

Feline Genetics: Clinical Applications and Genetic Testing

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DNA testing for domestic cat diseases and appearance traits is a rapidly growing asset for veterinary medicine. Approximately 33 genes contain 50 mutations that cause feline health problems or alterations in the cat's appearance. A variety of commercial laboratories can now perform cat genetic diagnostics, allowing both the veterinary clinician and the private owner to obtain DNA test results. DNA is easily obtained from a cat via a buccal swab with a standard cotton bud or cytological brush, allowing DNA samples to be easily sent to any laboratory in the world. The DNA test results identify carriers of the traits, predict the incidence of traits from breeding programs, and influence medical prognoses and treatments. An overall goal of identifying these genetic mutations is the correction of the defect via gene therapies and designer drug therapies. Thus, genetic testing is an effective preventative medicine and a potential ultimate cure. However, genetic diagnostic tests may still be novel for many veterinary practitioners and their application in the clinical setting needs to have the same scrutiny as any other diagnostic procedure. This article will review the genetic tests for the domestic cat, potential sources of error for genetic testing, and the pros and cons of DNA results in veterinary medicine. Highlighted are genetic tests specific to the individual cat, which are a part of the cat's internal genome. © 2010 Elsevier Inc. All rights reserved.

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A cat's appearance, its phenotype, and its health can be influenced by both genetic (inherited) and non-genetic (environmental or epigenetic) influences. The diseases and traits that have known mutations, hence clearly heritable and genetic, are generally called simple genetic traits, because the presentations are controlled mostly by a single specific mutation in a single specific gene. Because these traits are "simple," most of the initially discovered mutations in any species have been for clearly genetic traits that have high frequencies in specific populations. In the early and mid-1990's,^{1,2} the first mutations identified in cats were for lipid and lysosomal storage diseases as these diseases have well-defined phenotypes and known genes with mutations that were also found in humans (see reviews^{3,4}). Most of the common diseases, coat colors, and coat types have been deciphered in the cat after the same candidate gene approach, implying finding a replicate trait in another species and checking the same gene for causative mutations. Commercially available simple genetic traits and diseases with known mutations in the cat are presented in Table 1. Other disease mutations are presented in Table 2. To date, other than the muscular dystrophy mutation,¹ all other mutations in the cat are autosomal, not found on the X or Y chromosomes.

The coat color mutations are common to all cats and are effective for genetic typing in all breeds and populations. However, even though long fur is common in breeds and random-bred cats, long fur is an exception because 4 different mutations in *FGF5* can cause a cat to have long fur.^{5,6} One mutation is common to most all breeds and populations, suggesting this mutation to be the most ancient mutation, but the others are more specific to particular breeds.⁷ Thus, to determine accurately if a cat carries a mutation for long fur, all 4 mutations must be genotyped.

In contrast to coat colors and long fur, most of the identified disease tests in cats are very specific to breeds and populations. Most diseases are identified in cat breeds, which are a small percentage of the cat population of the world, perhaps at most 10% to 15% in the United States.⁸ Some mutations that were found in a specific breed, such as mucopolysaccharidosis in the Siamese,^{2,9} were found in a specific individual and the mutation is not actually prevalent in the breed (Table 2). These genetic mutations should not be part of routine screening by cat breeders and registries, but clinicians should know that genetic tests are available for diagnostic purposes, especially from research groups with specialized expertise, such as at the University of Pennsylvania (<http://research.vet.upenn.edu/penggen>). Other biomarkers are also available at these specialized laboratories to help decipher between the lysosomal storage and metabolism orders. Other diseases, such as polycystic kidney disease (PKD), are prevalent. PKD in Persians is estimated at 30% to 38% worldwide.¹⁰⁻¹² Because of cross breeding with Persians, many other breeds, such as British shorthairs, American shorthairs, and Scottish folds, also need to be screened for PKD.¹³⁻¹⁵ Thus, veterinarians need to be aware of cross-

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Table 1. Common Commercialized DNA Tests for Domestic Cats

Disease/Trait	MOI†	Phenotype	Breeds	Gene	Mutation
Agouti ⁴⁰	AR	Banded fur to solid	All breeds	<i>ASIP</i>	del122 to 123
Amber ⁴¹	AR	Brown color variant	Norwegian Forest	<i>MC1R</i>	G250A
Brown ^{42,43}	AR	Brown, light brown color variants	All breeds	<i>TYRP1</i>	b = C8G, b ^l = C298T
Color ^{37,43,44}	AR	Burmese, Siamese color pattern, full albino	All breeds	<i>TYR</i>	c ^b = G715T, c ^s = G940A, c = C975del
Dilution ⁴⁵	AR	Black to grey/blue, orange to cream	All breeds	<i>MLPH</i>	T83del
Gloves ⁴⁶	AR	White feet	Birman	<i>KIT</i>	c.1035_1036delinsCA
Hairless (naked) ⁴⁷	AR	Atrichia	Sphynx	<i>KRT71</i>	c.816+1G>A
Long fur ^{5,6}	AR	Long fur	All breeds‡	<i>FGF5</i>	c.356insT, C406T, c.474delT, A475C
Rexing (curly fur) ⁴⁷	AR	Curly hair coat	Devon Rex	<i>KRT71</i>	c.1108-4_1184del, c.1184_1185insAGTTGGAG, c.1196insT
AB blood type ³⁹	AR	Determines type B	All breeds	<i>CMAH</i>	18indel-53, G139A
Gangliosidosis 1 ⁴⁸	AR	Lipid storage disorder	Korat, Siamese	<i>GBL1</i>	G1457C
Gangliosidosis 2 ⁴⁹	AR	Lipid storage disorder	Burmese	<i>HEXB</i>	15bp del (intron)
Gangliosidosis 2 ⁵⁰	AR	Lipid storage disorder	Korat	<i>HEXB</i>	C39del
Glycogen storage disease IV ⁵¹	AR	Glycogen storage disorder	Norwegian Forest	<i>GBE1</i>	230bp ins 5' - 6kb del
Hypertrophic cardiomyopathy ²⁶	AD	Cardiac disease	Maine Coon	<i>MYBPC</i>	G93C
Hypertrophic cardiomyopathy ³¹	AD	Cardiac disease	Ragdoll	<i>MYBPC</i>	C2460T
Progressive retinal atrophy ⁵²	AR	Late-onset blindness	Abyssinian	<i>CEP290</i>	IVS50 + 9T>G
Progressive retinal atrophy ⁵³	AD	Early-onset blindness	Abyssinian	<i>CRX</i>	n.546delC
Polycystic kidney disease ¹⁵	AD	Kidney cysts	Persian	<i>PKD1</i>	C10063A
Pyruvate kinase def.*	AR	Hemopathy	Abyssinian	<i>PKLR</i>	13bp del in exon 6
Spinal muscular atrophy ⁵⁴	AR	Muscular atrophy	Maine Coon	<i>LIX1-LNPEP</i>	140kb del, exons 4 to 6

*Unpublished test, presented only as abstract.

†Mode of inheritance of the non-wildtype variant.

‡Long fur variants are more or less common depending on the breed.

breeding practices, which differ in different cat registry organizations, so that genetic tests can be placed as high or low priority for differentials and diagnostics.

Non-genetic components, including toxins, infections, infestations, sporadic damage and changes to the DNA, and environmental influences, such as diet, exercise, and social surroundings, can produce a phenotype that looks just like an inherited characteristic or diseases, a phenocopy. Detailed examinations of cats with heart murmurs may reveal different presentations of heart disease, one that may be genetic and one that may be environmentally induced, such as by low dietary taurine, which results in dilated cardiomyopathy.¹⁶ Some diseases may present differently in different tissues,

termed as pleiotropic effects of the same gene. For example, completely white cats can be just white, whereas others have blue eyes or odd-eyed color and some may be deaf.¹⁷⁻¹⁹ Genetic testing can assist the clinician to rule out the common and environmental causes of clinical presentations versus a condition caused by a heritable defect in the cat's DNA.

Hallmarks of Genetic Diseases

A cat's phenotype is a combination of the presence of diseases, visible traits, or morphological types. Attributes of the phenotype can be desirable or undesirable, especially in the case of diseases. Because phenotypes can be a result of a

Table 2. Other Mutations for Inherited Domestic Cat Diseases*

Disease	Gene	Mutation	Disease	Gene	Mutation
Gangliosidosis 2 ⁵⁵	HEXB	inv1467 to 1491	Mucopolysaccharidosis I ⁵⁶	<i>IDUA</i>	del1047 to 1049
Gangliosidosis 2 ⁵⁷	HEXB	C667T	Mucopolysaccharidosis VI ²	<i>ARSB</i>	T1427C
Gangliosidosis 2 ⁵¹	<i>GM2A</i>	del390 to 393	Mucopolysaccharidosis VI ^{9,58}	<i>ARSB</i>	G1558A
Hemophilia B ⁵⁹	F9	G247A	Mucopolysaccharidosis VII ⁶⁰	<i>GUSB</i>	A1052G
Hemophilia B ⁵⁹	F9	C1014T	Niemann-Pick C ⁶¹	<i>NPC</i>	G2864C
Hyperoxaluria ⁶²	<i>GRHPR</i>	G>A I4 acceptor site	Polydactyla ⁶³	<i>SHH</i>	A479G
Lipoprotein lipase def. ⁶⁴	<i>LPL</i>	G1234A	Polydactyla ⁶³	<i>SHH</i>	G257C, A481T
Mannosidosis, alpha ⁶⁵	<i>LAMAN</i>	del1748 to 1751	Vitamin D-resistant rickets ⁶⁶	<i>CYP27B1</i>	G223A, G731del
Mucopolidosis II ⁶⁷	<i>GNPTA</i>	C2655T	Vitamin D-resistant rickets†	<i>CYP27B1</i>	G637T

*The presented conditions are not prevalent in breeds or populations but may have been established into research colonies.

†Implies yet unpublished work.

single gene, the interaction of several genes, the accumulation of environmental exposures, or a combination of interactions, a veterinarian may choose different types of therapy and clinical management, or make different prognoses if a phenotype is known to be genetic. If the same condition is found in other species, a veterinarian may have opportunities to try novel approaches for health care by considering comparative medicine. Several characteristics are common to genetic diseases that will help decipher sporadic, idiopathic occurrences from inherited conditions.

The 6 common hallmarks for inherited diseases include: 1) early age of onset, 2) bilateral and/or multiple presentation, 3) presence in a closed or small population, 4) indications of inbreeding, 5) uniformity in presentation, and 6) advanced parental age at birth. Only advanced age of parents at birth has not been shown to have an effect in cat-inherited diseases. In humans, older mothers have a higher frequency of having children with trisomy 21,²⁰ and dwarfism is associated with advanced paternal age.²¹

Two examples of diseases that present as sporadic and inherited forms are PKD²² and lymphosarcoma.⁸ Each of the 5 characteristics that define genetic diseases can help identify PKD from cats with sporadic kidney cysts. Kidney cysts can occur in any cat, but not all cystic presentations are indicative of PKD. PKD can sometimes be detected as early as 6 to 8 weeks of age by ultrasound, consistently by 10 months of age.¹⁴ Both kidneys are normally affected and multiple cysts are generally present. The cysts are not similar in size but are similar in etiology. PKD is rampant in Persian cats, and thus must be considered to be present in related breeds, such as Exotic shorthairs and Himalayans. Surprisingly, this genetic problem has a very high frequency in one of the oldest and largest cat breeds, thus not a small or closed population, but the early onset, bilateral presentation, and high prevalence in a breed clearly demarcate this condition as a heritable problem. An older, random-bred cat with one kidney cyst in one kidney would not be a candidate for PKD and genetic testing.

Lymphosarcoma is also frequent in cats, but it is generally found in older cats and/or cats that have been infected and are positive for feline leukemia virus.²³ An unusual rate of mediastinal lymphosarcoma has been specifically identified in silver Oriental shorthairs that are feline leukemia virus negative and generally younger than 2 years of age. Not only is a breed influence suspected in this disease, but even a particular color or line. Other related breeds, such as Siamese, Colorpoint shorthairs, and the longhaired varieties of Siamese, should be suspected of having a prevalence of this lymphoma. Age of onset is 1 to 2 years and the tumors respond well to chemotherapy, but reoccur with a poor prognosis. Thus, this disease is found in a closed, inbred population, has an early onset and generally uniform presentation, and the tumor is found in areas not common to older onset forms of lymphosarcoma. These hallmarks define a heritable condition versus sporadic lymphomas occurring in older-aged cats.

Genetic Risk Factors and Complex Traits

A genetic problem with a high frequency can be found in nearly every breed, thus no one breed can generally be considered healthier than another. Detrimental genes are found in random-bred cats as well, but low inbreeding prevents an increased incidence in presentation of the disease. However, for recessive or dominant diseases that do not cause early clinical presentations, breeders can be completely unaware of the propagation of a deleterious gene. Some breeds may also have health problems, genetic and nongenetic, because of the conformation or “type” desired for the breed. Shortened skull structures and nasal canals cause Persians to have weeping eyes, asymmetric skulls, and poor bite.²⁴ Anecdotally, the fine, elegant structures of Abyssinians and Siamese exacerbate patellar luxation. The largest breed, the Maine Coon, is under investigation for hip dysplasia, a very common problem in large dog breeds. Likely, these more complex problems have a genetic component, but determining the number and the affect

of genes is difficult, thus recommendations for better breeding practices could be of more value than genetic testing.

All the mutations influencing a disease may not be identified at any given time, thus, usually the mutations that influence a condition the most have the highest heritability and are identified first. Because multiple mutations may act additively to cause a disease, each mutation may be said to confer a “risk” for disease development. Thus, some mutations may be considered risk factors, predisposing an individual to a health problem. These risk-conferring mutations are neither necessary nor sufficient for causing disease. An excellent example of mutations that confer a risk are the DNA variants associated with cardiac disease in cats.

Hypertrophic cardiomyopathy (HCM) is a recognized genetic condition in cats.²⁵ In 2005, Kittleson and colleagues published that a DNA alteration in the gene cardiac myosin-binding protein C3 (*MYBPC3*) was strongly associated with HCM in a long-term research colony of Maine Coon cats at University California–Davis.²⁶ The DNA mutation is commonly referred to as A31P, because this DNA mutation changes codon 31 from an alanine to a proline in the amino acid sequence (i.e., protein) of cMYBPC. The data clearly show that not all cats with the mutation had HCM and that some cats with HCM did not have the DNA mutation. Age of onset, variable expression, and disease heterogeneity were alluded to in this report. These aspects suggest that the identified DNA variant should be considered more of a “risk factor” than a directly causative mutation. Two recent articles have shown that not all Maine Coon cats with the A31P mutation get HCM,^{27,28} and one of those articles has mistakenly interpreted this lack of penetrance as being evidence that the A31P mutation is not causal.²⁸ This interpretation is misleading, causing debate as to the validity of the Maine Coon HCM test.

To date, most cat genetic tests have been for traits that have nearly complete penetrance, have little variability in expression, and are early in onset. However, some imperfect examples do exist in cats that have not caused as much controversy as the HCM test. The *CEP290* PRA mutation in Abyssinians has a late age of onset, and some cats with subclinical disease have been identified.²⁹ Some cats with the pyruvate kinase deficiency can have very mild and subclinical presentations.³⁰ The interplay of various coat color genes often muddle the determination of the true coat color of cats. As it is true in humans with cardiac disease, the finding that not all cats with the A31P mutation in *MYBPC3* get HCM is actually usual in the field of HCM genetic testing. Therefore, disease- or trait-causing mutations may not be 100% penetrant, thus they do not always cause clinically detectable disease. Presence of clinical disease in an individual cat and the severity of disease (expression) are likely affected by the known genetic aspects presented below.

Incomplete Penetrance

For some traits and diseases, even though a known causative mutation has been identified, an individual with that mutation does not present with the condition. Incomplete

penetrance is an extreme of variable expression (see below). In general, the reason as to why a condition would not present is unknown, but other genetic, biological, and environmental interactions certainly play a role in the overall appearance and health of an individual and its organs. The sensitivity of clinical diagnostics may also influence the determination of penetrance. In the case of HCM, echocardiography (cardiac ultrasound) can be considered an insensitive tool for detecting mild forms of HCM in cats, thus many cats with mild do not clinically appear to have cardiac disease. Experience and bias also play a role in diagnosis. For example, individuals who do not have expertise with ultrasonic examinations for HCM or PKD are less likely to be able to provide an accurate diagnosis for these diseases.

Age of Onset (Age-related Penetrance)

Some diseases have a slow progression and may not present until later in life. In humans, HCM due to *MYBPC* mutations is clearly a disease that has slow progression and commonly does not express until the individual is over 50 years of age. HCM in Maine Coon cats can also develop in older cats, especially in cats that are heterozygous for the mutation (carry only one copy of the mutated gene) and, for some unknown reason, in females. Often, an autosomal dominant disease may be more severe if 2 copies of the risk mutation are present in an individual, leading to earlier and more severe disease, which appears to be the case with the A31P mutation. The definitive age as to when a cat is clear of developing HCM is not precisely determined.

Variable Expression

Most traits and diseases have some amount of variable expression depending on the individual. For example, not all cats with the mutation for blue dilution have the same color of blue/gray. Obviously, the background genetics and environment of the individual influence the overall presentations of traits and diseases. Thus, the level of presentation can be variable in regard to left ventricular wall thickness in cats with HCM. Cats can have mild, moderate, or severe HCM. Only those cats with severe HCM show clinical signs, although a few cats with lesser severity of disease may die suddenly. Cats with HCM may fall in the “equivocal” range for wall thickness, thus definitive affected status is difficult to declare. These equivocal cats may progress to more severe disease with time, or the equivocal status may be as severe as the disease gets. Some cats with PKD have only a few cysts and never progress to renal failure; others have severe and fast progression of disease and succumb renal failure in a few years.

Disease Heterogeneity

Often, more than one mutation in the same gene, or mutations in different related genes can cause the same disease. Genetic heterogeneity for HCM in humans is well established, thus there is no reason not to think the same

situation is true for cats. Currently over 1000 mutations in over 10 genes are known to cause HCM in humans. Only 2 mutations have been identified that cause HCM in cats, the A31P mutation in Maine Coon cats and the R820W mutation in Ragdolls,^{26,31} which also causes disease in humans.³² Both mutations are in *MYBPC3*, the most commonly mutated gene in humans with HCM (see review³³). Other breeds of cats including Bengals, Siberians, Devon Rex, Sphynx, and mixed-breed cats either do not have or have an extremely low prevalence of the A31P or the R820W mutation. However, there are Maine Coon cats that have HCM that do not have the A31P mutation and so there has to be at least one more cause of HCM, most likely another mutation, in this breed. The long fur mutations in the cat are examples of trait heterogeneity.

Genetic Testing Accuracy

Even though a specific genetic mutation may be identified for a genetic trait or disease, research laboratories use different methods to assay for the mutation. Errors in genetic assays may produce inaccurate DNA results, leading to the confusion of genetic test interpretation. Direct DNA sequencing is considered the most robust method, the “gold standard,” but also one of the more costly methods of analysis. Because DNA primers must bind to the DNA sequence flanking a specific mutation, other, unimportant mutations may be in the areas where the primers bind, causing poor or no amplification of one or both alleles for a given individual. This condition is known as allelic dropout and all testing laboratories are aware of this potential source of error for a genetic test. Even direct DNA sequencing can suffer from allelic dropout, but because a larger portion of the gene which may have other DNA variants is generally amplified, a higher likelihood of detecting allelic dropout is available. Laboratories will place polymerase chain reaction primers in different locations surrounding the mutation of interest, which is often proprietary information, in attempts to lower the risk of allelic dropout. Thus, some laboratories have better assays than others, even if they are doing the same assay method and testing for the same mutation. The different DNA assay methods are usually developed to reduce cost and to fit the laboratory’s expertise and instrumentation. But some assays may have, in general, some increased risk of test failure. Different common methods for DNA testing include real-time polymerase chain reaction (TaqMan), restriction fragment length polymorphism, allele-specific oligos, or even now mass spectroscopy-based methods. Just as a veterinarian may want to know if a feline immunodeficiency virus test is performed by a SNAP test (IDEXX Laboratories, Westbrook, Maine, USA) versus an enzyme-linked immunosorbent assay (ELISA), versus a Western Blot because each method has different sensitivities and specificities, so too is it true for DNA testing methods. Veterinarians will need to become familiar with the different genetic testing approaches and not hesitate to

ask a testing laboratory about their methods and sensitivity and specificity for their approaches.

Inaccurate Clinical Diagnosis

Ultrasonic examination of the heart, echocardiography, is the most common and currently the only useful method for detecting cardiac disease in cats. Several studies have evaluated HCM presence in domestic shorthair and Maine Coon cats.^{34–36} Not all cardiac disease is HCM, and even the definition of HCM can be debated. A consistent definition for HCM is not always used by all cardiologists, thus there is some difficulty with correlating a genetic test result with an ultrasound report, especially if detailed diagnostic criteria are not presented in the report. Misinterpretations in ultrasound examinations may lead to different interpretations with disease status.

Overall, the only way to determine the true risk conferred by some mutations is to follow up with cats over the course of their lifetime with common diagnostic procedures and compare the genetic test results. Only time and continued follow-up will help determine the true relative risk that mutations convey for complex diseases. In the case of HCM, various studies have indicated higher or lower risks in different populations of cats, but none have been able to follow up with cats throughout their lifetime. These studies are important and are of great value to the community. Other mutations need to be found, and the cooperation of breeders must be positive and enthusiastic to have successful studies.

Breeding Recommendations

Cat breeders are very knowledgeable in regards to weighing different factors to produce healthy cats that are of good type and temperament. Many genetic tests help a breeder make a clearer, more educated decision. Cats with a positive genetic test for diseases should be screened by other diagnostics, such as ultrasound in the case of HCM and PKD, to determine disease status and this overall information used in breeding decisions. Other health, type, and behavioral attributes should certainly be considered in the overall breeding program. However, breeders need to work hard to reduce the risks with any health issue. With the HCM A31P mutation, every cat that has the mutation is at risk for developing HCM and every cat with the mutation will pass it on to some or all of its offspring. Cats that are homozygous for the A31P mutation will definitely pass the mutation to their offspring. The homozygous cats are at high risk of developing severe HCM. Cats that are heterozygous for the mutation should not be bred unless they have other qualities that are either highly beneficial or necessary to the breed. Kittens that test negative for the mutation should be used to replace them in the gene pool. A slow eradication of disease is recommended for highly prevalent diseases, such as HCM and PKD, as quick elimination of such a high number of cats could lead to other effects of inbreeding depression. Breeds with very low popu-

Table 3. Domestic Cat DNA Testing Laboratories

Lab/Webpage	Region	University Research		Cat Test*			
		Affiliate	ID	Disease	Color	Blood	Coat
Animal DNA Testing www.animalsdna.com	Australia		Yes	4	Some	Yes	No
Animal Health Trust www.aht.org.uk	UK	Animal Health Trust	Yes	PKD	No	No	No
Antagene Immeuble Le Meltem www.antagene.com	France		Yes	4	Color	Yes	No
BioAxis DNA Research Centre Ltd. www.dnares.in	India		Yes	PKD	No	No	No
DNA Diagnostics Center www.dnacenter.com	USA		No	PKD	No	No	No
GENINDEXE www.genindexe.com	France		Yes	7	5	Yes	No
Genoscooper www.genoscooper.com	Finland		Yes	No	No	Yes	No
Gribbles www.gribblesvets.com	Australia		No	PKD	No	No	No
IDEXX www.idexx.ca	Canada		No	PK def.	No	No	No
Laboklin www.laboklin.de/	Germany		Yes	9	5	Yes	Long
Langford Veterinary Services, Molecular Diagnostics Unit, Langfordvets.co.uk	UK	Bristol	No	3	No	No	No
PennGen research.vet.upenn.edu/ penngen†	USA	Pennsylvania	No	PK GSD	No	No	No
PROGENUS S.A. www.progenus.be	Belgium		Yes	HCM PKD	No	No	No
Van Haeringen Laboratory www.vhlgenetics.com	Netherlands		Yes	9	5	Yes	Long
Veterinary Cardiac Genetics Lab www.vetmed.wsu.edu/ deptsvcgl/	USA	Washington State	No	HCM	No	No	No
Veterinary Genetics Lab www.vgl.ucdavis.edu	USA	California, Davis	Yes	7	All	Yes	All
VetGen www.vetgen.com	USA	Michigan	Yes	No	Brown dilute	No	Long
Vetogene www.vetogene.com	Italy	Milan	Yes	HCM PKD	No	No	No

Abbreviations: PKD, Polycystic kidney disease; GSD, glycogen storage disease; HCM, hypertrophic cardiomyopathy.

*Tests reference to those listed in Table 1. If a laboratory offers only one or two tests, those tests are listed. PKD and the HCMs are the most popular tests to offer.

†PennGen also offers tests for diseases in Table 2 that are not of concern to the cat breeds or population in general.

lution sizes, such as Korats, have learned to manage the gangliosidoses in their breed, never breeding carriers together. Hopefully, all the disease mutations can be eventually eradicated, but good breeding decisions and balancing population diversity must be considered.

Genetic Testing Concerns in Different Breeds or Populations

Once a mutation is identified for a gene, which causes a particular coat color or disease, a service laboratory, either in

association with the investigator who found the mutation, or an independent commercial laboratory (Table 3), will establish a genetic test for that mutation to offer to the public. Nearly a dozen laboratories around the world now offer the genetic test for PKD in cats. All the laboratories may be technically very good and accurate, but, not all of them equally “know their cats.” Thus, some of the concerns with specificity and sensitivity of genetic tests, particularly in regard to testing in hybrid cat breeds, are due to a lack of knowledge of how cat breeds are developed and cat evolutionary relationships.

Table 1 shows all of the known genetic mutations in the cat that have been published and may be of concern for genetic testing. In the case of diseases, diseases usually present in a specific breed, and thus are only associated with that breed. However, some breeds are allowed to outcross with others and some are legal or illegally used to help refine the “look” of another breed. Siamese and Persians both have a host of other cat breeds that they have influenced. Hence, any mutation found in one breed can be found in others if cross breeding has occurred. In addition, cats are bred all over the world and the rules between registries and associations are not always the same. An outcross that may be acceptable for The International Cat Association in the United States may be unacceptable for the Cat Fanciers’ Association or perhaps the Governing Council of the Cat Fancy in the United Kingdom. Thus, testing laboratories need to understand some of these cat breed dynamics, so that they know a test is valid for a given breed in any part of the world and so that tests are freely offered for the breeds at risk.

Why does one care if a genetic test is valid in a different breed? The concern is disease heterogeneity. As owners, breeders, and veterinarians, we see a clinical presentation that is abnormal in the cat. However, any of us can quickly jump to conclusions. Cats have many causes of renal failure, and not all renal failure is caused by PKD. Likewise, cats have different types of cardiac disease, and not all cardiac disease is HCM. Even when a diagnosis of HCM is definitive, not all HCM is caused by the same mutation. Herein lies the concern. An unknowing veterinarian, owner, or breeder may want a cat to have a genetic test for HCM or PKD because the cat has clinical signs consistent with these diseases. If the test shows a negative result, this result does not mean the cat does not have HCM or PKD, if the test has not been proven in that selected breed. However, the result does imply that the cat does not have the mutation causing Maine Coon or Ragdoll HCM or Persian cat PKD. A laboratory may very well run the test, but laboratories have different capabilities and skills with genetic counseling. The veterinarian may be on his or her own to understand the meaning of a negative test, hence the reason why a test is generally listed for pertaining to a specific breed. Until enough cats from a particular breed come forward with clinical data, such as ultrasound diagnoses and genetic test results, a test cannot be valid for the breed unless clear outcrossing to the risk breeds is apparent.

Besides knowing cats, testing laboratories need to know their genetics as well. The protein sequence for the genetic

a) Protein sequence

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CSRLEEYNSRQALCDGTPEGPLLRNPGN Cat
.....H.S..N.....R.. Human
.....R. Siamese.....
...T..... Dog
    
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b) DNA Sequence

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CCTGGGAAT non-pointed Siberian
CCTAGGAAT Siamese pointed Siberian
    
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c) DNA Sequence

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CCCGGGAAT -
CCTAGGAAT -
    
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Fig. 1. Genetic sequence for the cat “points” mutation. (a) Protein sequence. The letters on the top line are the single letter codes for amino acids, for example, a P is proline, an N is asparagine, an R is arginine, and a G is glycine. The top line is the sequence for a normal, nonpointed cat. The second line is the sequence in humans, the third is the Siamese-pointed cat, and the bottom line is the sequence for the domestic dog. (b) The genetic sequence of a non-pointed cat on top and the sequence of a pointed cat below. The mutation is underlined and in bold. (c) A dominant white Siberian cat with yellow eyes had one allele for points, but the other allele had a normal DNA variant. This variant did not change the amino acid; this is silent. A genetic test that incorporates this sequence surrounding the mutation for points may fail in some cats, producing allelic dropout. The cat would have a result of homozygous for points. All pointed cats must have blue eyes, thus this variant was identified and the genetic assay changed to account for this possibility in the flanking genetic sequencing.

mutation in the gene *Tyrosinase*, which causes the “points” mutation common to Siamese cats,³⁷ is presented in Figure 1a. Some positions in the sequence have different amino acids between the species, more between cats and humans than between cats and dogs because cats and dogs have a closer evolutionary history. These protein alterations are normal differences between humans, dogs, and cats. It is the change of a glycine to an arginine within the cat that makes a cat have “points.” This simple mutation causes temperature sensitivity in the protein, the protein that produces the melanin, which then only functions in areas where the cat’s body temperature is lower: the face, paws, ears, and tail (the “points”). However, in the DNA sequence (Fig 1b), 3 nucleotides join together to code for one amino acid. Hence, the DNA sequence is 3 times longer than the protein sequence for a given protein. Four nucleotides (A is adenine, G is guanine, C is cytosine, and T is thymidine) make up DNA. The coding of amino acids has built-in redundancy. The amino acid glycine can be coded by the DNA sequence as GGG, GGC, GGA, or GGT. The single nucleotide change of the guanine to the adenine changes the amino acid from glycine to arginine, and this makes the cats have points when both copies of their DNA have the same change. A mutation can occur at any nucleotide site. If the CCT became CCC, the thymidine changes to a cytosine. This change does not alter the amino acid; it still codes for proline. This type of mutation is called

a silent mutation, because it does not affect the protein. When hunting for important mutations, the silent mutations are generally discounted, being normal genetic variation found between individuals or species. A mutation that does not change the amount expressing timing or structure of the protein generally will not have an effect on phenotype or health. However, these silent mutations and DNA variants can wreak havoc with a genetic test. For example, a Siberian cat from Italy with yellow eyes that was dominant white was presented for genetic testing to determine the underlying coat color alleles. The genetic test for the color (*TYR*) mutations suggested the cat was homozygous for the pointed mutation. If so, the cat had to have blue eyes, regardless of being dominant white. In this case, the cat had the normal, but silent, variant in the sequence around the mutation for points (Fig 1c). Notice the cat has only one allele for points, the bottom line, but the top line has the silent mutation. Because the sequence was not the normal sequence before the important mutation site, the test failed for that allele and the test result suggests the cat was homozygous for points. A good laboratory knows this can happen and has other ways to detect these anticipated problems. Because the laboratory had strong interactions with the breeders, the test was redesigned to account for this anomaly.

Genetic Testing Concerns in Hybrid Cat Breeds

The normal level of variation between cats is expected, being far less than 1% of a sequence that codes for a protein. Herein lies a problem for hybrid cat breeds. The evolutionary time between cat species is millions of years,³⁸ not hundreds to thousands between cat breeds and populations. An Asian leopard cat had a common ancestor with the domestic cat about 6 million years ago, the bobcat about 8 million years ago, the Serval about 9.5 million years ago. The Jungle cat is more closely related to a domestic cat than the leopard cat to the domestic cat. In addition, for some of these wild felid species, different subspecies have been incorporated into the breed. The DNA sequence between a domestic cat and one of these wild felid species will have many genetic differences, maybe a several percentage difference; less for the Jungle cat, more for Serval as compared with a domestic cat. The genetic differences are most likely silent mutations, but the variation will interplay with genetic assays and may cause more allelic dropout than would normally be anticipated. No genetic tests have been validated in the hybrid cat breeds, although they are typically used very frequently.

Most laboratories recognize that disease mutations are specific to breeds, but not the coat colors. The coat color mutations occurred during the early domestication of the cat before the breeds were developed, so all breeds tend to have the same mutation. This is true for all the coat color tests so far, but for the hybrid breeds, such as Bengals, Chausies, and Savannahs, some oddities in coat color and disease testing may occur. The normal DNA sequence around each one of

the mutations for coat colors needs to be evaluated in many individuals from each wild felid species to find the normal, silent mutations that occur between the wild felids and the domestic cats. At any given gene, in a Bengal, one never knows if you have one leopard cat sequence or 2 are present. Thus, the accuracy for any genetic test is not known for hybrid cat breeds. If the domestic cat alleles are present, the test will perform as expected. But one never knows when one allele or both are from the leopard cat. Generally, selection is favoring the wild felid colorations, so, inherently, the breed is selected for the DNA sequences that may cause the genetic tests to fail.

Other Inappropriate Genetic Testing

Genetic testing laboratories attempt to provide the best services for the lowest costs. Many of the newer technologies allow for higher throughput of samples, as well as performing more than one genetic test in one assay, greatly lowering costs of reagents and manpower. Many testing laboratories are seeking to be as complete as possible, providing all available genetic tests for any given species. However, in the zeal of competition, a few genetic tests that are offered in the cat do not have sufficient scientific support. Any genetic test should have a publication that can be referenced to determine the genetic sequence surrounding the mutation, and provide the statistical support for the accuracy of the mutation for conferring disease or the trait of interest in specific breeds and populations. Published abstracts are not peer-reviewed articles and do not have sufficient information to determine the accuracy of a test, thus abstracts are not appropriate references for genetic tests. Some universities or researchers have spin-off companies and their discoveries may be protected by licensure or patent, possibly never publishing the data for competitive advantage. Thus, some genetic tests can only be found with specific testing companies. Currently, the patented tests for cats include PKD, HCM, the mutations for *Tyrosinase* at the color locus that confer Siamese and Burmese style “points,” and type B blood type.³⁹ However, licensure is available for each of these tests and the patents only pertain to the United States. Some companies will violate these patents because the overall income to the university is generally very low, thus the likelihood that a university would enforce a patent would be low as the cost would be prohibitive. However, violation of genetic test patents is not encouraged and generally considered inappropriate.

Aside from patent violations, some laboratories will offer genetic tests that are not scientifically sound to appear to gain a competitive advantage. A mutation in *MYBPC3* for HCM was reported in an abstract, but was never presented in a peer-reviewed publication. No support for the risk this mutation confers for HCM in cats has been well documented, but some laboratories offer the test for this DNA variant. Laboratories will post disclaimers, often leaving the veterinarian, owner, and breeder to speculate as to the test’s overall influence to the cat’s help.

Conclusion

Genetic testing is an important diagnostic tool for the veterinarian, breeder, and owner. Genetic tests are not 100% foolproof, and the accuracy of the test procedure and the reputation and customer service of the genetic testing laboratory need to be considered. Some traits are highly desired and genetic testing can help breeders to more accurately determine appropriate breedings, potentially becoming more efficient breeders, thus lowering costs and excess cat production. Other traits or diseases are undesired, thus genetic testing can be used to prevent disease and potentially eradicate the concern from the population. Genetic tests for simple genetic traits are more consistent with predicting the trait or disease presentation, but, as genomics progress for the cat, more tests that confer risk will become more common. Veterinarians will have to weigh the relative risk of having a mutation versus having disease as part of their differentials, and breeders will have to consider risk factors along with the other important attributes of a cat for their breeding decisions.

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