

A31P DNA test and the HCM disease in Maine Coon

Introduction

HCM has long been known to affect cats, and it is the most common heart disease in cats. This is the case for both housecats and pedigree cats, and in some breeds there has been observed a higher prevalence of the disease, especially in Maine Coon (MCO) and three other breeds.¹

In 2005, veterinary Kathryn Meurs studied MCO and discovered a mutation in a gene that controls some processes in the heart, and that could be linked to the development of HCM (Meurs 2005). There has been several studies of this mutation since then. Some papers have concluded that the mutation does not have a strong connection to the disease, while others have observed stronger correlation. Many breeders test for this mutation, while others are critical and is of the opinion that it has no importance.

In this article I will try to systematize the published results, to evaluate the reasons for recommending DNA testing, as well as breeding recommendations of taking homozygous cats, and possibly also heterozygous ones, out of breeding.

The disease HCM

HCM, Hypertrophic Cardiomyopathy, is the name of a heart disease that among other symptoms show increased cardiac mass and a hypertrophied left ventricular. It can also be expressed as enlarged papillary muscles, and other symptoms. The heart will show increasing problems of working efficiently. Also observed are blood clots or small infarctions that can lead to death (Meurs 2005, s 3587).

The diagnosis can only be given through echocardiography, ultrasound, of the heart by a cardiologist, or post mortem through an autopsy.

Cats with HCM can display various degrees of symptoms, and many show no heart murmur, coughing, breathing difficulties, or any visible symptoms easy to detect. The prevalence of this illness increases with age; however it turns out that most cats who develop HCM show symptoms within 5 years of age. The disease can sometimes develop slowly and have a mild progression that can be treated, while other cases present aortic thromboembolism and sudden death (Godiksen 2013, p 95).

A final diagnosis of HCM can often only be given after autopsy. An enlarged heart can have other causes, and it is assumed that HCM also has several secondary forms, with different causes; among other examples this disease has been found in overweight cats, and fast growth can be a triggering factor in cats predisposed of HCM (Freeman 2012). Secondary HCM can be treated indirectly through the underlying illness, while primary HCM will not go into remission. It is the primary form of HCM which is linked to the DNA mutation.

¹ Persian/Exotic, Sphynx and Cornish Rex has a higher risk than other breeds, based on statistics from the PawPeds database (comparison study done by IG Herzgesunde katzen). For Maine Coon there are a much larger number of registered health results (ultrasound) since the health programme for HCM started already in 2004.

A healthy cat will normally have LV-measurement of under 5 mm, over 6 mm is a clear sign of illness (Gundler 2008), however with the precaution of reading the values in relation to the cat's size. A large cat of 6 kg and more will have an adjusted scale. HCM also seems twice as frequent in male cats as for females (Meurs 2005, Longeri 2013).

Hypertrophic Cardiomyopathy

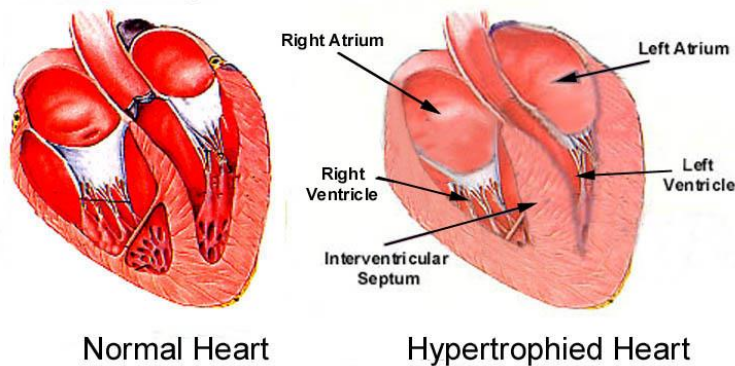


Illustration from Vetbook.org.

Prevalence of the disease in Maine Coon

It is difficult to find large enough overviews for how frequent the disease actually is in Maine Coon, but the knowledge of HCM in the Maine Coon breed is much larger than for most other breeds, because of the health programme and research. Godiksen refers to a total prevalence of HCM: between 9,5 % and 26,3 % of all MCO. Experienced cardiologist veterinaries discussed the issue at a work meeting, and estimated the number of affected MCO at approximately 5 % (Skålnes). Results from the PawPeds database also indicate that the number of cats with HCM diagnosis from breeders who participate in the health programme, has gradually decreased during 1998 to 2011, since the registrations started.

Possible sources of error that make the prevalence number very roughly estimated are the low number of cats in the research studies, and that some cats who die from clinical HCM are not correctly diagnosed. Since there is no death cause registry of cats, the numbers will always be the victim to some uncertainty.

Mutations in MyBP C3 in MCO and RAG

Kathryn Meurs discovered the A31P mutation through her studies of a colony of Maine Coon where many cats suffered from severe HCM. These cats had their DNA sequenced and analyzed, looking for changes that could explain the disease. Measuring protein levels in the cats with HCM, it was discovered that the level of myosin binding protein c (MyBPC) was lower than for healthy cats. Accordingly the MyBPC3-gene was target for the search for mutations, and in the affected cats changes were discovered as predicted, the A31P-mutation (Meurs 2005, p 3591).

From 2005 to 2013 several important papers have described the connection between A31P and HCM in Maine Coon. Wess studied German and Austrian cats, Mary studied mainly

French Maine Coon, but also cats from other European countries, Godiksen had Danish cats in her study, and Longeri covered American and Italian cats.

Differences appear in the gene frequency of A31P in Europe and the US: Approximately 20 % of American Maine Coons are registered with the mutation, whereas for the European cats over 35 % and perhaps up to 40 % seem to have it (Longeri 2013, s 6), with numbers being on the lower side in Germany, and higher in Italy and France. Fries tested 3310 cats and found a prevalence² of 34 % A31P in MCO. The A31P-mutation has never been found in other breeds or housecats.

For Ragdoll another mutation in the same gene MyBPC3 is discovered, R820W. This has not been studied as much; however it seems to have stronger penetrance, with lower gene frequency (Meurs 2007, Ohlsson). Unfortunately there are few numbers available here since research has been focusing on Maine Coon.

Inheritance

The disease is considered to be dominant with incomplete penetrance and variable expression. This means that it is highly variable and individual if, when and how the disease is expressed. For heterozygous cats not everyone will get HCM, and those who are affected will normally show symptoms much later than the homozygous, and in a milder form.

For the homozygous positive there will also be exceptions with cats who stay healthy for years, and a few cats seem to become old without showing any signs of HCM disease.

Since the gene frequency has been registered as high, breeding policies and advice have mainly been limited to advise against hetero-/hetero- matings, and to avoid using homozygous positives. In the 8 years since the test for A31P became available, it does not seem from the research that the breeders have tested and selected away from the mutation. One reason can be that the some have expressed doubt about how essential A31P is for developing HCM.

Mating 2 heterozygous N/A31P + N/A31P	N	A31P
N	N/N	N/A31P
A31P	N/A31P	A31P/A31P

Mating heterozygous N/A31P to free N/N	N	N
N	N/N	N/N
A31P	N/A31P	N/A31P

² «Prevalence» is here used about both hetero- and homozygous, and is not parallel to gene frequency.

Mating 2 N/N	N	N
N	N/N	N/N
N	N/N	N/N

Correlation between A31P and ultrasound diagnosis

Since Meurs published her article in 2005, several research studies have dealt with the possible correlation between A31P and diagnosed HCM. Common for all of them is that cats evaluated as *equivocal*³ in the echocardiography are not included in the analysis, in order to get more exact results. It is known that several of these *will* develop HCM later.

Carolina **Carlos-Sampedrano** published in 2009 a study bases on 96 Maine Coon both DNA-tested and examined with echocardiography. 17 % of these cats had HCM. They find that homozygous positive cats more often suffer from HCM, however with only 16 HCM-affected cats in the study it is too small for a final conclusion.

Dr. Gerhard **Wess** in Munich presented April 2010 a study of 84 Maine Coon, where the A31P-mutationen was tested in addition to a SNP⁴ called A74T or the «Koch-mutation». All cats were examined for HCM with ultrasound. Average age of the cats was 5 to 6 years. 12 of them were diagnosed with HCM. A total of only 18 (22 %) of the cats in this study had the A31P-mutation, significantly lower than the average for European MCO. Wess concluded that the mutations in the MyBPC3-gene did not have any significance for the development of HCM in Maine Coon.

Dr. Jérôme **Mary** in France published a few months later, June 2010, a larger study. 2744 MCO were tested for the A31P-mutation, and the gene frequency found to be as high as 41,5 % in these European cats. 164 of them were also examined by echocardiography. Average age for these cats were 2,6 years, young animals.

Results and conclusions differ substantially from Wess' study. Of the 164 cats there were a total of 109 negative N/N of which only 2 showed HCM through ultrasound. 5 of 7 homozygous positives were affected, as were 12 of the 48 heterozygous (HCM/N). Penetrance for the disease were calculated to 71 % for the homozygous cats. Compared to the cats tested free of A31P those who had the mutation were overrepresented in the affected cats with 83 %. "MYBPC3-A31P mutation was therefore significantly associated with

³ LV-thickness between 5-5,5/6,0 mm and thus ambiguous results

⁴ Single Nucleotide Polymorphism

an increased risk of HCM. The risk increased when cats were homozygous for the mutation” (Mary 2010, s 158) is their conclusion.

In 2011 Mia **Godiksen** published a study with 332 Maine Coons both DNA-tested and examined by echocardiography. They find that cats who develop A31P-related HCM before 6 years of age are mostly homozygous positive. The disease has low penetrance in young heterozygous cats, and half of the 21 HCM-affected cats in the study do not have the mutation. Summed up in the conclusion:

“Furthermore, due to the high probability of developing fHCM in the p.A31P cMyBP-C homozygous cats the ‘production’ of homozygous MC cats should be avoided. Thus, although genotyping of the p. A31P cMyBP-C mutation can not stand alone in limiting fHCM in MC, it is very important that breeders are aware of the genotype status and breeders should be informed of breeding recommendations.” (Godiksen 2011, p 10).

Veterinary Niels **Pedersen** DVM is a researcher at Veterinary Genetics Laboratory, University of California in Davis, the lab that took part in developing a test for the A31P-mutation. In 2011 he published a summary of the research on A31P published by then, and concludes:

“Therefore, cats with one copy of the mutant allele are 1.8 times more likely to develop HCM than cats carrying normal alleles. Cats with two copies of the mutant allele are 18 times more likely to develop HCM than cats carrying normal alleles and 10 times more likely to develop HCM than cats with one copy of the mutant allele” (Pedersen 2011).

The extensive research study led by Maria **Longeri** was published at the turn of 2012-2013, and presented even more important conclusions. In short 533 MCO in Italy and the USA were examined, 208 of these both DNA and ultrasound. Cats homozygous for A31P show a clearly increased risk of HCM and will usually develop the disease before middle age; heterozygous cats also have increased risk, but much smaller than the homozygous, and the heterozygous cats are normally affected later in life, over half being well at 5 years of age.

The study also demonstrates how penetrance is related to age; the homozygous cats over 36 months old were all diagnosed with HCM. “However, this study also demonstrated that Maine Coons homozygous for the A31P mutation usually develop HCM and almost all do by the time they are middle-aged. These results were strengthened and corroborated by performing a meta-analysis using data from previous studies.” (Longeri 2013, p 6).

The research team here is also clear that breeders must *both* echo and DNA-test their breeding animals:

“Based on this study and previous studies, the Maine Coon A31P heterozygotes usually lack evidence of HCM during the years at which they would most commonly be bred. Even homozygotes for the A31P mutation might not have evidence of HCM until they are closer to middle age. Consequently, echocardiographic screening, especially of young cats, should not be the sole diagnostic to identify HCM-potential cats because genetic screening is needed to identify cats with the HCM-associated mutations. At the very least, Maine Coon breeders should genotype their cats to make sure they are not breeding heterozygous to heterozygous cats and thereby producing cats homozygote for the A31P mutation.” (Longeri 2013, p 7)

Summarizing we see that the conclusions about A31P and causality of HCM-diagnosis become clearer for every study.

All the studies emphasize that there are exceptions, with cats homozygous for the mutation with no signs of disease, even at a high age, pointing to the variable penetrance of the

mutation. It is also important that some cats who get HCM do *not* have the A31P mutation, thus other causes also exists.

Table 1 show clearly how homozygous positive cats have a much higher risk than N/N-tested cats for HCM. The majority of the cats in the studies are young, so the real numbers would be higher if one followed the animals throughout life.

Comparing all numbers from the studies (Table 2), a more complete picture appears. The number of cats studied, gene frequency of A31P for these cats, and the number of affected cats can all influence the conclusions of each separate study.

Wess who concluded that A31P was without significance also had the smallest study, and gene frequency of A31P was remarkably lower than in the other studies. The number of HCM-diagnosed cats were also higher than assumed prevalence. This could have influenced the conclusion. Longeri who also did a meta-analysis from Mary and Wess had similar values for disease prevalence and gene frequency as seen in Table 2.

The first study	Cats tested: DNA and ultrasound	A31P-test above, HCM diagnosis below				Notes and remarks
		total	N/N	N/HCM	HCM/HCM	
Meurs 2005	23	23	7 0	10 10	6 6	16 affected and 7 healthy cats from a colony of related MCO.

Meurs' numbers are not included in the summary, as her study is not statistically representative.

Table 1 Summary of research papers. US = ultrasound/echocardiography

Main author Year	DNA US	A31P-test over, HCM diagnosis under				Age	Notes and remarks Conclusions
		total	N/N	N/HCM	HCM/HCM		
Longeri 2013	533 208	208	139 7	57 5	12 7	48 > 3 yr all homozygous over 3 yrs ill	Double A31P is high risk for HCM: develops disease before «middle-age», while heterozygous has low risk. A74T no influence on HCM.
Godiksen 2011	332	332	225 10	89 2	18 9	282 < 4 yr 50 > 4 yr	No heterozygous cats under 4 yrs, assessed to low risk. Breeders should know A31P-status never «produce» homozygous cats due to high risk. 2 of 2 homozygous over 4 yrs ill.
Mary 2010	2744 164	164	109 2	48 5	7 5	2,6 yr avr	A31P-mutation only in MCO (extensive) and of high importance for developing HCM. 164 of total 2744 also US-tested
Wess 2010	83	83	65 9	15 2	3 1	5,5 yr avr	Fewer A31P-cats in study than average for Europe.
Carlos Sampedrano 2009	96	96	52 2	38 4	6 4		10/38 N/HCM without HCM < 4 years, All w HCM < 5 yr, 45 < 2 yr
TOTAL cats	3788 883	883	590	247	46	Mostly young	Unfortunately few cats over 4 years in the studies. No equivocal cats included.
w HCM-diagn.		67	23	18	26		
HCM frequency by A31P status		all	N/N 3,90 %	N/HCM 7,03 %	HCM/HCM 56,52 %	NB: most cats < 4 yrs	Real numbers would be higher if cats were followed through lifespan.

Table 2: US = ultrasound

Main author year	DNA	US	A31P-test over, HCM diagnosis under				# cats with med HCM-diagnosis	Gene frequency A31P	# cats w A31P
			total	N/N	N/HCM	HCM/HCM			
Longeri 2013	533	208	208	139 7	57 5	12 7	9,13 %	19,47 %	33,17 %
Godiksen 2011	332	332	332	225 10	89 2	18 9	6,32 %	18,82 %	32,23 %
Mary 2010	2744	164	164	109 2	48 5	7 5	7,32 %	18,90 %	33,54 % (41,5 %)
Wess 2010	83	83	83	65 9	15 2	3 1	14,46 %	12,65 %	21,69 %
Carlos Sampedrano 2009	96	96	96	52 2	38 4	6 4	10,42 %	26,04 %	45,83 %
TOTAL cats	3788	883	883	590	247	46		19,20 %	33,18 %
w HCM-diagn.			67	23	18	26	7,59 %		

Summed up these studies show:

- Homozygous cats have a highly increased risk to get HCM, with few cats without signs of illness at 4-5 years of age.
- Heterozygous cats also have an increased risk, but the disease strikes much later and often after 5-7 years of age. Over half of the cats are healthy at 5 years of age.
- A large number of the HCM-afflicted Maine Coons do have this mutation.

The last chapter has not been written on this topic: A group of cardiologists, including several of the researchers behind the studies referred to here, collaborate in the *Feline Cardiomyopathy Consortium* (FCC). There will be further research from this group, and Dr. Jens Häggström in Sweden is expected to publish a larger study including many cat breeds.

Several researchers work on looking for further mutations that can cause HCM, both in Maine Coon and other breeds. All Maine Coon breeders and owners, and those with other breeds with increased risk for HCM,⁵ can contribute by submitting blood samples of their cats together with the results from echocardiography/ultrasound, to the veterinaries in FCC. This is especially important for Maine Coon with HCM who do not have the A31P-mutation, and other breeds with no known mutations correlated to HCM.

Health Programmes for Maine Coon

For Maine Coon there is a health programme organized by PawPeds: HCM echocardiography and HCM-A31P DNA test. Breeders with heterozygous breeding animals should select N/N offspring as future breeding stock to eliminate the mutation.

In FIFe both echocardiography and DNA-testing HCM-A31P are recommended. None of the other international registries have guidelines related to HCM MCO.

Ultrasound vs DNA-test as breeding tool to reduce risk

Research concludes that *both* ultrasound scans and DNA-testing are important to trace the cats with a high risk of becoming affected by HCM, to avoid breeding them. Both methods

⁵ Breeds with increased risk: EXO/PER, RAG, BRI, SPH (mentioned in FIFe); SIB, CRX, EUR, CHA, BEN, NFO and Am Shorthair (mentioned by Longeri 2013).

combined are necessary to lower the prevalence of HCM in the breed. However there are quite a few differences between these screening methods regarding accessibility, price level, reliability, and practical opportunity to follow up.

The DNA-test is only needed once, and offspring of parents tested free will stay free of the mutation. They do not need to be retested except sporadically as control for lab errors. The test is performed once per cat and will cost 25-70 €. The testing can be performed by any veterinary, who will submit the sample to one of several recognized laboratories. A DNA-test performed correctly and analyzed at a laboratory with high quality routines will be very reliable. Thus it is both very easy and inexpensive to eliminate HCM-risk by the known mutation, and there are no excuses not to know the status for one's breeding cats.

Echocardiography is more complicated for the breeder. The examination must normally be performed five times during the life of a breeding cat⁶ if the breed is to benefit from the scans, and an absolute *minimum* of three times if the worst cases are to be discovered and removed from the breeding stock: before the first mating, at appr 3-4 years age, and at an older age (at least over 5-6 years) since several cats who develop HCM have no symptoms until over 5 years old. A cat tested normal through ultrasound 4-5 years old can still develop HCM some months later. Many breeding cats are at that time neutered and placed in pet homes, and registration show that only few cats are actually examined that late in life.

The price of an echocardiography is € 100-250 and needs to be repeated several times in the cat's life: before being bred, and about every other year until old age. This adds up to a significant amount for each breeding cat.

Cardiologist veterinaries cannot be found everywhere, and are not easily accessible for breeders living in more remote locations. Ultrasound examinations can be subject of a certain degree of error, depending on the veterinary's equipment, competence and experience. Cardiologists specializing in HCM diagnosis warn about trusting results from veterinaries with no specialization in cardiology. Interpretation of ultrasound can thus be more subjective than a DNA-test performed correctly.

These factors together cause very few breeders to fully follow the health programme for HCM. With gradual elimination of the A31P-mutation, the total risk for HCM in Maine Coon will get lower, and focus can be moved to other causes of the disease.

Neither echocardiography nor DNA-testing can guarantee *any* cat "free of HCM". Breeders who claim this for their kittens can be accused of promising too much in their ads, and no matter what testing is done or not, breeders need to be careful in giving guarantees regarding kittens' future health. One can only do one's best, and following the health programme is a good way to do this.

⁶ Health programme PawPeds recommendations: before 1. mating/1 yr, 2 yr, 3 yr, 5 yr and 8 yr, thus normally 5 scans during the cat's life. If the cat is bred late one can «get away with» 3. Cardiologists point out that the worst cases are discovered at the first scan, and that ultrasound before first mating/at 1 year old is the most important. To catch all cats who develop HCM late, scanning at 8-9 years age is also highly important.

Identifying lines free of A31P, but with a higher risk of HCM, should be priority for future research. Blood samples of cats with HCM-diagnosis are used in research of other causes than the known mutations. Full genetic sequencing of these cats are done to map other genetic causes that might be tested in the future, both for Maine Coon and other breeds.

Summary and Conclusion

From the results of all the studies, it is clear that there is a strong correlation between the A31P mutation and development of HCM. It is also clear that HCM is found among cats without this mutation, so there are other causes in addition to A31P. *Gold standard: Both DNA-testing with a gradual selection away from the mutation from the gene pool, and repeated ultrasound scans (echocardiography) are necessary to limit the cases of HCM in the Maine Coon breed.*

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