Study of *MYBPC3* Gene A31P Mutation (c.91 G>C) Causing Hypertrophic Cardiomyopathy in Maine Coon and Other Breed Cats

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Abstract. *Hypertrophic cardiomyopathy (HCM) is one of the most common heart diseases among cats. The aim of the study was to find out the prevalence of the missense A31P mutation of the MYBPC3 gene in the feline population and its dependence on breed and sex. During the study, 130 individuals were tested by PCR-RFLP method. The 242 bp PCR product was digested with AvaI restriction enzyme and the fragments were separated by electrophoresis in 2.5% agarose gel. Besides that, retrospective analysis was performed based on data from the veterinary clinic, collected in a three years period. The mutation (G/C genotype) was detected only in the Maine Coon breed. Data analysis showed that frequency of heterozygous genotype in the tested Main Coon population was 0.23, which is equal to 13.85% of all the studied feline population and 23.1% of the tested experimental (Maine Coon) group. Allele frequencies were calculated in the experimental group (Maine Coon), the control group (feline of other breeds) and for all tested individuals. In all groups, the most frequently repeated allele was G, and in the control group, it was the only detectable allele. Frequency of the C (mutated) allele was calculated for all individuals (0.07) and for experimental (Maine coon) (0.115) groups. Statistically significant Main Coon breed dependence on the mutation was assessed ($P < 0.05$). Information analyzed retrospectively showed that HCM was more often diagnosed for males (87%) than females.*

Introduction

Hypertrophic cardiomyopathy (HCM) is characterized by an abnormal increase in myocardial mass that affects cardiac structure and function. HCM is the most common inherited cardiovascular disease in humans (0.2%) and the most common cardiovascular disease in cats (14.7%) (Gil-Ortuño et al., 2020). Domestic cats of any age from 3 months upward, of either sex and of any breed, can be affected. A higher prevalence in male and domestic shorthair cats has been reported (Kitz et al., 2019).

Feline hypertrophic cardiomyopathy (HCM) is defined by an unexplained thickening of the left ventricular wall without dilation of the chambers and in absence of any other cardiac or noncardiac disease that itself is capable of causing hypertrophy of the heart (Kittleson et al., 2021).

Feline HCM has long been called an idiopathic disease. Viral infections have been implicated as the cause of cardiomyopathy in several mammalian species (Machado et al., 2010). Recently, however, there has been a growing trend towards a different – genetic – diagnosis of HCM. Genetic HCM is thought to occur in American Shorthair, Maine Coon, Persian, Norwegian Forest, Ragdoll and Sphinx cats. Based on research in human medicine in the diagnosis of HCM, the genetic origin of feline HCM was first identified in 2005 by a sequence analysis of the gene encoding sarcomer proteins (Wess et al., 2010; Connolly et al., 2005). More than 1500 variants in *MYBPC3, MYH7* and other sarcomeric genes are associated with human HCM, while in cats, only two causative variants in *MYBPC3* are currently known (Schipper et al., 2019). For the first time, the A31P mutation in the *MYBPC3* gene was found for Maine Coons. It is supposed that about 34 percent world Maine Coon population has the missense A31P mutation (Mary et al., 2010). Frequent prevalence of the disease in Maine Coon population for which this mutation has not been identified indicates that it is probably not the only mutation causing HCM in this breed.

The A31P mutation (c.91 G > C). A change in the nucleotide sequence of the DNA was detected at codon 31, nucleotide 91 of the third exon of the *MYBPC3* gene on chromosome 11, in which the conserved guanine (G) was replaced by cytosine (C). Such a change in the nucleotide sequence at the codon resulted in a change in the GCC triplet, which encodes alanine, to the CCC triplet, which encodes proline. There are two ways to term this mutation: the first is A31P, which contains the codon number and exchanged amino acids, and the second is c.91G>C, which indicates the nucleotide number of the DNA

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and substituted nucleotides (Meurs et al., 2005).

The prevalence of the A31P mutation was observed among Maine Coon. In one study, 34% of the studied Maine coons had this mutation and 10% of them were homozygous. This study also suggests that the mutation is observed only in the Maine Coon breed cats (Fries et al., 2008). However, in 2010, an A31P mutation was also detected in one cat of British Shorthair (Mary et al., 2010). The prevalence of the A31P mutation in the *MYBPC3* gene has not been analyzed so far among Maine coons and other breeds of cats bred in Lithuania.

As in humans, the HCM causing mutation in cats may have different penetrantity. Mutation manifestation phenotypically was found to be in 6–8% cats with a heterozygous genotype and 58–80% with a homozygous genotype. Therefore, even one cat of the same origin may have minimal heart changes and others have a severely developing disease. Such differences between genotypes and phenotypes indicate that there may be other genetic and environmental factors that influence the development of a particular phenotype (Kittleson et al., 2015).

So far, the genetic alterations that cause HCM have been identified in the gene *MYBPC3*, which encodes the cardiac isoform of myosin-binding protein C (cMyBP-C). This isoform consists of 2% of myofibril proteins in the heart and is important in the regeneration of muscle cells. Decrease in cMyBP-C and myomezin protein levels were observed in cats affected by the mutation. cMyBP-C is a protein that regulates the heart muscle, which affects the power and the rate of heart contractions. It also contributes to cardiac systolic and diastolic function and the ability of the heart to increase contractions under the influence of an inotropic stimulus (Sadayappan et al., 2012).

Due to the frequent occurrence of HCM in cats, a clear mechanism for the onset of the disease is very relevant in veterinary medicine. Therefore, the aim of the research was to evaluate the prevalence of the A31P mutation in the *MYBPC3* gene in feline population, its dependence on different factors such as breed and sex. Detection of the mutation allows preventive measures to be taken to ensure the well-being and health of cats at a risk of HCM.

Material and methods

MYBPC3 gene polymorphism study

A total of 130 individuals were studied. Biological samples were taken from various breeds of cats (*Table 1*). Samples for research were collected from different regions of Lithuania. They were divided into two parts: control and experimental groups. The control group consisted of cats of various breeds (Scottish Fold, British Shorthair, Siamese, Devon Rex, Persian, Bengal and crossbreed), and the experimental group was comprised only of Maine Coon cats. DNA was isolated from buccal epithelial cells (Aidar et al., 2007). The *MYBPC3* gene polymorphism study was performed by the PCR-RFLP method. This test was used to identify a mutated allele that forms when a missense mutation occurs, guanine changes to cytosine at codon 31 of the third exon (c.91G>C). According to feline *MYBPC3* gene sequence (GeneBank accession No. NC_018732.3), using

Table 1. Distribution of studied individuals by breed and gender

No.	Breed	Number of tested individuals	Females	Males
1.	Maine Coon	78	57	21
2.	Scottish Fold	20	11	9
3.	Persian	3	1	2
4.	Devon Rex	1	0	1
5.	British Shorthair	10	1	9
6.	Crossbreed	13	3	10
7.	Bengal	3	2	1
8.	Siamese	2	0	2

Table 2. Primers, PCR reaction conditions, size of PCR product and restriction enzyme

Patin7Genetic defect	Primers	PCR profile			PCR product size, bp	Restriction enzyme
Hypertrophic cardiomyopathy (HCM)	F: 5'-agccttcagcaagaagcca-3' R: 5'-caaacttgaccttggaggagc-3'	95°C	3 min		242 bp	*AvaI*
		94°C	30 sec	35 cycles		
		56°C	30 sec			
		72°C	40 ses			
		72°C	7 min			

"Primer3" software, oligonucleotide primers were designed. The PCR mode used in the study is shown in *Table 2*. The primers amplify 242 bp fragment. The obtained PCR product was digested with a restriction enzyme. The appropriate restriction enzyme (*AvaI, Thermo Scientific*) was selected by the "CLC Sequence Viewer 8" program. 10 µL of the PCR product was digested with 10 µL of the restriction mixture. Samples were incubated in a thermostat overnight at 37°C. The digested PCR products, stained with 10 µL of ethidium bromide, (CS-300V Cleaver; Scientific Ltd), were fractionated by electrophoresis at 2.5% agarose gel (1 × TAE) for 45 min. Then they were analyzed under UV light (wavelength 300 nm) with a "MiniBisPro" video documentation device (Herolab). *MYBPC3* gene c.91G>C DNA fragments sizes after digestion with the restriction enzyme were: homozygous genotype for the normal allele (G/G) – 242 bp, heterozygous genotype (G/C) – 242, 179, 63 bp, homozygous genotype for the mutated allele (C/C) – 179 and 63 bp. (Fig. 1).

Data for retrospective analysis was collected at the veterinary clinic. Information was collected from patients who referred to the clinic for heart problems. A medical history of the animals was collected during the visit, including the conditions of animal keeping, preventive measures, changes in behavior, general well-being of the animal. Patients underwent general and specific tests.

Fig. 1. Analysis of the genotypes in the agarose gel. A– G/G genotype; B – G/C genotype; C – 50 bp DNA ruler (Thermo Fisher Scientific).

Statistical Analysis

IBM SPSS Statistics software was used to interpret the results. The frequencies of the obtained results were calculated using the Descriptive statistics FREQUENCIES function, averages with the Compute variable MEAN function. The Descriptive statistics Crosstabs CHI-SQUARE function was used to calculate the significance between the different variables. Results were considered significant, when $P < 0.05$.

Results

Genotype frequencies were calculated separately for the experimental (Maine Coon, n = 78) and for the control group (various breeds, n = 52). The calculated data show that in both cases the genotype (G/G) leading to the absence of the mutation is more common. However, comparing the frequencies of the experimental group and the control group genotypes, the frequency of the heterozygous genotype was higher in the experimental group (Table 3).

Breed dependence on the mutation was assessed. A heterozygous genotype was detected only in the experimental (Main coon) group. A statistically significant result was obtained ($P = 0.0002$, $P < 0.05$) (Fig. 2).

Allele frequencies were calculated in the experimental (Maine Coon), the control groups, and for all tested individuals. In all groups, the most frequently repeated allele was G, and in the control group, it was the only detectable allele. Frequency of the C allele was determined in the experimental (0.115) and all individuals (0.07) group (Table 4).

Another relevant aspect of outcome evaluation is the distribution of mutation by gender. Seventy-five females were examined, which accounted for 57.7% of all subjects, and 55 males, which accounted for 42.3% of all studied subjects. Heterozygous genotype (G/C) was identified for 18 cats: 14 females (78%) and 4 (22%) males. However, in the analysis of the data only for cats of the Maine Coon breed, the percentage of females with the heterozygous genotype was 24.56%, and the number of males was 19%, respectively, based on the number of individuals of each gender studied.

After collecting data at the veterinary clinic, it was determined that 15 cats were diagnosed with HCM in the three years period. It was observed that the disease was diagnosed more often in male individuals: 13 males (frequency 0.867). Less frequently, the disease was diagnosed for females (frequency 0.133).

The frequency of the age group at which HCM was diagnosed was calculated. The most common disease was diagnosed in the youngest cats' group (frequency 0.467) (Table 5), and less often in the older cats' group. The mean age of diagnosis with HCM was 4.8 years (Fig. 3).

Table 3. Genotypes frequencies table

Frequencies of experimental group genotypes		Frequencies of control group genotypes	
G/G	0.769	**G/G**	1
G/C	0.231	**G/C**	0
C/C	0	**C/C**	0

Table 4. The frequencies table of alleles

Allele	Experimental group	Control group	All tested individuals
G	0.885	1	0.93
C	0.115	0	0.07

Table 5. Age groups and frequency table

Age group, years	Number of individuals with HCM	Frequency
1–3	7	0.467
3–6	5	0.333
6–9	0	0
9–12	2	0.133
> 12	1	0.067

Discussion

Samples were collected from both males (55) and females (75) belonging to Maine Coon and other breed cats (Scottish Fold, British Shorthair, Siamese, Devon Rex, Persian, Bengal and crossbreed). The mutation (G/C genotype) was detected only in the Maine Coon breed. The prevalence of the A31P mutation was not determined in the other studied breed cats. That makes 13.85% of all studied feline population and 23.1% of the tested experimental (Maine Coon) group. Breed dependence on the mutation was assessed ($P = 0.0002$, $P < 0.05$). The same results are also confirmed by other researches. HCM is one of the most common diagnoses in cats, and thus far only two genetic variants have been reported to be associated with this disease (Ontiveros et al., 2019). According to O'Donnell et al., the lack of the MYBPC3 p.Ala31Pro and other mutation variants in feline HCM population suggests that the clinical utility of genetic testing is greatest in the specific cat breeds in which these causative variants of HCM have been identified (O'Donnell et al., 2021).

Compared with the results obtained by other authors, a similarity is visible. Mary et al. identified a breed dependence of the mutation. Of the 2744 Maine Coon cats tested, 41.5% had the A31P mutation detected. However, in this study, a mutation was also detected in one cat of the British Shorthair breed (Mary et al., 2010). Godiksen et al. indicate that the prevalence of HCM causing mutation in the Maine Coon population is between 9.5% and 26.3% (Godiksen et al., 2011).

When evaluating the distribution of the mutated allele according to the gender of the studied cats,

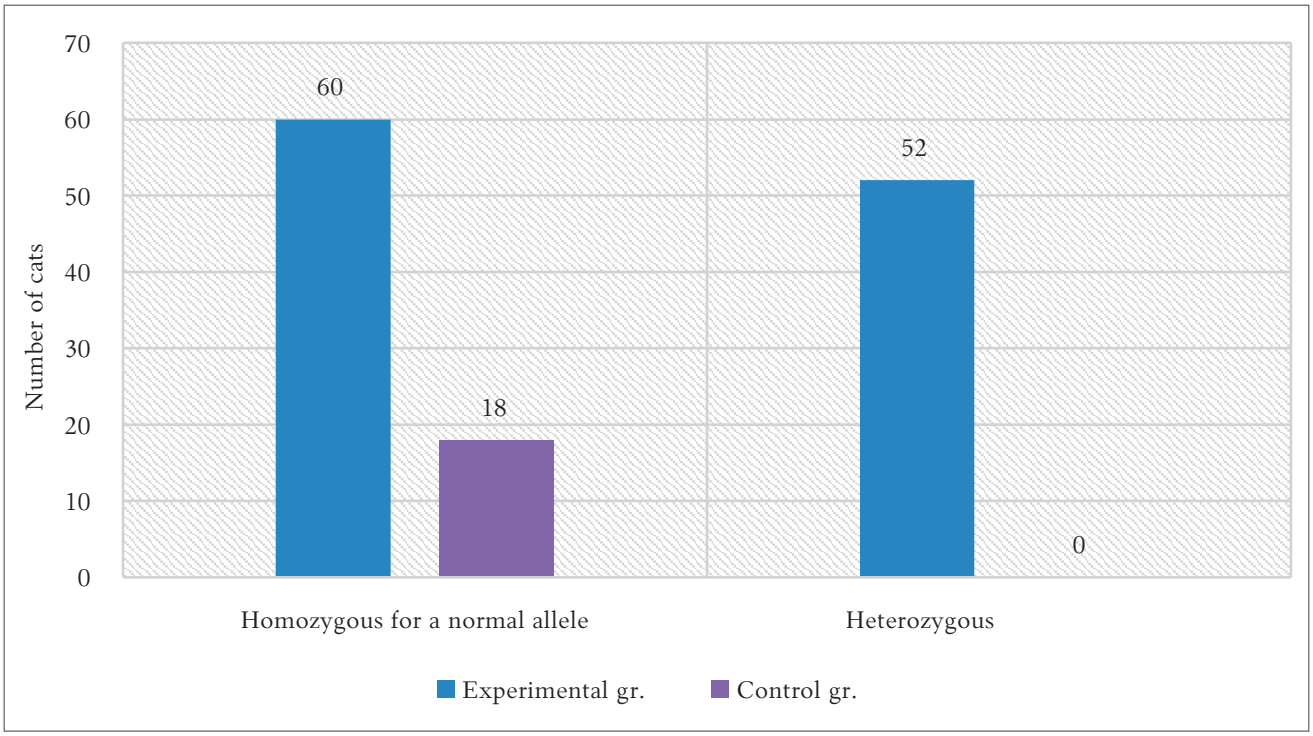

Fig. 2. Distribution of genotypes in experimental and control groups.

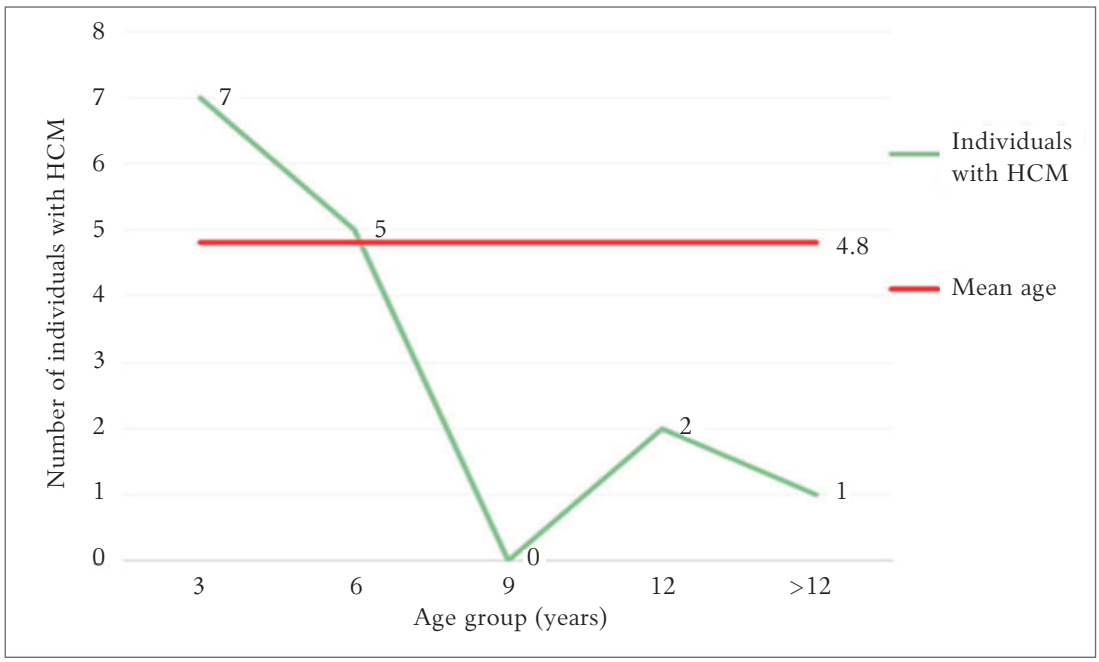

Fig. 3. The number of individuals diagnosed with HCM by age group and mean age.

only the experimental group was analyzed, since the mutated allele was detected only in it. Females with a heterozygous genotype accounted for 24.56%, and males for 19%, respectively, of all tested representatives of each gender.

After information analysis collected at the veterinary clinic, it was observed that the disease was diagnosed more frequently in males (frequency 0.867). This accounted for 87% of all samples. Less frequently, the disease was diagnosed in females (frequency 0.133).

According to Payne et al., the prevalence of the disease is higher in males (62.6%) than in females, suggesting that sex may be one of the predisposing factors, leading to the manifestation of the mutation (Payne et al., 2015). Analysis of Longeri et al. research results also showed the incidence of the disease by gender: HCM was found in 78.9% of males and only 21.1% of females (Longeri et al., 2013). According to Riesen et al., HCM is common in both sexes equally, but males develop symptoms earlier and often have a more severe form of the disease (Riesen et al., 2007).

The age dependence of HCM was assessed by analyzing data from veterinary clinic X. During a three-year period, the disease was the most often diagnosed in the youngest cats' group, aged 1–3 years (46.7%), and slightly less in the middle age cats' group, aged 3–6 years (33.3%). The mean age at which HCM was diagnosed was 4.8 years.

According to Payne et al., the prevalence of the disease was found in 4.3% of young cats (6–12 months of age) and 29.4% of older cats (⩾ 9 years). However, it is thought that age may vary depending on the breed. Maine Coon, Sphinx, British Shorthair and Rag cats have a diagnosis of the disease ranging from 5 months to 4.2 years. This is thought to have led to a higher incidence of younger cats in the study. According to Payne et al., the mean age of diagnosis of HCM in the feline population is 6 years (Payne et al., 2015).

Conclusions

The A31P mutation (c.91 G>C) was detected only in the Maine Coon breed. Based on the data of the study, it can be stated that the nucleotide substitution of the MYBPC3 gene that determines HCM is specific and prevalent only in cats of the Maine Coon breed. However, no significant relationship between the gender and the genotype of the mutated allele was found. On purpose to select cats for prophylactic testing to detect the disease at an early stage or to reduce the incidence of the mutant allele causing the disease, especially in the Maine Coon population, it is most appropriate to perform molecular genetic testing.

Additional information

All tests were performed in accordance with the requirements of national and European Union legal acts: Law of the Republic of Lithuania on the Care, Keeping and Use of Animals No. VIII –500/1997; and the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Conflict of Interest

The authors declare that there is no conflict of interest.

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