



BEGINNER'S GUIDE TO USING GENETIC TESTS IN CATS

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BACKGROUND

Just like any other mammalian species, including man, cats suffer from genetic diseases and have traits controlled by genetic mutations. Whilst considerably more is known about human genetics, there have been major advances in our understanding of feline genetics over recent years. As in humans, the feline genome has been sequenced (although there is still more work to be done in identifying all of the genes) and this has greatly helped the discovery of genetic mutations.

The ability to determine whether a cat is likely to suffer from a genetic disease by just taking and analysing a mouth swab has been of benefit to both disease diagnosis and breeding strategies. For example, before the genetic mutation responsible for polycystic kidney disease was discovered in 2004, the disease could only be diagnosed ante-mortem by ultrasound scanning of the kidneys. Whilst this was a reliable diagnostic technique, it could not be undertaken until the cat was at least 10 months of age, required the cat to be brought to the clinic and needed specialist equipment and operators. With genetic testing, a diagnosis can be made from a mouth swab taken from the cat by a vet or breeder and sent to a testing laboratory; in addition the swab can be taken from cats of any age. Samples are not normally accepted directly from owners, however, the advent of owner-submitted swabs for genetic testing is primarily used to inform breeding strategies rather than decisions regarding the health and welfare of individual cats. For cases that involve diagnosis, treatment or ongoing monitoring we would still recommend veterinary involvement. This revolution is even more important for genetic diseases that have a late-onset and/or are inherited in a recessive manner, e.g. retinal atrophy. Because clinical signs often do not appear until at least 3-5 years of age, affected cats may have been used for breeding extensively before they show clinical signs of disease



Fig. 1: An example of a familial inherited defect of which a genetic test has become available - episodic hypokalaemic polymyopathy, most often seen in Burmese and related breeds. This Burmese cat shows ventral neck flexion which is indicative of muscle weakness.

and carrier cats never show any signs of the disease. The ability to determine the genetic status of these cats before they are of breeding age significantly helps cat breeders to manage and plan their breeding programmes so they do not produce affected kittens.

AVAILABLE TESTS

Over recent years, many genetic tests for cats have become available (*see Table 1*). These cover a range of genetic conditions including diseases, coat colours and blood type. Some genetic mutations appear to be distributed throughout many breeds of cat (e.g. the coat colour mutations), whilst others are specific to only single cat breeds (e.g. Ragdoll hypertrophic cardiomyopathy). It is important to bear this in mind when requesting certain genetic tests on certain breeds of cat, since although the test can be run and a result obtained, the result may not be relevant to disease in that breed. Testing laboratories are always happy to discuss breed/test combinations if veterinary surgeons are unsure of their validity. Another issue to bear in mind is that certain cat breeds are allowed to use other breeds in outcross programmes, which means that some of the more breed specific mutations may start arising in other breeds if the outcross cats have not been tested. Researchers at Langford are continually working to find new mutations responsible for other feline genetic diseases and traits and due to the considerable amount of work this involves often participate in international studies, such as was the case recently with episodic hypokalaemic polymyopathy (*Fig. 1*).

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GENETIC TEST	BREEDS KNOWN TO HAVE THE MUTATION
Ragdoll hypertrophic cardiomyopathy	Ragdoll, Ragamuffin
Maine Coon hypertrophic cardiomyopathy	Maine Coon
Polycystic kidney disease	Persian, Chinchillas, Exotics, BSH, Ragdoll, Birman, Asians, Cornish Rex, Devon Rex, Snowshoes
Pyruvate kinase deficiency	Abyssinian, Somali, Bengal, Singapura, Egyptian Mau, La Perm, Maine Coon, Norwegian Forest Cat, Savannah, Siberian
Progressive retinal atrophy	Abyssinian, Somali, Ocicat, Siamese, Balinese/Javanese, Oriental Shorthair, Colourpoint Shorthair
Blood type	Any
Chocolate coat colour	Any
Cinnamon coat colour	Any
Dilute coat colour	Any
Agouti coat colour	Any
ALC Agouti colour	Bengal
Siamese colourpoint	Any
Burmese colouration	Any
Long hair/Short hair	M1 found in Ragdoll M2 found in Norwegian Forest Cat and Tiffanie M3 found in Ragdoll and Maine Coon M4 found in all breeds including the above
Glycogen storage disease type IV	Norwegian Forest Cat
Amber coat colour	Norwegian Forest Cat
Gangliosidosis (GM2)	Burmese
Episodic hypokalaemic polymyopathy	Burmese, Asian, Australian Mist, Bombay, Burmilla, Cornish Rex, Devon Rex, Singapura, Sphinx, Tiffanie, Tonkinese
Spinal muscular atrophy	Maine Coon

Table 1: Feline genetic tests available at Langford Veterinary Services www.catgenetics.co.uk

SAMPLES

In the early days of genetic testing, a blood sample was required to obtain an adequate amount of cat DNA. Technological improvements mean we can now routinely use mouth swabs, which are less invasive and can be taken by the breeder, although blood samples will still work fine. The vast majority of the ~6000 samples a year that we analyse are mouth swabs submitted by owners and breeders.

The quality of the mouth swab is of vital importance for reliable and accurate genetic testing, and in our experience the swabs that breeders collect are satisfactory more than 98% of the time. However, it is

still worth reiterating that the aim of a mouth swab is to collect cheek cells, which contain the DNA, and not to collect just saliva. It is also important that swabs are taken at least an hour after cats and kittens have fed. The swab tip is inserted between cheek and gum and twirled for a few seconds, ideally this should be repeated on the other side of the cat's mouth. Breeders are encouraged to swab cats one at a time to reduce the risk of mixing up the swabs and keep swabs separate to prevent cross contamination. Swabs should be sent to the laboratory as soon as possible since bacteria and fungi can grow on them if they are stored for too long.

SUBMISSION

All genetic testing laboratories now accept direct submission of mouth swabs from owners and breeders, as well as mouth swabs and/or blood samples from veterinary surgeons. The main aim of breeders who submit samples is not to determine disease status, but rather to inform their breeding programme, e.g. so that they do not mate two cats carrying a recessive genetic disease. In some circumstances (see below) there is a requirement for the veterinary surgeon to collect the mouth swab and confirm the cat's identity by its microchip number:

- **International Cat Care** (formerly Feline Advisory Bureau) run various negative registers for genetic diseases (e.g. polycystic kidney disease and hypertrophic cardiomyopathy) that require the cat's identity to be confirmed by a veterinary surgeon.
- **The Governing Council of the Cat Fancy (GCCF)** run various Active registers (e.g. Ragdoll hypertrophic cardiomyopathy) that require the cat's identity to be confirmed by a veterinary surgeon.

LABORATORY

When swabs arrive at the Langford, the DNA is extracted from the cheek cells and polymerase chain reaction (PCR) is used to copy a short section of DNA containing the genetic mutation millions of times. The DNA sequence surrounding the mutation is then determined by pyrosequencing, which is used to indicate whether the cat has the mutation, and if it does, whether it is a carrier. Turnaround time for genetic testing at Langford is 3-4 days.

INTERPRETATION

Interpretation of genetic test results, especially those for genetic diseases, can cause issues for both breeders and veterinary surgeons for several reasons:

- The absence of a mutation known to cause a genetic disease **does not** mean the cat can never suffer from that disease; it only means that the known mutation is not responsible for the disease. There are therefore limitations to using genetic tests for diagnosis of a particular condition. For example, the mutation that is known to cause hypertrophic cardiomyopathy (HCM), in Maine Coon cats is not the only factor involved in HCM due to the fact that some Maine Coon cats suffering HCM do not have that particular mutation. There may potentially be multiple mutations that play a part in the condition and to further complicate matters, HCM can occur secondary to other diseases such as hyperthyroidism.
- Whilst the presence of a particular genetic mutation may have been strongly associated with a particular disease in a cat breed (e.g. the development of cystic kidneys in Persians with the PKD mutation), whether the presence of the condition actually goes on to have clinical effects at all, the age of onset of clinical disease and its severity, will vary considerably from cat to cat. This is due to many

genetic and environmental factors, such as other genetic mutations that may have a protective effect, the effect of diet etc.



Fig. 2: Ultrasound image (courtesy of Kate Bradley) showing multiple cysts within a kidney of a Persian cat affected by polycystic kidney disease.



Fig. 3: Gross pathology of kidneys at post mortem from a Persian cat affected by polycystic kidney disease.

- Some genetic disease mutations appear to have greater penetrance than others. For example, cats with the PKD mutation will invariably develop cystic kidneys at some point in their lives (Figs. 2 & 3). However, cats homozygous for the known HCM mutations do not necessarily show phenotypic effects. Those that do often have very variable outcomes, with some dying at an early age and others living much longer.
- A genetic mutation normally found in one breed of cat (e.g. pyruvate kinase deficiency), may or may not be of concern when discovered in other breeds. In the case of pyruvate kinase deficiency, although Bengal and Singapura cats are descended from Abyssinian lines and the mutation has been identified in these breeds, clinical disease due to the mutation has not yet been documented in Bengals and Singapurans, possibly due to the low number of cats carrying the mutation and the episodic nature of the disease. However, at present it is not clear whether they actually suffer disease as a result of the mutation.

TO NEUTER, OR NOT?

Many breeders' initial response to being told they have a cat affected by, or carrying, a genetic disease is to neuter it. Whilst this is understandable and (over the long term) it is desirable to remove disease causing mutations from the feline population, this should be done slowly to preserve the gene pool. Many cat breeds are already quite in-bred and have small gene pools. If a significant number of cats are then neutered because they have a genetic mutation the gene pool will get even smaller. This is an even bigger problem in breeds where there are relatively few individuals and/or the mutation is present at a high frequency. This means that cats carrying recessive mutations and even some

homozygous recessive affected cats (which do not necessarily show severe disease), will probably have to be used in breeding programmes in order to maintain genetic diversity and the kittens genetically tested in order to be used for further breeding purposes. Out breeding to closely related breeds can also be undertaken if this is allowed by the Governing Council of the Cat Fancy (GCCF). However, care must be taken so as not to introduce undesirable mutations from the chosen breed by testing all out cross cats since this is probably how many genetic disease mutations have spread between breeds.

BREEDING

Recessive mutation (e.g. pyruvate kinase deficiency).

		AFFECTED		CARRIER		CARRIER	
		+	+	+	-	+	-
NORMAL	-	+/- Carrier	+/- Carrier	+/- Carrier	-/- Normal	+/- Carrier	-/- Normal
	-	+/- Carrier	+/- Carrier	+/- Carrier	-/- Normal	+/- Carrier	-/- Normal
CARRIER	+	+/- Carrier	+/- Carrier	+/+ Affected	+/- Carrier	+/- Carrier	+/- Carrier
	-	+/- Carrier	+/- Carrier	+/- Carrier	-/- Normal	+/- Carrier	-/- Normal

Fig. 4: Diagrams showing the results of mating between normal, affected or carrier cats with a recessive mutation.

For recessive traits it is relatively easy to gradually remove a mutation from a population over several generations. In general it is not recommended to use affected cats in breeding programmes, although if this has to be done due to high prevalence/low breeding numbers they should be mated to cats not having the mutation, which will result in all of the kittens being carriers. Carrier cats, which will show no clinical signs of the disease, can be used in breeding programmes as long as they are mated to cats not having the mutation, as breeding to other carrier cats introduces the risk of producing some affected kittens. In this scenario, none of the kittens will suffer from disease but some are likely to carry the mutation (*Fig. 4*). Litters can be screened for the genetic mutation and if necessary the carriers mated to other cats not having the mutation.

Dominant mutation (e.g. polycystic kidney disease).

For dominant traits, the situation is not quite as straightforward since there are only affected and normal cats to use for breeding (i.e. there are no carrier cats). If an affected cat is bred to a normal cat (*Fig.5*) there is a risk of producing more affected kittens together with normal kittens. These can be easily identified by genetic testing at an early age and the normal kittens used in future breeding programmes. Using affected cats in breeding programmes is controversial since more affected kittens are likely to be produced. However, if the prevalence of a mutation is high, as it was with PKD in the Persian breed in mid 2000, the alternative strategy of not using any affected cat for breeding would almost certainly have been much worse for the breed overall due to the dramatic reduction in the gene pool.

		AFFECTED	
		+	-
NORMAL	-	+/- Affected	-/- Normal
	-	+/- Affected	-/- Normal

Fig.5: Diagram showing the result of mating normal and affected cats with a dominant mutation.

CONCLUSION

Genetic testing has allowed breeders to identify cats carrying desirable traits (e.g. coat colours) and undesirable traits (e.g. genetic diseases) so that breeding programmes can be established.

An example of genetic testing and selective breeding at work is polycystic kidney disease in Persian and related breeds, which had a prevalence of 30% in 2005, but which had reduced to 3% in 2013 (Fig. 6).

This graph indicates that over 8 years the prevalence of this mutation has dropped by 90% in the cats tested, which is presumably a result of breeders using selective breeding to try to reduce the incidence of this mutation.

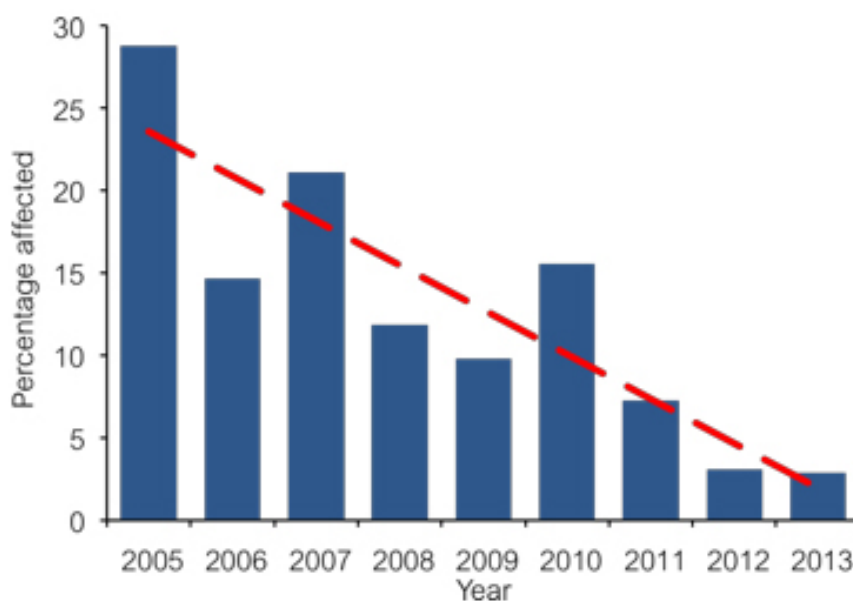


Fig. 6: The percentage of Persian and related cats testing positive for polycystic kidney disease over the years 2005 to 2013. The red dotted line shows the general trend.

Source - submission of over 3300 samples to Langford Veterinary Services.

THE FUTURE

New genetic tests are being developed rapidly in response to the discovery of new mutations - for example in 2012, the mutations responsible for hypokalaemia and a head defect in the Burmese breed were discovered and Langford Veterinary Services have since tested nearly 800 cats for this mutation. With new high-throughput DNA sequencing technologies becoming cheaper and more available it will soon be possible to use these techniques to search for mutations causing many other diseases or traits, although the analysis of the billions of base pairs of DNA sequence may become a limiting factor.