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LANGFORD AND FORT DODGE ANIMAL HEALTH - WORKING TOGETHER FOR THE BENEFIT OF CATS

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Genetic Tests for Cats: What the Practitioner Needs to Know

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Figure 1: A Persian cat having an ultrasound scan to look for renal cysts seen in polycystic kidney disease (PKD).

Introduction to Genetic Tests for Cats

There have been major advances in the ability to identify genetic mutations in recent years and this has led to ready availability of a number of genetic tests for cats. This article will provide some background information on genetic tests as well as guiding the practitioner on how specific tests can be used in practice.

The first specific gene mutations for feline inherited disease were identified in the first half of the 1990s for a form of muscular dystrophy and some forms of storage diseases, which were also well-known inborn errors of metabolism in humans. At this time the use of a candidate gene approach was the most realistic technique for identifying gene mutations in cats. This technique was based on looking for comparable mutations that had been identified as the underlying cause of similar genetic diseases in other species, often man; as human and cat gene sequences are over 70% identical. Some preliminary characterisation of feline chromosomes had been established at this stage using techniques such as chromosome banding and painting. Chromosome studies facilitated the location of particular genes in the cat genome. Linkage maps subsequently became available, which are based on microsatellite markers and large families of cats. This work was facilitated by genomic studies of hybrid cats, such as the Bengal, by differentiating genes derived from domestic cats and the Asian Leopard. Family-based linkage analyses have enabled the mapping of some genetic defects of cats such as spinal atrophy.

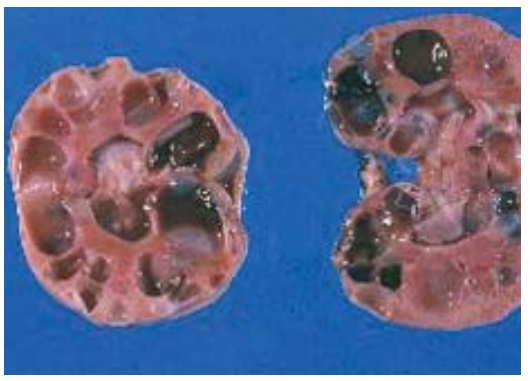

Figure 2: Kidneys from a Persian cat affected with PKD.

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More recently a rudimentary genomic sequence has been published and was developed from in an inbred Abyssinian cat called Cinnamon. Cinnamon came from a colony of cats that had been inbred as part of a study to identify the genetic basis of a form of progressive retinal atrophy (PRA) seen in Abyssinians. The genomic sequence provides a much more powerful tool for identifying genetic mutations in cats and has led to the availability of a number of new genetic tests for cats in recent years. The genetic sequence allows the identification of a large number (several hundred thousand) of single nucleotide polymorphisms (SNPs), that facilitate the identification of genetic traits. These resources are likely to lead to a further massive explosion in our knowledge of genetic diseases and availability of practical tools to deal with these in the coming years as outlined later in the article.

Are these tests of any relevance to our non-pedigree cats?

Inherited genetic diseases are inevitably more commonly encountered in pedigree animals due to selective breeding leading to some degree of inbreeding. However, a number of genetic diseases have been first identified in non-pedigree cats; such as some of the storage diseases. Genetic mutations are often more easily identified in a group of closely related cats of known ancestry, as in pedigree breeds,

and some genetic tests have been developed using pedigree cats. However, these tests may also prove of value in diagnosing some genetic mutations of non-pedigree cats.

Some of the more common and important genetic mutations that have been identified in recent years are based on autosomal dominant genes e.g. polycystic kidney disease (PKD) seen primarily in Persian cats *(figure 1 and 2)* and related breeds and familial hypertrophic cardiomyopathy (HCM), now characterised in Maine Coon *(figure 3 and 4)* and Ragdoll cats. These mutations appear to be prevalent in the high-risk breeds, present in up to 30-40% of individuals.

Figure 3: A Maine Coon cat, this breed, along with Ragdoll cats, is affected by hypertrophic cardiomyopathy (HCM) and a genetic test for the causal mutation is now available

Since these genetic disorders are based on autosomal dominant mutations, affected cats can arise from the first crossing of a pedigree cat carrying the gene with a non-pedigree. As a substantial number of mixed-breed pet cats come from such crossings the genetic tests can be of value in helping the practitioner to diagnose these genetic defects in non-pedigree as well as pedigree cats.

Whilst these genetic tests may be of value in diagnosing genetic diseases, the main indication for their use is to the breeder in avoiding genetic diseases by screening potential breeding cats which may be carrying the undesirable mutation.

What genetic tests are available for cats?

There is now a substantial list of genetic tests available. These tests fall into two main groups – tests for genetic diseases *(table 1)* and tests for genes which confer specific phenotypes i.e. coat colour *(table 2)*.

Table 1 shows the tests for genetic diseases currently available. Once a genetic mutation has been published, other laboratories are able to use this information to develop tests (although there may be differences in the methods used to detect the mutation) subject to any patent considerations which may vary from one country to another. Thus tests may soon become available through a range of laboratories. The leading laboratory for cat genetic tests is the Veterinary Genetics Laboratory (VGL) at the University of California, Davis (UC Davis) (http://www.vgl.ucdavis.edu/). The Diagnostic Laboratories at Langford Veterinary Services, Langford (http://www.langfordvets.co.uk/diagnostic_laboratories.htm) have a long tradition of specialising in diagnostic tests for cats and has been working closely with other researchers working in this area. These collaborations have led to the availability of a number of genetic tests for cats via the Diagnostic Laboratories *(see table 1)* and we are the leading laboratory in this field in the UK.

What samples are required for genetic tests?

Originally most genetic tests required a blood sample, however, as techniques for extraction of DNA from samples have improved and the tests have become more sensitive, buccal swabs are acceptable for most tests. The procedure for collecting samples is simple (see http://www.langfordvets.co.uk/lab_pkdsampling.htm for a video of the technique). Standard swabs including cotton buds/Q tips are generally suitable. Swabs should be packaged to ensure that no cross contamination can occur e.g. a plastic zip-lock bag or clean, unused envelope or by replacing the swab in the plastic sheath.

What other tests are available?

The UC Davis VGL offers a wide range of tests for different coat colour gene mutations and other related tests as shown in table 2. These are often used by breeders to identify if their cats are carrying certain coat colours to decide suitable matings. Another genetic test which breeders make use of is a test for blood group mutations. Practitioners will be familiar with the relevance of blood groups in cats and their importance in transfusions. It is recommended

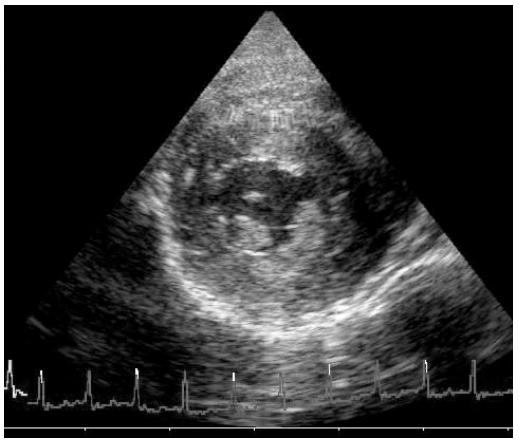

Figure 4: An echocardiographic image of the left ventricle of a Maine Coon cat with HCM illustrating the ventricular wall thickening seen in this disease

that both donor and recipient cats are blood typed prior to transfusion to avoid incompatibility which can lead to potentially fatal transfusion reactions. Testing cards are available to enable the blood group to be determined easily in practice, or samples can be submitted to a diagnostic laboratory. Awareness of blood groups is also important to avoid neonatal isoerythrolysis (NI). NI can occur when a group B queen produces group A kittens (if mated to a group A tom) resulting in a haemolytic crisis in the kittens that absorb anti-A antibodies from their mother's colostrum, often leading to death in the first few days of life. This is a particular concern to pedigree breeders who have breeds with a relatively high

Genetic Disease	Affected Breeds
Polycystic kidney disease*	Persians, British Shorthair, Exotic Shorthair, Himalayan, Scottish fold
Hypertrophic cardiomyopathy*	Maine Coon and Ragdoll
Pyruvate kinase deficiency*	Abyssinian, Somali
Storage diseases	
Gangliosidosis 1	Korat, Siamese
Gangliosidosis 2	Burmese
Gangliosidosis 2	Korat
Glycogen storage disease IV	Norwegian Forest
Progresive retinal atrophy	Abyssinian, Somali, Ocicat
Spinal muscular atrophy	Maine Coon

Table 1: Genetic disease mutation tests available for cats *= available at The Diagnostic Laboratories, Langford Veterinary Services.

Characteristic	Breeds Affected
Agouti	All breeds
Amber	Norwegian Forest
Brown	All breeds
Dilution	All breeds
Colour - Burmese colour pattern, Siamese colour pattern, Full Albino	All breeds
Long fur	All breeds
AB blood group	As indicated

Table 2: Genetic tests available for coat colour and other characteristics.

proportion of group B individuals, notably British Shorthair, Birmans and Rexs. Breeders can use blood grouping of breeding cats to avoid matings that could potentially lead to isoerythrolysis or to take preventative action if necessary. Use of the genetic test has the advantage of detecting not only group B cats but also cats that are carrying the B gene. The predominant blood group A, and the relatively rare AB are dominant to B so mating two type A cats that carry the B gene could result in type B kittens. The genetic test will not differentiate between group A and AB individuals.

Can owners submit samples?

This question has posed a dilemma for our diagnostic laboratory. In the past we have accepted only samples from veterinary surgeons. However, now that most tests can be performed on buccal swabs rather than blood samples it is feasible for owners to submit samples directly. The majority of breeders were choosing this option, sending samples to laboratories overseas rather than collecting a blood sample. To become more competitive we have decided to accept samples directly from owners. However, we stress the value of local veterinary input in counselling for interpretation of results. Tests for which the owners wish to include their cats in an official registry, such as the Feline Advisory Bureau (FAB) PKD and HCM registers (http://www.fabcats.org/breeders/registers.php), require samples to be submitted by a veterinary practice following verification of the cat sampled through a microchip.

An additional factor in accepting samples submitted directly from the owners is that this facilitates development of new genetic tests, which usually necessitates collection of samples from large numbers of cats both affected and unaffected. We frequently work with specific breed clubs, taking samples at shows, to facilitate this work.

How should results be interpreted and owners counselled?

Test interpretation and counselling will clearly depend on a number of factors including the importance of the mutation, prevalence, mode of inheritance and the relationship between phenotype and genotype (i.e. how likely is the genetic mutation to result in clinically significant disease). The practitioner has a crucial role in counselling clients on how to act on results.

For some defects the relationship between genotype and the likelihood of disease is not straightforward. This is particularly true of some of the important dominant genetic mutations such as PKD and HCM. Whilst availability of genetic tests has made an important contribution to advancing our knowledge of inherited disorders, some tests have raised important unanswered questions. For example considering HCM; the inheritance had been recognised as following an autosomal dominant pattern prior to development of a genetic test and it had been presumed that homozygotes were lethal and did not exist. However, since the development of genetic tests homozygotes have been identified, although at a lower frequency than might be anticipated compared to the heterozygotes (which are around 30%).

One explanation for this lower prevalence is that clinical disease may occur at an earlier age and more severely in homozygotes leading to early deaths. However, a significant number of old (>10 years) homozygous individuals have been identified that do not show any evidence of cardiomyopathy. It is also clear that clinical disease will not develop in all heterozygotes; although about 30% of the Maine Coon and Ragdoll populations appear to be heterozygotes the prevalence of clinical disease in these breeds is plainly considerably lower. The same applies to PKD, another autosomal dominant defect that has been shown to have a very high prevalence in Persians and closely related breeds. There is clear evidence from studies based on scanning Persians that once a cat carrying the gene mutation has reached 6-12 months of age it will invariably develop renal cysts. Yet not all, and probably a relatively small proportion of these cats will subsequently develop renal failure.

The most likely explanation for this finding is incomplete or variable expression of these genes, or there may be other genes which influence the likelihood of disease developing.

Can genetic tests help in the diagnosis of disease?

In most clinical cases diagnosis of genetic diseases is based on clinical investigations supported by appropriate diagnostic aids such as laboratory testing and imaging. However, genetic testing can play a role in diagnosis of some inherited diseases, particularly if clinical signs are variable and inconsistently present, such as pyruvate kinase deficiency in Abyssinians *(figure 5)* and Somali cats, which causes waxing and waning anaemia.

Figure 5: An Abyssinian cat, this breed, along with Somali cats, is affected by Pyruvate kinase deficiency which can result in anaemia; and for which mutation a genetic test is now available

Genetic tests can also be used to predict if an individual cat is likely to develop a specific genetic disease. However, as mentioned above, in some cases the genotype will not always predict disease and cats may remain unaffected throughout their lives. A Ragdoll or Maine Coon cat carrying the mutant gene for HCM may not develop cardiomyopathy, although the presence of the gene significantly increases the risk. Conversely a negative test does not guarantee a cat will not succumb to cardiomyopathy due to other, unidentified, gene mutations or secondary to another disease such as hyperthyroidism.

How should genetic tests be used as an aid to preventing inherited diseases?

Genetic tests now provide a realistic tool for breeding programs to eliminate specific inherited disorders. It is possible for owners to test all breeding cats for mutations for which a test is available and eliminate the mutation within a generation. However, there can be disadvantages with this approach. In the case of a dominant gene mutation, such as PKD and HCM, the prevalence within affected breeds is high and if those cats are eliminated from the breeding pool it may significantly restrict the genetic diversity within the breed with unpredictable consequences such as the emergence of a different genetic disease. A more pragmatic approach is to continue to use a restricted number of carrier individuals, which are of high breeding value for other reasons. These should be bred to cats negative for the mutation and their offspring should be tested, half of which would be expected to be negative and could be considered for future breeding.

Control of recessive gene mutations is generally more straightforward. The prevalence of recessive genes is usually relatively low in the population and therefore selecting against these genes will have a lesser effect on the gene pool. It is also possible to continue to use carrier cats without producing affected offspring by ensuring that they are mated only with individuals that have tested negative.

Future developments in genetic testing

There is likely to be further refinement of the genetic map and genomic sequence for cats which will facilitate further development of new genetic tests. There are also important technological developments that will contribute to further advances. A 'SNP chip' for cats is being developed supported by the Morris Animal Foundation which received a substantial donation from Hill's Pet Nutrition for this purpose. This 'chip' will greatly facilitate large-scale studies and will make it possible to look for genetic markers for more complex, multiple gene associated disorders. For example, studies are being initiated to search for genetic markers for diabetes mellitus in cats. Another potential application will be to investigate possible genetic susceptibility to feline infectious peritonitis (FIP). This may enable breeders to select cats with natural resistance in the future.

Genetic testing is now playing an increasingly important role in identifying people who are at particular risk of certain diseases for whom, routine screening is a priority; for example susceptibility to breast cancer. Similar approaches may make it possible to identify cats at increased risk of certain diseases and allow early interventions, for example avoiding obesity in cats at risk of diabetes mellitus.