

Feline Hypertrophic Cardiomyopathy (HCM) Genetic Testing

This statement is in regard to the genetic testing of HCM in domestic cat breeds. Some unnecessary and perhaps unintended controversy has arisen as of late, thus, this statement is being presented in the hopes that it will help clarify the role of genetic testing for HCM in cats.

Hypertrophic cardiomyopathy (HCM) is a recognized genetic condition in cats (1). In 2005, Drs. Meurs, Kittleson and colleagues published that a DNA alteration in the gene *cardiac myosin-binding protein C 3 (MYBPC3)* was strongly associated with hypertrophic cardiomyopathy (HCM) in a long-term research colony of Maine Coon cats at UC Davis (2). These cats were originally obtained from Maine Coon breeders for the study of HCM.

In their publication, the authors discuss the association of the DNA mutation with HCM. The DNA mutation is commonly referred to as A31P, as this DNA mutation changes codon 31 from an alanine to a proline in the amino acid sequence (i.e., protein) of cMYBPC. The authors clearly present 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 and are discussed in more detail below.

Two recent papers have shown that not all Maine Coon cats with the A31P mutation get HCM (3, 4) and one of those papers has mistakenly interpreted this lack of penetrance as being evidence that the A31P mutation is not causal (4). This interpretation is misleading, the statements below should help clarify the role of the A31P mutation in Maine Coon HCM.

To date, most cat genetic tests have had nearly complete penetrance, less variable expression and early onset. However, some imperfect examples include the *CEP290* PRA mutation in Abyssinians that has a late age of onset, some cats having subclinical disease. Some cats with the pyruvate kinase deficiency can have variable, including very mild and subclinical presentations. The interplay of various color genes often muddle the coat color of the cats. As true in humans with HCM mutations, the finding that not all cats with the A31P mutation get HCM is actually usual in the field of HCM genetic testing.

Mutations may not be 100% penetrant, thus do not always cause clinically detectable disease. Presence of HCM in an individual cat and HCM severity (expression) likely are affected by the aspects presented below:

1) Incomplete Penetrance – for some traits and diseases, even though a known causative mutation is present, 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. Probably the most simple explanation, however, is that echocardiography (cardiac ultrasound) is probably an insensitive tool for detecting mild forms of HCM in cats and so many cats with mild HCM look like they do not have HCM. Experience and bias also play roles here. Individuals who have not examined hundreds or even thousands of cats with HCM are less likely to be able to give an accurate diagnosis.

2). **Age of onset** (age-related penetrance) – some diseases have a slow progression and may not show until later in life. In humans, HCM due to *MYBPC* mutations is clearly a disease that has slow progression and commonly doesn't express until individuals are 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 two 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.

2). **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.

4) **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, hence, there is no reason to not think the same situation is true for cats. Currently there are over 1000 mutations in over 10 genes that are known to cause HCM in humans. There are only two mutations identified that cause HCM in cats, the A31P mutation in Maine Coon cats and the R820W mutation in Ragdolls (4). Both mutations are in *MYBPC3*, the most commonly

mutated gene in humans with HCM. Other breeds of cats including Bengals, Siberians, Devon Rex, and Sphynx and mixed breed cats either do not have or have an extremely low prevalence of the A31P or the R820W mutation. However, it is known that 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.

5) 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 the most costly. Because DNA primers must bind to the sequence of interest, 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 drop-out and all testing laboratories are aware of this potential source of error for a genetic test. Even direct DNA sequencing can suffer from allelic drop-out, but because a larger portion of the gene is generally amplified, which may have other DNA variants, there is a higher likelihood of detecting allelic drop-out. Laboratories will place PCR primers in different locations surrounding the mutation to lower the risk of allelic drop-out, which is often proprietary information. Thus, some laboratories have better assays than others, even if they are doing the same assay method. 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 PCR (TaqMan), restriction fragment length polymorphism (RFLP), allele-specific oligos (ASO), or even now mass spectroscopy-based methods.

6) Inaccurate Clinical Diagnosis – Ultrasonic examination of the heart (echocardiography) is the most common and the only useful method for detecting cardiac disease in cats. Several studies have evaluated HCM presence in domestic shorthair and Maine Coon cats (6-8). 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.

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 that have HCM on an ultrasound exam should be eliminated from the

breeding population. Cats with a positive HCM genetic test should be screened by ultrasound 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 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.

DON'T GIVE UP ON RESEARCH – the only way to determine the true risk is to follow cats over the course of their lifetime with ultrasound and comparing to the HCM genetic test results. Only time and continued follow-up will help determine the true relative risk that the mutations convey. Various studies have indicated higher or lower risks in different populations of cats, but none have been able to follow cats through-out 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.

References

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