make profound contributions towards our understanding of gene function and disease aetiology and should therefore be reported in the scientific literature at the earliest opportunity. But they present the scientist and the DNA test provider with a dilemma when it comes to deciding whether to make a DNA test available based on risk factors. Even when a mutation/variant is associated with disease, and reducing the frequency of that mutation within a breed would almost certainly lead to a reduction in the prevalence of the disease, we need to keep in mind that dogs are not cows. By this I mean they don't live and reproduce as a herd; rather they live in very small groups with individual breeders being responsible for the production of small numbers. Breeders tend to put very intense selective pressure on disease-associated mutations and generally choose not to breed with dogs that are known to carry a disease mutation, effectively reducing the frequency of the mutation within the breed quite swiftly. This is in contrast to the gradual and measured reduction that could be achieved if a small number of breeders were responsible for the entire breed/herd.

In reality there is a need for dog breeders to balance the desire to breed for genetic health by eliminating disease-associated mutations from their breed with the need to maintain genetic diversity. It is, in my opinion, irresponsible for DNA test providers to offer tests for isolated mutations that are only weakly associated with disease. These mutations, which may be very common within a breed, are likely to be minor modifiers of more major risk factors and eliminating them may reduce genetic diversity without dramatically reducing the prevalence of disease.

DNA tests should be restricted to those based on mutations that increase a dog's disease risk substantially, and certainly should not be offered for mutations that are very common within a breed unless they account for virtually all disease risk. Any DNA test that is offered for a complex disease needs to be accompanied by comprehensive and customised breeding advice, specific to the mutation and the breed. As risk factors become increasingly easy to identify, DNA test providers have a firm obligation to only offer DNA tests when it is truly in the relevant breed's bests interests; they must not allow themselves to bend to pressure from either the breed clubs that may have supported their research over the years or to the financial controllers who may have paid for it.

Breeding dogs cannot be divorced from responsibility, both to the individual dogs that are born or to the people who will take those dogs into their homes and love them for all their lives. The health of an individual dog can never be guaranteed, but there are many steps that can be taken to maximise the chance of breeding a healthy dog. DNA testing is one such step and scientists, DNA test providers, veterinarians and dog breeders must all their play their own role in ensuring full and effective use of this particular tool.

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