The prevalence and phenotypic progression of hypertrophic cardiomyopathy in domestic cats of New Zealand

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“The noblest pleasure is the joy of understanding.”

— Leonardo da Vinci

Abstract

Hypertrophic cardiomyopathy (HCM) is considered the most common heart disease in domestic cats (*Felis catis*). However, the prevalence of HCM in the general feline population is unknown outside the United Kingdom and United States of America. Furthermore, the cause of HCM is unknown in most cats, and the pattern of disease progression is markedly heterogeneous, so much so that some cats will die due to HCM within months of diagnosis, but many live for many years without developing any clinical signs.

To describe the prevalence of HCM in New Zealand and better understand the pattern of disease progression, five studies were conducted. The aims of these five studies were to 1) describe the prevalence of feline HCM in New Zealand, 2) describe the pattern of HCM phenotypic development, 3) describe the HCM phenotypic progression with a specific focus on the subtype of HCM where the blood flow is obstructed due to systolic anterior motion of the mitral valve (SAM), 4) describe the influence of psychological or physiologic stress as an environmental cause of feline HCM, and 5) investigate the pattern of HCM disease progression and a potential genetic cause of HCM in Sphynx cats in New Zealand.

Prospective screening of non-purebred cats in the Centre of Feline Nutrition, which serves as a convenience sample of the general feline population in New Zealand, showed that 15.2% (95% confidence interval: 9.5-22.4%) had echocardiographic evidence of subclinical HCM. However, a retrospective review of the 10-year mortality data showed that only 3.8% (95% confidence interval: 1.2-8.6%) cats died due to complications of HCM, highlighting that many cats with HCM do not develop evidence of heart disease.

Serial echocardiography of cats in the Centre of Feline Nutrition revealed that the anterior mitral valve leaflet increased in length by median 0.5 mm per year (95% CI: 0.4-0.7 mm per year, P <0.001), prior to developing HCM in cats. The anterior mitral valve leaflet length did not further increase in cats that had already developed HCM. Additionally, the left ventricular wall thickness increased both in cats with HCM, median 0.4 mm per year (95% CI: 0.2-0.5 mm per year, P <0.001) and in cats without HCM, median 0.5 mm per year (95% CI: 0.4-0.7 mm per year, P <0.001).

Some of the cats with HCM gained SAM over time, while some lost SAM over time, highlighting that SAM is a labile process in feline HCM. In a retrospective study

of 60 cats with HCM from two referral centres over a median 2.1 years of follow-up (minimum 1.0 years; maximum 5.9 years), the yearly incidence of gain or loss of SAM in cats with HCM was 8%. Cats with SAM at the initial diagnosis were more likely to develop congestive heart failure than cats without SAM at the initial diagnosis.

Cat-Stress-Score and hair cortisol concentration are proposed markers of psychological or physiologic stress. To determine a possible role of stress in the development of feline HCM, both markers were measured in a case-control study of 20 cats (10 with subclinical HCM and 10 without HCM) that were matched by sex, neuter status, and age. Neither of these markers were associated with the diagnosis of subclinical HCM in this study, suggesting that psychological or physiologic stress is not a cause of HCM in cats.

Prospective screening of Sphynx cats owned by breeders and pet owners revealed that subclinical HCM was common in Sphynx cats with a prevalence of 40% (95% confidence interval: 28.1-53.2%). A variant in the *ALMS1* gene (*ALMS1*:ENSFCAG00000008756:c.7384G>C;p.[G2462R]) had been previously suggested to be a potential cause of HCM in Sphynx cats. However, while the allelic frequency of *ALMS1* was found to be high at 70.9% in the population of Sphynx cats in New Zealand, the presence of *ALMS1* gene variant was not associated with HCM. This suggests that the *ALMS1* gene variant is unlikely to be the sole determining factor in the development of HCM and that testing for this variant is unlikely to be a successful way to reduce HCM in this cat breed.

Acknowledgement

I completed this thesis while working full time and this would not have been possible without the support from my four supervisors, John Munday and Hayley Hunt from Massey University, and Virginia Luis Fuentes and David Connolly from The Royal Veterinary College.

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Abbreviations

2	
2D	Two-dimensional
A	
ACVIM	American College of Veterinary Internal Medicine
ALMS1	Gene encoding Alström syndrome protein 1
AMVL	Anterior mitral valve leaflet
ARVC	Arrhythmogenic right ventricular cardiomyopathy
ATE	Arterial thromboembolism
ATP	Adenosine triphosphate
AUC	Area under the curve
B	
BCS	Body condition score
C	
CAM	Systolic anterior motion of the chordae tendineae
CFN	Centre for Feline Nutrition
CHF	Congestive heart failure
CI	Confidence interval
CMD	Coronary microvascular dysfunction
CSS	Cat-Stress-Score

D	
DCM	Dilated cardiomyopathy
DLVOTO	Dynamic left ventricular outflow tract obstruction
DRVOTO	Dynamic right ventricular outflow tract obstruction
E	
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
G	
GEE	Generalised estimating equations
H	
HCM	Hypertrophic cardiomyopathy
H/RC	Hypertrophic/ restrictive cardiomyopathy
I	
ICC	Intraclass correlation coefficient
ICD	Implantable cardiac defibrillator
IQR	Interquartile range
ISFC	International Society and Federation of Cardiology
IVS	Interventricular septum or interventricular septal
IVSd Max	Maximal interventricular septal thickness at end-diastole

L

LA	Left atrial or left atrium
LA/Ao	Left atrial to aortic ratio
LAD Max	Maximal left atrial diameter
LA FS%	Left atrial fractional shortening
LV	Left ventricle or left ventricular
LV FS%	Left ventricular fractional shortening
LVFW	Left ventricular free wall
LVFWd Max	Maximal left ventricular free wall thickness at end-diastole
LVH	Left ventricular hypertrophy
LVIDd	Left ventricular internal diameter at end-diastole
LVIDs	Left ventricular internal diameter at end-systole
LVOT	Left ventricular outflow tract
LVOT Vmax	Maximal left ventricular outflow tract velocity
LVWT	Left ventricular wall thickness
LVWT Max	Maximal left ventricular wall thickness at end-diastole

M

mTOR	Mechanical target of rapamycin
MR	Mitral regurgitation
MV	Mitral valve
MYBPC3	Gene encoding myosin binding protein C
MYH7	Gene encoding β myosin heavy chain

N	
NSC	Non-specific cardiomyopathy
NZ	New Zealand
P	
PM	Papillary muscles
R	
RA	Right atrium or right atrial
RCM	Restrictive cardiomyopathy
RPLax	Right parasternal long-axis view
RPSax	Right parasternal short-axis view
RV	Right ventricle or right ventricular
S	
SABP	Systolic arterial blood pressure
SAM	Systolic anterior motion of the mitral valve
SCD	Sudden cardiac death
SD	Standard deviation
SRX	Super-relaxed state
T	
TMT	Transient myocardial thickening
TNNT2	Gene encoding troponin T

U

UK	United Kingdom
USA	United States of America

V

Vmax	Maximal velocity

W

WHF	World Heart Federation
WHO	World Health Organisation

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List of presentations, publications, and grants

Peer reviewed publications

Seo, J., Loh, Y., Connolly, D. J., Luis Fuentes, V., Dutton, E., Hunt, H., & Munday, J. S. (2024). Prevalence of Hypertrophic Cardiomyopathy and ALMS1 Variant in Sphynx Cats in New Zealand. Animals, 14(18), 2629.

Seo, J., Owen, R., Hunt, H., Luis Fuentes, V., Connolly, D. J., & Munday, J. S. (2025). Prevalence of cardiomyopathy and cardiac mortality in a colony of non-purebred cats in New Zealand. New Zealand Veterinary Journal, 73(1), 1-9.

Seo, J., Novo Matos, J., Munday, J. S., Hunt, H., Connolly, D. J., & Luis Fuentes, V. (2024). Longitudinal assessment of systolic anterior motion of the mitral valve in cats with hypertrophic cardiomyopathy. Journal of Veterinary Internal Medicine, 38(6), 2982-2993.

Presentations (poster)

2025 ECVIM Congress ESVC-P-16: Seo J., Owen R., Hunt H., Chang Y.M., Connolly D.J., Luis Fuentes V., Munday J.S., Changes in left heart dimensions in non-purebred cats with or without hypertrophic cardiomyopathy.

Grants

Feline Cardiomyopathy in New Zealand Cats

Healthy Pets New Zealand 2021 (HPNZ-21-01)

$11,155.00

Chapter 1. Background and review of the literature

1.1 Background

A cardiomyopathy is a primary myocardial disorder that affects cardiac structure and function. Globally, cardiomyopathy is the most common heart disease diagnosed in domestic cats (*Felis catis*) in clinical practice.[1] The prevalence of cardiomyopathy in cats in New Zealand is unknown. However, two studies in the United Kingdom (UK) and United States of America (USA) reported that approximately 15% of apparently healthy cats in the general population have subclinical cardiomyopathy detectable using echocardiography.[2,3] In 2018, a retrospective multicentre study of 1730 cats from 21 countries across 5 continents estimated that 6% cats with cardiomyopathy die with cardiac symptoms within 1 year of diagnosis.[1] In the same study, the incidence of cardiac death increased each year and reached up to 28% in cats with cardiomyopathy during a >5 year follow-up period.[1] This high prevalence of cardiomyopathy and its potential to cause cardiac death emphasises that cardiomyopathy is a major health concern in domestic cats.

Several types of cardiomyopathies are reported in cats.[4] Of these, hypertrophic cardiomyopathy (HCM) is most common and constitutes more than 94% of subclinical cardiomyopathies diagnosed in apparently healthy cats.[2,3] Given the high prevalence of HCM, most research in feline cardiomyopathies is focused on HCM. There are now more than 900 peer reviewed articles on feline HCM, which have collectively improved the understanding of its pathobiology and natural disease history. However, these studies also highlight marked heterogeneity in disease manifestation between affected cats, including the age of disease onset, the speed of disease progression, and the anatomy of the affected heart on both echocardiography and postmortem examination. To date, there is no effective treatment that can reverse or delay the HCM progression.

Hypertrophic cardiomyopathy also occurs in humans with an estimated prevalence of 0.2%, with an annual incidence of cardiac death in 1-2% of the affected patients.[5,6] There are now more than 20,000 peer-reviewed publications describing human HCM. The large number of publications on human and feline HCM show that the disease in both species is remarkably similar, not only in pathobiology, but also in the variation in disease manifestation between patients. Currently, feline HCM is

viewed as a promising animal model for human HCM.[7-9] Likewise, studies of human HCM are considered beneficial for understanding HCM in cats.

Similar to feline HCM, there currently is no effective treatment that can reverse or delay the human HCM disease progression. A major challenge for finding an effective treatment that can reverse or delay the HCM progression is likely to be the heterogeneity of HCM disease manifestation in both species. To better understand the variation in disease manifestation between individuals and to advance the current understanding in human and feline HCM, this thesis contains descriptions of five studies that were conducted using domestic cats as subjects. In the first chapter, the literature on human and feline HCM is reviewed and the areas of knowledge gaps are highlighted.

1.2 History of cardiomyopathy classification system in humans and cats

Cardiomyopathies are a diverse group of primary myocardial disorders that affect cardiac structure and function. In humans, cardiomyopathies were first organised into groups in 1972 using a classification system proposed by Goodwin and Oakley.[10] Since then, human cardiomyopathies have been reclassified with revised definitions in 1980, 1996, 1998, 2006, 2008, 2013, and 2025 (Table 1.1).[11-16] The basis on which these classifications were made was different from one another, but each revision was supported by a growing understanding of cardiomyopathies and advancements in diagnostic imaging, genetic and molecular science.

In comparison, feline cardiomyopathies were traditionally classified using the 1996 World Health Organization /International Society and Federation of Cardiology scheme.[17] It is only recently that the first dedicated feline cardiomyopathy classification scheme was proposed by the American College of Veterinary Internal Medicine (ACVIM).[4] This 2020 classification scheme closely followed the 2008 European Society of Cardiology scheme in humans and divided feline cardiomyopathies into five types according to their major morphologic and functional features, or ‘phenotypes’ (Table 1.2).[4,16]

Table 1.1. History of human cardiomyopathy classification.

Year	Definitions and Classifications	References
1972	Cardiomyopathies defined as 'myocardial diseases of unknown origin'. This first classification system divided cardiomyopathies into three groups: Congestive cardiomyopathy (later became dilated cardiomyopathy) Hypertrophic cardiomyopathy Obliterative cardiomyopathy (later became restrictive cardiomyopathy)	Goodwin and Oakley[10]
1980	World Health Organisation (WHO) and International Society and Federation of Cardiology (ISFC) defined cardiomyopathies as 'myocardial diseases of unknown aetiology'. The classification system proposed by Goodwin and Oakley was used as the basis. A new type of cardiomyopathy called 'specific heart muscle disease' was introduced, which was characterised by 'unknown causes of myocardial affliction'.	WHO/ISFC Task Force[11]
1996	WHO/ISFC redefined cardiomyopathies as 'disease of myocardium associated with myocardial dysfunction' and introduced arrhythmogenic right ventricular cardiomyopathy and unclassified cardiomyopathies in the scheme. Specific heart muscle disease was removed from the classification system.	Richardson *et al.*[12]
1998	ISFC became World Heart Federation (WHF)	-
2006	American Heart Association redefined cardiomyopathies as 'diseases of myocardium associated with mechanical and/or electrical dysfunction, which usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilation due to a variety of causes that frequently are genetic'. An aetiology-based classification system was proposed, which classified cardiomyopathies into primary (disease solely or predominantly involving the heart) or secondary (multiorgan disease) groups. Primary cardiomyopathies were then subclassified by the aetiology into genetic, acquired, or mixed forms.	Maron *et al.*[15]
2008	European Society of Cardiology redefined cardiomyopathies as 'myocardial disorder in which the heart muscle was structurally and functionally abnormal'. The new classification system was proposed to maintain importance of phenotypic description before genetic classification for clinical practice. Cardiomyopathies were classified into 5 groups, with each group being subclassified into familial (genetic) or non-familial (non-genetic) forms: Dilated cardiomyopathy Hypertrophic cardiomyopathy Restrictive cardiomyopathy Arrhythmogenic right ventricular cardiomyopathy Unclassified cardiomyopathy	Elliot *et al*.[16]
2013	WHF proposed MOGE(S) classification system. In this system, cardiomyopathies are described according to their morphology and function (M), organ involvement (O), genotype and inheritance pattern (G), aetiology (E), and the optional functional stage (S).	Arbustini *et al.*[18]
2025	University of Padua redefined cardiomyopathies as 'diseases primarily affecting the myocardium which are characterised by permanent structural and functional heart abnormality'. This 'Padua classification' scheme removed Takotsubo cardiomyopathy, left ventricular non-compaction, and ion channelopathies and divided cardiomyopathies into 3 groups: Hypertrophic/ restrictive cardiomyopathy Dilated/ hypokinetic cardiomyopathy Scarring/ arrhythmogenic cardiomyopathy	Corrado *et al.*[19]

Table 1.2. Classification system of feline cardiomyopathies proposed by the American College of Veterinary Internal Medicine.[4] *In this classification system, cardiomyopathy is defined as 'a myocardial disorder in which the heart muscle is structurally and functionally abnormal in the absence of any other cardiovascular disease sufficient to cause the observed myocardial abnormality'. With this definition, cardiomyopathies are divided into five distinct phenotypic types regardless of the cause (e.g., endocrinopathies). Abbreviations: ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; RCM, restrictive cardiomyopathy; NSC, non-specific cardiomyopathy.*

Type	Definition
HCM	Diffuse or regional increased left ventricular wall thickness with a nondilated left ventricular chamber.
RCM	Endomyocardial form: Characterised macroscopically by prominent endocardial scar that usually bridges the interventricular septum and left ventricular free wall, and may cause fixed, mid-left ventricular obstruction and often apical left ventricular thinning or aneurysm; left atrial or biatrial enlargement is generally present. Myocardial form: Normal left ventricular dimensions (including wall thickness) with left atrial or biatrial enlargement
DCM	Left ventricular systolic dysfunction characterised by progressive increase in ventricular dimensions, normal or reduced left ventricular wall thickness, and atrial dilatation.
ARVC	Severe RA and RV dilatation and often, RV systolic dysfunction and RV wall thinning. The left heart may also be affected. Arrhythmias and right-sided congestive heart failure are common.
NSC	A cardiomyopathic phenotype that is not adequately described by the other categories; the cardiac morphology and function should be described in detail

This so-called 'phenotypic' classification system proposed by the ACVIM is supported by previous studies as it provides prognostic information.[1,20-22] For example, cats with cardiomyopathy that meets the ACVIM definition of RCM and DCM have a shorter lifespan than those with cardiomyopathy that meets the ACVIM definition of HCM.[1,20-23] However, some limitations are recognised. For example, morphologic and functional features of an abnormal heart in cats with cardiomyopathy are not always exclusive to a specific cardiomyopathy phenotype described by the ACVIM scheme.[4,24-26] Also, the cardiac phenotype can transition from one type to another during the cat's lifetime.[27] Lastly, this classification system is solely based on the morphology and function of the diseased heart and does not consider the underlying cause. There is an increasing number of genetic studies that suggest that feline cardiomyopathies are associated with certain genetic variants, but the final morphological and functional abnormalities can be caused by more than one genetic variant or even non-genetic disorders such as hyperthyroidism.[4,28-35] These limitations of using cardiac phenotypes to classify cardiomyopathies were also observed in humans,[16,36,37] and they provided the basis for a revision in the human cardiomyopathy classification system in 2025.[19] Unfortunately, this new classification system is not applicable to cats as it requires an ability to test the cat's genetics and assess the heart at the tissue level. At present, the genetic architecture of feline HCM

is poorly understood, and assessing the heart at the tissue level using advanced cardiac imaging is limited to research or academic institutes. Therefore, the 2020 ACVIM classification remains the most appropriate classification system for feline cardiomyopathies at the current time despite the limitations mentioned.

1.3 Hypertrophic cardiomyopathy in humans and cats

1.3a Definition and nomenclature

According to the 2008 European Society of Cardiology classification system, human HCM is defined as 'increased left ventricular wall thickness (LVWT) or mass in the absence of abnormal loading conditions such as systemic hypertension that are sufficient to cause the observed abnormality' (Figure 1.1).[16] However, the latest 2025 Padua classification proposed combining HCM and restrictive cardiomyopathy (RCM) into a single type of human cardiomyopathy.[19] Hypertrophic/restrictive cardiomyopathy (H/RC) was the proposed name, which was defined as 'a group of genetic heart muscle disorders from genetically defective sarcomeric and non-sarcomeric proteins and non-genetic phenocopies (storage and infiltrative diseases), resulting in left ventricular (LV) diastolic dysfunction, with no or variable degree of LV hypertrophy (LVH)'.[19] Some overlap in echocardiographic and microscopic abnormalities between HCM and RCM have also been recognised in cats, but the ACVIM classification system includes separate classification of HCM and RCM in feline medicine.[4,24,26] According to the ACVIM classification system, feline HCM is defined as 'diffuse or regional increased LVWT with a nondilated LV chamber in the absence of other disease sufficient to cause the observed myocardial abnormality' (Figure 1.1).[4] In contrast, RCM is defined into two forms: The endomyocardial form, which is characterised macroscopically by prominent endocardial scar that usually bridges the interventricular septum and left ventricular free wall, and may cause fixed, mid-left ventricular obstruction and often apical left ventricular thinning or aneurysm, and the myocardial form, which is characterised by normal LV dimensions (including LVWT) but with left atrial or biatrial enlargement (Figure 1.2).

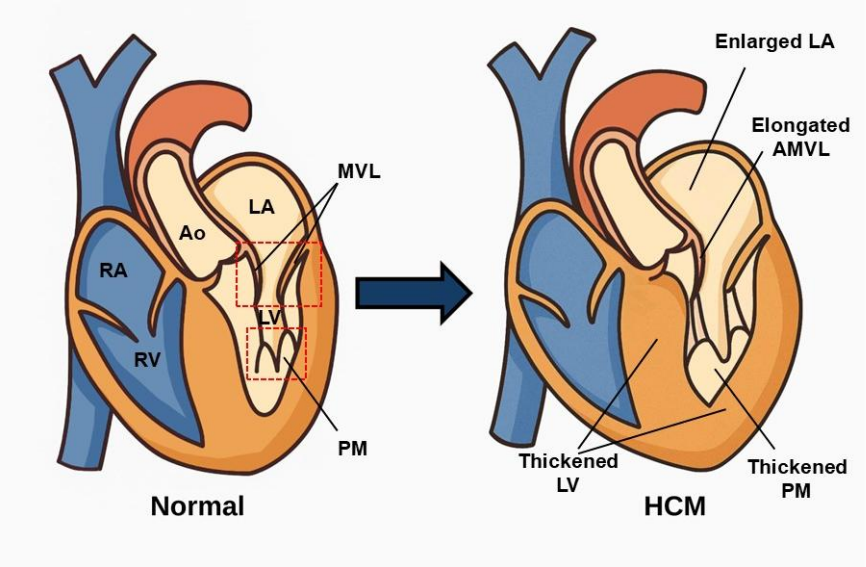

Figure 1.1. Schematic diagrams of a normal heart and a heart affected by hypertrophic cardiomyopathy (HCM). *In both humans and cats, HCM causes thickened left ventricular wall and reduced left ventricular internal chamber size. Additionally, humans and cats with HCM may develop thickened papillary muscles (PM), an elongated anterior mitral valve leaflet (AMVL), and enlarged left atrium (LA). Abbreviations: Ao, aorta; LV, left ventricle; MVL, mitral valve leaflets; RA, right atrium; RV, right ventricle*

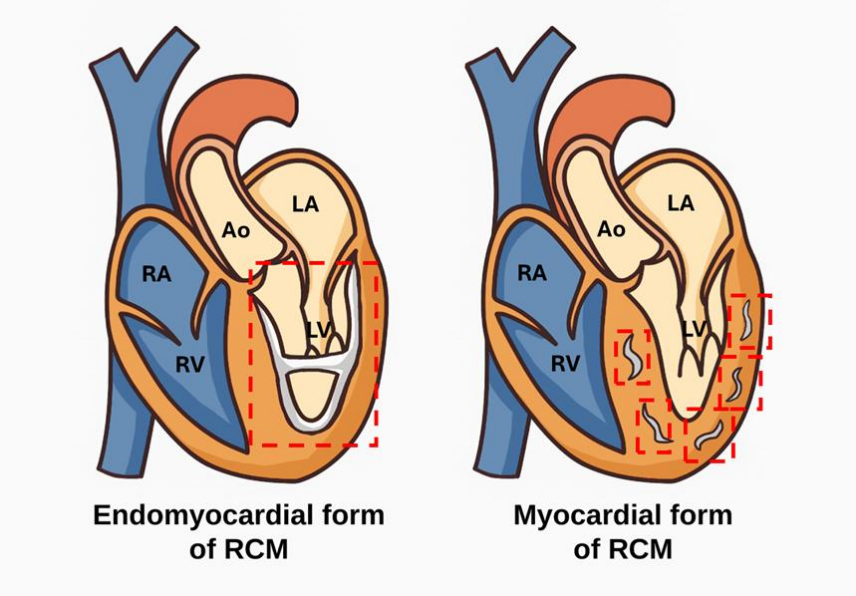

Figure 1.2. Schematic diagrams feline restrictive cardiomyopathy (RCM). *The current feline cardiomyopathy classification system[4] divides restrictive cardiomyopathy into two forms: The endomyocardial form is characterised macroscopically by a prominent endocardial scar that usually bridges the interventricular septum and left ventricular free wall (red box), and may cause fixed, mid-left ventricular obstruction and often apical left ventricular thinning or aneurysm; the myocardial form is characterised by normal LV dimensions (including LVWT) but with left atrial or biatrial enlargement, typically due to fibrosis within the LV myocardium (red boxes). Due to HCM being also capable of causing endocardial scarring and myocardial fibrosis, there is some overlap between HCM and RCM in cats. Abbreviations: Ao, aorta; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle*

Any pattern and distribution of increased LVWT or LVH can occur with human and feline HCM. Furthermore, hypertrophied papillary muscles, elongated mitral valve (left atrioventricular valve) leaflets, and right ventricular hypertrophy are additional morphologic abnormalities that are commonly observed in humans and cats with HCM.[38-42] These additional morphologic abnormalities may also occur due to other cardiac diseases in humans and cats but are considered an important part of the disease expression in both species. In humans, apically displaced papillary muscles, myocardial crypts (blood filled invaginations within the left ventricular myocardium), anomalous insertion of papillary muscle directly onto the anterior mitral valve leaflet (AMVL) in the absence of chordae tendineae, and myocardial bridging (a coronary artery that is tunnelling through the myocardium instead of lying over it) are also considered common features of HCM.[41-44]

In humans and cats with HCM, the blood flow may be obstructed across the LV outflow tract (LVOT) due to LVH and an abnormal motion of the elongated AMVL (Figure 1.3). This form of HCM is commonly described as obstructive HCM. However, the blood flow within the left ventricle is not invariably obstructed in patients with HCM, and the current human and feline guidelines recommend HCM as the disease name regardless of the presence of obstructed blood flow.[4,41] In cats, 'mitral dysplasia' is also used to describe HCM in some reports.[45,46] In this context, an elongated AMVL is considered a separate congenital abnormality, and that the LVH is secondary to the obstructed blood flow caused by the abnormal motion of the elongated AMVL (Figure 1.3). While this hypothesis was supported by a case report that demonstrated complete resolution of LVH following appropriate management of the abnormal mitral valve motion, this is not considered the likely pathogenesis for HCM in most cats.[46] The precise onset of AMVL elongation is currently unknown in cats with HCM, but an abnormal motion of an elongated AMVL is a common feature in cats with HCM, even in cats that were diagnosed with HCM at late age.[38,40]

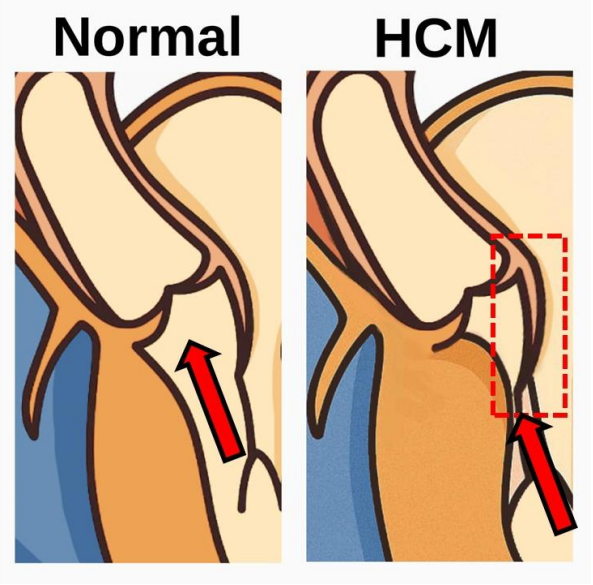

Figure 1.3. A schematic diagram showing an elongated anterior mitral valve leaflet (dotted box) in association with hypertrophic cardiomyopathy (HCM). *Elongation of the anterior mitral valve leaflet is a common feature of HCM in both humans and cats.[38,47] The direction of blood flow from the left ventricle toward the aorta is displayed using red arrows. In humans and cats with elongated AMVL, the tip of the mitral valve is dragged toward the left ventricular outflow tract during systole and obstructs the blood flow toward the aorta. This phenomenon is named 'systolic anterior motion of the mitral valve'. The precise cause and timing of anterior mitral valve leaflet elongation is unknown in both species.*

The 2020 ACVIM classification system for cats suggested using the word 'phenotype' when describing the morphology and function of a diseased heart.[4] For example, a heart with diffuse or regional increased LVWT with a nondilated LV chamber should be reported as an 'HCM phenotype' as there are many potential causes for the increased LVWT (Table 1.3). The diagnosis of 'HCM' can be made only after excluding other diseases that are capable of increasing LVWT with a careful clinical exam. The use of 'phenotype' when describing cardiomyopathies was also introduced in human medicine by the European Society of Cardiology in 2018.[16,48] In comparison, conditions that cause LVH and mimic the HCM phenotype but arise from alternative disease mechanisms can be reported as an 'HCM phenocopy' in both humans and cats.[49-51] Examples of 'HCM phenocopies' in humans include genetic disorders such as RASopathies (e.g., Noonan syndrome), metabolic or storage disease (e.g., Pompe and Danon disease), and non-genetic secondary causes (Table 1.3). An example of 'HCM phenocopy' in cats include hyperthyroidism, which may cause thickening of the LV wall and mimic HCM. However, the thickening of the LV wall is not due to a primary cardiac disease, and

the cardiac abnormality in this context should be reported as an 'HCM phenocopy'. If this cat did not have appropriate diagnostic tests to include or exclude hyperthyroidism, the cardiac abnormality should be described as an 'HCM phenotype'. The list of reported mimics of HCM in cats is presented in Table 1.3.

Table 1.3. Potential causes of a hypertrophic cardiomyopathy (HCM) phenotype in humans and cats. *An HCM phenotype is characterised by diffuse or regional increase in left ventricular wall thickness (LVWT) in a non-dilated left ventricular chamber, regardless of the cause. The diagnosis of 'HCM' can be made only after excluding other diseases that are capable of increasing LVWT with a careful clinical exam.*

Humans	**Cats**
Hypertrophic cardiomyopathy[52]	Hypertrophic cardiomyopathy[9]
RASopathies[53]	Hyperthyroidism[54]
Glycogen storage diseases: Danon disease[55] Cori disease[56] Pompe disease[57] PRKAG2 disease[58]	Systemic hypertension[59]
	Pseudohypertrophy[60]
	Acromegaly[61]
	Transient myocardial thickening or myocarditis[62]
	Infiltrative neoplasia[63]
Mitochondrial disease[64]	Aortic stenosis[65]
Friedreich's ataxia[66]	Systemic reactive angioendotheliomatosis[67]
Anderson-Fabry disease[68]	Obesity[51]
Cardiac amyloidosis[69]	Left ventricular non-compaction[70]
Cardiac sarcoidosis[71]	Endomyocardial disease[72]
Obesity[73]	
Drug induced[74]	
Systemic hypertension[75]	
Hyperparathyroidism[76]	
Hyperthyroidism[77]	
Pseudohypertrophy[78]	
Acromegaly[79]	
Mural thrombus mimicking increased LVWT[80]	
Left ventricular non-compaction[81]	
Athletic heart syndrome[82]	
Aortic stenosis[83]	
Endomyocardial disease[84]	
Acute myocarditis[85]	
Stress or Takotsubo cardiomyopathy[86]	

1.3b Aetiology of human HCM

A growing number of genetic studies suggest that human HCM is caused by genetic variants. In humans, a familial form of HCM was first reported in 1960.[87] Since then, >1700 pathogenic variants have been identified within 11 genes.[88] Most of these variants affected the sarcomere by encoding the myosin binding protein C (MYBPC3) and myosin heavy chain (MYH7).[88] Other variants account for only 1-5%

of human cases of HCM, and most rare variants were labelled as ‘private’, indicating that they are unique to an individual family.[88] However, a genetic cause is identified in only 50% of human HCM cases and the cause for the remaining 50% of cases are unknown.[89] Moreover, the final clinical phenotype can differ within a family or patients who share the same genetic variant, and it is currently impossible to predict the age that a person will develop HCM with any genetic variant.[89] These observed variations in disease penetrance and phenotypic expression may be explained by the presence of multiple pathogenic variants (polygenic) and other modifier genetic variants, epigenetic and environmental factors that will modify disease expression.[90] Some of the causes of an HCM phenocopy (Table 1.3), such as systemic hypertension and exercise, are also considered a possible trigger for pathogenic HCM gene expression, rather than causing reactive LVH via an independent pathway.[90] However, these possibilities remain unproven.

In humans, males develop HCM approximately 1.5 times more frequently than females.[91-93] However, this increased risk may be due to the possibility of males simply being over-diagnosed with HCM due to naturally having thicker LVWT than females.[94,95] Indeed, the current diagnostic criteria for human HCM uses a single LVWT cut-off without considering the biological sex of the patient.[41,96,97] The possibility of males being over-diagnosed is further supported by a meta-analysis of 9,427 HCM patients (3,719 females) where females were approximately 1.5 times more likely to progress and develop cardiac death compared to males.[98] This observation that HCM has a worse prognosis in females than males may suggest that males are diagnosed earlier in the disease than females as the normal LVWT is higher in males than females. Overall, the question whether being male truly increases the risk of developing HCM in humans remains unanswered at the time of literature review.

There are no other factors with sufficient evidence to show they cause HCM in humans. However, there is a popular belief that an intense or chronic psychological stress can cause heart disease of any type. This belief was critically reviewed by Levine in 2022, who concluded that this belief is biologically plausible but the number of studies that investigated this potential association is low.[99] From the available literature, a clear association between psychological stress and cardiac health is demonstrated in the context of ‘stress or Takotsubo cardiomyopathy’.[100,101] This unique cardiac condition usually develops within 1 week of an intense stress such as the death of a loved one, violent confrontation or attack, extreme anger,

profound financial loss, and natural disaster. On cardiac imaging, the apical segment of the left ventricle is hypokinetic while the other LV segments appear normal. Additionally, patients with stress cardiomyopathy may develop increased LVWT.[86] The abnormal LV morphology and function will usually return to normal within a few weeks, but the increased LVWT may be permanent in some patients.[102] Therefore, the potential effect of stress in HCM disease expression warrants further studies. However, studies of stress in humans are challenging to design as humans under stress will often develop unhealthy lifestyle including smoking, alcohol consumption, and reduced exercise and sleep. These unhealthy lifestyle factors are major confounders in cardiovascular studies as they increase the risk of systemic hypertension and obesity (which are potential causes of an HCM phenotype). Thus, studies of psychological stress on the risk of HCM diagnosis and disease progression may be easier to perform in cats as their husbandry is better controlled.

1.3c Aetiology of feline HCM

In cats, familial HCM was described in several breeds including Maine Coons, Ragdolls, Norwegian Forest Cats, British Shorthairs, and Domestic Shorthairs.[8,25,27,30,103-105] The reported inheritance pattern of HCM in these breeds was autosomal dominance with incomplete age-dependent penetrance.[28,30] In 2005, Meur *et al.,* described the first genetic variant that explained HCM in Maine Coons.[28] Since then, 5 additional genetic variants have been described, totalling 6 described genetic variants over 4 different genes in Maine Coons, Ragdolls, Sphynx cats, and a Domestic Shorthair with HCM.[28-33] Five of these genetic variants affected the sarcomere by altering *MYBPC3*, *MYH7*, or troponin T (*TNNT2*). The remaining *ALMS1*:ENSFCAG00000008756:c.7384G>C;p.(G2462R) affected the gene encoding a protein whose function is unknown but, in humans, abnormalities in this protein are known to result in Alström syndrome, which is characterised by childhood obesity, progressive vision and hearing loss, and cardiomyopathy.[29,106,107] It is important to note that most of these genetic variants are not validated by an independent study, despite being commercially available to test in cats. In a recent study that used the American College of Medical Genetics guidelines to critically appraised the 6 genetic variants associated with feline HCM, the authors concluded that only 2 genetic variants in *MYBPC3* (*MYBPC3*:XM_019812397.2;c.91G>C;p.[A31P {XP_019667956.2}] in the Maine Coon, and *MYBPC3*:XM_019812397.2:c.2453C>T

p.[R818W{XP 019667956.2}] in the Ragdoll, originally reported as *MYBPC3*:c.2060C>T;p.[R820W]) were pathogenic in cats.[108] In the same study, *MYH7*:XM_006932746.5:c.5647G>A;p.[E1883K{XP_006932808.1}], was classified as likely pathogenic while the other three genetic variants were labelled as variants of unknown significance.[108]

Due to the limited number of pathogenic gene variants identified in cats, and those being limited to Maine Coons and Ragdolls, the majority of feline HCM cases are considered unknown in aetiology. In Maine Coons with *MYBPC3*:c.91G>C; p.[A31P] and Ragdolls with *MYBPC3*:c.2453C>T;p.[R818W], the pattern of disease penetrance and phenotypic expression is still not predictable as the age cats develop HCM and the final clinical phenotype may vary between cats.[109,110] As in humans, this variation in disease penetrance and phenotypic expression may be explained by the presence of multiple pathogenic variants and other modifier genetic variants, epigenetic or environmental factors. As hypothesised in people, diseases that cause an HCM phenocopy in cats (Table 1.3) may also trigger the pathogenic HCM gene expression rather than causing reactive LVH via an independent pathway.[90]

Male cats are approximately three times more likely to be diagnosed with HCM than female cats.[1,2,23,105,111,112] Despite this widely recognised male predilection, the mechanism by which being male increases the risk of HCM is poorly understood. Like in humans, male cats naturally have slightly thicker LVWT than female cats.[113] Furthermore, male cats are usually larger than female cats and LVWT has a small but significant association with body weight in cats.[113] Therefore, it is possible that male cats are simply over-diagnosed with HCM due to their normal LVWT being higher than female cats. However, unlike in humans, male cats are equally or more susceptible to developing cardiac death than female cats.[23,112,114-116] Therefore, the male predilection of HCM remains a valid observation in cats that warrants further investigation. Studying the influence of sex hormone in HCM disease expression is probably easier in cats than humans as elective neutering is a common practice to reduce behavioural problems and prevent unintentional breeding. This provides a unique opportunity to assess not only the effect of sex genes but also the effect of reproductive cycles on the HCM gene expression. The possible effect of neutering on HCM gene expression was not evaluated in previous studies, probably as most pet cats are already neutered by the time they are seen by a cardiologist.

There are no other factors with sufficient evidence to show that they cause HCM in cats. However, intense psychological stress may be associated with an HCM

diagnosis in cats, as is suggested in people.[62,117] This potential association is highlighted in the study in 2018 by Novo Matos *et al.*, who reported 21 cats with 'transient myocardial thickening', a cardiac syndrome in which the LVWT increases but resolves over a few months.[62] In this study, the medical records of 15 cats (71%) contained a stressful event within the 2 weeks prior to the diagnosis of increased LVWT. Subsequently in 2023, Romito *et al.* reported 27 cats with transient myocardial thickening, and the medical record of 9 cats (33%) described a stressful event within 2 weeks of the diagnosis.[117] The mention of stressful events prior to the diagnosis of transient myocardial thickening in both studies suggests that there may be an association between psychological stress and increased LVWT in cats, as seen in people with stress cardiomyopathy. The mechanism by which the LVWT could increase with psychological stress in cats is unknown, but if the mechanism is similar between cats and humans, it is plausible to consider that increased LVWT may also be permanent in some cats as seen in people.[102] Indeed, the original study by Novo Matos *et al.* included 21 cats with HCM (permanently increased LVWT) as a control group, and the medical record of 6 cats (29%) with HCM also had mentions of a stressful event within 2 weeks of the initial diagnosis.[62] Given that the cause of HCM is poorly understood in cats, the potential association between stress and the HCM diagnosis warrants further investigation. Such a study should be best done using a colony of cats that share the same environment, as this would minimise the number of confounders associated with lifestyle.

1.3d Biomechanical, metabolic, and energy abnormalities associated with HCM in humans and cats

Hypercontractile left ventricle is a common feature of HCM in both species. The precise mechanism by which the pathogenic HCM genes cause increased left ventricular contractility is not understood. Many experimental studies suggest that it is due to the direct effect of the genetic variants associated with HCM; however, similar findings are also seen in human and feline HCM where the pathogenic genetic variant is not identified. For example, a study of bovine β cardiac myosin engineered to mimic abnormalities caused by the 178 *MYH7* variants associated with human HCM demonstrated that the number of active myosin heads available for force production is increased under the genetic influence and ultimately results in

excessive actin-myosin cross bridging.[118] In other experimental studies of transgenic mice with introduced variants associated with human HCM (α-cardiac actin; p.[E99K] and cardiac troponin; p.[I61Q] and p.[L48Q]), myofilaments had enhanced calcium sensitivity under the genetic influence.[119,120] Similarly, a study of engineered human pluripotent stem cells with *MYBPC3*:c.2453C>T; p.(R818W), a variant associated with HCM in cats, demonstrated hypercontractility compared to the isogenic control cell line.[121] However, these observations are also made in human and feline HCM without an identifiable pathogenic genetic variant. For example, a study that compared the sarcomeric protein function and calcium regulation in isolated LV tissue samples from 25 cats (18 with HCM, 7 without HCM) with 14 humans with HCM, had identifiable genetic variants in only 1 cat and 7 humans with HCM. Nonetheless, LV hypercontractility and increased myofibrillar calcium sensitivity were evident in all of the studied cats and humans with HCM.[122] This finding suggests that there are either more pathogenic genetic variants are yet to be discovered in humans and cats, or the hypercontractile left ventricle and increased myofibrillar calcium sensitivity may be a secondary effect of HCM disease expression.

In humans, HCM is also associated with inefficient or excessive use of energy. For example, a proportion of cardiac myosin heads should be in a super-relaxed (SRX) state, which means that they are restricted from binding to actin by the thin filament regulatory proteins (the troponin-tropomyosin complex). This SRX state represents a low metabolic rate of cardiac myocytes at rest. In studies of purified human β-cardiac myosin, the number of myosin heads in the SRX state was reduced under the influence of the genes associated with HCM and therefore had faster metabolic turnover.[123-125] Furthermore, mitochondrial dysfunction and a shift from fatty acid oxidation to increased glucose oxidation, which are features of various heart diseases, may also contribute to inefficient energy utilisation and energy depletion in HCM patients.[126] In clinical studies, humans with HCM had a reduced phosphocreatine-to-adenosine triphosphate (ATP) ratio compared to healthy controls, which further supports the concept that myocardial energy deficiency is a feature of HCM.[127,128] In comparison, the information on the energetics in feline HCM is very limited. A recent metabolomic study that compared 23 healthy cats to 60 cats with HCM demonstrated that circulating free fatty acid, 3-hydroxy fatty acid, acylcarnitine, cortisol, and 11-dehydrocorticosterone were all greater in concentrations in cats with HCM than healthy controls.[129] The implications of this finding require further investigation in cats.

Diastolic dysfunction is also a common feature of HCM in both species. The mechanism of diastolic dysfunction is easily explained by the increased left ventricular stiffness and reduced left ventricular chamber size due to progressive LVH. However, in two studies that used mice or human pluripotent stem cells to study the *MYH7* p.(R663) or alpha myosin heavy chain variants p.(R403Q), which are gene variants associated with HCM in humans, the calcium uptake by the sarcoplasmic reticulum was reduced compared to the control groups.[130,131] This observation suggests that dysregulation of calcium uptake may contribute to the diastolic dysfunction, possibly in both human and feline HCM. Furthermore, energy is required for the relaxation phase of the myocytes and myocardial energy deficiency may also contribute to the diastolic dysfunction.

1.3e Development of the HCM disease phenotype in humans and cats

The precise mechanism by which a gene variant important in HCM development causes an HCM phenotype is poorly understood in both species. The reason for the limited understanding is likely due to the scarce number of human embryos available to scientists, cultural and religious concerns of introducing a variant associated with HCM to humans, and the poor understanding of the genetic architecture in cats with HCM. Therefore, most studies used an experimental murine model, induced pluripotent stem cells, or isolated cardiac myocytes to study the influence of HCM gene expression.

In a study of mice with cardiac troponin C variants (p.[161Q] and p.[L48Q], which are variants associated with human HCM), the enhanced myofibrillar calcium sensitivity and prolonged tension of the myocytes caused myocyte hypertrophy via the ERK1/2 signalling pathway.[119] However, certain features of HCM are observed even earlier than myocyte hypertrophy in humans and cats. For example, a study of mice with a knocked out *MYBPC3* gene (mimicking the variants associated with human and feline HCM) demonstrated that certain abnormalities of the heart are present during organ development in animals unable to produce normal MYBPC3 protein. Specifically, cardiac myocyte disarray and the presence of blood-filled invaginations (named ‘left ventricular crypts’, a common feature of HCM in humans) were visible in the left ventricle in mice prior to birth.[132,133] In contrast, hypertrophy of the cardiac myocytes was not visible prior to birth.[132,133] Similarly, a study of isolated

adult feline cardiac myocytes with introduced modified gene associated with human HCM (*MYH7*:MYH7NM_000257.4:c.1208G>A;p.[Arg403Gln]) by site-directed mutagenesis using polymerase chain reaction showed that myofilaments created by cardiac myocytes were unable to assemble into normal sarcomeres, causing disarray in myofibre alignment within 5 days of the modified gene being introduced.[134]

In human HCM, enhanced myocardial contractility, diastolic dysfunction, LV crypts, elongated AMVL and reduced LV end-systolic cavity size are all considered to occur prior to LVH.[44,135] In feline HCM, elongated AMVL and impaired left atrial (LA) function, which may suggest diastolic dysfunction in cats, are thought to occur prior to LVH. For example, a prospective study of 107 cats and a retrospective study of 55 cats demonstrated that elongated AMVL and impaired left atrial function precede the development of LVH in cats with HCM.[40,136] In another study of 21 Maine Coons with *MYBPC3*:c.91G>C;p.[A31P], 6 cats had echocardiographic evidence of diastolic dysfunction even in the absence of LVH.[137] Overall, these observations suggest that LVH is a relatively late manifestation of HCM in both humans and cats, despite LVH being the defining feature of HCM in both species.

1.3f Antemortem diagnostic criteria in humans

Current guidelines recommend the use of two-dimensional (2D) echocardiography to diagnose HCM (Figure 1.4).[41,96,97] In human adults, all guidelines recommend the use of end-diastolic LVWT ≥15 mm at any LV segment for the diagnostic criterion of HCM.[41,96,97] However, a lower cutoff of ≥13 mm is recommended when the patient has a familial history or genetic variant associated with HCM.[41,96,97] Due to the impact of growth on normal cardiac dimensions, the diagnosis of HCM in children requires the use of regression analysis and adjusting LVWT to the body surface area.[41,96,97] The recommended diagnostic criteria for HCM in children varies between current guidelines, and the suggested LVWT cutoffs ranged between >2.0 and ≥2.5 standard deviations above the mean adjusted to the body surface area on the regression analysis.[41,96,97] None of the guidelines suggested a different LVWT cutoff for male versus female.

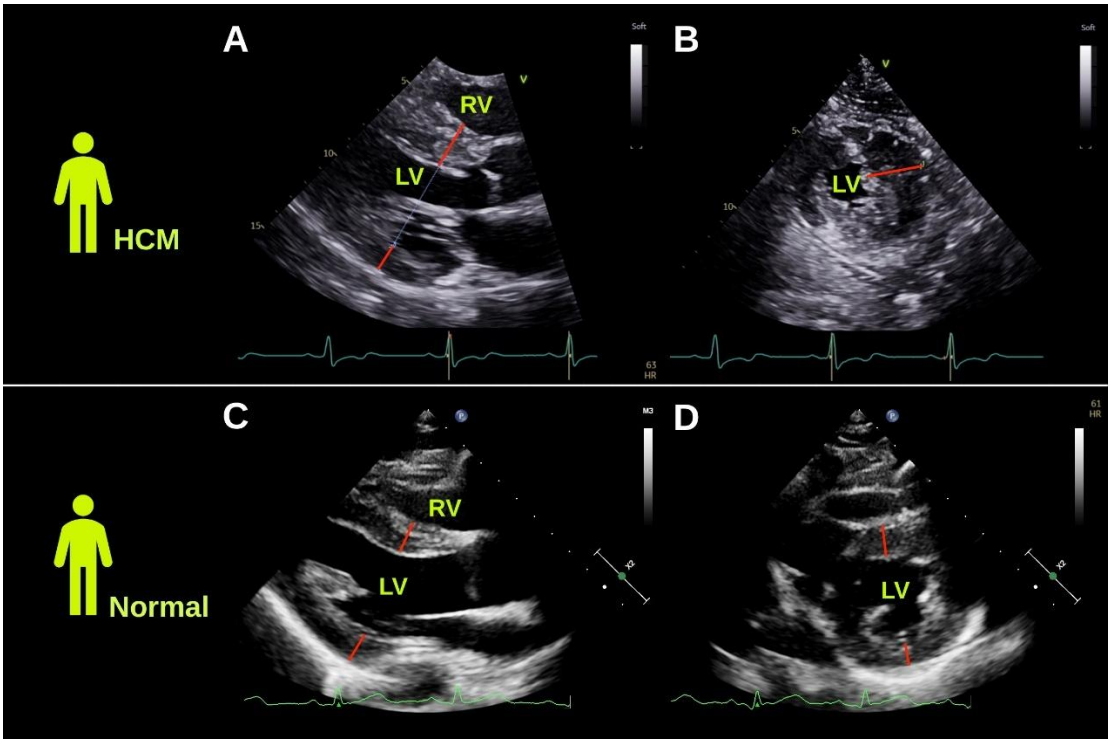

Figure 1.4. Echocardiographic images of two human adults. *The still images were acquired at the end-diastole using the right parasternal long-axis (A, C) and short-axis views (B, D). The red line shows left ventricular wall thickness. The patient with hypertrophic cardiomyopathy (HCM) had asymmetric hypertrophy of the interventricular septum with an end-diastolic interventricular septal thickness measuring 18-27 mm (A and B). In comparison, the patient with normal echocardiogram had a normal end-diastolic left ventricular wall thickness of 11 mm (C and D). Abbreviations: LV, left ventricle; RV, right ventricle. Figure materials used with permission from Erika Hong, Princess Alexandra Hospital, Queensland, Australia.*

Asymmetric hypertrophy of the interventricular septum is considered the most common pattern of LVH but any pattern or distribution of increased LVWT or LVH can occur with HCM.[138-141] The pattern of LVH does not appear to be prognostically important but it may be associated with the underlying genetic cause of HCM.[142]

A careful physical examination and a 12-lead electrocardiogram (ECG) may assist with the diagnosis of HCM.[143] However, the diagnosis of HCM is ultimately based on the morphology of the diseased heart and a form of cardiac imaging is necessary for the antemortem diagnosis of HCM. When the echocardiographic diagnosis of HCM is inconclusive, complementary cardiac imaging such as speckle tracking echocardiography, transoesophageal echocardiography, computed tomography angiography or cardiac magnetic resonance imaging can be performed.[41,96,97] These complementary imaging techniques not only provide

additional assessment of the LVWT but also the myocardial function, mitral valve anatomy, coronary blood flow, and the presence of myocardial ischemia, fibrosis, and aneurysm.[41,96,97,144]

1.3g Antemortem diagnostic criteria in cats

Two-dimensional (2D) echocardiography is recommended to diagnose HCM in cats (Figure 1.5).[4] An increase in end-diastolic LVWT is required for the diagnosis of HCM, but the current ACVIM guidelines do not suggest a specific LVWT cutoff for the diagnosis of HCM as there is no universally accepted reference interval for normal LVWT in cats.[4] The lack of accepted normal LVWT may be due to a number of reasons including that the studies that described normal LVWT were small in sample size, did not standardise the method to measure LVWT, or lacked rigorous health screening to determine that the reference cats were indeed healthy. For example, Häggström *et al.* described normal left heart dimensions using 56,169 apparently healthy purebred cats that presented for pre-breeding cardiac screening.[113] While this study included large numbers of cats, LVWT were measured by 151 examiners without a standardised method. In other studies, different methods of measuring end-diastolic LVWT were shown have a difference as wide as 1.6 mm, which is larger than the normal range of LVWT in some studies.[145,146] Standardising the method of measuring LVWT is critical in cats due to the small overall size of their hearts.

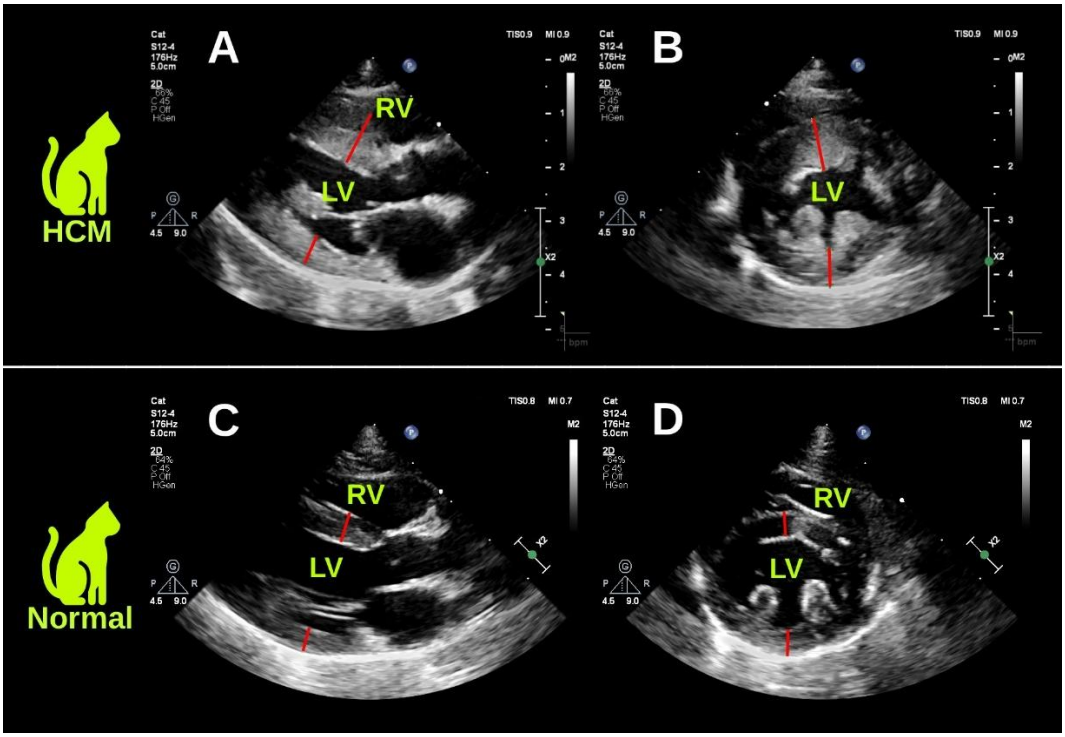

Figure 1.5. Echocardiographic images of a 3-year-old Ragdoll cat with hypertrophic cardiomyopathy (HCM) and a 4-year-old Sphynx cat with normal heart. *The still images were acquired at the end-diastole using the right parasternal long-axis (A, C) and short-axis views (B, D). The red line shows left ventricular wall thickness. The Ragdoll with HCM had asymmetric hypertrophy of the interventricular septum with end-diastolic interventricular septal thickness measuring 9 mm. In comparison, the Sphynx cat had a normal end-diastolic left ventricular wall thickness of 4.5 mm. Abbreviations: LV, left ventricle; RV, right ventricle*

In the literature, feline HCM was defined using echocardiography with either a simple cutoff of end-diastolic LVWT that ranged between >5.5 mm to ≥7 mm, end-diastolic LVWT exceeding the normal range according to published reference intervals, or end-diastolic LVWT exceeding the thickness of the adjacent LV segment by more than 50%.[8,9,112,145,147-153] Among these criteria, a simple cutoff of maximal end-diastolic LVWT ≥6 mm in any of the LV segments was most used and studied.[8,9,112,147-151] This ≥6 mm cutoff was first introduced by Fox *et al.* in 1995 as a similar concept to the ≥15mm cutoff in humans.[9] Since then, the ≥6 mm cutoff was shown to be a highly repeatable diagnostic criteria of HCM among veterinary cardiologists (kappa >0.84).[145] However, the ≥6 mm cutoff is not suitable for growing kittens, very small, and very large cats as LVWT is affected by the growth phase in kittens and body weight in adult cats. For example, a retrospective study of 56,169 purebred cats suggested that healthy small cats weighing <4.5 kg should have LVWT less than 5.0 mm, while healthy large cats weighing ≥9 kg may have LVWT that

naturally exceeds 6 mm.[113] Despite this limitation, most cats in the general population are <9 kg and the current practice guidelines suggest that the ≥6 mm cutoff for the diagnosis of HCM is probably appropriate to use, especially in purebreds whose LVWT are better described.[4] However, cats with LVWT 5-6 mm may not be normal and these cats may be described as equivocal HCM.[4]

As in humans, any pattern of LVH can occur with feline HCM. However, asymmetric hypertrophy of the interventricular septum is the most common pattern of LVH in cats with HCM.[9,26,154,155] There is no study investigating a potential relationship between the pattern of LVH and genetic cause of HCM, probably due to the poor understanding of the genetic cause of HCM in cats. However, studies reported breed variation with the pattern of LVH in cats with HCM. For example, Trehiou-Sechi *et al.*, investigated echocardiographic features of 340 cats from 5 breeds (Chartreux, Persian, Sphynx, Domestic Shorthairs, and Maine Coons) and reported that 20 out of 28 (71.4%) Maine Coons had either symmetric LVH or asymmetric hypertrophy of the LV free wall, whereas only 8 out of 28 (28.6%) had asymmetric hypertrophy of the interventricular septum or a discrete upper septal thickening. [155] This pattern of LVH in Maine Coons were different to that of Chartreux, Persian, Sphynx, and Domestic Shorthairs as HCM in these breeds were mostly associated with asymmetric hypertrophy of the interventricular septum.[9,26,154,155] Similarly, a study of 16 Maine Coons with HCM showed that the LVH was mostly confined to the LV free wall and papillary muscles. [8] Given the breed variation, the pattern of LVH with HCM may be associated with the causative genetic variants in cats, as it is in humans.

Like in humans, the pattern of LVH does not appear prognostically important in cats with HCM. For example, a retrospective study of 255 cats with HCM over a >2-year follow-up period showed that the pattern of LVH was not associated with development of cardiac complications or death in cats with HCM.[156] Possibly due to the lack of clear indication to report the pattern of LVH, most feline studies do not report the pattern of LVH.

The signalment of the cat and physical examination may assist with the diagnosis of HCM. For example, being male, of older age, with cardiac auscultatory findings of an audible gallop sound, loud heart murmur, and arrhythmias, as well as increased blood markers of myocardial wall tension (N-terminal pro-B-type natriuretic peptide) and injury (cardiac troponin I) suggest an increased likelihood of HCM in cats.[2,150,157-159] However, the diagnosis of HCM is ultimately based on the morphology

of the diseased heart and a form of cardiac imaging is necessary for the antemortem diagnosis of HCM.

In summary, the current literature supports the use of a simple end-diastolic LVWT cutoff of ≥6 mm in any LV segment for an antemortem diagnostic criterion of feline HCM, unless the cat is not fully matured, very small, or very large. Cats with end-diastolic LVWT of 5-6 mm and cats with LVWT <5.0 mm that have an elongated anterior mitral valve leaflet, reduced left atrial function, SAM, DLVOTO, diastolic dysfunction, or hypertrophied papillary muscles should be classified as equivocal HCM.

1.3h Postmortem diagnostic criteria in humans

There are two major guidelines that established the minimum standards and approach for cardiac autopsy in people who suffered sudden unexplained death.[160,161] In these guidelines, authors highlight a considerable overlap in macroscopic and microscopic abnormalities between HCM and other cardiomyopathy types, secondary cardiac changes due to an abnormal loading condition such as systemic hypertension, and athletic heart syndrome. However, authors also highlight that a 'highly probable' diagnosis of HCM can be made by appropriately identifying the key features of HCM, which are left ventricular hypertrophy, myocyte hypertrophy, myofibre disarray, and interstitial fibrosis (Figure 1.6).[160,161]

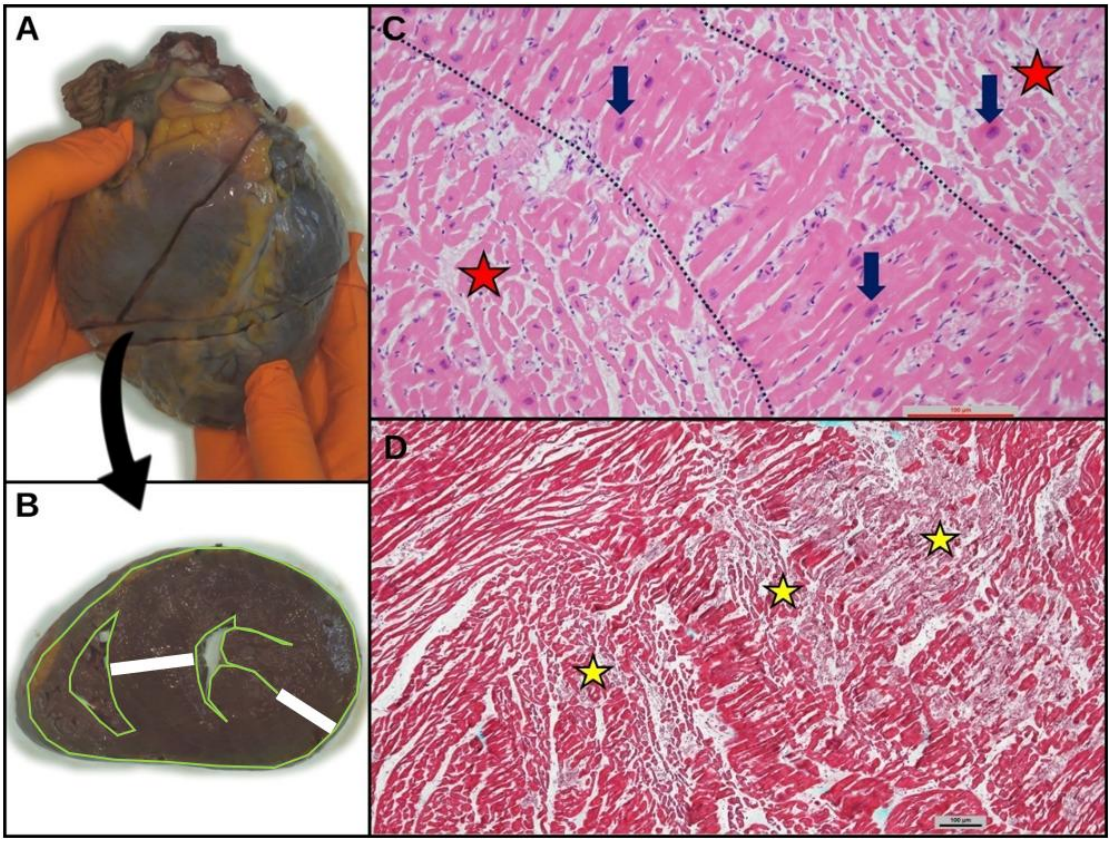

Figure 1.6. An example of postmortem diagnosis of hypertrophic cardiomyopathy in humans. *A. On macroscopic examination, the heart weighed 369 grams following evacuation of blood clot. This weight of the heart is heavier than the expected normal interval based on the body weight (153-327 g) and for the height (109-348 g).[162] B. A transverse section of the heart at the level of papillary muscles showed hypertrophied interventricular septum (white line) with reduced internal diameter of the left ventricle. C. Microscopic examination of the hypertrophied septum using haematoxylin and eosin stain showed prominent nuclear enlargement and hyperchromasia (blue arrows), which are features of HCM. The myocytes are aligned perpendicular to one-another at the location of dotted lines, which is described as myofibre disarray. There were superimposed changes of myocyte loss secondary to reperfusion injury following cardiac arrest and cardiopulmonary resuscitation (red stars). D. Masson's trichome stain further highlights the misalignment of myocytes and myocyte damage (due to reperfusion injury, yellow stars). There was no increase in interstitial fibrosis in this case. Figure materials used with permission from J Ciaran Hutchinson, Great Ormond Street Hospital for Children NHS Foundation Trust, United Kingdom.*

In humans, LVH is defined as increased cardiac weight and LVWT relative to the body weight, height, age, and sex.[160-162] Myocyte hypertrophy is characterised by increased size of individual cardiac myocytes that is often accompanied by enlarged, hyperchromatic, and pleomorphic nuclei.[163,164] In humans, healthy cardiac myocytes in haematoxylin and eosin stained paraffin sections have transverse diameter of up to 15 μm.[165-167] The best method of measuring the diameter of the cardiac myocyte is on cross section where the nucleus is centrally located.[167] Up to 20 μm diameter is

considered mild hypertrophy and most studies of human HCM use ≥20 μm to define a diagnosis of HCM.[165,166,168] In some studies, erythrocytes were used as an internal size marker. A human erythrocyte is 6-8 μm in diameter, meaning that HCM will result in a transverse myocyte being thicker than 3 erythrocytes.[168] The severity of myocyte hypertrophy can vary within the myocardium and one study reported that myocyte hypertrophy is greater in the subendocardial region.[164] Myofibre disarray is characterised by an oblique or perpendicular alignment of myocytes to each other at varying patterns including pinwheel or herringbone configurations. Lastly, interstitial fibrosis is characterised by excessive accumulation of extracellular matrix proteins. Collectively, these three microscopic features are frequently labelled as the hallmarks of HCM in humans, with an emphasis put on myofibre disarray.[8,9,169-179]

However, there are several controversies surrounding the postmortem diagnosis of HCM in people. Notably, several authors did not agree with the macroscopic or even the three hallmark microscopic features of HCM, identified discrepancies between macroscopic and microscopic exam findings, and questioned the severity of microscopic features that was required to diagnose HCM. For example, studies of the human heart showed that changing the plane of orientation of the tissue block can ‘create’ myofibre disarray in regions of a normal heart where the myofibre connects at an acute angle.[164,173,176,179] However, recent studies and practice guidelines appear to have addressed this concern by simply avoiding the assessment of myofibre arrangements in regions where it may appear disarrayed in a normal heart. Another controversy was that myofibre disarray was present in some patients without overt HCM, including those with congenital heart defects.[180] This raised a debate that myofibre disarray may not be a unique feature of HCM. This debate is not completely resolved but can be addressed by two hypotheses that 1) myofibre disarray is an early feature of HCM that develops during embryogenesis under the influence of a genetic cause of HCM,[132] and that 2) a patient can have co-existing cardiovascular disease in addition to HCM. These two hypotheses are supported by recent genetic studies that showed correlations between microscopic features of HCM with the known pathogenic gene variants of human HCM,[181] and that myofibre disarray was present pre-birth in an experimental murine model of human HCM.[132] With the growing data on human HCM, it is now widely accepted that that not all features of HCM occur at the same time, and myofibre disarray is considered one of the earliest feature of HCM in humans. However, there remains a debate on the severity of myofibre disarray that is required to diagnose HCM. Some

studies described ‘extensive myofibre disarray’ without specific definition while others calculated the area affected by myofibre disarray from the relevant tissue section and used a varying cutoff that include >5%,[180] >10%,[175] and >20%.[164] The debate appears unresolved in the human literature, but pathologists appear to agree that LVH with minimal myofibre disarray (<5%) should be classified separately as ‘idiopathic LVH’ instead of HCM. In humans, studies suggest idiopathic LVH is linked to arrhythmogenesis and sudden death syndrome.

Myocardial fibrosis is common in humans with HCM. In the study by Liu *et al.,* where 38 human hearts with HCM were examined, interstitial or replacement fibrosis was present in 33 out of 38 hearts (86%).[171] Of these 38 patients, fibrosis was considered moderate to severe in 15 patients (39%). However, myocardial fibrosis is a non-specific change seen in a variety of different heart diseases that result in myocardial necrosis.

Intramural arteriosclerosis, which is characterised by thickened intramural coronary arteries due to proliferation of intimal and medial smooth muscle cells and collagen with or without resultant luminal narrowing, is also a common finding in human HCM. In the study by Liu et al., 25 out of 38 patients had some degree of intramural arteriosclerosis (66%).[171] In the same study, intramural arteriosclerosis was commonly found in the region of moderate to severe myocardial fibrosis (in 12 out of 29 tissue sections showing moderate to severe fibrosis versus in 5 out of 85 tissue sections showing mild fibrosis; P <0.001).[171] This observation suggested that intramural arteriosclerosis plays a potential role in the development of myocardial fibrosis by increasing the risk of myocardial ischemia and infarction.[171] In human HCM, the term coronary microvascular dysfunction (CMD) syndrome is used to describe compromised myocardial microcirculation. For example, the presence of intramural arteriosclerosis, severe LVH, left ventricular systolic and diastolic dysfunction, obstructive blood flow secondary to SAM and DLVOTO can all reduce myocardial perfusion by creating resistance or direct force against the coronary blood flow.[182-184] Additionally, the hypercontractile myocardial function and DLVOTO will increase the myocardial oxygen demand. The resultant mismatch between myocardial oxygen demand and supply can lead to myocardial ischemia, myocardial death, and replacement fibrosis. The irreversible stage of myocardial ischemia that leads to myocardial death and replacement fibrosis is described as myocardial fibrosis. At present, CMD is a highly supported mechanism for the increased risk of myocardial ischemia and infarction in humans with HCM.[182-184]

In humans with SAM, the constant contact between the AMVL and LV outflow tract can cause reactive thickening and fibrosis of the MV leaflet and endocardial layer of the LV outflow tract. This lesion is described as an 'impact lesion' in the literature.[185] An impact lesion of the MV and LV outflow tract is not pathognomonic for HCM but increases the suspicion of HCM on postmortem exam.[168]

The current practice guidelines recommend that when HCM is diagnosed by postmortem examination, genetic testing to detect variants known to cause HCM is performed. Detection of a genetic variant supports the diagnosis of HCM as well as allowing medical counselling of first-degree relatives.[41,97] Additionally, complementary postmortem cardiac imaging using cardiac magnetic resonance, computed tomography angiography, or micro computed tomography can be considered.[186-190] This imaging can provide additional information on the location and severity of myocardial infarction, fibrosis, and other diseases that the patient may have had prior to death.

1.3i Postmortem diagnostic criteria in cats

There are no published guidelines for the diagnosis of HCM in cats by postmortem examination. However, most feline studies used similar criteria to each another. In the literature, studies defined feline HCM by LVH on macroscopic exam, and myocyte hypertrophy, myofibre disarray, and interstitial fibrosis on microscopic exam (Figure 1.7). As in humans, myocyte hypertrophy, myofibre disarray, and interstitial fibrosis were commonly described as hallmarks of HCM in the feline literature.

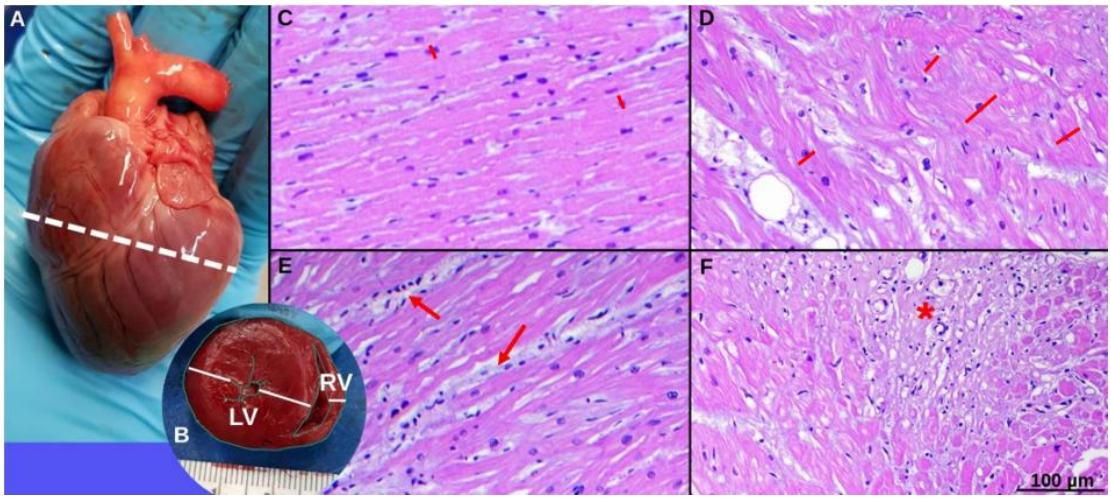

Figure 1.7. An example of postmortem diagnosis of feline hypertrophic cardiomyopathy. *A. On macroscopic examination, the feline heart affected by HCM typically weighs ≥19 grams after removing blood clots. B. On a transverse section at the level of the mid-left ventricle (white dash line, A), the left ventricular wall thickness (excluding the papillary muscles) is greater than 3 times the right ventricular wall thickness. C. Photomicrograph of the myocardium of a cat without HCM for comparison (HE). D. Compared to the section from the normal myocardium, myocytes in cats with hypertrophic cardiomyopathy are hypertrophied (red line marks the diameter of the cardiac myocytes), wavy, and perpendicularly aligned to each other (myofibre disarray). E. Additionally, there is excessive accumulation of extracellular matrix proteins, which defines interstitial fibrosis (red arrows). F. In some cases, the fibroblasts can replace the cardiomyocytes, which is described as replacement fibrosis (red asterisk).*

Postmortem findings described for hearts with HCM defined LVH as cardiac weight ≥19 g with increased LVWT.[116,171,191-193] This ≥19 g cutoff originated from the descriptive postmortem studies of feline HCM by Liu *et al.*, in 1984 and 1993.[171,194] In these two studies, 51 feline hearts that resembled human HCM were described, and cats with HCM had a cardiac weight that ranged from 19 to 33 g.[171,194] However, the diagnosis of feline HCM in these studies was made on postmortem examination of the heart, not by echocardiography prior to death. In 2023, Janus *et al.* described macroscopic and microscopic features of HCM using 13 cats with echocardiographic diagnosis of HCM prior to death and compared these with 10 control cats that died of non-cardiac reasons.[195] In this study, the cardiac weight of cats with HCM ranged from 15 to 45 g, while cats without heart disease had a cardiac weight that ranged from 13 to 15 g.[195] This observation suggests that cats with HCM may have a lower cardiac weight than 19 g. However, in the absence of echocardiographic information prior to death, using the cardiac weight ≥19 g still appears appropriate to use for the postmortem diagnosis of HCM, as most cats with echocardiographic diagnosis of HCM had a cardiac weight ≥19 g in the study by Janus *et al*..[195]

In addition to using an unadjusted cardiac weight, a cardiac-to-body-weight ratio can be calculated by dividing the cardiac weight (in grams) to the body weight (in kilograms). In healthy cats, the cardiac-to-body-weight ratio ranges from 3 to 5 g/kg.[171,196,197] In cats with HCM, the cardiac-to-body-weight ratio ranges from 3 to 12 g/kg, but the average ratio is usually 6.5 g/kg.[171,198] Based on these data, a >4 g/kg cutoff was suggested for the postmortem diagnosis of HCM in a recent review article.[191] However, adjusting the cardiac weight to the body weight appeared less popular in studies of feline HCM. A possible reason for this is that the body weight in cats is generally similar to one another, and this limits the need of adjusting the cardiac weight to the body weight. Furthermore, the use of cardiac-to-body-weight ratio may potentially be erroneous when examining an obese or emaciated cat.

In cats with an increased cardiac weight, the hypertrophy needs to be localised to the left ventricle for a diagnosis of HCM. In the literature, the assessment of the LVWT at postmortem examination is commonly done by comparing the LVWT to the right ventricular free wall thickness on a transverse section of the heart at the level of a mid-left ventricle (Figure 1.7). In cats without HCM, the LVWT is typically 3 times greater than the right ventricular free wall thickness, regardless of the cardiac cycle. Therefore, an increase in LVWT more than 3 times the right ventricular free wall is suggestive of LVH.[116] However, a subjective assessment of LVH by visually assessing the left ventricle is also mentioned. With appropriate training in anatomic pathology, either approach is probably suitable. Alternatively, the LVWT can be measured using a calliper. Studies report that cats with HCM generally have LVWT greater than 8 mm, while most normal cats have an average LVWT of 5-6 mm.[171,199,200] However, defining HCM using a single LVWT measurement at postmortem examination has never been reported in the literature, probably because the measurements are dependent on the angle of the transverse section, stage of rigor mortis, and fixing technique. For example, the heart is usually fixed at a varying stage of systole due to rigor mortis, and the length of fixing with formalin were shown to impact the postmortem cardiac measurements in cats.[186,201]

The macroscopic diagnostic criteria for feline HCM were generally well agreed in the literature. In contrast, the microscopic criteria for the diagnosis of feline HCM was poorly agreed in the literature. For example, the presence of myocyte hypertrophy is commonly used to define HCM on microscopic exam, but the transverse diameter of a healthy cardiac myocyte in cats was poorly described. In the study by Janus *et al.*, the mean transverse diameter of cardiac myocytes was 20 μm

(99% confidence interval: 16-24 μm) in 13 cats with echocardiographic diagnosis of HCM, which was greater than the mean transverse diameter of 14 μm (99% confidence interval: 11-17 μm) in 10 control cats who died of non-cardiac reasons.[195] Based on this work, using the size of erythrocytes as an internal metric system, as in human pathology, may be appropriate in cats. The transverse diameter of feline erythrocytes is 5-6 μm. Given this, a cardiac myocyte that exceeds 3 times the size of the erythrocytes likely suggests myocyte hypertrophy. However, Kershaw *et al.*, reported that there was no difference in the transverse diameter of the cardiac myocytes in 15 cats with macroscopically diagnosed HCM (mean diameter 23.3 μm [99% confidence interval: 17.0-29.5 μm]) and that of 10 control cats that died of non-cardiac disease and had no evidence of LVH on macroscopic examination (22.9 μm [99% confidence interval: 11.1-34.6 μm], independent t-test, unadjusted P = 0.779).[193] The conflicting results between these two studies is likely due to the different inclusion criteria, as none of the cats in the study by Kershaw *et al.* had echocardiography prior to death. By incorporating the clinical information prior to death, Janus *et al.* highlighted that the commonly used macroscopic criteria for HCM (unadjusted cardiac weight >19 g) may be less specific as some cats with HCM have a cardiac weight as low as 15 g. It is likely that some of the control cats in the study by Kershaw *et al.* had subclinical HCM.

Several studies describe myofibre disarray as the gold standard of microscopic diagnosis of HCM in cats (Figure 1.7D).[116,163] However, the frequency of myofibre disarray is inconsistent between studies of feline HCM. First, Liu *et al.*, reported that only 15 out of 51 cats (30%) with macroscopic postmortem diagnosis of HCM had myofibre disarray.[172] Second, Fox et al., reported that 8 out of 13 cats (62%) with echocardiographic diagnosis of HCM had myofibre disarray.[9] Third, Kittleson et al., reported that all 8 Maine Coons with echocardiographic or macroscopic postmortem diagnosis of HCM had myofibre disarray.[8] Fourth, a study by Kershaw *et al.* investigated the pattern of myofibre branches and failed to demonstrate a difference in myofibre branching pattern between 15 cats with macroscopic postmortem diagnosis of HCM versus 15 control cats that died of non-cardiac reasons and had no evidence of HCM on macroscopic postmortem examination (cardiac weight <19 g).[193] Last, Janus *et al.* reported that 11 out of 13 cats (85%) with echocardiographic diagnosis of HCM had myofibre disarray while none of the 10 cats that died of non-cardiac reasons had myofibre disarray.[195] The disparity between these studies is likely explained by the difference in breeds and

diagnostic criteria used to define HCM. For example, some studies only examined cats with echocardiographic diagnosis of HCM, while others diagnosed HCM using macroscopic postmortem examination alone. Additionally, a genetic influence on myofibre disarray is possible, and the genetic cause of HCM may be different between breeds. For example, the family of Maine Coon cats reported by Kittleson *et al.* had a genetic cause identified 6 years later.[8,28] In an experimental study that infected isolated adult feline cardiac myocytes with a modified gene that encodes β myosin heavy chain (gene associated with HCM in both humans and cats),[108] myofibre disarray occurred within 5 days of genetic modification, suggesting that the myofibre disarray is likely explained by the underlying genetic variants in cats.[134]

Interstitial fibrosis and replacement fibrosis are common microscopic findings in feline HCM (Figure 1.7E and 1.7F). In a study by Liu *et al.*, 27 out of 51 cats (53%) with HCM had evidence of interstitial or replacement fibrosis.[171] Additionally, intramural arteriosclerosis was identified in 38 out of 51 cats (74%) and like in humans, the abnormal intramural coronary arteries were commonly seen within or at the margins of areas of moderate to severe fibrosis. This observation also suggested a possible role of CMD in cats.[171] Similarly, moderate to severe intramural arteriosclerosis and myocardial fibrosis were identified in all 8 Maine Coons with HCM in another study.[8] However, interstitial and replacement fibrosis are not a unique feature of HCM, and 2 separate studies by Kershaw *et al.* and Janus *et al.* failed to demonstrate an increased area of myocardial fibrosis in cats with an echocardiographic or postmortem diagnosis of HCM compared to control cats that died of non-cardiac reasons.[193,195]

In cats with SAM, reactive thickening and fibrosis of the MV leaflet and endocardial layer of the LV outflow tract is also common due to the constant contact between the AMVL and LV outflow tract.[9] This lesion is not pathognomonic for HCM but increases the suspicion of HCM on postmortem exam.

A method of postmortem cardiac imaging was recently reported.[186,190] Specifically, the use of micro-computed tomography was shown to provide a comprehensive overview of the cardiac anatomy, which includes the myofibre architecture that mirrored the traditional microscopic examination by anatomic pathologists.[186,190] Micro-computed tomography is currently limited to research units, but an increasing availability will likely improve the quality of postmortem assessment of HCM in cats.

Overall, the evidence suggests that a probable diagnosis of HCM can be made on postmortem examination. The current literature supports the use of an unadjusted cardiac weight ≥19g with evidence of increased LVWT on subjective examination by an experienced pathologist or an increased LVWT that is 3 times greater than the right ventricular free wall thickness for the macroscopic diagnosis of HCM. Microscopic criteria are less agreed but common features include myocardial hypertrophy, myofibre disarray, and interstitial fibrosis.

1.3j Phenotypic progression and prognostic factors of HCM in humans and cats

In cats and humans with HCM, the appearance of the heart continues to change as the disease progresses.[27,136,202] This includes progressive LVH, which is prognostically important, especially when the LVH is considered 'massive' or 'extreme'.[9,41,115] For example, a study of 480 humans with HCM by Spirito *et al.* demonstrated that approximately 40% of patients with end-diastolic LVWT ≥30 mm (described as 'massive LVH') suffered sudden death, whereas approximately 11% of patients with end-diastolic LVWT <30 mm suffered sudden death during >20 years of follow-up after the initial diagnosis of HCM.[203] In comparison, a study of 282 cats with HCM by Payne *et al.*, demonstrated that end-diastolic LVWT ≥9.0 mm (described as 'extreme LVH') was an independent predictor of death, and cats with end-diastolic LVWT ≥9.0 mm were 4.5 (95% confidence interval: 2.2-9.2) times more likely to develop a complication of HCM and die over a median 2 year follow-up period (minimum 0 days – maximum 7.5 years).[115]

Systolic anterior motion of the mitral valve (SAM; Figures 1.2 and 1.8) is a common feature of HCM in both cats and humans. In both species, the modified-Bernoulli equation is commonly used to estimate the pressure gradient (PG) across the narrowest point in blood flow created by SAM to quantify the severity of the obstructive blood flow. According to the Bernoulli principle, a higher PG would suggest greater obstructive blood flow. In humans, PG ≥30 mmHg is conventionally described as 'dynamic LV outflow tract obstruction' (DLVOTO).[41,96,97] However, the severity of DLVOTO is influenced by the heart rate, vascular tone, and myocardial function, which means that DLVOTO can be severe only during exercise but resolve upon resting.[47] To accurately assess the severity of DLVOTO in each patient, the current guidelines recommend a 'provocative manoeuvre' to physiologically

challenge the heart during echocardiography and test if the patient develops severe DLVOTO (Figure 1.8). Commonly performed provocative manoeuvres in humans include standing up, treadmill exercise, and a 'Valsalva manoeuvre', which involves straining while breath holding.[41,96,97] With a provocative manoeuvre, up to 60-70% humans are diagnosed with SAM and DLVOTO at the time of HCM diagnosis. [47] A careful assessment of the DLVOTO is considered important as resting or provoked PG ≥50 mmHg is associated with increased risk of symptoms related to myocardial ischaemia and warrants therapeutic intervention.[41,96,97,204,205]

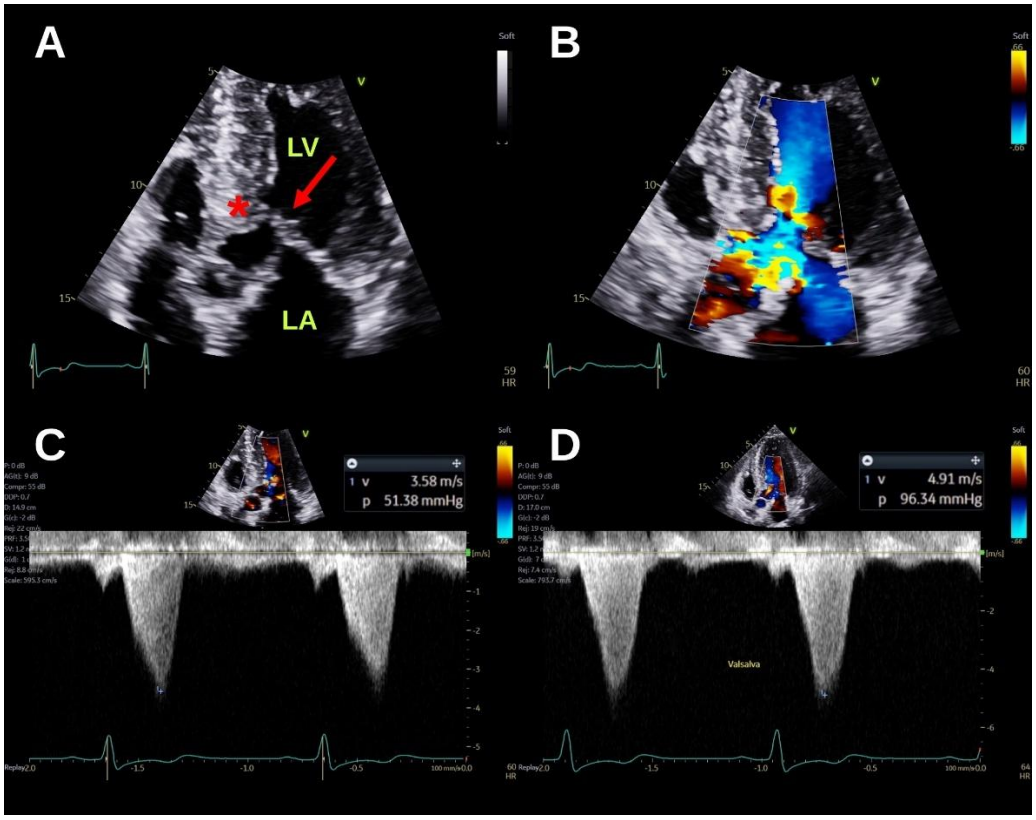

Figure 1.8. Echocardiographic still images of a human adult with hypertrophic cardiomyopathy with systolic anterior motion of the mitral valve (SAM) and dynamic left ventricular outflow tract obstruction (DLVOTO). *A. The SAM is characterised by the displacement of the tip of the mitral valve leaflets toward the left ventricular outflow tract (asterisk) during systole (red arrow). SAM is a common feature of HCM in humans and is considered to develop due to the complex changes in the left ventricular geometry, secondary to the asymmetric left ventricular hypertrophy, elongated mitral valve leaflet, and increased myocardial contractility. B. In patients with SAM, the abnormal positioning of the mitral valve obstructs the blood flow to the aorta, which is displayed as mosaic colour flow on colour Doppler assessment. This obstructed blood flow is reported as DLVOTO. C. In this patient with HCM and SAM, a DLVOTO was diagnosed with resting pressure gradient of 51 mmHg. D. However, a provocative manoeuvre (Valsalva manoeuvre), which aims to physiologically enhance the left ventricular function, heart rate, and vascular tone, demonstrated that DLVOTO can worsen with a pressure gradient of up to 96 mmHg. In humans, resting or provoked PG ≥50 mmHg warrants therapeutic intervention.[41,96,97,204,205] Abbreviations: LA, left atrium; LV left ventricle. Figure materials used with permission from Erika Hong, Princess Alexandra Hospital, Queensland, Australia.*

In cats, a clear association between DLVOTO and the risk of cardiac death is not established. For example, in five retrospective studies with sample sizes that ranged between 46 to 1008 cats with heart disease, the presence of SAM or DLVOTO was not associated with earlier death.[1,9,23,114,115] However, it is important to emphasise that these five studies were retrospective in design, and cats with SAM or DLVOTO are expected to be diagnosed earlier than cats without SAM or DLVOTO. This is because, the presence of SAM and DLVOTO typically creates a loud heart murmur that can be readily detected on routine health checks, which prompts a referral for echocardiography. Indeed, cats with SAM or DLVOTO were younger than those without in these studies, which further suggests a potential selection bias. Moreover, the presence of SAM and DLVOTO were assessed only at a single time-point in these studies. The level of stimulation, heart rate, vascular tone, and myocardial function can change over time and some cats with HCM were shown to either gain or lose SAM in two prospective studies of 32 and 21 cats with HCM over a ≥6 month follow-up period.[136,206] Neither of these prospective studies was designed to report the incidence rate and clinical importance of the gain and loss of SAM over time, but highlighted the importance of serial echocardiography when examining SAM and DLVOTO in cats. Separate to these studies, two retrospective studies demonstrated that the plasma or serum concentration of troponin I is increased in cats with SAM or DLVOTO.[207,208] Given that increased plasma troponin I concentration is usually associated with earlier cardiac death in cats with HCM, one may consider that SAM and DLVOTO are harmful in cats with HCM.[209] Nevertheless, neither of the two studies that measured troponin I in cats with SAM or DLVOTO directly evaluated the risk of cardiac death to confirm this possibility. Overall, the question on whether SAM and DLVOTO are harmful in cats with HCM remains, and further studies are needed to answer this question.

Mitral regurgitation (MR) is common in humans and cats with SAM due to the anterior displacement and distortion of the mitral valve during systole. The resultant MR typically peaks during mid-to-late systole and is directed posteriorly and laterally.[210] In humans, MR can be severe and worsen the prognosis of HCM.[211] In contrast, the MR in cats with HCM is typically mild. In two retrospective studies of 255 and 282 cats with HCM, similar to DLVOTO, the presence of MR was not associated with clinical signs or death associated with HCM, which further supports the general observation that the MR is mild in cats with HCM.[115,156]

The diastolic dysfunction is also progressive in humans and cats with HCM. The mechanism of progressive diastolic dysfunction in HCM is considered a combination of increased left ventricular internal pressures, reduced left ventricular size and compliance from hypertrophy and fibrosis, altered energetics, myocardial ischemia, and impaired calcium recycling during cardiac cycle.[137,212] In humans, diastolic dysfunction can contribute to decreased exercise capacity and adverse prognosis independent of DLVOTO.[213,214] In cats, an association between diastolic dysfunction and development of clinical signs or death associated with HCM was assessed by Payne *et al.* In this study of 282 cats with HCM, the hazard ratio of cats with a restrictive filling pattern (the highest grade of diastolic dysfunction) for developing a complication of HCM during a median follow-up period of 2 years was 3.1 (95% confidence interval: 1.7-5.7).[115] This study highlights the importance of careful assessment of the myocardial function in cats with HCM. However, the assessment of diastolic function using echocardiography is generally difficult in cats due to their fast heart rate, as all variables of diastolic function are influenced by the heart rate. Probably due to this difficulty, only 114 out of 282 cats in the study by Payne *et al.* had the diastolic function assessed, and the restrictive filling pattern was not a significant variable in their multivariable analysis. In comparison, a measurement of left atrial function can provide a crude assessment of the left ventricular diastolic function. The left atrial function is considered particularly important in cats with HCM as stiff left ventricles are more dependent on healthy atrial function to provide an adequate ventricular filling and stroke volume. Payne *et al.* also investigated the impact of left atrial function on the development of complications of HCM, and every 1% drop in the percentage change of the left atrial diameter over each cardiac cycle (named ‘left atrial fractional shortening’) increased the hazard ratio of developing a complication of HCM by 1.1 (95% confidence interval: 1.1-1.2).[115] The prognostic influence of left atrial fractional shortening remained significant in their multivariable analysis.

The hypercontractile left ventricle is a common feature of HCM in humans and cats. According to the biomechanical studies, the increased myofibrillar calcium sensitisation, reduced SRX state, and excessive actin-myosin cross bridging appear to be the major mechanisms for the hypercontractile left ventricle.[118-125,215] However, the hypercontractile left ventricle eventually loses its contractility and develops systolic dysfunction over time. In humans with HCM, left ventricular systolic dysfunction is associated with increased risk of arrhythmia development and sudden

death, as well as symptoms related to heart failure and myocardial ischemia.[213,216,217] In cats with HCM, those with systolic dysfunction were 4.5 (95% confidence interval: 2.2-9.2) times more likely to die within 2 years of the diagnosis of HCM in one retrospective study of 282 cats with HCM.[115]

In advanced cases, the myocardium becomes necrotic and is replaced by fibrosis. The resultant cardiac morphology is a dilated left ventricle with thin and hypokinetic wall segments. This late stage of HCM can occur in both humans and cats with HCM, and is commonly described as 'end-stage HCM'.[4,27,103] In humans, up to 15% of patients with HCM can progress into end-stage HCM.[218,219] In comparison, the exact incidence of end-stage HCM in cats is unknown, but it is also regularly identified by veterinary cardiologists. Histologic features of end-stage HCM in humans and cats are similar, with focal or multifocal loss of cardiac myocytes and replacement fibrosis being the predominant findings.[103]

1.3k Prevalence and clinical outcome of human HCM

The prevalence of human HCM across the globe is approximately 0.2%, but the prevalence increases with age and reaches up to 0.4% in humans >60 years old.[5,220-223] Despite this relative common diagnosis, HCM is not detrimental in every patient, as only 34% of patients develop symptoms related to heart failure, arterial thromboembolism (ATE) or stroke, or suffer a sudden cardiac death (SCD) event.[6] The remaining 66% patients with HCM are expected to live a normal life without developing major symptoms or requiring cardiac treatments.[6]

The prevalence of myocardial ischemia diagnosed using cardiac magnetic resonance, echocardiography, and nuclear imaging in humans with HCM is approximately 55%.[184] Not all patients with myocardial ischemia develop major symptoms. In fact, some patients will be completely asymptomatic, and these ischemic events are described as 'silent ischemia'. The high prevalence of myocardial ischemia associated with HCM is explained by a decrease in coronary microcirculation and increased myocardial oxygen consumption by the presence of intramural arteriosclerosis, severe LVH, LV hypercontractility, LV diastolic dysfunction, and obstructive blood flow secondary to SAM and DLVOTO.[182-184] Additionally, 23-41% HCM patients have myocardial bridging compared to 5-7% in the general population. Myocardial bridging is a condition where a coronary artery is

tunnelling through the myocardium instead of travelling over it, and the intramyocardial section of the coronary artery can become compressed by the heart muscle during contraction.[43,184,224] As a result, patients with myocardial bridging can experience transient myocardial ischemia. Possibly due to the compromised coronary circulation and increased myocardial oxygen consumption, many human HCM patients develop symptoms of myocardial ischemia including angina, exercise intolerance, exertional dyspnoea, and nausea.

Heart failure is broadly used to describe symptoms caused by the heart failing to meet the physiologic demand of the body. With this definition, the prevalence of heart failure in human HCM is as high as 50% as it includes symptoms associated with myocardial ischemia.[225] Congestive heart failure (CHF) is a subset of heart failure that develops when the heart cannot propel blood forwards adequately due to impaired ventricular filling or emptying. Congestive heart failure results in circulatory volume overload, pulmonary venous congestion, lower extremity oedema, and renal dysfunction. In humans, CHF is considered an uncommon consequence of HCM that develop from a complex interplay of progressive diastolic dysfunction and reduced compliance caused by progressive LVH, myocardial ischemia, arrhythmias, and other cardiometabolic syndromes including obesity, hypertension, sleep apnoea and diabetes.[225]

Arterial thromboembolism (ATE) in HCM is characterised by development of an intracardiac thrombus, which embolises and circulates with blood flow until the diameter of the distal artery becomes smaller than the thrombus. The blood flow where the distal artery normally supplies is blocked, and ischemic injury results in the area where the distal artery normally supplies to. When the ATE results in ischemic brain injury, the syndrome is conventionally described as a ‘stroke’. In humans, a stroke is a dreaded consequence of HCM due to its high risk of causing death or permanent disability.[41,226] Therefore, an appropriate risk stratification and prevention of ATE is paramount. The yearly incidence of ATE in patients with HCM is 0.8% but increases up to 1.9% in patients >60 years old.[227] The outcome of patients with HCM and ATE is best described in a study by Maron *et al.*, where 44 out of 51 patients with ATE (86.3%) had a stroke and the remaining 7 patients (13.7%) developed ATE in the limbs, kidneys, or spleen. In the 44 patients with a stroke, 10 patients (22.7%) did not survive and 11 out of 34 surviving patients (32.4%) had a varying degree of permanent neurologic impairment such as aphasia and hemiparesis. In comparison, none of the patients that had ATE in the kidneys, limbs, or spleen died or suffered

permanent disability due to the ATE event. Currently established risk factors for ATE in human HCM patients include older age, concurrent vascular disease, greater maximal LV wall thickness, previous ATE events, and the presence of heart failure, myocardial ischemia, and atrial fibrillation.[227,228] In humans at high risk of ATE, anticoagulant therapy, such as low-molecular weight heparin or warfarin, is strongly recommended.[41,97] In one retrospective study of 4821 HCM patients, 172 patients developed ATE within 10 years of the HCM diagnosis, with anticoagulant therapy reducing risk of ATE by approximately 50%.[228]

In humans, sudden cardiac death (SCD) is defined as death due to a cardiovascular cause that occurs within an hour of the onset of symptoms, or if unwitnessed, within 24 hours of last being seen alive.[229,230] The yearly incidence of SCD in HCM patients is approximately 1% in adults and 1.5% in children.[231,232] Most SCD events secondary to HCM are caused by the development of malignant ventricular arrhythmias. Currently established risk factors for the development of malignant ventricular arrhythmias and SCD include older age, left atrial enlargement, greater maximal LV wall thickness, history of fainting episodes, history of SCD in the family, and the presence of DLVOTO, left ventricular systolic dysfunction, myocardial ischemia, heart failure, and atrial fibrillation.[161,233,234] Some of these risk factors were used to develop scoring systems, which allow appropriate patient selection for an early implantable cardioverter-defibrillator (ICD).[41,97] With the increasing use of such scoring systems and prevention of SCD by early ICD implantation, the incidence of SCD has decreased in human HCM patients, with the yearly incidence of SCD reduced to 0.3% in the current era.[233]

1.3l Prevalence and clinical outcome of feline HCM

The prevalence of feline HCM has only been investigated in the United Kingdom (UK) and United States of America (USA) but the prevalence was nearly identical at both countries.[2,3] Paige *et al.,* screened 103 apparently healthy cats using echocardiography in the USA, and 15 cats (14.6%) had echocardiographic evidence of subclinical HCM.[3] In that study, most cats were young adults aged between 1 and 5 years old, and 91.3% were non-purebred. The small number of purebred cats (8.7%) included the Himalayan, Siamese, Ocicat, and Maine Coon. In comparison, Payne *et al.*, screened 780 apparently healthy cats using echocardiography in the UK

(the CATSCAN study), and showed that 115 cats out of 780 (14.7%) had echocardiographic evidence of subclinical HCM.[2] In that study, most were also young, and aged between 1 and 5 years old. Similarly, most cats were non-purebred (97.2%), with the 2.8% purebred cats being Bengals, Exotic Shorthairs, and Ragdolls. In contrast to the study by Paige *et al.*, Payne *et al.* also demonstrated that the prevalence of HCM increased with age and reached 29% in cats >9 years old.[2]

The prevalence of HCM outside the UK and USA is mostly unknown. However, HCM is a global disease,[1] and HCM is regularly diagnosed in cats in New Zealand. Therefore, it is tempting to assume that HCM is similarly prevalent in New Zealand as in the UK and USA. Nevertheless, a geographic difference is still possible and, as part of this thesis, a dedicated study that investigates the prevalence of HCM in New Zealand cats is reported. If the prevalence of feline HCM in New Zealand is shown to be different from that of the UK and USA, the New Zealand veterinarians may need a different HCM screening strategy to that suggested in the UK and USA.

Similar to humans, not all cats with HCM will develop a cardiac complication or die from it. For example, a multicentre retrospective study of 1730 cats across the globe (the REVEAL study), demonstrated that 69.5% cats with HCM were alive without developing any complications from HCM, while the remaining 30.5% cats developed congestive heart failure, arterial thromboembolism (ATE), or sudden cardiac death (SCD) over a >5 year follow-up period.[1] Similarly, in a prospective study that serially screened 107 cats with echocardiography and blood tests (the CATSCAN II study), 79.4% cats with HCM did not develop any cardiac complications, but the remaining 20.6% developed CHF, ATE, or SCD over a median follow-up period of 3.6 years.[136] Despite the likelihood of cats with HCM living a normal life, the clinical manifestations of CHF, ATE, SCD are very distressing, and many studies highlighted the importance of appropriate risk stratification for initiating preventive therapy and counselling pet owners.[1,23,27,114,115,136]

Cats with HCM are also considered to be susceptible to developing myocardial ischemia, as echocardiographic and histologic features of myocardial ischemia and infarction are frequently identified.[27,103,209,235,236] However, most cats do not appear to develop clinical signs related to myocardial ischemia as seen in humans. For example, there is only one report of cats that developed clinical signs during the period of probable myocardial ischemia.[190] In this report, cats displayed depressed mentation, panting, and drooling and these clinical signs were deemed to limit the quality of life of cats and resulted in humane euthanasia in two cats out of

three.[190] The absence of other reports of clinical signs associated with myocardial ischemia suggest that the clinical signs of myocardial ischemia are either too subtle for cat owners and veterinarians to detect, or that many cats genuinely do not develop symptoms ('silent ischemia'). Furthermore, the long-term consequence of myocardial ischemia in cats is poorly understood, and the question whether those that develop myocardial ischemia will progress faster is unknown.

In cats, heart failure, specifically CHF, is a common consequence of HCM with a yearly incidence of 2-6%.[1,136] Most cats with CHF present in severe respiratory distress and require prompt stabilisation and long-term diuretic therapy. In a retrospective study by Payne *et al.*, 44 out of 255 (17.3%) cats with HCM developed CHF and died within 2 years of the initial diagnosis of HCM.[156] In the same study, older age, presence of arrhythmias, enlarged left atrium, diastolic dysfunction, left atrial dysfunction, increased LVWT were associated with the risk of death related to CHF.[156] Heart failure other than conventional CHF is poorly recognised in cats, likely due to their sedentary lifestyle and tendency to hide symptoms.

The incidence of ATE is 2-3% per year in cats with HCM.[1,136] Affected cats usually present with severe pain and paralysis of one or more limbs due to the ischemic injury to the muscles and nerves. However, ATE to other organs including the kidneys is also possible. The development of thrombus can be explained by the Virchow's triad, which consists of blood stasis, endothelial dysfunction, and hypercoagulable state.[237] In context of feline HCM, blood stasis is explained by the progressive left atrial enlargement, arrhythmias, and loss of the left atrial function; [156,238] endothelial injury is explained by endothelial disruption by severe left atrial enlargement and upregulation of procoagulant glycoproteins that promote formation of a thrombus;[239,240] and hypercoagulability is explained by platelet activation, decreased fibrinolysis, presence of immunothrombins, and enhanced thrombin generations.[237] Identifying some of these risk factors (i.e., moderate LA enlargement) and starting preventive medications, such as clopidogrel or dual therapy (e.g., clopidogrel and rivaroxaban), is currently recommended.[4] Prevention of ATE is important as >50% of cats with ATE are euthanised by veterinarians due to severe pain and perceived poor prognosis.[241] However, it is important to note that not all cases of ATE are life-threatening or caused by advanced HCM, and some cats that develop ATE have hyperthyroidism or an unidentifiable cause.[242] There is no study that describes the long-term prognosis in cats that developed ATE due to

hyperthyroidism or an unidentifiable cause, but these cats are anecdotally described to have a good to excellent long-term prognosis following the treatment of ATE.

In cats, SCD is usually defined as an unexpected death without any clinical signs or known trauma within 24 hours of death, and when the death was assumed to be cardiac in origin.[1,115,136,156] The incidence of SCD in cats with HCM is 0.5-1.4% per year.[1,136] However, there is a possibility that the incidence of SCD is underestimated in cats with HCM, as not all pet owners would update vets after their cat experiences SCD. In a retrospective study by Payne *et al.*, 12 out of 255 cats (4.7%) were believed to have suffered SCD within 2 years after the diagnosis of HCM.[156] In this study, a history of fainting episodes, regional wall hypokinesis, left atrial enlargement, left ventricular systolic dysfunction, and the presence of spontaneous echocardiographic contrast were associated with the risk of SCD.[156]

The reason for the higher incidence of complications and death associated with HCM in cats compared to humans is likely multifactorial. First, HCM may simply progress more rapidly in cats compared to humans. Second, the timing of the HCM diagnosis in relation to the disease progression may be late in some cats, as the decision to perform echocardiography usually depends on the cat to either have a loud heart murmur or arrhythmia, a familial history of severe HCM, breed predisposition to developing HCM, a recent death of a sibling due to heart disease, or that the cat is already displaying clinical signs of HCM. If these cats are diagnosed with HCM at later severity than in humans in terms of the disease progression, the yearly incidence of developing complications related to HCM will be higher in cats compared to humans. Third, medicating cats can be difficult, and currently available antithrombotic medications, namely clopidogrel and rivaroxaban, are very bitter in taste. Due to the limited compliance of cats, some ATE events may not be prevented, further increasing the incidence of ATE in cats compared to humans.

The site of ATE is also different between cats and humans, as ATE typically involves limbs in cats, whereas it typically causes a stroke in humans. This difference may be explained by the biological difference in the vascular anatomy, but a more plausible explanation is that ATE in the limbs is very easy to identify in cats compared to the ATE in the brain. For example, a cat presenting to a veterinary clinic with painful, cold, pulseless limb can be diagnosed with ATE in the limb if there is no history information or physical examination abnormality to suggest trauma.[243,244] In comparison, ATE in the brain requires an advanced imaging such as magnetic resonance imaging, which is not commonly performed in cats compared to that in

humans due to the limited availability, requirement of general anaesthesia, and cost involved. Another difference between ATE in cats and humans is that the survival rate in cats with ATE is much lower than in humans. The lower survival rate in cats with ATE is likely because many veterinarians choose to euthanise cats with ATE due to the perceived poor prognosis associated with ATE.[241] However, a selection bias is also possible, as severe ATE in cats is easily diagnosed on clinical examination, while mild ATE in cats can be missed by owners and veterinarians.

1.3l Therapeutic options for human and feline HCM

There are several therapeutic options that target the haemodynamic, arrhythmic, and thrombogenic consequences of HCM. For example, medical or surgical intervention to relieve the obstructive blood flow is recommended for a human HCM patient with symptoms related to DLVOTO. Similarly, a human at risk of SCD due to arrhythmia may undergo an ICD implantation, and those at risk of ATE or stroke are recommended to receive anticoagulant therapy. In cats, those at risk of ATE are initiated on long-term preventive treatments of antiplatelet or anticoagulant medications, and those with symptoms related to CHF are initiated on diuretic therapy. These therapeutic strategies are outlined in the current practice guidelines for human and feline HCM.[4,41,96,97]

Despite these therapeutic options that can prevent or treat the pre-existing sequela of HCM, there is no treatment that has been clearly demonstrated to prevent or reverse the HCM process such as LVH, myofibre disarray and myocardial fibrosis in humans and cats. However, the recent advancement in genetic and molecular studies provides unique treatment options that may possibly prevent or delay the LVH, myofibre disarray, and myocardial fibrosis in humans and cats. The current information on these emerging treatments is summarised below.

Mavacamten and aficamten are cardiac myosin inhibitors, which inhibit the cardiac myosin ATPase and reduce the excessive actin-myosin cross-bridging component of HCM.[245] The cardiac myosin inhibitor was first identified by Green *et al.* in 2016, who demonstrated that mice treated with cardiac myosin inhibitors had less severe myofibre disarray, myocardial fibrosis, and LVH compared to the untreated mice.[245] Since then, mavacamten and aficamten have been developed by pharmaceutical companies and extensively studied in people with HCM. Both

mavacamten and aficamten demonstrated reduced disease severity and improved symptomatic scores in studies of human HCM;[246-262] however, it is important to note that these clinical trials only enrolled patients with obstructive HCM (e.g., patients with DLVOTO), and whether the benefits of mavacamten and aficamten would extend to patients with non-obstructive HCM is currently unknown. Small clinical trials of mavacamten and aficamten in patients with non-obstructive HCM showed similar promise.[263,264] However, the larger scale phase III clinical trials of mavacamten and aficamten are still undergoing to review the benefits in patients with non-obstructive HCM. A recent press-release from the sponsor of one of the trials (ODYSSEY-CM) suggested that mavacamten failed to improve the clinical severity score in patients with non-obstructive HCM.[265] However, the data analysis for this study has not been completed. In comparison, cardiac myosin inhibitors have only been investigated in a small number of cats. In these cats, cardiac myosin inhibitors (aficamten and other prototype) demonstrated a dose-dependent improvement in the LV hypercontractility as shown in people.[266-268] Given that LV hypercontractility is a common feature of feline HCM, cardiac myosin inhibitors are a promising avenue in the management of feline HCM, especially in cats with SAM and DLVOTO. However, despite the potential benefit of cardiac myosin inhibitors, there currently is no ongoing clinical trial for feline HCM due to the lack of sponsors. Furthermore, mavacamten and aficamten cannot be dispensed by veterinarians and be used for cats at present, as these medications are protected for human use by the drug companies and regulatory bodies in countries where they are available in. Additionally, compounding mavacamten and aficamten by independent pharmaceutical companies is illegal due to the drug patent in all countries where these medications are available, and this further prevents veterinarians from further researching and using these medications in feline HCM.

EDG-7500 is a selective cardiac sarcomere modulator that works similarly to the cardiac myosin inhibitors but preferentially works during low calcium conditions.[269] Compared to cardiac myosin inhibitors, EDG-7500 increases the rate of both the engagement and disengagement of the actomyosin and decreases the contractility only in the beginning of the myocardial contraction. EDG-7500 is currently being investigated in an open-label phase II trial in humans with both obstructive and nonobstructive HCM (CIRRUS-HCM; NCT06347159).

Rapamycin, also known as sirolimus, is an inhibitor of the mechanical target of rapamycin (mTOR) kinase enzyme. At low intermittent dosing frequency,

rapamycin was shown to reduce reactive LVH in response to mechanical stimulation without the risk of adverse effect, such as insulin resistance and glucose intolerance in mice.[270-272] Given that reactive LVH is a protective response by the cardiac myocyte to reduce the amount of mechanical force applied to each cardiac myocyte, preventing the compensatory LVH could potentially be harmful. Furthermore, the mTOR pathway is currently not considered to be the primary mechanism of LVH in HCM in humans and cats. However, LVH has been shown to predict earlier cardiac death in some feline studies of HCM, and the potential benefit of rapamycin by suppressing the LVH was evaluated in a clinical trial of 43 cats with subclinical HCM (RAPACAT study).[273] In this clinical trial, cats treated with rapamycin had a reduced maximal LVWT (mean 7.4 mm, 95% CI: 6.9-7.9 mm) compared to those treated with placebo (mean 8.4 mm, 95% CI: 7.9-8.9 mm) at a 6 month recheck (P = 0.01).[273] However, this study was small in size and demonstrated a very small reduction in LVH, so whether this subtle reduction in LVWT will lower the risk of HCM complications is in question. Overall, the level of evidence to support the use of rapamycin in treatment of feline HCM is low, especially given the potential harm of suppressing reactive LVH. However, a second clinical trial for rapamycin for feline HCM is currently undergoing recruitment phase, with plans to follow cats for one year (the HALT study).[274] If the HALT study clearly demonstrates that rapamycin is safe and can delay the clinical sequalae of HCM beyond suppressing LVH, it will likely become the standard treatment option for feline HCM as rapamycin is relatively cheap and widely available in most countries. Rapamycin is not being investigated as a treatment to prevent progression of human HCM.

In humans, there are several clinical trials for gene therapy that are either being developed or in the recruitment phase. For example, the MyPEAK-1 trial is investigating the use of TN-201 DNA to treat HCM patients with *MYBPC3* variants (gene associated with HCM in humans and cats), and early results showed the treatment safety increased the number of MYPBC3 protein in treated patients.[275] Similarly, a gene therapy for patients with HCM associated with TNNI3 variant (LX2022) is currently under development.[276] In cats, there are no planned or in progress clinical trials for gene therapy for HCM. The cost for gene therapy is very high and the cause of feline HCM is mostly unknown, which likely will limit the benefit of specific gene therapy in cats.

Perhexiline is a carnitine palmitoyl transferase-1 inhibitor that shifts the myocardial metabolism from fatty acid to glucose utilisation. In one human study,

patients with nonobstructive HCM had improved phosphocreatine-to-adenosine triphosphate ratio, exercise capacity and other measures of HCM severity after a mean duration of 4.6 months of treatment.[277] In comparison, trimetazidine, which inhibits oxidation of fatty acids, was not shown to improve the peak oxygen consumption in patients with nonobstructive HCM after 3 months of treatment. The lack of benefit with trimetazidine was hypothesized to be due to trimetazidine being a weaker class of drug compared to perhexiline or having an insufficient duration of therapy.[278] Ninerafaxstat is a cardiac mitotrope pFOX inhibitor, which partially inhibits fatty acid oxidation through the mitochondrial long-chain fatty acid beta-oxidation pathway, thus reducing the amount of oxygen required for ATP generation and increasing myocardial efficiency.[279,280] In a clinical trial of human HCM, ninerafaxstat did not improve the peak oxygen consumption in treated patients,[279] but this drug is expected to be further evaluated in a phase IIb trial. None of these drugs are being studied in feline HCM currently.

1.4 Summary and hypotheses

This literature review identified many remarkable similarities between human and feline HCM. Specifically, echocardiographic, macroscopic, and microscopic features of HCM and the potential sequela of HCM were very similar between the two species. However, some differences were also noted, which include a higher prevalence of subclinical HCM in cats (1 in 7 cats) compared to humans (1 in 500 humans), and higher incidence of CHF and ATE in cats with HCM compared to humans. In both species, several treatments have been investigated to directly target the molecular and genetic component of HCM; however, none of the studies revealed a clearly effective treatment for HCM. Furthermore, whether SAM and DLVOTO are harmful in cats with HCM is unknown. In addition, the causes of HCM remain unknown in cats and, as in humans, it is unclear the role of environmental factors such as psychological stress on the development of HCM. Finally, the prevalence of HCM has only been studied cats in the UK and USA, despite the disease being diagnosed globally.

In this thesis, five studies were designed to address some of these knowledge gaps and advance the understanding of feline HCM. The objectives of these five studies were to 1) describe the prevalence of feline HCM in New Zealand, 2)

describe the pattern of HCM phenotypic development, 3) describe the HCM phenotypic progression with a specific focus on SAM and DLVOTO, 4) describe the influence of psychologic or physiologic stress as an environmental cause of feline HCM, and 5) investigate the pattern of HCM disease progression and a potential genetic cause of HCM in Sphynx cats in New Zealand. Based on these five aims, the following eight hypotheses were generated.

Hypotheses

- The prevalence of subclinical HCM in non-purebred cats in New Zealand is 14-15%.
- Cats with HCM can spontaneously gain or lose SAM over time.
- Cats with HCM and SAM or DLVOTO have faster disease progression than cats without SAM or DLVOTO.
- AMVL elongation is an acquired process that occurs prior to the development of an HCM phenotype in cats.
- An elongated AMVL, upper limit of normal LVWT, and reduced LA FS% are predictors of an HCM phenotype in cats.
- Measurable markers of psychologic or physiologic stress are associated with the subclinical diagnosis of HCM in cats.
- Sphynx cats in New Zealand have a higher prevalence of HCM than non-purebred cats (>15%).
- *The ALMS1* gene variant *(ALMS1*:ENSFCAG00000008756: c.7384G>C;p.[G2462R]) is not associated with the HCM diagnosis in Sphynx cats in New Zealand.

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Chapter 2. Labile nature of SAM

Note, the experiments reported in this chapter were published in Journal of Veterinary Internal Medicine.[1] The published manuscript has only undergone minor modifications for this thesis chapter.

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2.1 Introduction

Hypertrophic cardiomyopathy (HCM) is defined as left ventricular hypertrophy (LVH) due to an unknown cause or a sarcomeric mutation.[2] Systolic anterior motion of the mitral valve (SAM) is present in 30-50% of cats at the time of HCM diagnosis,[3,4] and is characterised as abnormal movement of the mitral valve towards the interventricular septum, narrowing the left ventricular outflow tract (LVOT) and causing dynamic LVOT obstruction (DLVOTO).

Severe DLVOTO is associated with increased risk of symptoms and death in people with HCM.[5,6] In studies of prognosis in cats with HCM, SAM has not been reported to be a risk factor for heart failure or death.[4,7-10] However, most studies of SAM in cats have been retrospective, with cats only evaluated at a single time-point. Recent prospective studies reported that some cats gained or lost SAM over time.[11,12] If the presence of SAM is evaluated at only a single time point in a study, any cats that had previously lost SAM would be mistakenly classified as non-obstructive HCM. Any association of SAM with the frequency of subsequent progression of cardiac disease would then be underestimated.

Several morphological and functional abnormalities of the left heart have been associated with SAM in humans.[13-15] These include increased mitral valve leaflet length and area; reduced mitral-aortic angle; reduced distance between mitral valve coaptation point and interventricular septum (IVS); septal bulge; and abnormal papillary muscle position and orientation.[14,15] Additionally, increased left ventricular (LV) systolic function and increased adrenergic tone alter the flow vector and drag the mitral valve leaflets towards the LVOT in humans.[16] In cats, increased anterior mitral valve leaflet (AMVL) length, papillary muscle hypertrophy, and increased numbers of false tendons in the LVOT have been associated with the presence of SAM.[17] Many of these factors require specific views for optimal imaging, so should ideally be studied either prospectively, or using computerized tomography or cardiac magnetic resonance imaging. However, echocardiographic details of left heart dimensions such as LV wall thickness, LV fractional shortening and left atrial (LA) size can be reported in retrospective studies.

Hypertrophic cardiomyopathy can take years to progress.[10,11] The aims of this study were to describe the incidence of change in the presence of SAM between baseline and follow-up assessment, and any clinical and echocardiographic features

associated with the gain or loss of SAM. The hypotheses were 1) some cats with HCM will gain or lose SAM between assessments ≥12 months apart, 2) the gain or loss of SAM are associated with cat's age, scan-interval, changes in the LV dimensions, and 3) cats with SAM will show more disease progression than cats without SAM. Greater understanding of SAM will better define HCM and help design future treatment trials or outcome studies in cats with HCM.

2.2 Materials and Methods

2.2a Study design

This was a retrospective cohort study involving two referral centres (Animal Referral Centre, New Zealand and Queen Mother Hospital for Animals, The Royal Veterinary College, United Kingdom). Ethical approval for data collection in the United Kingdom was provided by the Royal Veterinary College Ethics Committee (URN: M20170143). Ethical approval for data collection in New Zealand was not required as this data was collected retrospectively.

Electronic medical records between October 2009 to January 2018 from Queen Mother Hospital for Animals, and between July 2020 to January 2023 from Animal Referral Centre, were searched for cats referred to a cardiologist for cardiac screening prior to breeding or blood donation, or for investigation of a heart murmur. Cats were included if they were diagnosed with HCM and had at least two echocardiograms ≥12 months apart performed by a board-certified cardiologist or supervised cardiology resident. Cats were excluded if they met any of the following criteria at baseline or recheck: incomplete clinical records, echocardiographic studies with low 2D temporal resolution (<60 frames per second), use of sedation, beta blockers or pimobendan in between or during the 2 time points, presence of clinical dehydration, hyperthyroidism, diabetes mellitus, cardiac neoplasia, congenital heart disease, anaemia (packed cell volume <20%), and systemic hypertension. Systemic hypertension was defined as Doppler systolic arterial blood pressure (SABP) measurement of ≥180 mmHg, or ≥160 mmHg if the cat had creatinine ≥140 µmol/L or was ≥6 years old.

Clinical and echocardiographic data were collected at baseline and last recheck. For the clinical data, the age, sex, breed, body weight, reason for cardiac

exam, medication, physical examination findings, Doppler SABP recordings, presence of arrhythmias, electrocardiogram findings, and clinical signs were recorded.

Each echocardiogram was reviewed and measured over 3 different cardiac cycles by a single observer (Joonbum Seo, a board-certified cardiologist). The diagnosis of HCM was confirmed when end-diastolic left ventricular wall thickness (LVWT Max) measured ≥6 mm, and other known differentials of an HCM phenotype ruled out based on the clinical summary.[4] The measurements of two-dimensional echocardiography (2D) variables were LV internal diameter in end-diastole and end-systole measured using a right parasternal short-view axis (RPSax) view;[18] LV fractional shortening calculated based on LV internal diameters;[18] maximal thickness of the IVS and LV free wall (LVFW) in end-diastole in right parasternal long axis (RPLax) 4-chamber, 5 chamber and RPSax views;[19] maximal LA diameter using a RPLax 4-chamber view,[19] LA to aortic ratio using a RPSax view,[20] and AMVL length from a RPLax 5-chamber view.[21] The RPSax view at the aortic valve level was used to guide M-mode for measurement of LA fractional shortening.[8] Maximal LVOT velocity (LVOT Vmax) was measured from a left parasternal apical 5-chamber view using spectral Doppler echocardiography guided by color Doppler.[4] Maximal LV wall thickness was recorded based on the IVS and LVFW measurements (whichever was greater).[11,22] Additionally, the presence of dynamic right ventricular outflow tract obstruction (defined as turbulence of right ventricular outflow tract in the absence of an anatomical abnormality with blood flow velocities exceeding 1.7 m/s);[23] DLVOTO (defined as LVOT Vmax exceeding 2.5 m/s);[4,17] mid-LV obstruction (defined as the presence of turbulence on colour Doppler during systole);[24] end-systolic cavity obliteration (defined as the disappearance of the LV cavity at end-systole on 2D imaging),[18] and regional LV wall hypokinesis (defined as reduced segmental LV wall motion)[25] were recorded. The heart rate was calculated from the echocardiographic loop used to diagnose SAM.

The presence of SAM was defined as the anterior movement of the AMVL towards the LV outflow tract during systole using 2D imaging (Figure 2.1).[15,17,22] In addition to the primary observer, a second observer (Virginia Luis Fuentes, a board-certified cardiologist) independently assessed the presence of SAM. The second observer was not provided with a clinical summary, timing of the echocardiogram, or the assessment from the primary observer (Joonbum Seo). When there was

disagreement between the primary and second observer, the echocardiogram was viewed together, and the final consensus was used for the statistical analysis.

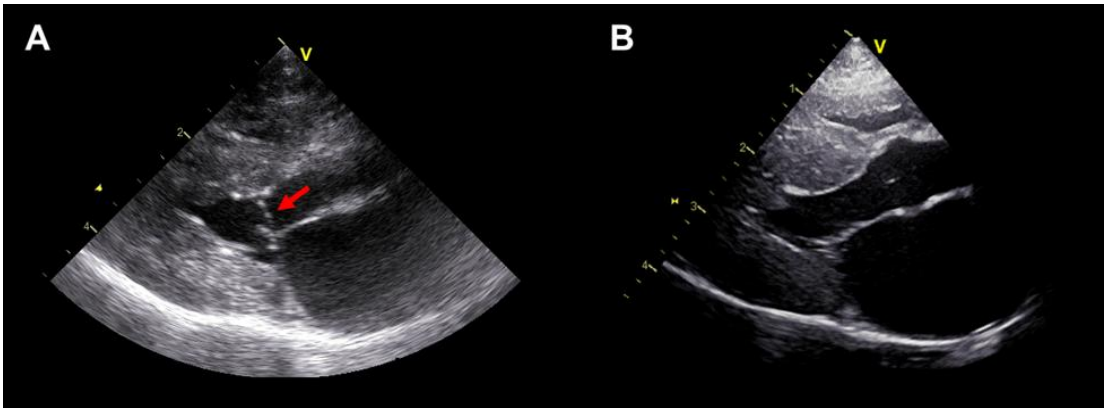

Figure 2.1. Still images of the right parasternal long-axis five chamber view at end-systole in two cats with hypertrophic cardiomyopathy. *The left (A) image shows systolic anterior motion of the mitral valve (arrow), with the anterior mitral valve leaflet touching the interventricular septum during systole. The right (B) image shows a cat without systolic anterior motion of the mitral valve.*

2.2b Statistics

Normality of continuous data was tested by visual assessment and Shapiro-Wilk test. Normally distributed data were presented as mean (95% confidence interval [95% CI]) and non-normally distributed data were presented as median (range). The baseline and recheck data of the study population were compared using a paired t-test or a Wilcoxon signed ranked test depending on the normality of the data. The study population was then further analysed by dividing them into two groups: those that had SAM at baseline and those that did not. Continuous variables in these two groups were compared using independent t-test or a Mann-Whitney test. Categorical demographic variables were compared by Pearson Chi-Square or Fisher's Exact test, as appropriate. The baseline characteristics, time interval between two echocardiograms, reasons for the second cardiac scan, and the percentage change in heart rate, body weight, and all echocardiographic variables were compared between cats with SAM at baseline and follow-up versus those that lost SAM at recheck, and between cats without SAM at baseline and follow-up versus those that gained SAM at recheck. The level of agreement between 2 observers on diagnosing SAM was assessed using Cohen's kappa (κ). 95% CI was calculated by

1.96 x standard error. The frequency of cats gaining or losing SAM was presented as incidence rate, where the numerator equalled the number of cats that gained or lost SAM during the follow-up period and the denominator equalled the number of cat-years at risk. Statistical significance was set at <0.05.

2.3 Results

A total of 130 cats met the inclusion criteria from the retrospective data search. Of these, 87 cats were excluded for the following reasons: 15 for incomplete medical records, 4 for SABP ≥180 mmHg, 2 for unavailable SABP with creatinine ≥140 µmol/L (≥1.6 mg/dL), 10 for hyperthyroidism, 6 for diabetes mellitus, 1 for packed cell volume <20%, 35 for receiving atenolol, 5 for receiving sedation, 8 for receiving pimobendan, 1 for possible cardiac neoplasia, 1 for limited echocardiographic study, and 4 for having low temporal resolution (<60 frames per second). This resulted in 38 cats. An additional 22 cats were recruited by being prospectively invited for a recheck at the Queen Mother Hospital for Animals. The final sample size was 60 cats. There was no statistical difference between the baseline variables between the original study group and those of the 22 additional cats.

The median temporal resolution of the cineloop used to diagnose SAM was 112 frames per second (minimum 60; maximum 317). There was good agreement between two observers on the diagnosis of SAM (κ = 0.831, 95% CI: 0.727-0.934). The 9 out of 120 scans where the disagreement arose was thought to be due to end-systolic cavity obliteration, which made the accurate observation of mitral valve leaflets difficult. These scans occurred in 2 cats at both time points and 5 cats at a single time point. Both observers ultimately agreed not to have these cats classified as having SAM.

At baseline, 38 cats (63%; 95% CI: 50-75%) had SAM and 22 cats (37%; 95% CI: 25-50%) did not have SAM. Two cats without SAM had congestive heart failure. Three cats were receiving medications: furosemide and clopidogrel (n = 1), furosemide and benazepril (n = 1), and aspirin (n = 1). One cat without SAM had ventricular premature complexes. Cats with SAM were younger, weighed less, and had higher LVOT Vmax, longer AMVL length, and greater LV hypertrophy than those

without SAM. A summary of the baseline clinical and echocardiographic characteristics of the two groups is presented in Table 2.1, Table 2.2, and Figure 2.2.

Table 2.1. Baseline characteristics of the study population in a study to evaluate the spontaneous gain or loss of systolic anterior motion of the mitral valve (SAM) in cats with hypertrophic cardiomyopathy. Cats were divided into groups according to the presence of SAM at baseline, and their baseline characteristics were compared.

	Whole group (n=60)	SAM (n=38)	No SAM (n=22)	*P* value
Age (years) [cont]	5.6 [0.3-14.2]	3.9 [0.3-11.2]	8.0 [1.5-14.2]	<0.001
Body weight (kg) [cont]	4.7 [2.8-8.8]	4.3 [2.8-7.6]	5.7 [3.8-8.8]	0.008
Body condition score (/9) [cat/o]	5 [4-9]	5 [4-8]	6 [4-9]	0.266
Sex				
Female:male [cat/b]	14:46	11:27	3:19	0.219
Entire:neutered [cat/b]	2:58	2:36	0:22	0.251
Purebred:Non-purebred [cat/b]	22:38	11:27	11:11	0.163
Balinese		1		
Bengal		1	1	
British Shorthair		1	1	
Burmese				
Exotic Shorthair			1	
Himalayan			1	
Maine Coon		2		
Norwegian Forest Cat			1	
Persian		1	3	
Ragdoll			1	
Russian Blue		1	2	
Siberian		1	1	
Sphynx		3		
Presenting complaint [cat/n]				0.041
Cardiac screening [cat/b]	12	4	8	0.022
Lethargy [cat/b]	2	1	1	1.000
Heart murmur [cat/b]	43	32	11	0.007
Respiratory signs [cat/b]	3	1	2	0.548
Heart rate (per minute) [cont]	182.6 [131-236]	181.1 [133-236]	183.5 [131-236]	0.921
Murmur (%) [cat/b]	49 (81.7%)	36 (94.7%)	13 (59.1%)*	0.001
Murmur grade [cat/o]	3 (2-4)	3 (2-4)	2 (2-4)	<0.001
Gallop (%) [cat/b]	5 (8.3%)	3 (7.9%)	2 (9.1%)	0.872
Medication	3 cats*	None	3 cats**	-
SABP (mmHg) [cont]	128.9 (121.1-136.8) (n=32)	126.8 (118.5-135.1) (n=18)	131.6 (115.8-147.5) (n=14)	0.565

*Variable types are annotated using superscripts: [cont] for continuous, [cat/n] for nominal categorical, [cat/o] for ordinal categorical, and [cat/b] for binary categorical variables. Normally distributed data are presented as mean (95% confidence interval) and non-normally distributed data as median [range]. *One cat without SAM had dynamic right ventricular outflow tract obstruction and 3 had midventricular obstruction. The cause of a heart murmur was not identified in the remaining 9 cats without SAM. **One cat was receiving aspirin, one cat was receiving furosemide and clopidogrel, and one cat was receiving furosemide and benazepril.*

Abbreviations: SABP, systolic arterial blood pressure; SAM, systolic anterior motion of the mitral valve.

Table 2.2. Baseline echocardiography results in a study to evaluate the spontaneous gain or loss of systolic anterior motion of the mitral valve (SAM) in cats with hypertrophic cardiomyopathy. Cats were divided into groups according to the presence of SAM at baseline, and their baseline echocardiography results were compared.

	Study group (n=60)	SAM (n=38)	No SAM (n=22)	*P* value
ATE (%) cat/b	0	0	0	-
CHF (%) cat/b	2	0	2	0.131
Arrhythmias (%) cat/b	1	0	1	0.373
Scan Interval (years) cont	2.1 [1.0-5.9]	2.4 [1.0-5.7]	1.8 [1.0-5.9]	0.067
Left atrium				
LA/Ao cont	1.4 [1.1-2.1]	1.4 [1.1-2.1]	1.3 [1.2-2.0]	0.730
LAD Max (mm) cont	16.2 [14.7-25.7]	15.5 [14.6-25.7]	16.5 [12.8-21.7]	0.586
LA FS% cont	29.5 (27.2-31.8)	28.7 (26.0-31.4)	30.9 (26.3-35.4)	0.379
Left and right ventricle				
IVSd Max (mm) cont	6.6 [5.4-9.7]	6.9 [6.0-9.7]	6.3 [5.4-9.1]	0.024
LVFWd Max (mm) cont	6.1 [4.3-8.5]	6.2 [4.3-8.5]	5.9 [4.7-7.5]	0.079
LVWT Max (mm) cont	6.7 [6.0-9.7]	6.9 [6.0-9.1]	6.5 [6.0-9.1]	0.027
LVIDd (mm) cont	14.9 (14.4-15.4)	14.8 (14.1-15.5)	15.0 (14.2-15.9)	0.686
LVIDs (mm) cont	5.0 (4.5-5.6)	4.6 (3.9-5.2)	5.8 (5.0-6.7)	0.020
LV FS% cont	66.5 (63.2-69.7)	69.5 (65.7-73.4)	61.2 (55.7-66.7)	0.012
End-systolic cavity obliteration cat/b	41 (68.3%)	29 (76.3%)	12 (54.5%)	0.081
Regional wall hypokinesis cat/b	0	0	0	-
Mid-LV obstruction cat/b	11/23 (47.8%)	7/12 (58.3%)	4/11 (36.4%)	0.292
LVOT Vmax (m/s) cont	1.9 [0.7-5.4]	2.9 [0.9-5.4]	1.0 [0.7-2.1] (n=18)	<0.001
DLVOTO (%) cat/b	25 (41.7%)	25 (65.8%)	0	<0.001
DRVOTO (%) cat/b	9/54 (16.7%)	8/35 (22.9%)	1/19 (5.3%)	0.137
Mitral valve apparatus				
AMVL length (mm) cont	11.1 [7.0-18.4]	12.2 [9.7-18.4]	10.7 [7.0-14.2]	0.002

Variable types are annotated using superscripts: cont for continuous, cat/o for ordinal categorical, and cat/b for binary categorical variables. Normally distributed data are presented as mean (95% confidence interval) and non-normally distributed data as median [range].

Abbreviations: AMVL, anterior mitral valve leaflet; ATE, aortic thromboembolism; CHF, congestive heart failure; DLVOTO, dynamic left ventricular outflow tract obstruction; DRVOTO, dynamic right ventricular outflow tract obstruction; IVSd Max, maximal interventricular septal thickness in end-diastole; LA/Ao, left atrium to aortic ratio; LAD Max, maximal left atrial diameter; LA FS%, left atrial fractional shortening; LV FS%, left ventricular fractional shortening; LVFWd Max, maximal left ventricular free wall thickness in end-diastole; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVOT Vmax, maximal left ventricular outflow tract velocity; LVWT Max, maximal left ventricular wall thickness in end-diastole; SAM, systolic anterior motion of the mitral valve.

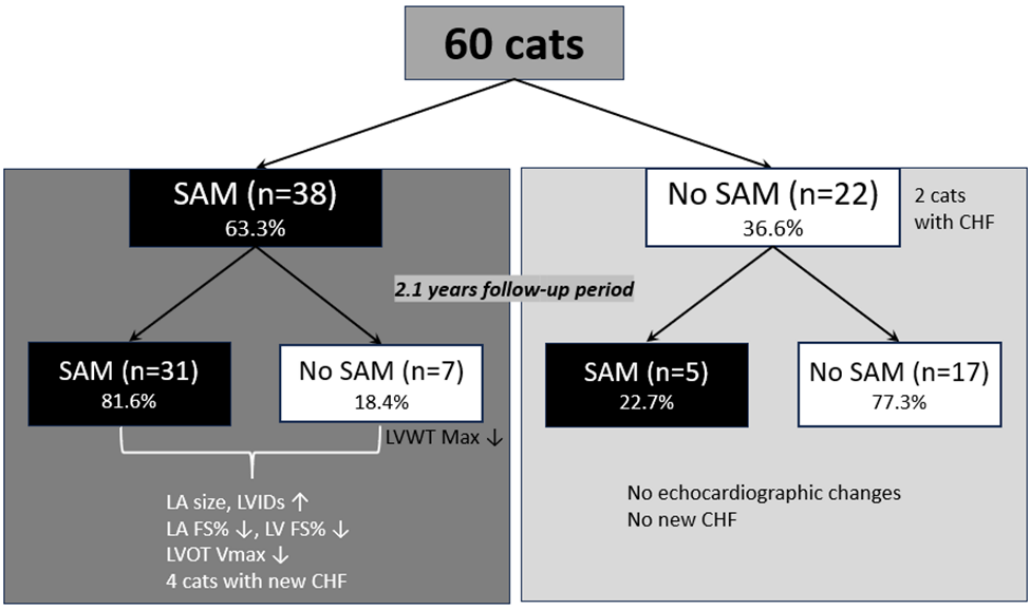

Figure 2.2. Summary of the study population and the results in a study to evaluate the spontaneous gain or loss of systolic anterior motion of the mitral valve (SAM) in cats with hypertrophic cardiomyopathy. *Out of 60 cats, 38 cats had SAM and 22 did not at baseline. After a median follow-up period of 2.1 years, 7 cats lost SAM and 5 cats gained SAM. The loss of SAM was associated with reduced maximal left ventricular wall thickening (LVWT Max). Only the cats with SAM at baseline showed progression in disease severity. These included increase in left atrial (LA) size and left ventricular internal diameter in end-systole (LVIDs), reduced left atrial fractional shortening (LA FS%), reduced left ventricular fractional shortening (LV FS%), reduced left ventricular outflow tract obstruction (LVOT Vmax). Four cats that had SAM at baseline developed congestive heart failure (CHF). Two cats without SAM at baseline had congestive heart failure. But none of the cats without SAM developed new congestive heart failure or changes in the echocardiographic parameters.*

The median follow-up period was 2.1 years (minimum 1.0 years; maximum 5.9 years), which was not different between the 2 groups (No SAM at baseline, median 1.8 years [minimum 1.0 year; maximum 5.9 years] versus SAM at baseline, 2.4 years [minimum 1.0 year; maximum 5.7 years], $P = 0.067$). With the entire study population assessed as a single group, at recheck the LA size had increased (LA to aortic ratio, mean 1.4 [95% CI: 1.4-1.5] to 1.6 [95% CI: 1.4-1.7]), LA function reduced (LA fractional shortening, mean 29.5% [95% CI: 27.2-31.8] to 27.1% [95% CI: 24.1-30.1]), and the LV diameter in end-systole increased (mean 5.0 mm [95% CI: 4.5-5.6] to 5.8 mm [95% CI: 5.1-6.4], Table 2.3). Further analysis showed that this progression in left heart dimensions was only seen in cats with SAM at baseline. At recheck, cats with SAM at baseline had increased LA size (LA to aortic ratio, mean 1.4 [95% CI: 1.4-1.5] to 1.4 [95% CI: 1.4-1.7]; LA maximal diameter, mean 15.5 mm [95% CI: 14.9-17.4] to 16.0 mm [95% CI: 16.3-19.3]), reduced LA function (LA fractional shortening, mean 28.7% [95% CI: 26.0-31.4] to 25.1% [95% CI: 21.7-

28.5]), increased LV diameter in end-systole (mean 4.3 mm [95% CI: 3.9-5.2] to 5.3 mm [95% CI: 5.1-6.5]), decreased LV fractional shortening (mean 69.5% [95% CI: 65.7-73.4] to 61.3% [95% CI: 57.5-65.0]), and lost any mid-left ventricular obstruction (58.3% [95% CI: 27.7-84.8%] to 24.0% [95% CI: 9.4-45.1]). There was no change in left heart parameters in cats without SAM at baseline.

Table 2.3. Comparison of baseline and recheck characteristics of the entire study population in a study to evaluate the spontaneous gain or loss of systolic anterior motion of the mitral valve (SAM) in cats with hypertrophic cardiomyopathy.

	Baseline	Recheck	*P* value
Age (years) [cont]	5.6 [0.3-14.2]	8.0 [2.5-18.0]	-
Body weight (kg) [cont]	4.7 [2.8-8.8]	4.9 [3.2-9.8]	0.409
Body condition score (/9) [cat/o]	5 [4.0-9.0]	5.0 [2.0-9.0]	0.891
Medication	3 cats	14 cats	-
ATE (%) [cat/b]	0	0	-
CHF (%) [cat/b]	2 (3.3%)	6 (10.0%)	0.272
Arrhythmias (%) [cat/b]	1 (1.7%)	8 (13.3%)	0.032
Murmur (%) [cat/b]	49 (81.7%)	42 (70.0%)	0.154
Murmur grade (/6) [cat/o]	3 (2-4)	3 (2-4)	0.595
Gallop sound [cat/b]	5 (8.3%)	9 (15.0%)	0.255
Heart rate (bpm) [cont]	182.6 [131-236]	186 [140-246]	0.125
SABP (mmHg) [cont]	128.9 (121.1-136.8) (n=32)	138.9 (130.3-147.5) (n=35)	0.178
Left atrium			
LA:Ao [cont]	1.4 [1.1-2.1]	1.4 [1.0-3.0]	0.024
LAD Max (mm) [cont]	16.2 [14.7-25.7]	16.0 [11.5-30.7]	0.060
LA FS% [cont]	29.5 (27.2-31.8)	27.1 (24.1-30.1)	0.043
Left and right ventricle			
LVWT Max (mm) [cont]	6.7 [6.0-9.7]	7.2 [5.6-10.2]	0.297
LVIDd (mm) [cont]	14.9 (14.4-15.4)	15.0 (14.4-15.6)	0.561
LVIDs (mm) [cont]	5.0 (4.5-5.6)	5.8 (5.1-6.4)	0.038
FS% [cont]	66.5 (63.2-69.7)	62.1 (58.6-65.6)	0.051
End-systolic cavity obliteration [cat/b]	41 (68.3%)	33 (55.0%)	0.133
Regional wall hypokinesis [cat/b]	0	3 (5.0%)	0.244
Mid-left ventricular obstruction [cat/b]	11/23 (47.8%)	9/33 (27.3%)	0.114
LVOT Vmax (m/s) [cont]	1.9 [0.7-5.4]	1.6 [0.7-5.2]	0.076
DLVOTO (%) [cat/b]	25/60	19/56	0.391
DRVOTO (%) [cat/b]	9/54	16/52	0.087
Mitral valve apparatus			
SAM (%) [cat/b]	38 (63.3%)	36 (60.0%)	0.707
SAM group [cat/b]	38/38 (100%)	31/38 (81.6%)	0.012
No SAM group [cat/b]	0/22 (0%)	5/22 (22.7%)	0.048
AMVL length (mm) [cont]	11.1 [7.0-18.4]	11.7 [8.9-16.0]	0.880

Variable types are annotated using superscripts: [cont] for continuous, and [cat/o] for ordinal and [cat/b] for binary categorical variables. Normally distributed data are presented as mean (95% confidence interval) and non-normally distributed data as median [range].
Abbreviations: AMVL, anterior mitral valve leaflet; ATE, aortic thromboembolism; CHF, congestive heart failure; DLVOTO, dynamic left ventricular outflow tract obstruction; DRVOTO, dynamic right ventricular outflow tract obstruction; LA/Ao, left atrium to aortic ratio; LAD Max, maximal left atrial diameter; LA FS%, left atrial fractional shortening; LV FS%, left ventricular fractional shortening; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVOT Vmax, maximal left ventricular outflow tract velocity; LVWT Max, maximal left ventricular wall thickness in end-diastole; SABP, systolic arterial blood pressure; SAM, systolic anterior motion of the mitral valve

Four cats with SAM at the initial scan subsequently developed congestive heart failure (Table 2.4). Two of these cats had lost SAM at recheck. Seven cats with SAM at the initial scan developed arrhythmias. All cats with arrhythmias had an electrocardiogram performed using the echocardiographic machine or a separate 6 lead electrocardiogram. The new arrhythmias consisted of ventricular premature complexes (n = 4), atrial premature complexes (n = 1), atrial fibrillation (n = 1), and supraventricular tachycardia (n = 1). None of the cats without SAM at initial scan developed new congestive heart failure or arrhythmias.

Table 2.4. Comparison of baseline and recheck characteristics in separate groups of cats with and without systolic anterior motion of the mitral valve (SAM) in a study to evaluate the spontaneous gain or loss of SAM in cats with hypertrophic cardiomyopathy.

	SAM at baseline (n=38)			No SAM at baseline (n=22)		
	Baseline	Recheck	*P* value	Baseline	Recheck	*P* value
Age (years) ^{cont}	3.9 [0.3-11.2]	6.8 [2.5-14.9]	-	8.0 [1.5-14.2]	10.8 [3.3-18.0]	-
Body weight (kg) ^{cont}	4.3 [2.8-7.6]	4.4 [3.2-8.4]	0.378	5.7 [3.8-8.8]	5.4 [3.3-9.8]	0.917
Body condition score (/9) $^{cat/o}$	5 [4-8]	5 [3-7]	0.639	6 [4-9]	5 [2-9]	0.824
Medication	0 cats	9 cats	-	3 cats	3 cats	-
ATE (%) $^{cat/b}$	0	0	-	0	0	-
CHF (%) $^{cat/b}$	0	4 (10.5%)	0.115	2 (9.1%)	2 (9.1%)	1.000
Arrhythmias (%) $^{cat/b}$	0	7 (18.4%)	0.012	1 (4.5%)	1 (4.5%)	1.000
Heart murmur (%) $^{cat/b}$	36 (94.7%)	32 (84.2%)	0.051	13 (59.1%)	10 (45.5%)	0.554
Murmur grade (/6) $^{cat/o}$	3 [3-4]	3 [2-4]	0.469	2 [2-4]	3 [2-4]	0.459
Gallop sound (%) $^{cat/b}$	3 (7.9%)	6 (15.8%)	0.480	2 (9.1%)	3 (13.6%)	1.000
HR (bpm) ^{cont}	181.1 [133-236]	184.0 [140-245]	0.528	183.5 [131-236]	193.5 [146-246]	0.102
SABP (mmHg) ^{cont}	126.8 (118.5-135.1) (n=18)	137.4 (126.4-148.3) (n=23)	0.363	131.6 (115.8-147.5) (n=14)	141.8 (125.7-157.8) (n=12)	0.331

Variable types are annotated according to their type using superscripts: ^{cont} for continuous, and $^{cat/o}$ for ordinal and $^{cat/b}$ for binary categorical variables. Normally distributed data are presented as mean (95% confidence interval) and non-normally distributed data as median [range].

Abbreviations: ATE, aortic thromboembolism; CHF, congestive heart failure; SABP, systolic arterial blood pressure; SAM, systolic anterior motion of the mitral valve.

At recheck, the 2 cats with congestive heart failure at baseline were also receiving aspirin and spironolactone. Additionally, 10 new cats were on medications, all of which had SAM at baseline. Medications administered were furosemide, benazepril, aspirin, and spironolactone (n = 1); furosemide, benazepril, and aspirin (n = 1); furosemide and clopidogrel (n = 1); furosemide alone (n = 1); benazepril (n = 2); and clopidogrel (n = 4).

The incidence rate of cats to either gain or lose SAM was 8 per 100 cat-years. The incidence rate of cats gaining SAM was 10 per 100 cat-years. The incidence rate of cats losing SAM was 7 per 100 cat-years. None of the cats that gained or lost SAM had disagreement of the SAM diagnosis between 2 observers.

Cats that gained SAM at recheck had a higher baseline LVOT, thinner LVFW and a greater ratio of maximal IVS to maximal LVFW thickness than those that did not. None of the baseline characteristics were associated with cats that later lost SAM (Table 2.5).

Table 2.5. Baseline characteristics that were statistically different between cats that gained systolic anterior motion of the mitral valve (SAM) and cats that did not gain SAM during the follow-up period

Baseline characteristics	Gained SAM at recheck (n = 5)	Unchanged at recheck (n = 17)	*P* value
LVOT Vmax (m/s)	1.6 (0.7-2.4)	1.0 (0.9-1.2)	0.017
LVFW (mm)	5.5 (4.8-6.3)	5.9 (5.7-6.4)	0.009
IVS/ LVFW	1.2 (1.0-1.6)	1.1 (1.0-1.1)	0.004

All data are normally distributed and are presented as mean (95% confidence interval). IVS, maximum interventricular septum in end-diastole

Abbreviations: LVFW, maximum left ventricular free wall thickness in end-diastole; LVOT Vmax, maximum left ventricular outflow tract velocity.

Gaining SAM was associated with increased LVOT Vmax and decreased maximal IVS to maximal LVFW thickness ratio (Table 2.6). There was no statistical difference between maximal IVS and maximal LVFW as individual variables between two time points.

Table 2.6. Variables that were statistically different between cats that gained systolic anterior motion of the mitral valve (SAM) and cats that did not gain SAM during the follow-up period

% change from baseline to recheck	Gained SAM at recheck (n = 5)	Unchanged at recheck (n = 17)	*P* value
△% LVOT Vmax	+135% (-305-+574%)	+6.7% (-18.0-+31.3%)	0.012
△% IVS/ LVFW	-0.3% (-21.4-+0.1)	6.5% (-5.2-+11.2)	0.036

All data are normally distributed and are presented as mean (95% confidence interval). IVS, maximum interventricular septum in end-diastole

Abbreviations: LVFW, maximum left ventricular free wall thickness in end-diastole; LVOT Vmax, maximal left ventricular outflow tract velocity.

Losing SAM was associated with reduction in maximal LVFW thickness and loss of DLVOTO (Table 2.7). The change in LA size, LA function, LV internal diameter in end-systole, LV fractional shortening, loss of end-systolic cavity obliteration, and development of new congestive heart failure or arrhythmia were not associated with the loss of SAM (*P* >0.05). The changes in scan interval, LV dimensions, and LVOT Vmax in cats that gained or lost SAM are shown in Figure 2.3 and Figure 2.4.

Table 2.7. Variables that were statistically different between cats that lost systolic anterior motion of the mitral valve (SAM) and cats that did not lose SAM during the follow-up period

% change from baseline to recheck	Lost SAM at recheck (n = 7)	Unchanged at recheck (n = 31)	*P* value
△% LVFW	-10% (-18-+9%)	+5.8% (-1.5-+9.4%)	0.040
△% Loss of DLVOTO*	71.4% (56.5-89.7)	22.6% (3.7-71.9)	0.032

*All data are normally distributed and are presented as mean (95% confidence interval). *95% confidence interval is also reported for the categorical data.*

Abbreviations: DLVOTO, dynamic left ventricular outflow tract obstruction; LVFW, maximum left ventricular free wall thickness in end-diastole.

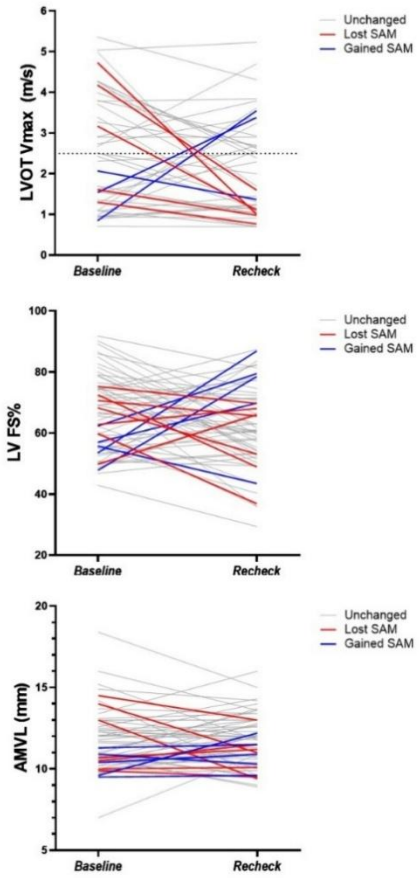

Figure 2.3. Changes observed in the maximal left ventricular outflow tract velocities (LVOT Vmax), anterior mitral valve leaflet (AMVL) length and left ventricular fractional shortening (LV FS%) in 60 cats with hypertrophic cardiomyopathy over a median follow-up period of 2.1 years (minimum 1.0 years; maximum 5.9 years). Cats that have lost systolic anterior motion of the mitral valve (SAM) at recheck are shown in red. Cats that gained SAM at recheck are shown in blue. Cats that either persistently had SAM or never had SAM at either time point are shown in grey. A horizontal line in LVOT Vmax shows 2.5 m/s, which was used as a cutoff to define dynamic left ventricular outflow tract obstruction. 16 Cats that gained SAM had faster baseline LVOT Vmax than those that did not (*P* = 0.017).

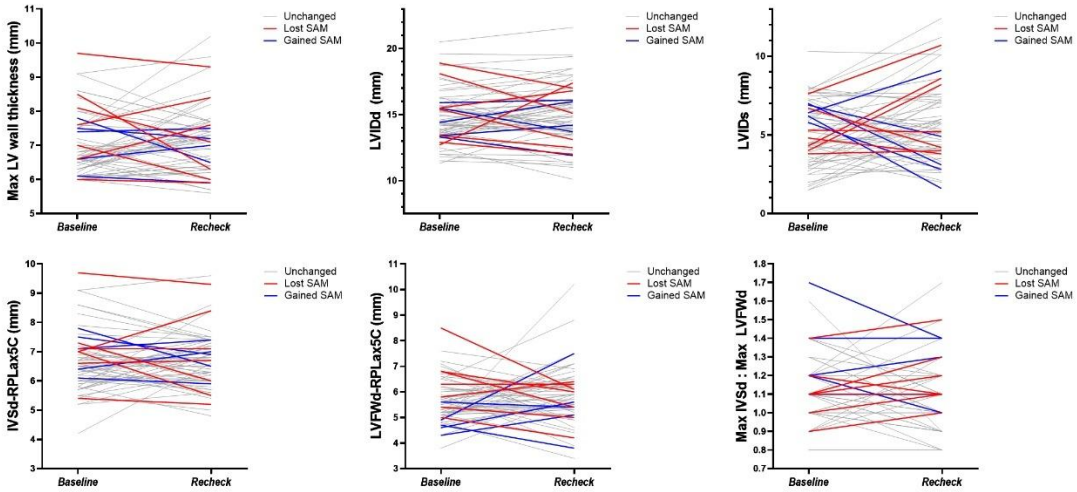

Figure 2.4. Change observed in the maximal left ventricular (LV) wall thickness, LV internal diameters in end-diastole (LVIDd) and end-systole (LVIDs), maximal interventricular septal thickening in right parasternal long axis 5 chamber view (IVSd-RPLAX5C), maximal left ventricular free wall thickness in right parasternal long axis 5 chamber view (LVFWd-RPLAX5C), and a ratio of maximal interventricular septum to maximal left ventricular free wall (Max IVS : Max LVFW) in 60 cats with hypertrophic cardiomyopathy over a median follow-up period of 2.1 years (minimum 1.0 years; maximum 5.9 years). *Cats that lost systolic anterior motion of the mitral valve (SAM) at recheck are shown in red. Cats that gained SAM at recheck are shown in blue.* Cats that either persistently had SAM or never had SAM at either time point are shown in grey. *Cats that gained SAM had thinner LVFWd-RPLAX5C (P = 0.009) and a greater Max IVS : Max LVFW at baseline (P = 0.004).*

2.4 Discussion

In this retrospective cohort study, the incidence rate of a change in the presence of SAM in cats with HCM was 8 per 100 cat-years. That is, in the order of 8 out of every 100 cats with HCM are expected to experience a change in the presence of SAM over a 12-month period. This high number of cats that gained or lost SAM further demonstrates that SAM is labile, and its presence can change over time. In a previous study, 3 of 14 cats were found to gain SAM (21.4%) in a median follow-up period of 5.6 years, while 4 of 7 cats lost SAM (57.1%) during the same time period.[11] In another study, 1 of 8 cats lost SAM (12.5%) and 1 of 8 cats gained SAM (12.5%) during a 6-month time period.[12] The results of the present study support these previous observations that cats with HCM can gain or lose SAM.

An unexpected finding was that cats with SAM at the initial scan were more likely to show disease progression than cats without SAM. In previous studies of feline HCM, cats with obstructive HCM are reported to have a similar or more benign outcome to non-obstructive cats.[4,8-10] Possible reasons for a different result in our study to previous studies include selection bias. In previous studies, cats with SAM have tended to be younger than those without.[4,8-10] Cats with obstructive HCM would be more likely to present with a heart murmur than cats with non-obstructive HCM, and this might lead to investigations at a younger age in cats with SAM than those without. Cats with non-obstructive HCM are more likely to remain undiagnosed until the development of clinical signs.

Furthermore, the labile nature of SAM would likely have influenced the result. Young cats might be more excitable than old cats, and more likely to exhibit SAM in a stressful environment. It is difficult to predict whether cats diagnosed with obstructive HCM remain obstructed at home, further biasing outcomes data of these studies. However, in our study the heart rate during echocardiography was similar between cats with SAM and those without SAM, suggesting that cats with or without SAM were similarly stimulated during echocardiography. Further studies are required to determine if more accurate identification of latent SAM will reveal an association between SAM or DLVOTO and disease outcome in cats.

The labile nature of SAM is well described in human cardiology. To accurately assess the clinical significance of SAM, pharmacologic and physiologic provocative methods have been proposed.[26] These methods stimulate adrenergic tone,

myocardial contractility, and vascular tone to induce SAM and potentially worsen DLVOTO.[16] For example, a treadmill exercise test can allow detection of patients with latent SAM, where SAM is neither present nor causing DLVOTO at rest.[27,28] Documenting patients with latent SAM is important as they can still develop significant DLVOTO with activity and require therapy. In contrast, there is no standardized method of assessing SAM and DLVOTO in cats with HCM. Furthermore, a clear distinction between SAM and DLVOTO are not always made in the literature. One could consider that the echocardiography in cats is, by itself, a provocative manoeuvre. However, cats can still display varying degrees of stress in each hospital visit. Provocative manoeuvres could not be used in this study as all baseline data and most of the follow up data were collected retrospectively. The authors acknowledge that this is a limitation of this study.

Cats that had faster LVOT Vmax, thinner LVFW, and a greater IVS to LVFW ratio at baseline were significantly more likely to develop SAM. These features support the hypothesis that underlying anatomical and functional substrates of the heart predispose to the development of SAM.[13,15,17] No other baseline variables predicted the subsequent development or loss of SAM. This highlights the difficulty of predicting the gain or loss of SAM using a single echocardiogram.

Age, time between the two scans, and indices of LV systolic function were not associated with the gain or loss of SAM. This occurred despite the decline in LV systolic function over time in cats with SAM. However, most of these cats were still hyperdynamic in LV systolic function when compared to an established normal reference interval in cats.[29] Hyperdynamic LV systolic function is an important factor for developing SAM in humans and likely cats.[15,30] A longer follow-up period could have documented further reduction in LV systolic function and the number of cats that lose SAM may have been similar to a previous study.[11] Similarly, cats in this study were relatively young at recheck due to a relatively short follow-up period for a disease with a long pre-clinical period. The presence of SAM is often associated with young cats with HCM.[4,10,22,31] It is possible that these young cats lose SAM as they get older. A study with a longer follow up period is needed to further test this hypothesis.

The presence of SAM has been reported to be associated with greater LV hypertrophy.[17] In the present study, cats that lost SAM had reduced LVFW thickness. However, it is unknown if the reduction in LVFW thickness was the cause or result of the loss of SAM. Furthermore, a reduction in LV wall thickness was also seen in cats

that maintained SAM. Recently, LV thinning was shown to represent intramural myocardial fibrosis in cats with HCM.[25] Regional wall hypokinesis was not common in the present study. It is unclear whether the loss of LV wall thickness is a result of myocardial fibrosis in some cats. Additionally, an increase in LV hypertrophy were seen in both cats that maintained SAM and cats that lost SAM. Increase in LV hypertrophy is an expected disease progression in most cats with HCM.[11]

As previously mentioned, the lack of standardized protocol for provoking SAM during echocardiography is a limitation of this study. Day-to-day variation in stimulation and adrenergic tone is an important confounder, although echocardiography performed at a veterinary clinic is arguably a provocative manoeuvre in itself. Secondly, the relatively short-term follow up period did not allow many cats with advanced heart disease to be included in the study. Thirdly, the sample size in each group was small. The small sample size was the result of strict exclusion criteria at both time points. This was necessary for carefully assessing the presence of SAM over time. Nevertheless, some features associated with gain or loss of SAM could have been missed due to type II error. A larger sample size would also allow a multivariable analysis since many anatomical and functional features of HCM are thought to cause SAM.[13] A multivariable analysis would allow identification of most important variables associated with SAM by ranking variables according to their magnitude of effect on the development of SAM. Fourthly, the study involved only two time points. The presence of SAM may change multiple times in some cats. Lastly, certain anatomical features, including aortic-septal angle, and papillary muscle morphologies were not studied in the present study. Many of these variables would be better assessed using an advanced cardiac imaging such as cardiac magnetic resonance imaging.[15]

The incidence rate was calculated to describe the frequency of gain or loss of SAM while accounting for the inconsistent follow-up period among cats. Interpreting the incidence rate requires an understanding of the interval censoring problem, where the disease or event could occur anywhere in between 2 time points. A common approach to account the interval censoring problem is by using a ‘mid-point’ incidence rate, where the event is assumed to have occurred half-way between the 2 time points.[32] However, this approach could underestimate the incidence near the end of the observation period.[32,33] Alternatively, random-point and Poisson binomial methods have been described to potentially reduce underestimation and bias.[32,33] An

ideal approach to account the interval censoring problem was not investigated in the present study.

Inter-observer agreement of SAM diagnosis was not 100% in this study. To overcome an observation error, the consensus between two board certified cardiologists (Joonbum Seo and Virginia Luis Fuentes) was used to diagnose SAM. Nine cineloops (7.5%) needed a review but the final consensus did not increase the number of cats that gained or lost SAM. If both cardiologists made an error on the echocardiographic diagnosis of SAM, the number of cats that gain or lose SAM would further increase.

Lastly, there is an argument that cats with SAM and LV hypertrophy have MV dysplasia instead of HCM. Indeed, cats with SAM and LV hypertrophy have elongated AMVL and changes to the papillary muscles that could suggest MV dysplasia. However, these changes to the AMVL and papillary muscles are a recognised feature of HCM in both cats and humans.[17,21] Based on the results of experimental studies, and longitudinal studies of humans and cats, abnormalities of the MV appear to occur after birth, but before LV hypertrophy.[21,34-37] The argument on the disease definition remains unresolved, but the current literature supports the use of HCM as the disease name in these cats.

2.5 Conclusion

The incidence rate of cats with HCM to gain or lose SAM was 8 per 100-cat years. Age and scan interval between 2 time points were not associated with the gain or loss of SAM. Cats that had faster LVOT Vmax, thinner LVFW, and greater IVS to LVFW ratio at baseline were significantly more likely to develop SAM. Cats with SAM at baseline had more advanced disease progression than those without SAM at baseline.

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Chapter 3. The prevalence of feline HCM in New Zealand

Note, the experiments reported in this chapter were published in New Zealand Veterinary Journal.[1] The published manuscript has only undergone minor modifications for this thesis chapter.

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3.1 Introduction

Cardiomyopathy is a primary myocardial disorder that changes the structure and function of the heart muscle in the absence of other disease sufficient to cause the observed myocardial abnormality. Currently, there are no studies evaluating the prevalence of cardiomyopathy in cats in New Zealand (NZ). However, studies of cats in the United Kingdom (UK) and United States of America (USA) suggest that approximately 15% of clinically healthy cats in the general population have cardiomyopathy that is detectable using echocardiography.[2,3] Several types of cardiomyopathy have been described in cats.[4] Of these, hypertrophic cardiomyopathy (HCM) is most common, constituting > 94% of apparently healthy cats diagnosed with cardiomyopathy.[2,3]

The clinical definition of HCM is left ventricular (LV) concentric hypertrophy caused by a genetic abnormality or an unknown aetiology.[4,5] Therefore, HCM is diagnosed by ruling out other diseases such as hyperthyroidism and systemic hypertension, which can mimic HCM (termed 'HCM phenotype').[4] Cats with HCM can remain asymptomatic for years (i.e. have subclinical disease) after diagnosis and may live a normal lifespan.[6] However, the disease may progress in some cats and result in congestive heart failure, aortic thromboembolism, myocardial infarction, and sudden death.[4,6,7]

A retrospective multicentre study of 1,730 cats with HCM reported the incidence rate of all cardiovascular deaths in cats with HCM as 63.4 per 1,000 cat-years.[6] Therefore, HCM is a serious health concern in cats. The prevalence of subclinical HCM in New Zealand has not previously been investigated. If the prevalence of HCM in New Zealand is similar to that of other countries, approximately 180,000 of the estimated 1.2 million pet cats in NZ may have subclinical HCM.[8] Furthermore, approximately 11,400 cats may die due to HCM in the next 5 years.

The Centre for Feline Nutrition (CFN) at Massey University (Palmerston North, NZ) was created in 1992 by breeding 10 cats obtained from rehoming centres in NZ. Since then, a population of 130–150 cats has been maintained by introducing four new breeding cats from rehoming centres every 5 years to maintain genetic diversity. The aim of the CFN is to represent the non-purebred feline population in NZ for nutritional studies. In the CFN, each large pen houses an average of eight cats.

Cats in each pen interact with staff members daily and receive weekly enrichment time in a playroom where cats are played with one-on-one, weighed, groomed, and undergo a health check. Cats are given water *ad libitum* and fed a varied combination of canned and dry cat foods that are approved by the Association of American Feed Control Officials.

The aim of this study was to use the CFN colony cats as a convenience sample of non-purebred cats in NZ to estimate the prevalence of cardiomyopathy in this population. The secondary aim was to estimate the proportions of cats in NZ that die due to cardiomyopathy and that die with subclinical HCM by comparing the rates of cardiomyopathy to the causes of death of cats within the colony.

3.2 Materials and Methods

3.2a Study design

The first part of this study (describing the prevalence of HCM in NZ) was prospective and observational in design. The second part (describing cardiac mortality) was a retrospective study. Ethics approval was provided by Massey University Animal Ethics Committee (MUAEC protocol 21/47).

3.2b Part 1 – Prevalence of cardiomyopathy

3.2c Animals

A convenience sample of cats from the CFN was prospectively screened for the presence of cardiomyopathy. Inclusion criteria were all cats at the CFN (n = 149). Exclusion criteria were neonates, queens that were nursing, clinically unwell cats, and cats that were unable to be examined due to their behaviour. All enrolled cats underwent physical examination and then echocardiography by a board-certified veterinary cardiologist (Joonbum Seo). Oral treats were provided to keep the cats still during data collection. No cats were sedated.

Medical histories from the CFN records and CFN staff were also reviewed. Information on age, sex, neuter status, current body weight, and current medications

were retrieved from the CFN record. From the physical examination, body condition score (scale 1–9), heart rate, and presence of a heart murmur, gallop sound, or arrhythmias were recorded. When present, the heart murmur intensity was graded on a scale of 1–6.

3.2d Echocardiography

Right parasternal two-dimensional (2D) echocardiography was performed using a general ultrasound machine (Xario 200; Toshiba, Tochigi, Japan) equipped with a sector array probe (6S3, frequency 4.4–6.2 MHz) as previously described.[9] All images were then analysed using image processing software (TOMTEC Imaging Systems, Unterschleissheim, Germany). The presence of systolic anterior motion of the mitral valve (SAM) or chordae tendineae (CAM) were examined subjectively using 2D imaging.[10,11] Each echocardiographic variable of interest was measured over three consecutive cardiac cycles and averaged.

The maximum left atrial diameter was measured using a right parasternal, long-axis, four-chamber view by bisecting the left atrium parallel to the mitral annulus, one frame before the mitral valve opening. The left atrial to aortic ratio (LA/Ao) was measured using a right parasternal, short-axis view at the level of the aortic valve, one frame after the aortic valve closing. The thickest sections of the interventricular septum and LV free wall were measured at end-diastole in three views: right parasternal, long-axis, four-chamber view, right parasternal, long-axis, five-chamber view, and short-axis view at the level of papillary muscles in end-diastole. The greatest value from the measurements in all three views was recorded as maximum LV wall thickness (LVWT Max). Descriptive data on which segment of the LV wall was thickened was not collected. The LV internal diameters in end-diastole (LVIDd) and end-systole (LVIDs) were recorded by bisecting the left ventricle using the right parasternal short-axis view at the level of the papillary muscles.

A basic assessment of left atrial and LV systolic function was performed by calculating the fractional shortening as follows:

$$Fractional\ shortening = \frac{(Maximum\ diameter - Minimum\ diameter)}{Maximum\ diameter} * 100$$

The maximum and minimum left atrial diameter was measured using the same view as LA/Ao in end-systole and end-diastole. The maximum and minimum LV diameter was already measured as LVIDd and LVIDs.

3.2e Echocardiographic classification of cardiomyopathy

Cardiomyopathy was classified based on the current consensus guidelines from the ACVIM.[4] Arbitrary cut-offs were used in this study when objective cut-offs were unavailable or inconsistently used in the literature. Briefly, an HCM phenotype was defined as LVWT Max ≥ 6 mm.[5] To reach the diagnosis of HCM, other differentials of an HCM phenotype were excluded. In cats that were < 6 years old, careful physical examination and review of the CFN record were used to exclude the differentials of an HCM phenotype. In comparison, cats that were ≥ 6 years underwent blood pressure measurements using the Doppler technique, with systemic hypertension defined as systolic arterial blood pressure > 160 mmHg.[12] To avoid inadvertently missing clinical signs of hyperthyroidism and hypersomatotropism in cats of any age, CFN staff performed weekly assessment of appetite, thirst, and weight checks as part of the colony protocol; any significant changes in the 2 years following echocardiography were flagged to the investigator, and appropriate blood tests were performed.

For this study, an RCM phenotype was defined as left or biatrial enlargement (LA/Ao > 1.6 mm and maximal LA diameter in long-axis > 16 mm, right atrial enlargement on subjective assessment) with either normal or equivocal LV thickening (LVWT Max < 6 mm) or the presence of a fibromuscular band crossing the LV. A dilated cardiomyopathy phenotype was defined as an increased LVIDd (> 18 mm) and reduced LV fractional shortening (< 30%). An arrhythmogenic right ventricular cardiomyopathy phenotype was defined as right atrial and right ventricular enlargement on subjective assessment in the absence of features of pulmonary hypertension. Lastly, cats were categorised as having non-specific cardiomyopathy if the features of cardiomyopathy did not meet any of the above criteria.

3.2f Part 2 – Prevalence of cardiac mortality

The 10-year mortality data was collected by reviewing the records of the CFN and the Pathology Database of the School of Veterinary Science (Massey University) between February 2012 and February 2022. From these records, the sex of the cat, age at death, reason for euthanasia (if applicable), postmortem findings, and the recorded cause of death were extracted. In this study, cardiac death was defined by the presence of congestive heart failure (CHF), arterial thromboembolism (ATE), or sudden cardiac death (SCD) with gross or histologic evidence of cardiac disease without any other explainable cause. In the pathologist's report, the left ventricle was assessed to be grossly thickened if the LVWT was more than three times the right ventricular free wall thickness.[13] The heart was considered enlarged if it weighed > 20 g, or if the relative heart-weight to bodyweight ratio was > 4 g/kg.[14,15] The presence of congenital heart defects was assessed subjectively. Histology of the heart was routinely performed.

3.2g Statistical analysis

SPSS Statistics (v 26, IBM; Armonk, NY, USA) was used for statistical analysis. Graphs were produced in GraphPad Prism (v 8.4.2; GraphPad, Boston, MA, USA). Normality of data was tested by visual assessment and the Shapiro–Wilk test. Normally distributed data was presented as mean (SD) and non-normally distributed data was presented as median (IQR). Categorical variables were presented as proportions (percentage). The proportions or summary means or medians that apply only to the sample are presented without CI. The prevalence of cardiomyopathy and cardiac mortality is intended to be an estimate of the wider population so 95% CI are also reported.

All comparisons were unadjusted for the presence of potential confounders. Cats from the echocardiographic study were divided into groups based on the presence or absence of cardiomyopathy. Unadjusted comparisons of continuous variables were made using an independent t-test or a Mann–Whitney test depending on the distribution, and unadjusted comparisons of categorical variables were made by Pearson's χ^2, or Fisher's exact test when the cell count was < 5. For contingency tables larger than 2 x 2, Fisher's exact test was used. Significance was considered where $P < 0.05$.

3.3 Results

3.3a Part 1 – Prevalence of cardiomyopathy

The outcome of the study is summarised in Figure 3.1. Of 149 cats in the CFN colony, 17 were excluded due to being neonates (n = 11), queens that were nursing (n = 2), clinically unwell (n = 2), or non-compliant (n = 2), leaving 132 cats that underwent cardiac examination. Five (3.8%) cats were from rehoming centres, and 127/132 (96.2%) cats were born in the colony. The median age was 4.1 (IQR 3.0–8.0) years (Figure 3.2). There were 65 (49.2%) female cats and 67 (50.8%) male cats. Many female cats were intact (42 intact *vs.* 23 neutered) whereas most male cats were neutered (3 intact *vs.* 64 neutered). The median body weight was 3.5 (IQR 2.9–4.1) kg. Eleven (8.3%) cats had co-morbidities. These were two cats each with chronic diarrhoea, controlled hyperthyroidism, dental disease, and lameness, and one cat each with an abdominal mass, skin mass, or nasal mass. The cats with controlled hyperthyroidism were receiving carbimazole (Vidalta; MSD Animal Health, Wellington, NZ). The cats with dental disease, lameness, and the nasal mass were receiving meloxicam (Metacam; Boehringer Ingelheim Animal Health NZ Ltd., Auckland, NZ).

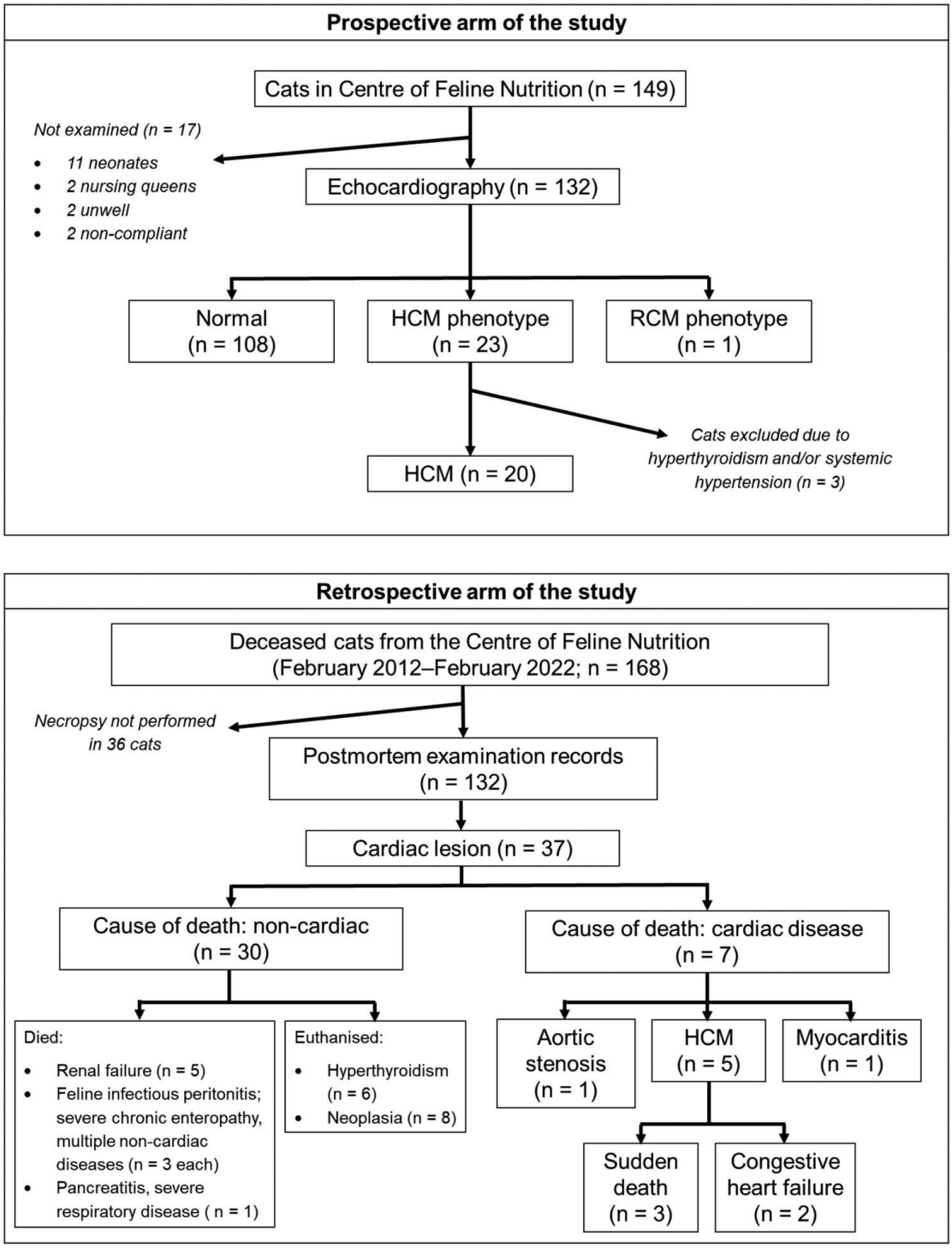

Figure 3.1. Flow chart summarising the design of a study to evaluate the prevalence of subclinical hypertrophic cardiomyopathy (HCM) in a colony of non-purebred cats. *Of 17 cats that were not examined, two were nursing kittens, 11 were neonates, two were non-compliant, and two were unwell. HCM = hypertrophic cardiomyopathy; RCM = restrictive cardiomyopathy.*

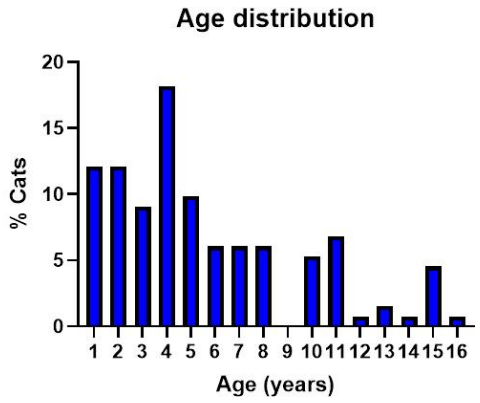

Figure 3.2. Age of cats (n = 132) prospectively enrolled in a study to evaluate the prevalence of subclinical HCM in a colony of non-purebred cats using echocardiography

Thirty-two of the 132 (24.2%) cats had a heart murmur, and 3/132 (2.3%) cats had an audible arrhythmia on auscultation. No cats had a gallop sound. Twenty-four of the 132 (18.2%) cats had an abnormal echocardiogram. Of these, 23/132 (17.4%, 95% CI: 11.4–25.0) cats had an HCM phenotype and 1/24 (0.8%, 95% CI: 0.0–4.1) cats had a RCM phenotype. Seven of the 23 cats with an HCM phenotype were older than 6 years old. Systemic hypertension was diagnosed in three of these cats, and one of these three also had hyperthyroidism. These three cats were excluded from the final HCM diagnosis. The final number of cats with HCM was 20/132 (15.2%; 95% CI = 9.5–22.4%). The cat with an RCM phenotype also had hyperthyroidism.

The results of statistical comparison between normal cats and cats with HCM is summarised in Tables 3.1 and 3.2. Of the 20 cats with HCM detected by echocardiography, 8 (40%) cats had a murmur detected on physical examination while 12 (60%) did not have a detectible murmur. Conversely, of the 108 normal cats that did not have evidence of heart wall changes detected on echocardiography, 23 (21.3%) had a murmur detected on physical examination. Five of the 20 (25%) cats with HCM had SAM, and 3/20 (15%) had CAM without SAM. None of the cats with a normal echocardiogram had SAM or CAM. The proportion of cats that had a moderate-to-loud heart murmur was greater in cats with HCM (4/20; 20%) than in cats with a normal echocardiogram (3/108; 2.8%; P =0.038). Similarly, the proportion of cats receiving medication was greater among cats with HCM (3/20 (15%) receiving meloxicam) than those with a normal echocardiogram (3/108 (2.8%) receiving meloxicam or carbimazole; P = 0.048). In female cats, HCM was positively

associated with neutered status (*P* = 0.042). Cats with HCM had a lower LVIDd and LVIDs, greater LV fractional shortening, and greater LVWT Max than normal cats.

Table 3.1. Categorical variables compared between cats with a normal echocardiogram and those diagnosed with hypertrophic cardiomyopathy (HCM)[a] in a study to evaluate the prevalence of subclinical HCM in a colony of non-purebred cats (n=132) using echocardiography.

	Normal (n = 108)	HCM (n = 20)	*P* value
Sex: female (n; %)	57 (52.8%)	7 (35%)	0.223
Proportion neutered (%)			
Female	17/57 (29.8%)	5/7 (71.4%)	0.042
Male	49/51 (96.1%)	12/13 (92.3%)	0.500
BCS (/9)			0.062
1	0	0:	
2	2	0	
3	0	0	
4	19	2	
5	71	10	
6	16	8	
7	0	0	
8	0	0	
9	0	0	
Heart murmur (n; %)	23 (21.3%)	8 (40.0%)	0.090
Soft : moderate–loud	18:5	4:4	0.038
Arrhythmia	1 (0.9%)	2 (8.7%)	0.063
Co-morbidities[a]	7 (6.5%)	3 (15.0%)	0.190
Medications[b]	3 (2.8%)	3 (15.0%)	0.048

[a]Diagnosed after excluding other differentials for an HCM phenotype. [b]Co-morbidities for normal cats were chronic diarrhoea (2), and abdominal mass, cutaneous mass, hyperthyroidism, lameness, and dental disease (1 each). Co-morbidities for cats with HCM were nasal mass, lameness, and dental disease (1 each). [c] Normal cats: meloxicam (2 cats) and carbimazole (1 cat). HCM group: meloxicam (3 cats).

Abbreviation: BCS, body condition score

Table 3.2. Continuous variables[a] compared between cats with a normal echocardiogram and those diagnosed with hypertrophic cardiomyopathy (HCM)[b] in a study to evaluate the prevalence of subclinical HCM in a colony of non-purebred cats (n=132) using echocardiography.

	Normal (n = 108)	HCM (n = 20)	*P* value
Age (years)	4.5 (2.1–8.0)	4.0 (3.2–5.3)	0.728
Body weight (kg)	3.42 (2.9–4.1)	3.80 (3.1–4.3)	0.152
LAD Max (mm)	13.1 (± 1.3) (n = 105)	12.8 (± 1.4)	0.276
LA/Ao	1.2 (1.1–1.3)	1.2 (1.1–1.2)	0.165
LVIDd (mm)	13.6 (± 1.8) (n = 107)	12.1 (± 2.0)	0.001
LVIDs (mm)	6.6 (± 1.3) (n = 107)	5.2 (± 1.1)	< 0.001
LV FS%	51.0 (± 7.8)	56.3 (± 8.8)	0.007
LVWT Max (mm)	4.7 (4.4–5.1)	6.3 (6.2–6.6)	–

[a]Normally distributed data are presented as mean (± SD) and non-normally distributed data are presented as median (IQR). Where data was not available for all members of a group, the number of cats for which the variable was available is shown in brackets. [b]Diagnosed after excluding other differentials of an HCM phenotype.

Abbreviations: LA/Ao, left atrial to aortic ratio; LAD Max, maximal left atrial size; LV FS%, left ventricular fractional shortening %; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVWT Max, left ventricular maximal thickness

3.3b Part 2 – Prevalence of cardiac mortality

A total of 168 cats from the CFN died between February 2012 and February 2022, with 132 cats subject to full postmortem examination (Figure 3.1). The median lifespan of the cats was 11.3 (IQR 9.5–12.8) years. There were 55 females and 73 males, with sex not documented in four neonatal kittens. Eighteen of 132 (13.6%) cats were from rehoming centres and 114/132 (86.4%) were born in the colony.

Thirty-seven of the 132 (28%; 95% CI = 17.2–32.5%) cats were reported to have cardiac abnormalities on either gross examination or histology. Of these 37 cats, 30 were reported to have hearts that were grossly thickened or enlarged on quantitative or subjective assessments, suggestive of cardiomyopathy. Seven of the 37 cats had cardiac lesions that were only detected by routine histology. These included 5/7 cats with LV fibrosis and 2/7 with fatty infiltrates in the left and right ventricles.

While gross evidence of cardiac abnormality was common, only 7/132 (5.3%, 95% CI = 2.2–10.6%) cats were considered to have died of heart disease. Three of these seven cats (42.9%) died suddenly and the only postmortem findings were a

grossly thickened LV and histologic lesions suggestive of HCM (myocardial hypertrophy, myofibre disarray, and interstitial fibrosis). Two of the seven (28.6%) cats that died of heart disease had gross and histological lesions of HCM and clinical evidence of congestive heart disease prior to death. Therefore, the total number of cats that died probably due to HCM during the study period was 5/132 (3.8%; 95% CI = 1.2–8.6%). Of the remaining two cats with a cause of death of heart disease, one cat was considered to have suffered arrhythmogenic sudden death due to aortic stenosis, and the other had myocarditis and pulmonary oedema. No cats died due to ATE.

Thirty cats had gross or histological evidence of heart disease, but these changes were not considered to have contributed to the death of the cat. Fourteen of these cats were euthanised (eight due to neoplasia and six due to uncontrolled hyperthyroidism) and 16 died from the following causes: renal failure (n = 5), feline infectious peritonitis (n = 3), chronic severe enteropathy (n = 3), multiple non-cardiac diseases (n = 3), pancreatitis (n = 1) and respiratory disease (n = 1).

3.4 Discussion

In the present study, 15% of cats in the colony had cardiomyopathy, specifically HCM, that was detected by echocardiography. Less than half of these cats had either a murmur or arrhythmia detectable on physical examination. Conversely, a murmur or arrhythmia was detected in cats that did not have cardiomyopathy. The lack of consistent association between findings of the physical examination and the presence of cardiomyopathy have been reported previously and highlight the need for echocardiography to diagnose cardiomyopathy.[2,3]

The detection of subclinical cardiomyopathy in cats, specifically HCM, is important because this condition may increase the risks associated with sedation, general anaesthesia, fluid therapy, or steroid medication. For example, many anaesthetic agents affect cardiovascular function, and the presence of cardiac disease can alter the anaesthetic plan. [15] Additionally, fluid therapy and steroid medication may increase blood volume, resulting in congestive heart failure in cats affected by HCM.[17-19] Furthermore, HCM can be progressive and can predispose cats to ATE and CHF.[6,7] Therapies such as clopidogrel can be used in cats with moderate to severe heart enlargement to reduce the risk of ATE and cardiac death.[20]

Not all cats with subclinical HCM will subsequently die of this disease, with previous studies reporting an incidence rate for all cardiovascular deaths in cats with HCM of 63.4 per 1,000 cat-years.[6] The probability that many cats with subclinical HCM will die of an unrelated cause was supported in the present study by the observation that only 3.8% of cats in the colony died due to HCM while 15% of cats in the colony were identified as having subclinical HCM. These two populations cannot be directly compared due to the difference in the method of data collection and age of the cats. However, if cardiac mortality represented 4% of deaths in the CFN during the period under study and assuming a 15% prevalence of HCM based on echocardiography, this would correspond to 25.3% of the cats with HCM dying of the disease. This is consistent with the literature.[6,7] Further evidence that HCM can remain subclinical over the lifetime of a cat was derived from the observation that 30 cats in our study died of non-cardiac causes but were found to have gross lesions consistent with HCM at postmortem exam. The increase in frequency of HCM diagnosis on postmortem examination compared to echocardiography is likely due to the difference in age between two study populations, as the frequency and severity of HCM increases with age.[3,7] As the median age of cats at death in this study was 11.3 years, more cats would be expected to be diagnosed with HCM in this group than in the group that underwent echocardiography (median age 4.1 years). Other than genetics, it is largely unknown why some cases of HCM progress faster than others.[6,7,21] It is possible that the current criteria used to diagnose HCM on echocardiography or by postmortem examination are not narrow enough, and a wide range of diseases are inadvertently grouped into a single disease entity. Further studies are needed to evaluate factors associated with the progression of HCM and disease classification.

The median age of the colony cats (4.1 years) is similar to cat populations used to investigate subclinical HCM in the UK and USA.[2,3] However, a recent survey showed the median age of cats in NZ may be closer to 7 years old.[8] As the prevalence of HCM increases with age,[3] the true proportion of cats with subclinical HCM in NZ may be higher than the 15% detected in the cat colony.

The CFN is considered to contain cats that are representative of non-purebred cats in New Zealand. However, CFN is not a randomly selected population, and the genetic diversity in the colony has not been assessed. Given that HCM is likely to have a genetic basis, we acknowledge that there is some uncertainty in how representative the colony data is to the wider NZ cat population. A recent survey

suggested that 20% of pet cats in NZ are purebred.[8] As there are genetic differences between breeds and non-purebred cats, the findings of this study may not be applicable to the NZ population if purebred cats are included. The prevalence of HCM and cardiac death in purebred cats in NZ is currently unknown. Furthermore, only 23/65 (35.4%) female cats in the colony were neutered, which may differ from the wider NZ population. Therefore, a 95% CI was provided, which suggests 95% certainty that the true prevalence of subclinical HCM lies between 9.5% and 22.4%.

The proportion of cats that died due to heart disease in the present study was similar to that observed in a study of cats presenting to first opinion veterinary clinics in the UK (4.2%).[22] Cardiac deaths were not further classified into SCD, CHF, or ATE in that study. However, CHF and ATE are most common causes of cardiac death in cats with HCM.[6,7] In contrast, SCD was the most common cause of cardiac death in the present study at postmortem examination. The reason for this difference is uncertain, although it may have been due to chance considering the relatively small sample size and the small number of cats dying due to HCM in the present study. Furthermore, postmortem diagnosis of other causes of sudden death, including asthma, neurological causes, anaphylaxis, and pulmonary embolism can be challenging. Due to the high prevalence of cardiomyopathy, pathologists may inadvertently attribute the cause of sudden death to subclinical cardiomyopathy. Alternatively, not all cases of SCD may have been recorded in previous studies, as they rely on owners to provide the data. In humans with HCM, SCD is the most common cause of death and is also more common in young patients.[23,24] It is possible that the incidence of SCD in cats with HCM is higher than previously described.

Loud heart murmurs, neutering in female cats, and receiving medications were associated with the detection of HCM using echocardiography in the present study. A loud heart murmur was previously shown to predict diagnosis of HCM.[3] In the present study, neutered female cats had higher rates of HCM than intact female cats. While male cats have been previously reported to be at risk for HCM, to the authors' knowledge no predisposition in neutered female cats has previously been reported. While this association may have been due to random chance, it is possible that oestrogen could provide some protection against HCM. It is possible that previous studies did not observe a reduced rate of HCM in intact females as cats in the UK and USA are typically spayed and therefore surveys may have contained few intact queens. The present study also showed that cats receiving medication had

higher rates of HCM than cats not receiving medications. Medications may predispose to HCM either directly (certain non-steroidal anti-inflammatory drugs cause in systemic hypertension in humans)[25] or the stress of being medicated may increase blood pressure. Alternatively, concurrent illnesses that require medication may predispose cats to HCM, perhaps by increasing blood pressure. Due to the small number of cats receiving medications in this study, further statistical analysis of the effect of individual medication was not possible.

There are some limitations in this study. First, a general sonographic machine used to perform echocardiography was not a cardiac-specific machine. This was adequate in quality but limited the cardiac assessment of the cats to 2D imaging. Second, not all differentials of an HCM phenotype were ruled out using blood tests. However, each cat in the colony unit undergoes a yearly veterinary examination and weekly evaluation of appetite, thirst and body weight. It is possible some cats may have had early hyperthyroidism or hypersomatotropism. However, none of the cats in the HCM group developed any clinical signs in the 2 years following the data collection, making this unlikely. Third, the echocardiographic prevalence of cardiomyopathy was assessed by a single cardiologist at a single time point. Although cardiac measurements from 2D imaging are considered highly repeatable among cardiologists, the objective criteria used to define types of feline cardiomyopathy can vary among cardiologists. Fourth, cardiac biomarkers are commonly used to screen cardiomyopathies in clinical practice. The utility of cardiac biomarkers was not tested in this study. Lastly, the 10-year mortality data from the CFN was collected retrospectively, and many pathologists were involved in the postmortem examinations. There may therefore be some unaccounted variation in approaches to assessing cardiac abnormalities postmortem in this study. A prospective study would be required to test repeatability and standardise the postmortem diagnosis of cardiac abnormalities among veterinary pathologists.

In summary, the results of this study suggest that around 15% (95% CI = 9.5–22.4%) of non-purebred cats in NZ may have HCM without clinical signs of disease. This is important for clinicians to be aware of because subclinical HCM may complicate anaesthesia and increase the risks associated IV fluid therapy and corticosteroid therapy. Additionally, if subclinical HCM is identified in cats, preventative treatments can be started which may prolong their lifespan. Many cats in this study died of non-cardiac diseases despite having evidence of HCM on

postmortem examination. This suggests that subclinical HCM may have a more favourable prognosis in cats than has previously been believed.

3.5 References

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Chapter 4. Phenotypic progression of cats with or without hypertrophic cardiomyopathy

4.1 Introduction

Hypertrophic cardiomyopathy (HCM) is characterised by progressive left ventricular hypertrophy (LVH) in the absence of abnormal loading conditions.[1] In cats, subclinical HCM is identified in up to 15% of the general population and causes death in up to 6 out of 100 affected cats per year. [2-5] Given the high prevalence of HCM and its potential to cause death, appropriate screening and risk stratification are recommended in the current veterinary guidelines.[1]

An antemortem diagnosis of HCM requires echocardiography to demonstrate increased left ventricular wall thickness (LVWT), which is a surrogate marker of LVH. However, LVH is a gradual process and can be preceded by other features of HCM including elongated anterior mitral valve leaflet (AMVL), reduced left atrial fractional shortening (LA FS%), and possibly abnormal left ventricular (LV) systolic and diastolic function.[6-9] These early features of HCM can also be assessed using echocardiography and allow identification of cats at risk of HCM.[10]

Prognostic information in cats with pre-existing HCM can also be assessed using echocardiography. Specifically, left atrial enlargement, reduced LA FS%, extreme LVH, and LV systolic dysfunction were shown to increase the risk of cardiac death.[5,11-15] However, the left heart dimensions in cats with HCM can change over time,[6,16-18] and there is limited information on the rate of changes in left heart dimensions in cats in the literature. Furthermore, there is paucity in data of incidence of cardiac death in the general feline population.

The primary aims of this study were 1) to describe the magnitude of change in left heart dimensions in cats per year and 2) to assess the predictive ability of cardiac dimensions in cats without HCM on the development of HCM in the following year. The secondary aim was to describe the incidence of cardiac death, specifically the incidence of sudden cardiac death (SCD), in a closely monitored convenience sample of the general population. The hypotheses were 1) LVWT increases in cats with or without HCM and 2) baseline LVWT and AMVL length are predictive of a diagnosis of HCM in the following year.

4.2 Materials and Methods

4.2a Study Design

This was a prospective observational study conducted at the CFN, Massey University, Palmerston North. This colony of cats served as a convenience sample of the general non-purebred feline population in New Zealand.[4] The prevalence of cardiomyopathy in the colony was recently described and was similar to that of the general feline population in the United Kingdom and United States of America.[2-4] Ethical approval was provided by Massey University Animal Ethics Committee (MUAEC protocol 21/47).

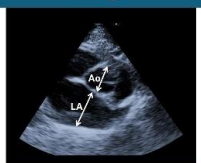
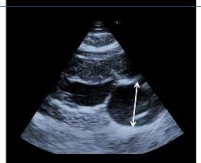
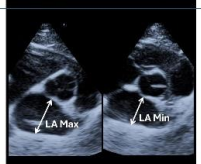
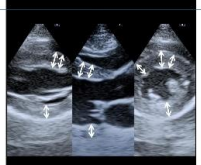
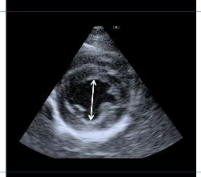
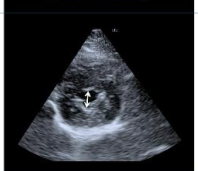
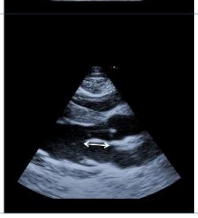
Inclusion criteria were apparently healthy non-purebred cats in the colony. Exclusion criteria were neonates, queens that were nursing, sick cats that required hospitalization, and non-compliant cats.

4.2b Clinical Data Collection

Each eligible cat underwent physical examination and right parasternal two-dimensional echocardiography by a board-certified cardiologist (Joonbum Seo) every 12 months between 2022 and 2024 (3 time points). None of the cats received sedation or oral anxiolytics: however, oral treats were provided to improve the compliance of cats. Age, sex, body weight, body condition score (on a scale of 9), and current medications were recorded at the time of echocardiography.

A general ultrasound machine (Xario 200; Toshiba, Tochigi, Japan) equipped with a sector array probe (6S3, frequency 4.4–6.2 MHz) was used for echocardiography. All images were exported and analyzed using image processing software (TOMTEC Imaging Systems, Unterschleissheim, Germany). The description of each echocardiographic variable is presented in Figure 4.1. To assess the intra-observer reliability with the general ultrasound machine, 12 randomly selected cats by a supporting technician (Rebecca Owen) were rescanned 24-48 hours later by the same cardiologist (Joonbum Seo) and left heart measurements were repeated.

Figure 4.1. Studied echocardiographic variables. Images acquired during the study are shown with annotations to describe each variable.

Variable	Image	Method
LA/Ao (unitless)		View: RPSax at the level of the aortic valve Timing: Beginning of diastole, the first frame of aortic valve closure Ao: From the blood-tissue interface at the midpoint of the right aortic sinus to the commissure between the noncoronary and left coronary aortic cusps LA: Extension of the aortic line to the blood tissue interface of the left atrial wall, immediately lateral to the pulmonary vein
LAD Max (mm)		View: RPLax4C Timing: Beginning of diastole, the last frame before the mitral valve opening From the mid-interatrial septum to the leading edge of the pericardium, bisecting the left atrium parallel to the mitral valve annulus.
LA FS% (%)		View: RPSax at the level of the aortic valve Maximal LA size: Identical to LA measurement used in LA/Ao Minimal LA size: Identical to LA measurement used in LA/Ao but at end-systole LA FS% = (Maximal LA size − Minimal LA size)/Maximal LA size x 100
IVSd, LVFWd and LVWT Max (mm)		View: RPLax4C, RPLax5C, and RPSax at the level of the papillary muscles Timing: End-diastole From the thickest segment of the interventricular septum (IVSd) and left ventricular free wall (LVFWd) using the leading-to-leading method. The greater number between IVSd and LVFWd were recorded as LVWT Max.
LVIDd (mm)		View: RPSax at the level of the papillary muscles Timing: End-diastole Blood tissue interface to blood tissue interface, bisecting the left ventricle in end-diastole
LVIDs (mm)		View: RPSax at the level of the papillary muscles Timing: End-systole Blood tissue interface to blood tissue interface, bisecting the left ventricle in end-systole
LV FS% (%) **AMVL (mm)**		LV FS% = (LVIDd−LVIDs)/LVIDd x 100 View: RPLax5C avoiding chordae tendineae Timing: Early diastole From the tip of the anterior mitral valve leaflet to the aortic hinge-point

Each echocardiographic variable was measured over 3 consecutive cardiac cycles and averaged.

Abbreviations: AMVL, anterior mitral valve leaflet; Ao, aorta; IVSd, interventricular septal thickness in end-diastole; LA/Ao, left atrium to aortic ratio; LAD Max, maximal left atrial size; LA, left atrial; LA FS%, left atrial fractional shortening; LVIDd, left ventricular internal diameter in end-diastole; LV FS%, left ventricular fractional shortening; LVFWd, left ventricular free wall thickness in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVWT Max, maximal left ventricular wall thickness; RPLax4C, right parasternal long axis four chamber view; RPLax5C, right parasternal long axis five chamber view; RPSax, right parasternal short axis view

4.2 c Classification of cardiomyopathy using echocardiography

Types of cardiomyopathies were determined following the current guidelines.[1] Arbitrary cutoffs were used when objective cutoffs were unavailable or inconsistently used in literature. Hypertrophic cardiomyopathy was defined as maximal end-diastolic LVWT (LVWT Max) ≥6.0 mm in the absence of systemic disease or systemic hypertension that can mimic HCM.[1,15] To recognise such systemic disease, each cat underwent weekly general health checks to document their body weight, appetite, drinking, and energy level as part of the colony protocol. Appropriate blood tests were performed when any of these parameters became abnormal. Doppler blood pressure was routinely measured in all cats with HCM phenotype that were >6 years old following the current guidelines.[19] Systemic hypertension was defined as systolic arterial blood pressure >160 mmHg.[19]

In this study, an equivocal HCM phenotype was defined as LVWT Max 5.0-5.9 mm. Normal LVWT was defined as LVWT Max <5.0 mm. A dilated cardiomyopathy phenotype was defined as increased LV internal diameter in end-diastole (>18 mm) and reduced LV fractional shortening percentage (LV FS%; <30 %).[1,4] A restrictive cardiomyopathy (RCM) phenotype was defined as left or biatrial enlargement (left atrial to aortic ratio [LA/Ao] >1.6 and maximal left atrial diameter [LAD Max] >16 mm) with either normal or equivocal LVH (LVWT <6.0 mm) or the presence of a fibromuscular band crossing the left ventricle.[1,4] An arrhythmogenic right ventricular cardiomyopathy (ARVC) phenotype was defined as right heart enlargement on subjective assessment. Finally, non-specific cardiomyopathy was defined as features of cardiomyopathy that did not meet any of the above criteria.

4.2d Outcome and postmortem examination

Cats that became ill during the study period were appropriately treated at the Massey University Veterinary Teaching Hospital (Massey University, Palmerston North, New Zealand). Deaths during the study period were recorded as spontaneous or assisted if humane euthanasia was performed due to life limiting illness. Spontaneous death was classified as sudden unexplained death if the cat did not show any clinical signs of illness within 12 hours prior to death.

All cats that died underwent complete postmortem exam by a board-certified anatomic pathologist. On completion, the likely cause of death or primary clinical sign that led to the decision of euthanasia was recorded by the attending pathologist. Cats with multiple abnormalities that likely contributed to death were recorded as having 'multiple' causes. Cats with sudden unexplained death were recorded to have suffered SCD when the only abnormalities were seen in the heart.

For consistent reporting of the cardiac postmortem exam findings, each heart was stored in 10% formalin and independently examined by a board-certified anatomic pathologist (Hayley Hunt). Each heart was examined grossly, then sectioned transversely at the level of the papillary muscles to facilitate examination of the left and right ventricles and valve leaflets. A thin section at this level was processed routinely for histology, and cats with focal echocardiographic or gross abnormalities had additional sections trimmed and processed to incorporate the abnormal regions. To avoid unintentional bias, this pathologist was not provided with the echocardiographic diagnosis until the end of data collection.

4.2e Classification of cardiomyopathy on postmortem exam

There are no accepted criteria for postmortem diagnosis of cardiomyopathies in cats. Therefore, the criteria used in this study were adopted from previous studies. Cardiac hypertrophy was defined by increased absolute or body weight corrected heart weight by >20 g or >4 g/kg, respectively.[15,20,21] Hypertrophic cardiomyopathy was characterised by cardiac hypertrophy with an increased ratio of LV free wall (LVFW) or interventricular septum (IVS) to right ventricular free wall thickness by >3.0 on macroscopic exam, and evidence of cardiac myocyte hypertrophy, myofibre disarray and interstitial fibrosis on microscopic exam.[15,20] The myocardial form of RCM was characterised by cardiac hypertrophy with minimal LVH (LVFW or IVS to right ventricular free wall ratio of <3.0) with the same microscopic features of HCM.[20,22] The endomyocardial form of RCM was defined as segmental or diffuse endocardial thickening composed of stellate, spindle-shaped or elongated mesenchymal cells surrounded by fibrous connective tissue on microscopic exam.[23,24] Dilated cardiomyopathy was defined as cardiac hypertrophy with severe and generalized dilation of left ventricle and microscopic features of thin or normal sized myofibres with nuclear hypertrophy and pleomorphism on microscopic

exam.[20,25] Arrhythmogenic cardiomyopathy was defined as thin right ventricular wall with microscopic features of myocardial replacement by adipose and fibrous tissue, extending from the epicardium to endocardium.[20,26] Finally, non-specific cardiomyopathy was characterised by evidence of cardiomyopathy that does not fall into any of the above criteria.

4.2f Statistical analysis:

Statistical analysis was performed using commercial software (SPSS Statistics). Normality of continuous data was tested by visual assessment and Shapiro-Wilk test. Normally distributed data were presented as mean (standard deviation) and non-normally distributed data were presented as median (interquartile range [IQR]).

The intra-observer reliability of image acquisition and left heart measurements using the general ultrasound machine was assessed using paired t-test and intraclass correlation coefficients (ICC). Reliability was deemed poor when ICC was <0.5, moderate when 0.5-0.75, good when 0.75-0.9, and excellent when >0.9.[27]

For the first aim of the study, cats were grouped based on their cardiac phenotype in the preceding year. Data from cats with two rechecks were entered twice using the preceding year's examination as the new baseline. The baseline characteristics between each group and yearly changes in left heart dimensions within each group were compared using linear mixed-effects models with post hoc Bonferroni correction. For binary categorical comparison, generalized estimating equations (GEE) were used. *P* value and 95% confidence interval (CI) were used for inferential statistics. Bootstrapping method was used to calculate 95% CI for median. Significance was set <0.05.

For the second aim of the study, multivariable analysis using GEE was performed to identify variables that can predict the development of HCM in the subsequent year. The Spearman rank correlation between variables with *P* <0.20 was first tested, and if the correlation was >0.9, collinearity was confirmed. For collinear variables, only one variable with higher ICC, lower *P* value, or clinically practical was selected in each model. Variables with >50% missing data were not included in the multivariable analysis. After the first multivariable analysis, LVWT was

excluded, and the analysis was repeated as even a small increase in LVWT could result in the HCM diagnosis in the subsequent year in cats with upper limit of normal LVWT. The variables were entered manually, and non-significant variables were removed manually in a backward stepwise manner. For each predictor of HCM, a receiver operating characteristic analysis was performed. Area under the curve (AUC), sensitivity and specificity were calculated using Youden's index as an ideal cutoff.

The frequency of cats suffering cardiac death was presented as incidence rate, where the numerator equalled the number of cats that died due to cardiac reasons and the denominator equalled the number of cat-years at risk.

4.3 Results

4.3a Intra-observer repeatability

The results of intra-observer reliability of left heart dimension measurements are shown in Table 4.1. Intra-observer reliability was good (ICC 0.75-0.9) for LAD Max, LA/Ao, LA FS%, LV internal diameter in end-diastole and end-systole, LV FS%, and maximal LVFW thickness in end-diastole; and excellent (ICC >0.9) for AMVL, maximal IVS thickness in end-diastole (IVSd Max), and LVWT Max.

Table 4.1. Intra-observer reliability of image acquisition and left heart measurements by a board-certified cardiologist in a study conducted to describe the yearly change in cardiac phenotypes in cats (n=121). The intra-observer reliability was assessed using paired t-test and intraclass correlation coefficients (ICC). Reliability was deemed poor when ICC was <0.5, moderate when 0.5-0.75, good when 0.75-0.9, and excellent when >0.9.[27]

	Mean diff (95% CI)	*P* value	ICC (95% CI)	*P* value	ICC Interpretation
LAD Max (mm)	0.1 (-0.4 – +0.6)	0.784	0.861 (0.518-0.960)	0.001	Good
LA/Ao	0 (0 – 0.1)	0.093	0.883 (0.594-0.966)	<0.001	Good
LA FS%	0 (-3 – +3)	0.980	0.789 (0.265-0.939)	0.008	Good
IVSd Max (mm)	0.1-0.3 (-0.1 – 0.4)	0.158	0.967 (0.884-0.990)	<0.001	Excellent
LVFWd Max (mm)	0 (0.5 – 0.1)	0.920	0.791 (0.273-0.940)	0.008	Good
LVWT Max (mm)	0.2 (0.1 – 0.4)	0.114	0.963 (0.872-0.989)	<0.001	Excellent
LVIDd (mm)	-0.3 (-0.9 – +0.3)	0.317	0.862 (0.519-0.960)	0.001	Good
LVIDs (mm)	-0.8 (-1.6 – 0)	0.051	0.845 (0.460-0.955)	0.002	Good
LV FS%	4.8 (-0.5 – 10.2)	0.073	0.838 (0.437-0.953)	0.003	Good
AMVL (mm)	0 (-0.1 – +0.1)	0.727	0.982 (0.938-0.995)	<0.001	Excellent

Abbreviation: AMVL, anterior mitral valve leaflet; ICC (intraclass correlation coefficient); IVSd Max, maximal interventricular septal thickness in end-diastole; LA/Ao, left atrium to aortic ratio; LAD Max, maximal left atrial size; LA FS%, left atrial fractional shortening; LVIDd, left ventricular internal diameter in end-diastole; LV FS%, left ventricular fractional shortening; LVFWd, Max maximal left ventricular free wall thickness in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVWT Max, maximal left ventricular wall thickness

4.3b Study population

A total of 145 cats were scanned between 2022 and 2024. Of these cats, 24 cats were excluded prior to the first recheck for reasons including death (10 cats), hyperthyroidism (4), systemic hypertension (4), non-compliance (1), nursing kittens (1), recent general anaesthesia (1) and a technical issue where echocardiographic images failed to store (1). Subsequently, 29 cats were excluded prior to the second recheck due to 17 deaths, 6 systemic hypertension, 3 non-compliance, and 1 each for hyperthyroidism, rehomed, and a technical issue where echocardiographic images failed to store. Therefore, 121 cats had at least two scans, and 92 had all three annual scans.

All 121 cats were non-purebred domestic shorthair cats. There were 61 females (23 neutered) and 60 males (56 neutered). The median age was 4.0 years (IQR 2.1-6.2). The median body weight was 3.6 kg (IQR 2.9-4.1). The median body condition score was 5 out of the scale of 9 (IQR 5-5). One cat who had two repeat scans had previously been successfully treated for nasal squamous cell carcinoma. None of the other cats had any known pre-existing illness and appeared clinically

healthy. None of the 121 cats were receiving medications at the time of the cardiac examination.

4.3c Changes in left heart dimensions and cardiac phenotype

The baseline cardiac phenotypes and the changes seen from 121 cats over the study period are presented in Figure 4.2. The characteristics of each cardiac phenotype over combined timepoints are presented in Table 4.2. The diagnosis of HCM was associated with older age, greater body weight, hyperdynamic systolic function (reduced LV internal diameter in end-systole and increased LV FS%), and longer AMVL length.

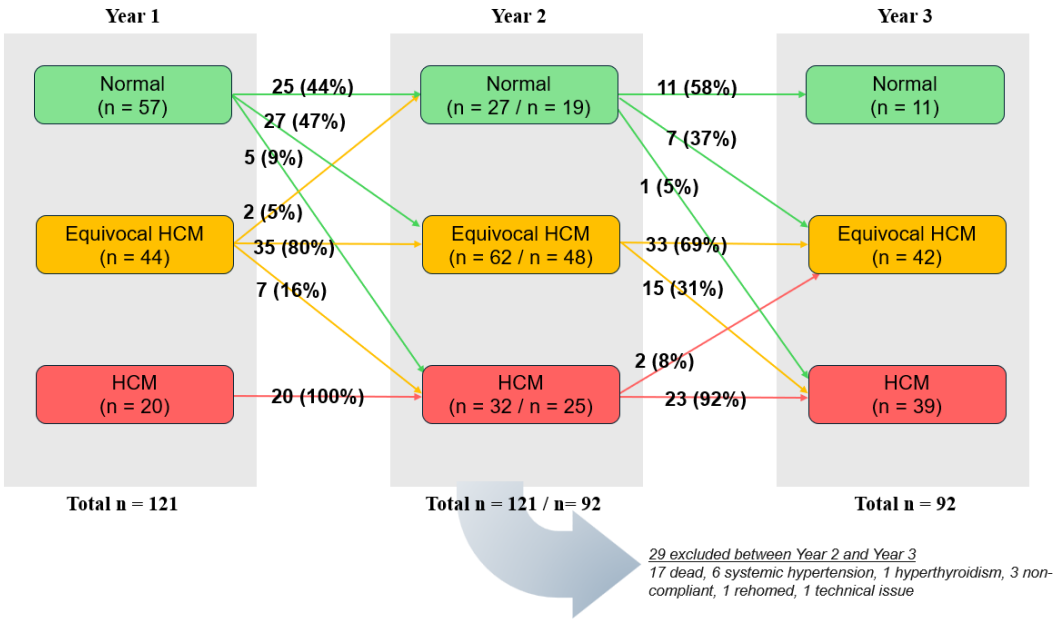


Figure 4.2. Flow chart summarising the changes in cardiac phenotypes in a study conducted to describe yearly changes in cardiac phenotype in 121 cats. *All 121 cats were examined at least twice (year 1 and year 2). Subsequently, 92 cats were examined again (year 3) as 29 were excluded due to 17 deaths, 6 new systemic hypertension, 1 new hyperthyroidism, 3 loss of compliance, 1 rehoming, and 1 technical issue. Cardiac phenotypes were divided accordingly to normal, equivocal hypertrophic cardiomyopathy (HCM), and HCM. The number of cats with each cardiac phenotype is listed under the heading of each cardiac phenotype. In year 2 the numbers of cats were separated to distinguish cats that were re-examined at year 1 and 2, and those that were examined at year 2 and year 3.*

Table 4.2. Comparison of signalment and echocardiographic measurements of left heart dimensions at baseline between cats with normal, equivocal hypertrophic cardiomyopathy (HCM), and HCM in a study conducted to describe yearly cardiac phenotype in 121 cats. In this table, 213 exams were used as a baseline, as the data from cats with two rechecks (92 cats) were entered twice using the preceding year's examination as the new baseline. If the cardiac phenotype had changed from the original baseline to the new baseline in any of the 92 cats with repeated data entry, the cardiac phenotype at the new baseline was used at the time of second data entry.

	Normal (n = 76)	Equivocal HCM (n = 92)	HCM (n = 45)	*P* value
Sex (female /male)	51 (67.1%) /25 (32.9%) [a]	37 (40.2%) /55 (59.8%) [a]	20 (44.4%) /25 (55.6%)	0.035
Neutered female	14/51 (27.5%)	16/37 (43.2%)	15/20 (75.0%)	0.369
Neutered male	23/25 (92.0%)	52/55 (94.5%)	25/25 (100%)	-
Age (years)	4.0 (±2.5) [a, b]	4.7 (±3.0) [a, c]	6.1 (±2.9) [b, c]	<0.001
Body weight (kg)	3.2 (2.7-3.7) [a, b]	4.0 (3.4-4.5) [a]	4.0 (3.4-4.6) [b]	<0.001
BCS (/9)	5 (4-5)	5 (5-6)	5 (5-6)	
LAD Max (mm)	12.3 (11.5-13.7) (n=74)	12.9 (12.1-13.6) (n=91)	13.1 (12.1-14.0)	0.153
LA/Ao	1.2 (1.2-1.3)	1.2 (1.2-1.3)	1.2 (1.1-1.3)	0.160
LA FS%	32.1 (27.0-36.5) (n=73)	31.1 (27.2-35.0) (n=90)	29.3 (25.9-34.3)	0.191
IVSd Max (mm)	4.6 (4.4-4.8)	5.3 (5.1-5.6)	6.3 (6.1-6.6)	-
LVFWd Max (mm)	4.2 (3.9-4.5)	4.6 (4.3-4.9)	5.0 (4.7-5.4)	-
LVWT Max (mm)	4.6 (4.4-4.8)	5.3 (5.1-5.6)	6.3 (6.1-6.6)	-
LVIDd (mm)	13.1 (±1.5)	13.6 (±1.9)	12.8 (±1.8)	0.123
LVIDs (mm)	6.5 (±1.4) [a]	6.3 (±1.6) [b]	5.3 (±1.3) [a, b]	<0.001
LV FS%	50.5 (±8.5) [a]	53.4 (±9.3) [b]	57.9 (±8.0) [a, b]	<0.001
AMVL (mm)	7.9 (7.4-8.4) (n=54) [a, b]	8.3 (7.8-8.8) (n=77) [a]	8.3 (7.8-8.9) (n=42) [b]	<0.001

Cats are grouped into those with normal echocardiogram, equivocal hypertrophic cardiomyopathy (HCM, left ventricular wall thickness 5.0-5.9 mm), and HCM (left ventricular wall thickness ≥6.0 mm). Normally distributed data are presented in mean (± standard deviation). Non-normally distributed data are presented in median (interquartile range). For variables with missing data, a total number of each variable was listed. Significance from the post-hoc analysis is annotated using superscript letters. Statistical comparison of neuter status in male cats could not be run due to majority of cats being neutered.

Abbreviations: AMVL, anterior mitral valve leaflet; BCS, body condition score; IVSd Max, maximal interventricular septal thickness in end-diastole; LA/Ao, left atrial to aortic ratio; LAD Max, maximal left atrial diameter in long-axis; LA FS%, left atrial fractional shortening; LV FS%, left ventricular fractional shortening; LVFWd Max, maximal left ventricular free wall thickness in end-diastole; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVWT Max, maximal left ventricular wall thickness in end-diastole

The changes seen in left heart dimensions are presented in Table 4.3. In cats with or without HCM, the IVSd Max and LVWT Max increased per year. In cats with normal echocardiograms at baseline, the median IVSd Max increased by 0.5 mm (95% CI: 0.4-0.7, *P* <0.001) and median LVWT Max by 0.5 mm (95% CI: 0.4-0.7, *P* <0.001) per year. In cats with equivocal HCM at baseline, the median IVSd Max increased by 0.4 mm (95% CI: 0.3-0.5, *P* <0.001) per year and median LVWT Max

by 0.3 mm (95% CI: 0.3-0.5, *P* <0.001) per year. In cats with HCM at baseline, the median IVSd Max increased by 0.4 mm (95% CI: 0.2-0.5, *P* <0.001) and LVWT Max by median 0.4 mm (95% CI: 0.2-0.5, *P* <0.001) per year. Additionally, AMVL length increased in cats with normal echocardiogram and equivocal HCM by median 0.5 mm (95% CI: 0.4-0.7, *P* <0.001) and median 0.4 mm (95% CI: 0.2-0.6, *P* = 0.013) per year, respectively. The AMVL length did not change in cats with pre-existing HCM (median difference 0.1 mm; 95% CI: -0.2- +0.3, *P* = 0.162).

Table 4.3. Yearly changes in echocardiographic measurements of the left heart dimensions in a study conducted to describe yearly cardiac phenotype in 121 cats. Cats were divided according to the echocardiographic evidence of a normal heart, equivocal hypertrophic cardiomyopathy (HCM), and HCM from the baseline. In this table, 213 exams from 121 cats were used as a baseline, as the data from cats with two rechecks (92 cats) were entered twice using the preceding year's examination as the new baseline. If the cardiac phenotype had changed from the original baseline to the new baseline in any of the 92 cats with repeated data entry, the cardiac phenotype at the new baseline was used at the time of second data entry.

	Normal (n = 76)			Equivocal HCM (n = 92)			HCM (n = 45)		
	Baseline	Recheck	*P* value	Baseline	Recheck	*P* value	Baseline	Recheck	*P* value
LAD Max (mm)	12.3 (11.5-13.7)	12.7 (11.8-13.7)	0.178 (n=74)	12.9 (12.1-13.6)	13.1 (12.4-14.0)	0.033 (n=88)	13.1 (12.1-14.0)	13.3 (12.7-14.5)	0.085 (n=44)
LA/Ao	1.2 (1.2-1.3)	1.3 (1.2-1.4)	<0.001	1.2 (1.2-1.3)	1.3 (1.2-1.4)	0.034 (n=91)	1.2 (1.1-1.3)	1.3 (1.1-1.3)	0.179
LA FS%	32.1 (27.0-36.5)	32.5 (28.5-36.6)	0.719 (n=73)	31.1 (27.2-35.0)	31.5 (28.6-35.8)	0.119 (n=90)	29.3 (25.9-34.3)	29.1 (25.2-33.2)	0.426
LVIDd (mm)	13.1 (±1.5)	13.8 (±1.8)	0.006	13.6 (±1.9)	13.7 (±1.7)	0.384	12.8 (±1.8)	12.6 (±1.5)	0.526
LVIDs (mm)	6.5 (±1.4)	6.6 (±1.9)	0.687	6.3 (±1.6)	6.2 (±1.6)	0.311	5.3 (±1.3)	5.3 (±1.3)	0.818
LV FS%	50.5 (±8.5)	52.6 (±10.2)	0.044	53.4 (±9.3)	55.2 (±8.7)	0.068	57.9 (±8.0)	58.1 (±8.4)	0.908
IVSd Max (mm)	4.6 (4.4-4.8)	5.1 (4.5-5.4)	<0.001	5.3 (5.1-5.6)	5.6 (5.3-5.9)	<0.001	6.3 (6.1-6.6)	6.7 (6.2-7.1)	<0.001
LVFWd Max (mm)	4.2 (3.9-4.5)	4.4 (3.9-4.6)	0.006	4.6 (4.3-4.9)	4.7 (4.3-4.9)	0.670	5.0 (4.7-5.4)	5.0 (4.7-5.4)	0.889
LVWT Max (mm)	4.6 (4.4-4.8)	5.1 (4.6-5.4)	<0.001	5.3 (5.1-5.6)	5.6 (5.3-5.9)	<0.001	6.3 (6.1-6.6)	6.7 (6.2-7.1)	<0.001
AMVL (mm)	7.9 (7.4-8.4)	8.1 (7.7-8.5)	<0.001 (n=54)	8.3 (7.8-8.8)	8.4 (7.9-8.9)	0.013 (n=77)	8.3 (7.8-8.9)	8.5 (8.0-9.0)	0.162 (n=42)

Normally distributed data are presented in mean (± standard deviation). Non-normally distributed data are presented in median (interquartile range). For variables with missing data, a total number of each variable was listed.

Abbreviations: AMVL, anterior mitral valve leaflet length; HCM, hypertrophic cardiomyopathy; IVSd Max, maximal interventricular wall thickness in end-diastole; LA/Ao, left atrial to aortic ratio; LAD Max, maximal left atrial diameter; LA FS%, left atrial fractional shortening; LV FS%, left ventricular fractional shortening; LVFWd Max, maximal left ventricular free wall thickness in end-diastole; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVWT Max, maximal left ventricular wall thickness in end-diastole.

Cats with normal echocardiograms at baseline also had an increase in left atrial to aortic ratio by median 0.1 per year (95% CI: 0.1-0.2, *P* <0.001) and LV internal diameter in end-diastole by mean 0.6 mm per year (95% CI: 0.2-1.1, *P* = 0.006). In cats with equivocal HCM, there was negligible increase in the left atrial size per year (left atrial to aortic ratio by median 0.001 per year [95% CI: 0.03-1, *P* = 0.033] and maximal left atrial size by 0.1 mm [95% CI: -0.2-0.4, *P* = 0.034] per year).

4.3d Prediction of HCM

Out of 213 baseline scans, 168 echocardiograms were consistent with a normal heart or equivocal HCM. In the subsequent year, 28 out of 168 cats developed HCM.

The results of the univariable analysis for the prediction of HCM in the subsequent year are presented in Table 4.4. Twelve variables had *P* <0.2 (previous age, previous neuter status in female cats, previous body condition score, previous LAD Max, previous IVSd Max, previous maximal LVFW thickness in end-diastole, previous LVWT Max, previous LV FS%, previous AMVL length, and previous changes in the IVSd Max, LVWT Max, and LV internal diameter in end-diastole). Neuter status in female cats and yearly changes in left heart dimensions were not included in the multivariable analysis due to the small sample size. Multicollinearity was identified among the variables of LVWT (correlation coefficient > 0.985; *P* < 0.001). After considering the intra-observer repeatability, IVSd Max was chosen over other variables of LVWT in the multivariable analysis. In summary, 6 variables (age, body weight, body condition score, LAD Max, IVSd Max, and AMVL) were selected for the first GEE analysis and 5 variables (age, body weight, body condition score, LAD Max, and AMVL length) in the second GEE analysis.

Table 4.4. Results of univariable analysis for the prediction of hypertrophic cardiomyopathy (HCM) diagnosis in the subsequent year using the baseline characteristics in a study conducted to describe yearly cardiac phenotype in 121 cats. The baseline constituted data from the previous year. In cats with two rechecks, the data was entered twice using the preceding year's examination as the new baseline. In this table, the total sample size was 168 as this only included the data of cats with normal or equivocal HCM at either the original (n=101) or new baseline in cats with two rechecks (n=67). In cats with two rechecks, the changes in cardiac dimensions observed from the previous examinations was also calculated and entered as new variables with annotation '△' before the variable name.

	No HCM at recheck (n=140)	HCM at recheck (n=28)	*P* value
Sex (female /male)	76 / 64	12 / 16	0.282
Neuter female	23 (30.3%)	7 (58.3%)	0.054
Neutered male	60 (93.8%)	15 (93.8%)	0.996
Previous Age (years)	4.0 (2.1-6.0)	4.9 (3.0-7.1)	0.115
Previous Body weight (kg)	3.6 (3.0-4.1)	3.6 (3.2-4.4)	0.215
Previous BCS (/9)	0/ 0 /0/ 28/ 85/ 27/ 0/ 0/ 0	0/ 0 /0/ 4/ 14/ 9 / 1/ 0/ 0	0.070
Previous LAD Max (mm)	12.7 (±1.3) (n=137)	13.1 (±1.2)	0.099
Previous LA/Ao	1.2 (1.2-1.3) (n=139)	1.3 (1.2-1.4)	0.334
Previous LA FS%	31.4 (±6.0) (n=135)	31.0 (±5.4)	0.743
Previous IVSd Max (mm)	4.9 (4.6-5.3)	5.4 (5.1-5.7)	0.001
Previous LVFWd Max (mm)	4.4 (4.1-4.7)	4.6 (4.2-4.9)	0.017
Previous LVWT Max (mm)	5.0 (4.6-5.3)	5.4 (5.1-5.7)	<0.001
Previous LVIDd (mm)	13.3 (±1.8)	13.6 (±1.5)	0.339
Previous LVIDs (mm)	6.5 (±1.5)	6.2 (±1.6)	0.364
Previous LV FS%	51.2 (45.6-57.3)	53.2 (46.3-64.0)	0.058
Previous AMVL (mm)	8.0 (±0.7) (n=106)	8.4 (±0.8) (n=25)	0.022
Previous △ Body weight (kg)	0 (±0.3)	-0.1 (±0.4)	0.649
Previous △ BCS (-1/ 0/ +1/ +2)	15/ 32/ 4/ 0	6/ 8/ 1/ 1	0.895
Previous △ LAD Max (mm)	+0.1 (-1.0-+1.2) (n=51)	+0.2 (-0.7-+0.5) (n=16)	0.689
Previous △ LA/Ao	+0.1(0-+0.2) (n=51)	+0.1 (0-+0.2) (n=16)	0.836
Previous △ LA FS%	-0.2 (-3.8-+7.7) (n=51)	+0.7 (-8.0-+4.2) (n=16)	0.365
Previous △ IVSd Max (mm)	+0.3 (0-+0.6) (n=51)	+0.4 (+0.3-+0.9) (n=16)	0.028
Previous △ LVFWd Max (mm)	+0.1 (-0.2-+0.4) (n=51)	+0.1 (-0.3-+0.4) (n=16)	0.747
Previous △ LVWT Max (mm)	+0.3 (0-+0.5) (n=51)	+0.4 (+0.3-+1.0) (n=16)	0.017
Previous △ LVIDd (mm)	+0.5 (±2.2) (n=51)	-0.3 (±1.2) (n=16)	0.120
Previous △ LVIDs (mm)	0 (±1.8) (n=51)	-0.6 (±1.6) (n=16)	0.224
Previous △ LV FS%	2.6 (±10.1) (n=51)	4.1 (±10.8) (n=16)	0.611
Previous △ AMVL (mm)	0.1 (±.4) (n=31)	0.3 (±0.8) (n=12)	0.555

Normally distributed data are presented in mean ± standard deviation). Non-normally distributed data are presented in median (interquartile range). For variables with missing data, a total number of each variable was listed.

Abbreviations: AMVL, anterior mitral valve leaflet length; HCM, hypertrophic cardiomyopathy; IVSd Max, maximal interventricular wall thickness in end-diastole; LA/Ao, left atrial to aortic ratio; LAD Max, maximal left atrial diameter; LA FS%, left atrial fractional shortening; LV FS%, left ventricular fractional shortening; LVFWd Max, maximal left ventricular free wall thickness in end-diastole; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVWT Max, maximal left ventricular wall thickness in end-diastole

In the first GEE analysis (*P* <0.001), all but IVSd Max were excluded (Table 4.5). The receiver operating characteristic analysis suggested an optimal cutoff of ≥5.3 mm for IVSd Max to predict HCM in the subsequent year (area under the curve: 0.743 [95% CI 0.637-0.850], *P* < 0.001) with sensitivity of 71.4% and specificity of 74.3%. The odds ratio of cats with the IVSd Max ≥5.3 mm developing HCM within 1 year was 5.8 (95% CI: 2.5-13.9).

Table 4.5. Final models for predicting the development of HCM in the subsequent year using the generalized estimating equations in a study conducted to describe yearly cardiac phenotype in 121 cats. After manually removing all non-significant variables in a backward stepwise manner, a single variable remained in each model, resulting in the same results to that of the univariable analysis.

Model 1	Odds ratio (95% CI)	*P* value
IVSd Max	7.0 (2.1-22.8)	0.001
Model 2	Odds ratio (95% CI)	*P* value
AMVL	2.1 (1.1-3.9)	0.022

Abbreviations: AMVL, anterior mitral valve leaflet; CI, confidence interval; IVSd Max, maximal interventricular septal thickness in end-diastole

In the second GEE analysis, all but AMVL were excluded (*P* <0.001; Table 4.5). The receiver operating characteristic analysis suggested an optimal cutoff of ≥8.5 mm for AMVL to predict HCM in the subsequent year (area under the curve: 0.623 [95% CI:0.506-0.758], *P* = 0.040) with sensitivity of 52% and specificity of 24.5%. The odds ratio of cats with the AMVL length ≥8.4 mm developing HCM within 1 year was 2.3 (95% CI: 0.9-5.6).

4.3e Clinical outcome in cats with cardiomyopathy

In addition to the 27 described deaths, 8 additional deaths occurred within 12 months of the last echocardiogram. Therefore, a total of 35 deaths occurred during the study period. Two deaths were spontaneous, and the rest were assisted. Two cats with spontaneous deaths had no preceding clinical signs within 12 hours of death, and postmortem exam revealed macroscopic and microscopic evidence of HCM without any other meaningful abnormality on complete postmortem exam. Thus, these 2 deaths were ultimately recorded as SCD. Additionally, congestive heart failure (CHF) was recorded as the cause of 3 assisted deaths. One of these cats had macroscopic and microscopic features of HCM, another had macroscopic

and microscopic features of ARVC, and the last remaining cat had severe dilation of the right atrium and right ventricle but with microscopic features of chronic lymphocytic myocarditis. In these 5 cats with cardiac death, the most recent cardiac examination showed equivocal HCM in 2 that suffered SCD and 1 that died of CHF due to ARVC, HCM in 1 that died of CHF due to HCM, and normal echocardiogram in 1 that died of CHF due to chronic myocarditis. The incidence rate of SCD from the entire colony was 1 per 100 cat-year. Clinical variables associated with cardiac deaths were not statistically assessed due to only a small number of cats suffering cardiac death during the study period.

Of the remaining 30 assisted deaths, 9 cats were euthanised due to neoplasia, 7 due to multiple causes (any combination of advanced chronic kidney disease, neoplasia, and hepatopathy), 6 due to chronic kidney disease, 5 due to feline infectious peritonitis, and 1 each due to penetrating eye injury, bronchopneumonia, and feline gastrointestinal eosinophilic sclerosing fibroplasia. Of these 30 cases, 2 hearts were not stored for an independent review by another pathologist (Hayley Hunt). Excluding these 2 cases, the postmortem cardiac exam of 28 hearts revealed HCM in 8 cats, non-specific cardiomyopathies in 7, and normal hearts in 12, with one heart revealing suspected myocardial lymphoma. The 7 non-specific cardiomyopathies included 4 hearts with LVH but without microscopic features of HCM, and 3 cats with normal macroscopic exam findings but with microscopic features of HCM/RCM (2 cats) or ARVC (1 cat). The complete list of deceased cats, their echocardiographic and postmortem diagnoses, age at death, and the time interval from echocardiography to postmortem exam is presented in Table 4.6.

Table 4.6. A list of deceased cats in a study conducted to describe yearly cardiac phenotype in 121 cats. The antemortem and postmortem cardiac diagnoses, age at death, time interval from last antemortem cardiac exam to postmortem exam, and the primary suspected cause of death by pathologists on complete postmortem exam are listed. Cats that were excluded from yearly echocardiographic monitoring still underwent complete postmortem exam, hence some cats had >12 months of time interval between the antemortem and postmortem cardiac exams. Cats that suffered cardiac deaths are highlighted in red.

#	Last antemortem cardiac diagnosis	Postmortem cardiac diagnosis	Age at death (years)	Duration since the last echocardiogram (days/ months)	Suspected primary cause of death on complete postmortem exam
1	HCM	HCM	15.9	300 days / 10 months	Renal (chronic kidney disease + renal secondary hyperparathyroidism)
2	HCM	HCM	12.6	140 days / 4.7 months	Renal (chronic kidney disease)
3	HCM	NSC (normal macroscopic exam, ARVC changes on microscopic exam)	12.3	55 days / 1.8 months	FIP
4	HCM	NSC (LVH without microscopic features of HCM)	12.9	612 days / 20.4 months	Neoplasia (Intestinal lymphoma)
5	HCM	HCM	2.8	357 days / 11.9 months	Cardiac (CHF due to HCM)
6	HCMp (SHT)	HCM	12.7	171 days / 5.7 months	Neoplasia (renal carcinoma with metastasis)
7	HCMp (SHT)	HCM	11.5	144 days / 4.8 months	Neoplasia (mammary carcinoma)
8	HCMp (SHT)	NSC (LVH without microscopic features of HCM)	14.8	44 days / 1.5 months	Neoplasia (pancreatic adenocarcinoma and urothelial carcinoma)
9	HCMp (hyperthyroidism)	HCM	16.5	125 days / 4.2 months	Multiple (pyelonephritis, chronic kidney disease, HCM phenotype with hyperthyroidism)
10	Equivocal HCM	HCM	4.6	169 days / 5.6 months	Eosinophilic sclerosing fibroplasia
11	Equivocal HCM	Infiltrative disease (suspect lymphoma)	12.4	448 days / 14.9 months	Neoplasia (suspect lymphoma in the kidneys, intestines and the heart)
12	Equivocal HCM	HCM	5.2	441 days / 14.7 months	FIP
13	Equivocal HCM	NSC (normal macroscopic exam but microscopic features of HCM/RCM)	7.7	169 days / 5.6 months	FIP
14	Equivocal HCM	NSC (LVH without microscopic features of HCM)	14.9	208 days / 6.9 months	Neoplasia (mammary carcinoma)
15	Equivocal HCM	Normal	13.9	149 days / 5.0 months	Multiple (Intestinal adenocarcinoma, chronic kidney disease, focal hepatic necrosis, pancreatitis)
16	Equivocal HCM	Normal	15.5	195 days / 6.5 months	Renal (chronic kidney disease)
17	Equivocal HCM	Normal	13.8	172 days / 5.7 months	Multiple (inflammatory bowel disease, chronic kidney disease, chronic cholangitis)
18	Equivocal HCM	Normal	12.4	20 days / 0.7 months	Respiratory (bronchopneumonia)
19	Equivocal HCM	NSC (LVH without microscopic features of HCM)	3.6	192 days / 6.4 months	FIP
20	Equivocal HCM	Normal	9.7	182 days / 6.1 months	Multiple (inflammatory bowel disease and chronic kidney disease)
21	Equivocal HCM	Normal	11.7	175 days / 5.8 months	Neoplasia (intestinal lymphoma)
22	Equivocal HCM	NSC (Normal cardiac weight (15g) but severe RV thickening with microscopic features of ARVC)	5.5	172 days / 5.7 months	Cardiac (sudden cardiac death due to cardiomyopathy)
23	Equivocal HCM	NSC (Normal cardiac weight (18g) but severe right heart dilation and thinning with microscopic features of ARVC)	3.8	168 days / 5.6 months	Cardiac (congestive heart failure due to cardiomyopathy)
24	Equivocal HCM	HCM	6.4	159 days / 5.3 months	Cardiac (sudden cardiac death due to HCM)
25	Equivocal HCMp (hyperthyroidism)	HCM	15.7	297 days / 9.9 months	Multiple (hepatocellular carcinoma with pulmonary metastasis, chronic kidney disease, and hyperthyroidism)
26	Equivocal HCMp (hyperthyroidism)	Normal	17.1	58 days / 1.9 months	Multiple (intestinal lymphoma and chronic kidney disease)
27	Equivocal HCMp (hyperthyroidism)	Normal	13.4	13 days / 0.4 months	Renal (chronic kidney disease)
28	Normal	Normal	11.9	300 days / 10.0 months	FIP
29	Normal	Normal	12.4	41 days / 1.4 months	Multiple (intestinal lymphoma and lymphoplasmacytic cholangiohepatitis)
30	Normal	Normal	10.3	755 days / 25.2 months	Neoplasia (renal carcinoma)
31	Normal	Normal	1.1	28 days / 0.9 months	Neoplasia (suspect lymphoma)
32	Normal	Myocarditis (chronic, lymphocytic, multifocal)	2.6	189 days / 6.3 months	Cardiac (CHF due to myocarditis)
33	RCM	NSC (normal macroscopic exam but microscopic features of HCM/RCM)	10.4	417 days / 13.9 months	Renal (chronic kidney disease)
34	HCM	N/A	4.7	243 days / 8.1 months	Ocular (full autopsy not performed but euthanised due to penetrating eye injury)
35	Equivocal HCM	N/A	15.5	209 days / 7.0 months	Renal (chronic kidney disease and renal secondary hyperparathyroidism)

Abbreviations: ARVC, arrhythmogenic right ventricular cardiomyopathy; CHF, congestive heart failure; FIP, feline infectious peritonitis; HCM, hypertrophic cardiomyopathy; HCMp, hypertrophic cardiomyopathy phenotype; NSC, non-specific cardiomyopathy; SHT, systemic hypertension

4.4 Discussion

This prospective observational study reports the rate of yearly changes in left heart dimensions in cats with or without HCM. Furthermore, this study highlights important features of HCM including 1) AMVL elongation precedes overt LVH in cats with HCM, and 2) baseline measurements of IVSd Max and, to a lesser extent, AMVL length can be used to identify cats at risk of echocardiographic diagnosis of HCM in the subsequent year.

Hypertrophic cardiomyopathy is an acquired disease that increases in prevalence with age.[2,6] Elongation of AMVL is a common feature of HCM in cats and can precede LVH in cats with HCM.[7,28] However, since the onset of AMVL elongation was unknown, cats were often diagnosed with congenital elongation of AMVL as a component of mitral dysplasia, rather than as an early manifestation of HCM. In an experimental study of mice with knocked out *MYBPC3* gene, which results in LVH and histologic features similar to spontaneous feline HCM, certain features of HCM including myofibre disarray, increased number of LV crypts, and increased LV trabecular complexity appeared during embryogenesis.[29,30] However, LVH and AMVL elongation were not present pre-birth and the authors hypothesised that these features are acquired processes that occur post-birth.[30,31] In a recent prospective study of 107 non-purebred cats from rehoming centres with a median follow up period of 5.6 years (CATSCAN II), cats with normal echocardiograms had a progressive increase in LVWT over time. The present study showed similar increase in LVWT to CATSCAN II, as well as an increase in AMVL length per year. Overall, these results support the hypothesis that AMVL elongation and LVH are acquired processes in cats with HCM and that HCM is a more appropriate disease name than mitral dysplasia in these cats.

Notably, a previous study by the same investigator (Joonbum Seo) did not report a gradual increase in AMVL length over time in 55 apparently healthy cats.[7] However, that study was retrospective in design, and measurements of AMVL used echocardiograms acquired by several cardiologists and supervised residents.[7,18] It is likely that a greater variation in measurements explained the lack of difference in AMVL length over time. As shown in the present study, serial echocardiography for AMVL measurements were highly reliable when performed by a single observer. Even a fraction of a millimetre in variation can impact the analysis due to the small heart size in cats. The cause of AMVL elongation in HCM is unknown in cats. Current

hypotheses include primary phenotypic expression of an underlying HCM causing gene variant and the secondary effects to chronic increase in LV pressure.[30,32-34] Further studies are required to evaluate the pathogenesis of AMVL elongation in cats.

In previous studies, upper limit of normal LVWT, elongated AMVL, and reduced LA FS% were predictors of HCM in 5 to 6 years.[6,7] In the present study, upper limit of normal LVWT (specifically IVSd) and elongated AMVL were predictors of HCM in 1 year, but not LA FS%. A likely reason for the lack of LA FS% as a predictor of HCM in the present study is the difference in study design. For example, the present study assessed variables that could predict HCM in 1 year, which is a common recommended time for a recheck by veterinarians in cats at risk of HCM or for breeding purposes. In comparison, the previous study that described the use of LA FS% for predicting HCM had a median follow up period of 5.6 years.[6] The usefulness of LA FS% with shorter follow up period was not reported but the data suggested that it is less useful for a short follow up period such as 1 year.[6] Furthermore, LA FS% was measured using an M-Mode in the previous study, which is different to the present study. Measurements of LA FS% using different types of imaging are not interchangeable and may have influenced the results.[35] Overall, the results of the present study suggest that measuring IVSd and AMVL may be more appropriate than LA FS% when assessing the risk of HCM within 1 year of the exam.

The previous retrospective study of 55 cats suggested that AMVL >8.7 mm (odds ratio 11.5, 95% CI: 2.3-57.7) can be used to identify cats at risk of developing HCM within median 5.4 years. In the present prospective study, AMVL ≥8.4 mm was an optimal cutoff for predicting HCM development, and the odds ratio of developing HCM within 1 year at this cutoff was 2.3 (95% CI: 0.9-5.6). The previous study was retrospective in design, involved multiple echocardiographers, and had a longer follow up period, which likely explains the difference in results. The concept that AMVL elongation occurs prior to LVH in cats with HCM is important but due to the poor specificity of 24.5%, echocardiographic AMVL measurements should not be used as a sole criterion for an earlier diagnosis of HCM.

The present study identified that cats with IVSd Max ≥5.3 mm were 5.8 times more likely to develop HCM in the subsequent year (95% CI: 2.5-13.9, $P < 0.001$). Similarly, two previous studies reported that the upper limit of normal LVWT were risk factors of HCM diagnosis within 5-6 years.[6,7] These results suggest that cats with upper limit of normal LVWT are closer to reaching the diagnostic threshold of HCM

than those with lower limit of normal LVWT and should have yearly follow-up echocardiography to review the cardiac phenotype.

It is possible that the LVWT may decrease in some cats with HCM due to myocardial infarction and intramural fibrosis.[16,17,36] None of the cats in the present study developed obvious myocardial thinning or segmental hypokinesia, which may be a feature of myocardial infarction and intramural fibrosis.[36,37] However, myocardial thinning appears to be a late manifestation of HCM and may have been missed by the short follow-up period.[16,17] In contrast, some cats with previously diagnosed HCM may have complete resolution of LV thickening due to a syndrome termed 'transient myocardial thickening'.[38,39] Previous publications defined transient myocardial thickening as reduction in LVWT below 5.5 mm and resolution of other cardiac abnormalities such as left atrial enlargement, typically over weeks to months after the initial diagnosis.[38,39] In the present study, 2 cats with HCM had reduced LVWT in the subsequent year but LVWT remained >5.5 mm and did not meet the current criteria of transient myocardial thickening in cats.[38,39]

The secondary aim of this study was to evaluate the incidence of cardiac death in cats due to HCM in this closely monitored convenience sample of the general feline population. In this study, the incidence rate of SCD from the entire colony population was 1 in 100-cat year. Interestingly, both cats that suffered SCD were diagnosed with equivocal HCM prior to death. However, the cardiac postmortem exam was consistent with HCM in 1 cat and non-specific cardiomyopathy in another as this cat had normal cardiac weight but thickened RV with microscopic features of ARVC. These deaths occurred 5-6 months after the echocardiogram, and it is possible that their cardiac phenotype naturally changed since the echocardiogram was performed. The agreement between a cardiologist and pathologist on the diagnosis and classification of feline cardiomyopathies was not assessed as cats were allowed to live a normal life and there was a considerable time between the time of echocardiography and postmortem examination. Nevertheless, many cats with echocardiographic or postmortem diagnosis of cardiomyopathies never displayed symptoms of cardiac disease and died of non-cardiac reasons. This observation that cats with cardiomyopathies can potentially live a normal life was also described in previous studies.[5,11]

There are limitations of this study. First this study used a strong assumption that the colony population at the CFN is a representative sample of the wider general feline population. This assumption is supported by published baseline characteristics

of the colony population; however, the diversity has not been genetically tested.[4] Second, a general ultrasound machine was used, which limited the measurements to simple left heart dimensions. Third, the yearly changes in left heart dimensions could not be assessed in multivariable analysis due to a small sample size. However, there was no strong association of these variables on univariable analysis. Fourth, clinical variables associated with cardiac deaths were not statistically evaluated due to only a small number of cats suffering cardiac death. Fifth, blood tests were only performed in unwell cats and systemic blood pressure was only measured in cats that have already developed an HCM phenotype and were >6 years old. Each cat was closely monitored following the colony protocol, but it is still possible that early systemic disease and underlying systolic hypertension may not have been identified if cats were hiding symptoms. Last, the reliability of measurements was tested by a single observer. Previous studies showed that both AMVL and LVWT measurements are repeatable across multiple observers. However, the effect of image acquisition on inter-observer repeatability is unknown. The variables with good reliability (ICC 0.75-0.9) still had wide 95% CI for ICC. More specifically, there was a wide variation in the LA/Ao, LVIDs, and LV FS%. The greater variation in LA/Ao was also demonstrated in dogs[40] and may be due to the multiplication of variations from two measurements. In comparison, LVIDs and LV FS% are indices of systolic function, which may change with varying degrees of stimulation. Given the mercurial temperament of cats, it is possible that the different moods at each scan may have contributed to the variations in indices of systolic function. Despite these notable limitations, the present study highlights important features of feline HCM and provides valuable background information for clinical management and future clinical research of feline HCM.

4.5 Conclusion

Left ventricular wall thickness and AMVL length increase in cats with age. Both LVH and AMVL elongation are acquired processes in cats with HCM. Elongation of the AMVL can precede LVH in cats with HCM. LVWT at the upper limit of normal and elongated AMVL were predictors of HCM in 1 year.

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Chapter 5. Influence of stress on the diagnosis of HCM

5.1 Introduction

Hypertrophic cardiomyopathy (HCM) is a common cardiac disease in domestic cats that affects approximately 15% of the general population.[1-3] Hypertrophic cardiomyopathy can remain asymptomatic in cats; however, some affected cats develop congestive heart failure, arterial thromboembolism, or sudden cardiac death. Progression of heart disease and death occurs in 6 per 100 cats with HCM every 12 months.[4] Given its high prevalence, HCM is a serious medical concern in cats.

The cause of HCM is unknown in most cats. Studies have identified several genetic variants associated with HCM.[5-9] However, cats with these genetic variants can show a wide range of phenotypic expression (pleiotropy). Furthermore, HCM has been observed in cats without a recognised genetic variant.[8,10,11] There are several hypotheses for the wide pattern of disease expression in cats: Firstly, there likely are many unrecognised genetic variants associated with HCM in cats, and these genetic variants may collectively manifest as HCM in affected cats (polygenetic inheritance).[12] Secondly, environmental or epigenetic factors can modulate the gene expression, and possibly increase or decrease the risk of an HCM phenotype in an individual.[13] Lastly, there may be exogenous or environmental factors that result in left ventricular hypertrophy that imitates an HCM phenotype, without the presence of an associated genetic variant. This phenomenon, termed a ‘phenocopy’, may explain unknown causes of HCM in some cats.[14] To authors’ knowledge, possible environmental or epigenetic influences in HCM pathogenesis has not been investigated in cats.

Stress is a normal biological response to physiological and psychological changes from noxious or unpleasant stimuli. In cats, an intense or chronic stress can cause vomiting, diarrhoea, and anorexia, and has been reported to increase the risk of diseases including upper respiratory infection and feline interstitial cystitis.[15-17] Specific to cardiac health, life-threatening respiratory signs have been reported in previously healthy cats after presumed stressful events as the result of transient myocardial thickening and dysfunction.[18,19] However, stress in these studies was subjectively defined and not measured using laboratory tests or a semi-quantitative

scoring system.[18,19] Therefore, the effect of measurable stress on myocardial thickness and the diagnosis of subclinical HCM warrants further exploration.

Stress levels in cats can be measured using a visual scoring system or a biochemical assay to measure components of the hypothalamic-pituitary-adrenal axis or sympathoadrenomedullary system.[15-17,20-27] Of the available visual scoring systems, Cat-Stress-Score (CSS) has been most studied and is currently most widely accepted.[25-27] This scoring system assesses cat behaviour and is highly repeatable among trained observers.[25] However, CSS does not provide information on the chronic level of stress in cats, and the score may be affected by age, sex, and neuter status.[27,28] In comparison, endogenous cortisol concentrations can be measured from blood, urine, faeces, and hair in cats.[20,22,23,27,29] Of these sample types, hair is easy to collect and provides an average measurement of cortisol concentration during the period of hair growth, thereby giving an estimate of cortisol within the last 2 months.[20,29]

The aim of this study was to determine if either acute (as observed by CSS) or chronic (measured by hair cortisol concentrations) stress were associated with subclinical HCM in cats. To the authors knowledge, this is the first time that stress has been investigated as a factor in the development of HCM in cats.

5.2 Materials and Methods

5.2a Study design

This was a prospective case-control study. Ethical approval was provided by Massey University Animal Ethics Committee (MUAEC protocol 21/47). The population source were cats in a colony used for nutritional studies (Centre for Feline Nutrition, Massey University), which serves as a convenience sample of non-purebred cat population in New Zealand.[3] The inclusion criteria for the affected group were cats with subclinical HCM as detected by complete echocardiogram performed by a board-certified cardiologist (Joonbum Seo). The inclusion criteria for the control group were apparently healthy cats with normal echocardiogram and physical examination that matched in age, sex and neuter status with the HCM group. Control cats were excluded if they had a history of chronic disease other than HCM or were receiving medications at the time of data collection.

5.2b Matching

All apparently healthy, non-pregnant, non-neonatal cats first underwent screening echocardiography by a board-certified cardiologist (Joonbum Seo) to define their cardiac phenotypes 1 year prior to the current study.[3] Using this published data, the control group was created by adding healthy cats with normal echocardiogram, one at a time, while matching sex, neuter status and age to a cat in the previously selected HCM group. Age was rounded to one decimal point for the matching process. Cats in the HCM group were excluded if there was no control cat that matched in age, sex, and neuter status within the colony.

To ensure that the cardiac phenotypes have not changed over the 12-month period, all eligible cats in both HCM and control groups underwent repeat echocardiography by the same cardiologist (Joonbum Seo) at the time of data collection. To ensure that cats in the control group were healthy, the medical records of the colony were further reviewed, and their weekly appetite, body weight, and behaviour were observed for 6 months following repeat echocardiography.

5.2c Echocardiography

Right parasternal two-dimensional echocardiography was performed using a general ultrasound machine (Toshiba Xario 200, Tochigi, Japan) equipped with a sector array probe (6S3, frequency 4.4-6.2 mHz). At minimum, right parasternal long-axis four chamber (RPLax4C), right parasternal long-axis five chamber (RPLax5C), and two right parasternal short axis (RPSax) views at the levels of papillary muscles and aortic valve were acquired. An HCM phenotype was confirmed when the maximal left ventricular wall thickness in end-diastole (LVWT Max) from any of the right parasternal views were ≥6 mm.[9,30,31]

Cats with an HCM phenotype that were ≥6 years old underwent Doppler blood pressure measurements following the current guideline.[32] The final diagnosis of HCM was made if cats with an HCM phenotype had no sign of systemic hypertension on Doppler blood pressure measurements and no evidence of hyperthyroidism, acromegaly, or neoplasia, as excluded by observing the cats' weekly appetite, body weight, and behaviour for 6 months following the data collection. Any cats with

subclinical HCM that developed any signs of intercurrent hypertension or other disease in the following 6 months of data collection were excluded from the study.

5.2d Cat-Stress-Scores

The CSS was measured as described by Kessler and Turner.[25] In this scoring system, behavioural and postural changes of the cat (11 systems comprised of the body, belly, legs, tail, head, eyes, pupils, ears, whiskers, vocalisation, activity) were assessed using a 7-scale system. A high score (7) suggested extreme stress, and a low score (1) suggested fully relaxed state. Scores from each system were then averaged to produce the final score for each cat. Sleeping cats were excluded unless they woke up during data collection for observers to record CSS.[33]

Prior to collecting CSS from two groups, an inter-observer variability of assessing CSS was first tested by two observers (Joonbum Seo and Megan Williams) by assessing 120 cats in the colony twice in a single day. The coefficient of variability between M.W. and J.S was 95%. Once this acceptable interobserver repeatability was established, an observer (Megan Williams) independently measured CSS at four-time points. These time points were morning and afternoon over two separate days. The observer was unaware of the echocardiographic findings of each cat. Once completed, an average CSS from each day and an average CSS of all four time points were included in the statistical analysis.

5.2e Hair cortisol assay

An approximately 5 cm by 5 cm area was clipped from the lumbar region from each cat on the first day of CSS measurements. The hair clipping was acquired after CSS measurements to avoid inadvertently influencing the CSS. Each clip was made carefully to include the entire length of hair, and the clippers were cleaned in between cats.

The method of preparing the hair sample for hair cortisol assay was adapted from previous publications.[20,21,29] The entire length of clipped hair was first cut into short fragments of <2 mm, and 20 mg of trimmed hair was mixed with 2 mL of methanol and incubated at 80 °C for 24 hours. Following incubation, the entire

aliquot was transferred to a new tube using a filtered pipette. The methanol was left to evaporate for 48 hours in a laboratory fume hood. Once the tube was completely dry, 1 ml of phosphate-buffered saline was added to create the test sample. The test sample was then vortexed for >1 minute to ensure complete mixing of the sample.

Cortisol was measured using a human salivary cortisol enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions (DRG International Inc, Springfield, New Jersey, United States of America). This assay was chosen as it has been previously used to measure hair cortisol in cats.[21,34,35] On completion of the ELISA protocol, the mean absorbance at 450nm was measured from each test sample, standard samples, and controls using a microplate reader (BioTek Epoch 2 Microplate Spectrophotometer, Aligen, Santa Clara, USA). Finally, a 4-parameter standard curve was produced by plotting the mean absorbance obtained from each standard against their concentration using an online software (MyCurveFit, mycurvefit.com). The curve fit was validated using the high- and the low-cortisol control samples provided in the kit. The concentration of each test sample was recorded using the standard curve.

5.2f Statistical analysis

SPSS Statistics (Version 26, IBM) was used for statistical analysis. GraphPad Prism 8 (Version 8.4.2) was used for producing graphs. Normality of data was tested by visual assessment and Shapiro-Wilk test. Normally distributed data was presented as mean (95% confidence interval [CI]) and non-normally distributed data was presented as median (range). Categorical variables were presented in value (percentage).

Continuous variables were compared using an independent t-test or a Mann-Whitney test depending on the distribution. Categorical variables were compared using Pearson Chi-Square or Fisher's exact test when the cell count was <5. Significance was set at <0.05.

5.3 Results

Of 132 cats in the colony that were evaluated using echocardiology, 20 cats were diagnosed with HCM. None of these cats showed clinical evidence of heart failure or any other symptoms attributable to HCM. Ten of these cats were excluded due to the lack of a control cat that matched in age, sex, or neuter-status. The final sample size was 20 cats, 10 cats with subclinical HCM, and 10 healthy controls that were matched in age, sex, and neuter status. Each group had a mean age of 6.3 (±0.9) years and was comprised of 1 entire male, 5 neutered males, 2 entire female, and 2 spayed females. There was no difference in the body weight (HCM group: mean 4.1 kg [95% CI: 3.4-4.7] versus control group: mean 4.2 kg [95% CI: 3.6-4.9], *P* = 0.674) and body condition score (HCM group: median 5.0 [range: 4.0-6.0] versus control group: median 5.5 [range: 3.0-6.0], *P* = 0.375) between 2 groups.

The summary of the results is presented in Table 5.1, Figure 5.1 and Figure 5.2. There was no difference in average CSS between the HCM (median: 1.9, range: 1.5-3.1) and control groups (median: 2.2, range: 1.9-3.4, *P* = 0.280). Similarly, there was no difference in hair cortisol concentration between the HCM (median: 0.19, range: 0.09-0.29) and control groups (median: 0.25, range: 0.15-0.35, *P* = 0.267).

Table 5.1. Comparison of continuous variable[a] between 10 cats with hypertrophic cardiomyopathy (HCM) and 10 healthy controls matched in age, sex, and neuter status within the same research colony, in a pilot study to describe the association between measures of psychologic stress and subclinical HCM diagnosis in cats. Physiological stress was assessed by measuring hair cortisol concentration and visually assessing the Cat-Stress-Score (CSS).

	Normal (n=10)	HCM (n=10)	*P* value
Body weight (kg)	4.1 (95% CI: 3.5-4.7)	4.2 (95% CI: 3.6-4.9)	0.694
LVWT (mm)	4.9 (range: 4.6-5.1)	6.4 (range: 6.0-6.7)	<0.001
AMVL (mm)	8.3 (range: 7.8-8.9)	8.8 (range: 8.4-9.3)	0.124
CSS Day 1	2.4 (range: 2.1-3.5)	2.3 (range: 1.5-4.1)	0.529
CSS Day 2	2.0 (range: 1.3-3.2)	1.8 (range: 1.0-2.3)	0.165
CSS average of 2 days	2.2 (range: 1.9-3.4)	1.9 (range: 1.5-3.1)	0.280
Hair cortisol (ng/mL)	0.25 (range: 0.15-0.35)	0.19 (range: 0.09-0.29)	0.267

[a]Normally distributed data are reported as mean (95% confidence interval). Non-normally distributed data are reported as median (range).

Abbreviations: AMVL, anterior mitral valve leaflet length; LVWT, left ventricular wall thickness; CSS, Cat-Stress-Score

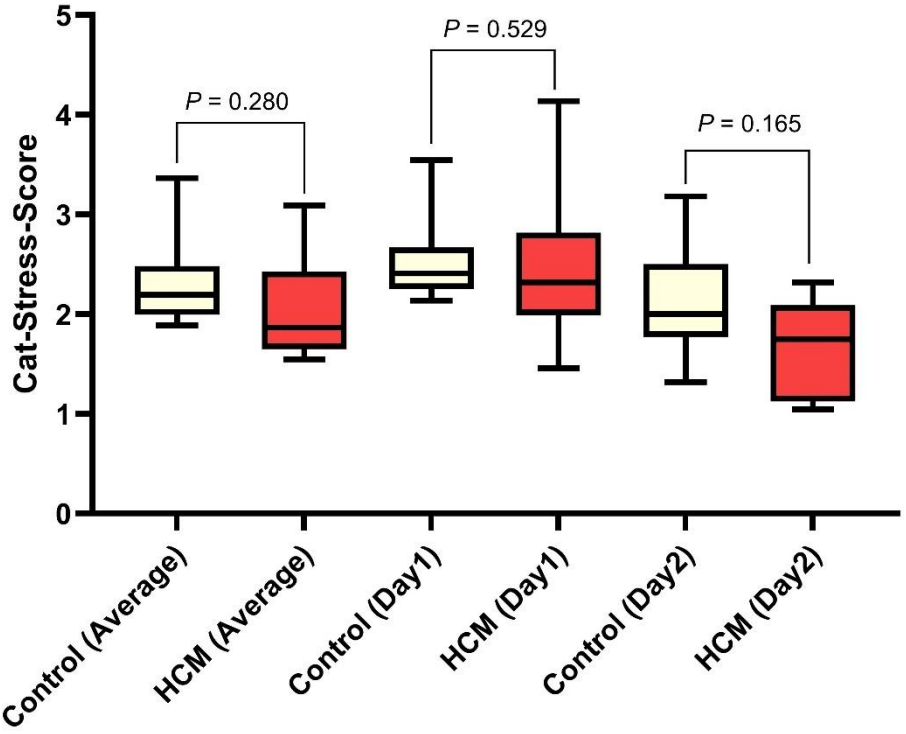

Figure 5.1. Comparison of Cat-Stress-Score measurements in 10 cats with HCM (red) and 10 healthy controls matched in age, sex, and neuter status (yellow) within the same research colony, in a pilot study to describe the association between measures of psychologic stress and subclinical HCM diagnosis in cats. *A high score suggests a higher level of stress. Cat-Stress-Score was recorded in the morning and afternoon on two separate days (a total of four time points). An average of all four time points, and average of morning and afternoon measurements in each day are presented. There was no statistical difference in Cat-Stress-Score between the two groups.*

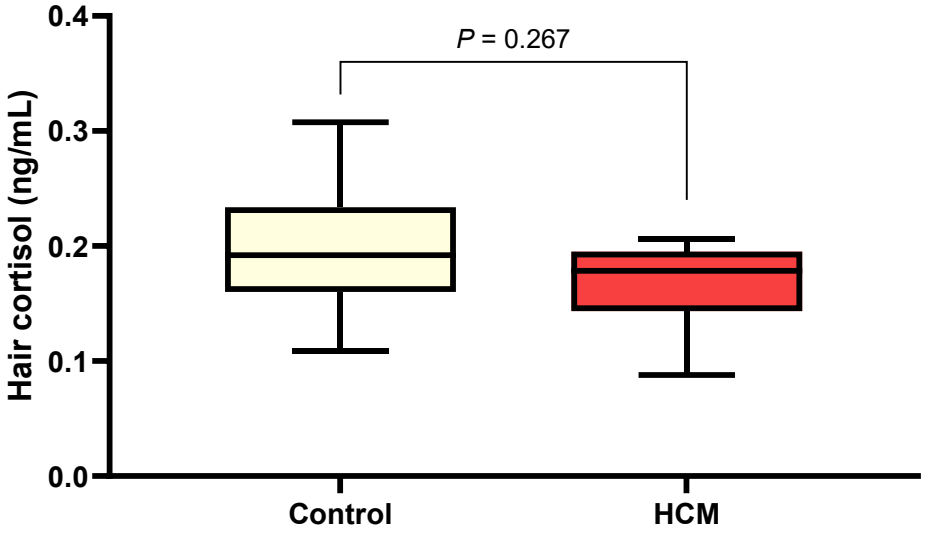

Figure 5.2. Hair cortisol concentrations in 10 cats with HCM (red) and 10 healthy controls matched in age, sex, and neuter status (yellow) within the same research colony, in a pilot study to describe the association between measures of psychologic stress and subclinical HCM diagnosis in cats. *There was no statistical difference in hair cortisol concentrations between the two groups.*

5.4 Discussion

Neither CSS scoring nor hair cortisol concentrations were significantly associated with subclinical HCM in this prospective case-control study. There are several possible explanations for this lack of association. Firstly, stress may not play a role in HCM pathogenesis in cats. To authors' knowledge, there is no literature that describes stress as a possible epigenetic or environmental factor for the development of HCM in cats. However, it is possible that cats need to be chronically stressed to develop negative cardiac effects of stress and subsequent HCM. A recent experimental study in mice showed that chronic intense stress for 28 days causes ventricular dilation and arrhythmias, and alters methylation at specific genes associated with dilated cardiomyopathy and adrenergic signalling of cardiomyocytes.[36] Cats in the present study had a similar distribution of CSS and hair cortisol concentrations to previous reports of shelter cats.[25,27,35] However, CSS and hair cortisol in pet cats in the general population may be more widely variable.[21,34] Experimentally inducing chronic intense stress to the cats in this study was not possible due to the welfare of the cats.

Secondly, difficulties in objectively defining and measuring stress could explain the lack of association between subclinical HCM and stress in this study. The methods of measuring stress were carefully selected based on the current literature. However, each method has limitations. For example, CSS is repeatable among observers but only allows brief assessment of stress, and cats can vary in CSS within a short period.[25,27] In contrast, hair cortisol measurements provide an average level of stress over several months but lack information on the intensity and frequency of stressful events.[21,27] Furthermore, as HCM is thought to take years to develop in cats, even a measurement of stress over the 2-month period of hair growth may not be long enough to detect chronic stress as a cause of HCM.[37,38] To accurately assess the effect of stress on pathogenesis of HCM, years of data on measurable stress are likely needed. Difficulties in defining and measuring stress may also explain the lack of literature on possible effect of stress on the development of HCM in both humans and cats.

Thirdly, some confounders including the genotype and familial relationship of cats were not considered and may have influenced the lack of association. Visual scoring systems such as CSS can be influenced by genotypes, as seen in several studies where kittens sired by friendly cats are friendlier toward humans compared to kittens from unfriendly sires.[24,39,40] Furthermore, there are genetic variants that can induce HCM in cats.[7,10] A variant in the myosin heavy chain 7 gene was described as the potential cause of HCM in one Domestic Shorthair.[41] However, this genetic variant was not identified in other studies, and causal genetic variants of HCM in non-purebred cats remain poorly described.[8,42] Despite this, the lack of genotypic and familial information is a limitation of this study.

Lastly, the small sample size may have been insufficient to identify significant differences. The small sample size was the result of strictly matching the two groups in their husbandry, age, sex, and neuter status. Furthermore, all cats share a similar environment as they are fed the same diet at the same schedule and are provided with the same toys and have similar interaction from the staff due to the nature of colony population. However, a type II statistical error is a possible explanation for the lack of association. Due to the lack of studies of hair cortisol and CSS in cats with HCM, a priori sample size calculation was not possible.

The same cortisol assay was previously used to measure hair cortisol in cats.[21,34,35] These publications originated from a single research centre; however, the results were consistent with the expected level of stress, and the distribution of

cortisol data in one of these studies was similar to that of the present study.[35] Nevertheless, this cortisol assay was not validated against other measures of stress, and the performance and variation in results compared to other assays are unknown. In the present study, the hair cortisol concentration was not compared with CSS as they were considered to measure stress of different chronicity. However, all hair samples were analysed as a batch to account for the possible day-to-day variation.

It is important to note that the effect of stress on the progression of subclinical HCM into clinical stage was not investigated as this was not the aim of this study. It is reported that stress can trigger symptoms in pre-existing HCM in both cats and humans.[43,44] The current veterinary practice is to minimise stress in cats with HCM, and the results of the present study should not be used to counter this recommendation. Furthermore, the hypothesis that intense or chronic stress can cause transient myocardial thickening in cats was not tested. Transient myocardial thickening mimics both subclinical and clinical HCM but the myocardial thickening and associated symptoms will resolve, typically over several months following diagnosis.[18,19] In the present study, inadvertent inclusion of cats with transient myocardial thickening is unlikely as cats with HCM were included if they had persistent echocardiographic evidence of HCM over a 12-month period. Therefore, the results of this study are not applicable for cats with transient myocardial thickening.

5.5 Conclusion

In summary, neither CSS nor hair cortisol concentrations was associated with the echocardiographic diagnosis of subclinical HCM in cats. Further studies are warranted to explore environmental and epigenetic factors associated with HCM in cats. An ideal future study would be to follow siblings over a long period with repeat measurements of chronic stress and cardiac phenotype.

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Chapter 6. Prevalence of HCM in Sphynx cats in NZ

Note, the experiments reported in this chapter were published in Animals.[1] The published manuscript has only undergone minor modifications for this thesis chapter.

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6.1 Introduction

Hypertrophic cardiomyopathy (HCM) affects approximately 15% of domestic cats.[2,3] In purebred cats, some evidence suggests that Sphynx cats may be predisposed to HCM, with a prospective screening study in France showing that 20% Sphynx cats are affected in this population.[4] Additionally, there is some evidence that HCM develops earlier in Sphynx cats compared to other breeds.[5,6]

A variant in the gene encoding Alström syndrome protein 1 was recently identified in Sphynx cats with HCM (*ALMS1*:ENSFCAG00000008756:c.7384G>C; p.[G2462R]).[7] The function of ALMS1 in cats is currently unknown. In humans, a variant in the *ALMS1* gene causes Alström syndrome (or Alström–Hallgren syndrome), which consists of early childhood obesity, type 2 diabetes mellitus, retinal degeneration, hearing loss, and dilated or restrictive cardiomyopathy.[8] Hypertrophic cardiomyopathy is not an identified consequence of *ALMS1* variant in humans.

Hypertrophic cardiomyopathy is an age-dependent disease, with its prevalence increasing with age.[2,3] This age-dependence can compromise a breeding program when cats are bred at a young age, as a normal pre-breeding cardiac examination does not preclude the cat from developing HCM in late adulthood. Therefore, a practical method to accurately predict the development of HCM in young cats would be ideal to avoid inadvertently breeding cats at high risk of HCM. Low left atrial fractional shortage (LA FS%), elongated anterior mitral valve leaflet (AMVL) length, and the left ventricular wall thickness (LVWT) at upper limits of normal, may all be present prior to manifestation of left ventricular hypertrophy (LVH) in cats with HCM.[9,10] However, the predictive utility of these parameters for earlier diagnosis of HCM has not been investigated in purebred cats, which limits their use in the clinical setting.

Screening for a causal genetic variant would be an ideal method to identify cats at risk of HCM prior to breeding. One study suggested that a variant in the *ALMS1* gene is associated with HCM in Sphynx cats.[7] However, this result was not replicated in another study.[11] It is now known that the *ALMS1* variant is widespread in Sphynx cats, with allelic frequency exceeding 50% in several populations.[11-13] Therefore, the potential effect of *ALMS1* on the development of HCM in Sphynx cats needs further investigation, prior to utilizing *ALMS1* as a genetic marker in breeding programs.

The aims of this study were 1) to describe the prevalence of HCM and the frequency of the *ALMS1* variant in Sphynx cats in New Zealand, 2) to assess the association between *ALMS1* and HCM in this population, 3) to assess the association between *ALMS1* and previously documented risk factors of HCM (LA FS%, AMVL and LVWT), and 4) to describe the cardiac changes in Sphynx cats with or without the *ALMS1* variant over time.

6.2 Materials and Methods

6.2a Study design

This was a prospective longitudinal study. Ethics approval was provided by Massey University (MUAEC Protocol: 21/48). The inclusion criterion was Sphynx cats greater than 6 months of age. Exclusion criteria were known pre-existing cardiac disease and confirmed or suspected pre-existing disease that could result in an HCM phenotype.[14]

An email invitation for free cardiac screening was sent to Sphynx breeders registered with a national cat club (New Zealand Cat Fancy). Breeders who accepted the invitation were asked to forward the invitation to members of the public who had bought cats from them.

Physical examination was performed by the primary investigator (Joonbum Seo). The body weight, body condition score (out of 9), presence of gallop sounds, arrhythmias, and a heart murmur were recorded. Systolic arterial blood pressure was measured using Doppler technique following the current guidelines.[15] Each cat underwent transthoracic echocardiography by the primary investigator. Each scan was performed using Philips EPIQ 7C equipped with 12-4 sector array probe (Philips New Zealand Limited, Auckland, New Zealand). At a minimum, two-dimensional (2D) and colour Doppler images of the right parasternal long-axis four chamber (RPLax4C), right parasternal long-axis five chamber (PPLax5C) and right parasternal short axis (RPSax) views at the levels of papillary muscles and aortic valve, were acquired.[16] When possible, the left and right ventricular outflow tracts were also assessed with spectral Doppler using the left parasternal apical five chamber and RPSax views, respectively.[16]

The following echocardiographic variables were measured over 3 consecutive cardiac cycles and averaged using offline software (TomTec, TomTec Imaging Systems GMBH, Unterschleissheim, Germany). These were the maximum left atrial (LA) diameter (measured by bisecting the left atrium parallel to the mitral valve using 2D images of the RPLax4C view), LA to aortic ratio (measured using 2D images of the RPSax view at the level of the aortic valve as described by Hansson *et al.*),[17] left ventricular (LV) internal diameters at end-diastole (LVIDd) and end-systole (LVIDs; measured by bisecting the left ventricle using 2D images of the RPSax at the level of the papillary muscles),[18] and LVWT (measured from the thickest end-diastolic LV segment using 2D images of the RPLax4C, RPLax5C and RPSax views). The LA FS% and LV fractional shortening (LV FS%) were calculated using the acquired maximum and minimum diameters of the LA and LV.[10,18] The presence of SAM was assessed using 2D imaging.[19,20] The papillary muscle morphology was subjectively assessed. The AMVL length was measured using a RPLax5C view by tracing the AMVL from the to the aortic hinge point.[9] Heart rate was calculated from the cineloop of the RPLax5C view.

A HCM phenotype was defined as LVWT ≥6 mm. The diagnosis of HCM was made when cats with a HCM phenotype did not have an identifiable cause of LV thickening or hypertrophy on careful history and clinical exam.[14] In cats that had a HCM phenotype that were ≥6 years old, hyperthyroidism, systemic hypertension (systolic arterial blood pressure ≥160 mmHg)[21] and diabetes mellitus were excluded by measuring blood pressure and performing appropriate blood tests.

In cats with HCM, the distribution of LVH was characterised by calculating the interventricular septal (IVS) to LV free wall (LVFW) ratio using the LVWT measurements.[4,22] The distribution of LVH was classified as generalized if the ratio was 0.7-1.3, asymmetric septal hypertrophy if the ratio was >1.3, and asymmetric LVFW hypertrophy if the ratio was <0.7.[4,22]

Buccal swabs were collected from each cat and submitted to a genetic laboratory to test for the *ALMS1* variant (*ALMS1*: ENSFCAG00000008756: c.7384G>C;p.[G2462R], Genetic Testing Service, North Carolina State Veterinary Hospital, USA).[7] The genetic results were unknown by the primary investigator (Joonbum Seo) until all baseline data had been collected from each cat.

The cats were invited back for repeat physical examination 1.5 years after the initial assessment. At this time, repeat blood pressure measurements, and

echocardiography were performed by the same investigator (Joonbum Seo). The investigator was blinded to the previous cardiac measurements and the *ALMS1* test results when performing the repeat echocardiographic examination to avoid unintentional observer bias.

For related cats, a pedigree line was produced using the information provided by the breeders. Cats that developed clinical signs during the study period were managed as required by either general practitioners or specialists of an appropriate field. Hearts were examined during postmortem examination of all cats that died by a board-certified cardiologist (Joonbum Seo) and board-certified anatomic pathologists (Hayley Hunt and John Munday).

6.2b Statistical Analysis

Normality of the data was assessed using histogram and Shapiro-Wilk test. Continuous variables were displayed as mean (± standard deviation) or median (range) depending on the distribution of the data. Categorical variables were displayed as number (%). Continuous variables were compared across 2 groups (*ALMS1*-negative versus *ALMS1*-positive, and HCM diagnosis versus normal echocardiogram) using independent t-test or Mann-Whitney test. Additionally, continuous variables were compared across 3 groups (wild-type versus *ALMS1* heterozygous versus *ALMS1* homozygous) using one-way ANOVA or Kruskal-Wallis test. Categorical variables were compared using Fisher's Exact test for cell counts 5 or lower, and Pearson's Chi-Squared test for cell counts greater than 5. Significance was set < 0.05.

A multivariable analysis was performed by creating a general linear model for each echocardiographic variable reported to precede LV hypertrophy in cats with HCM (maximal LVWT, AMVL length, and LA FS%).[9,10] Variables were log-transformed if the distribution was severely skewed, and the residuals of each model were visually inspected by quantile plots, ensuring the assumption of normal distribution of linear models. The association between the dependent variables (maximal LVWT, AMVL length, LA FS%) and other variables was first tested by univariable analysis, by either Pearson's or Spearman's test for continuous variables; an independent t test or Mann-Whitney test for bivariate categorical variables, and a one-way ANOVA or Kruskal-Wallis test for >2 categorical variables. For cats that had

multiple scans, the data from the most recent study was selected. For each model, all variables with $P < 0.25$ from the univariable analysis were considered. Non-significant variables were removed 1 at a time in a backward stepwise manner until only significant variables remained ($P < 0.05$). Collinearity statistics were checked, and multicollinearity was eliminated.

6.3 Results

The e-mail invitation to participate in the study was sent to all 6 Sphynx breeders registered with national cat club in New Zealand (New Zealand Cat Fancy). Two breeders declined the invitation without a specific reason. Another breeder was unable to participate due to the distance. A total of 55 cats were enrolled. This included 24 cats directly owned by the three participating breeders as well as 31 cats that had been acquired from the breeders but were now owned by other people. The median age at the initial scan was 4.0 years (range: 0.5-11.1, Figure 6.1). The mean body weight was 4.8 kg (±1.3) with a median body condition score of 5 (range: 4-5). Thirty-two cats were female (10 entire, 22 neutered) and 23 were male (3 entire, 20 neutered).

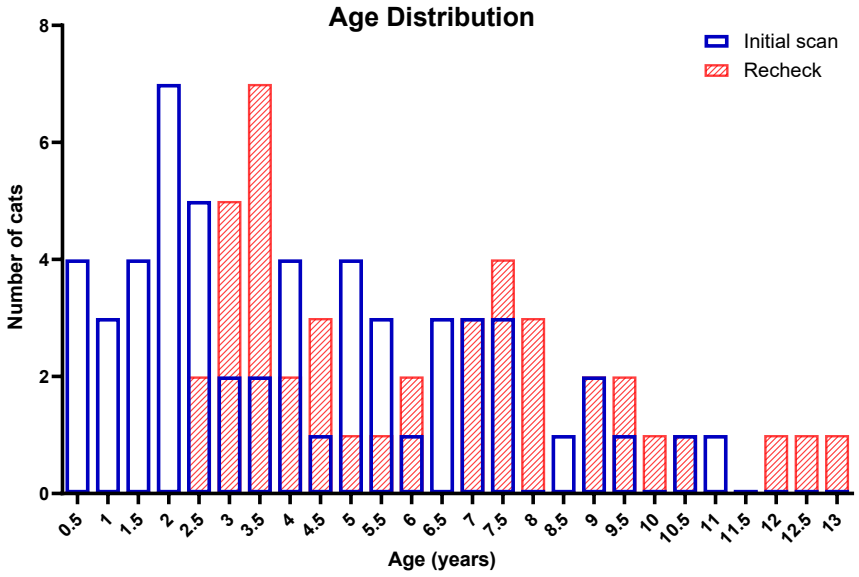

Figure 6.1. Age distribution of the study population at the initial scan (55 cats, blue) and recheck (42 cats, red crossed) in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats.

Twelve cats (21.8%) were diagnosed with HCM at the initial examination. The diagnosis of HCM was associated with being male, presence of a heart murmur, greater heart murmur intensity, larger LA size, reduced LA function, hyperdynamic LV function, and longer AMVL (Table 6.1). Two cats with HCM had SAM. One cat with HCM had congestive heart failure (CHF) at the initial evaluation.

Table 6.1. Comparison of the signalment, physical exam, and echocardiographic findings between cats with normal echocardiogram and those diagnosed with hypertrophic cardiomyopathy (HCM) in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats.

	Normal echocardiogram (n=43)	**HCM (n=12)**	***P* value**
Age (years)	3.3 (0.5-11.1)	5.3 (1.9-9.7)	0.089
Follow-up period (years)	1.8 (1.7-2.2)	1.8 (1.6-2.2)	0.210
Sex (Male : Female)	15 : 28	8 : 4	0.048
Neutered males (%)	13/15 (86.7%)	7/8 (87.5%)	0.955
Neutered females (%)	18/28 (64.3%)	4/4 (100%)	-
Weight (kg)	4.8 (±1.4)	5.0 (±1.2)	0.573
BCS (/9)	5 (4-6)	5 (4-5)	0.076
Murmur (%)	4/43 (9.3%)	8/12 (66.7%)	<0.001
Murmur grade (1/2/3/4/5/6)	0/3/1/0/0/0	0/5/3/0/0/0	<0.001
Arrhythmia (%)	0	1/12 (8.3%)	-
Heart rate (/min)	224.3 (±20.8)	211.1 (±30.7)	0.183
LA/Ao	1.4 (±0.1)	1.5 (±0.2)	0.132
LA FS%	30.5 (±5.5)	24.8 (±7.0)	0.019
LAD Max (mm)	14.7 (11.4-17.3)	15.5 (13.3-19.8)	0.026
LVWT (mm)	4.9 (3.8-5.9)	6.7 (6.0-7.7)	-
LVIDd (mm)	16.0 (±2.1)	15.5 (±2.4)	0.493
LVIDs (mm)	7.0 (±1.9)	5.8 (±1.9)	0.080
LV FS%	56.6 (±9.2)	63.2 (±7.7)	0.020
LVOT Vmax (m/s)	1.3 (0.9-1.9)	1.4 (1.1-4.2)	0.122
RVOT Vmax (m/s)	1.2 (0.7-2.0)	1.4 (0.8-1.9)	0.212
AMVL (mm)	7.8 (±0.9)	8.9 (±0.8)	<0.001
SAM	0	2/12 (16.7%)	-
Fused papillary muscles	4/43 (9.3%)	1/12 (8.3%)	0.683
DUST	1/43 (2.3%)	2/12 (16.7%)	
ALMS1 Positive (%)	31/43 (72.1%)	8/12 (66.7%)	0.714
ALMS1 (WT/Het/Hom)	12/22/9	4/7/1	0.605

Normally distributed continuous data are presented in mean (±SD). Non-normally distributed continuous data are presented in median (range).

Abbreviations: AMVL, anterior mitral valve leaflet; BCS, body condition score; DUST, discrete upper septal thickening; Het, heterozygous; Hom, homozygous; LA/Ao, left atrium to aortic ratio; LA FS%, left atrial fractional shortening; LAD Max, maximal left atrial diameter; LVWT, maximal left ventricular wall thickness; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LV FS%, left ventricular fractional shortening; LVOT Vmax, maximal left ventricular outflow tract velocities; RVOT Vmax, maximal right ventricular outflow tract velocities, SAM, systolic anterior motion of the mitral valve; WT, wild type.

Follow-up echocardiography was performed in 42 cats (76.4%). Echocardiography could not be repeated for 13 of the original 55 cats, with 3 cats dying prior to the rescan (2 due to CHF, and 1 euthanised due to non-cardiac disease), 8 rehomed or relocated, and 1 was made unavailable by the owner. The median follow-up period was 1.8 years (range: 1.6-2.2). The follow-up period was not different in cats with or without HCM (Table 6.2). The median age at recheck was 5.8 years (range: 2.4-13.1). At recheck, an additional 11 cats had HCM. Additionally, 1 cat with an HCM phenotype at the initial scan had reduced LVWT from 6.2 mm to 5.6 mm and all other echocardiographic measurements were normal at recheck. This cat was removed from the final HCM group due to possible transient myocardial thickening. Therefore, the overall prevalence of HCM in this cohort was 40% (22 out of 55 cats; 95% CI: 28.1-53.2%). Lastly, one cat that was clinically asymptomatic initially had a normal echocardiogram but developed a dilated cardiomyopathy phenotype at recheck (Figure 6.2F).

Table 6.2. Changes of clinicopathological data in 42 cats that had a follow-up data collection in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats.

	Normal echocardiogram at baseline (n = 35)			HCM at baseline (n = 7)		
	Baseline	Recheck	*P* value	Baseline	Recheck	*P* value
Age (years)	3.3 (0.5-11.1)	5.0 (2.4-13.1)	-	5.5 (±2.9)	7.3 (±3.1)	-
Weight (kg)	4.4 (±1.3)	4.7 (±1.3)	0.086	4.5 (±1.1)	4.6 (±0.8)	0.968
BCS (/9)	5 (4-6)	5 (4-8)	0.003	5 (4-5)	5 (5-5)	-
Murmur (%)	3/35 (8.6%)	2/35 (5.7%)	0.166	4/7 (57.1%)	1/7 (14.3%)	1.000
…Grade (1-6)	0 (0-3)	0 (0-2)	0.021	2 (0-3)	0 (0-2)	0.459
Heart rate (/min)	225.6 (±22.3)	217.9 (±22.8)	0.054	206.7 (±33.2)	206.6 (±15.1)	0.990
LA/Ao	1.4 (±0.1)	1.4 (±0.2)	0.086	1.5 (±0.2)	1.6 (±0.3)	0.125
LA FS%	30.7 (±5.4)	28.9 (±6.5)	0.148	26.8 (±4.7)	28.3 (±5.1)	0.543
LAD Max (mm)	14.4 (±1.7)	15.3 (±1.9)	<0.001	16.1 (±2.2)	17.0 (±3.9)	0.366
LVWT (mm)	5.1 (±0.5)	5.3 (±0.8)	0.063	6.3 (6.0-7.7)	6.1 (5.6-9.4)	0.866
LVIDd (mm)	15.7 (11.7-19.6)	15.6 (13.0-23.9)	0.902	15.6 (±2.9)	15.8 (±3.8)	0.832
LVIDs (mm)	6.6 (3.9-10.6)	7.2 (2.9-18.0)	0.093	5.8 (±2.0)	5.1 (±2.5)	0.579
LV FS%	56.9 (±7.7)	53.5 (±10.6)	0.070	63.5 (±6.9)	58.5 (±12.0)	0.121
LVOT Vmax (m/s)	1.3 (0.9-1.9)	1.2 (0.8-1.8)	0.003	1.3 (1.1-4.2)	1.3 (1.0-2.0)	0.091
RVOT Vmax (m/s)	1.2 (0.8-2.0)	1.2 (0.7-1.6)	0.131	1.3 (±0.3)	1.2 (±0.3)	0.091
AMVL (mm)	7.7 (±1.0)	7.5 (±0.6)	0.186	8.9 (±0.6)	9.0 (±1.1)	0.915
HCM (%)	0	11/35 (31.4%)	-	7/7 (100%)	6/7 (85.7%)	-
HCM (LVH-IVS)	0	4/11 (36.4%)	-	5/7 (71.4%)	2/6 (33.3%)	-
HCM (LVH-LVFW)	0	5/11 (45.5%)	-	1/7 (14.3%)	1/6 (16.7%)	-
HCM (LVH-Generalized)	0	2/11 (18.2%)	-	1/7 (14.3%)	3/6 (50.0%)	-
SAM (%)	0	0	-	1/7 (14.3%)	2/7 (28.6%)	-

Cats are grouped according to the presence of hypertrophic cardiomyopathy (HCM) or normal echocardiogram at initial scan. Normally distributed continuous data are presented in mean (±SD). Non-normally distributed continuous data are presented in median (range).

Abbreviations: AMVL, anterior mitral valve leaflet; BCS, body condition score; LA/Ao, left atrium to aortic ratio; LA FS%, left atrial fractional shortening; LAD Max, maximal left atrial diameter; LVWT, maximal left ventricular wall thickness; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LV FS%, left ventricular fractional shortening; LVH-Generalized; generalized left ventricular hypertrophy; LVH-IVS, interventricular septal hypertrophy; LVH-LVFW, left ventricular free wall hypertrophy; LVOT Vmax, maximal left ventricular outflow tract velocities; RVOT Vmax, maximal right ventricular outflow tract velocities, SAM, systolic anterior motion of the mitral valve.

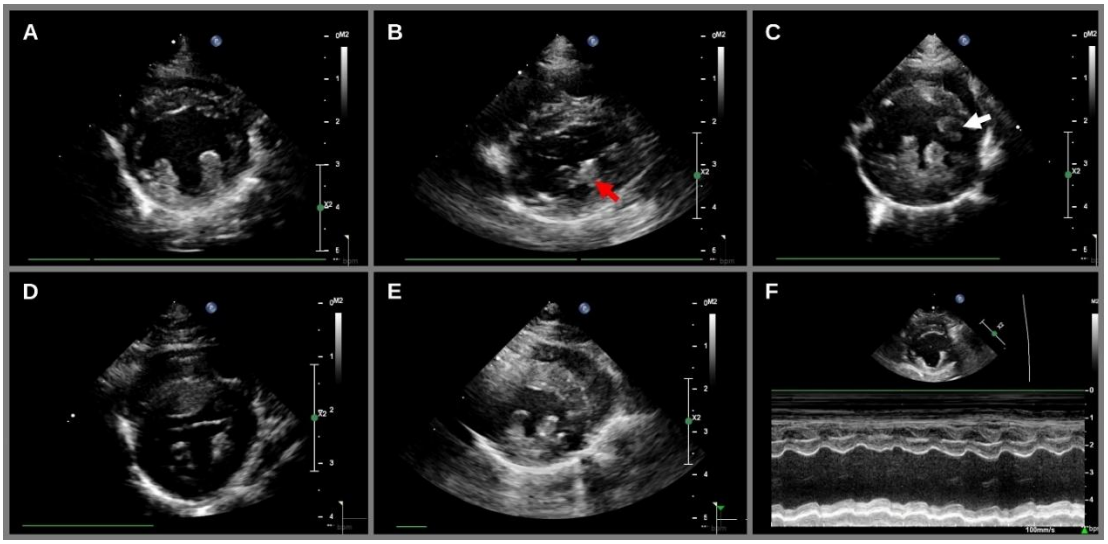

Figure 6.2. Observed cardiac phenotypes in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats . *From the top left, normal cardiac morphology (A), fused papillary muscles (red arrow, B), a hypertrophic cardiomyopathy phenotype with left ventricular free wall hypertrophy (C), a hypertrophic cardiomyopathy phenotype with interventricular septal hypertrophy (D), a hypertrophic cardiomyopathy phenotype with generalized hypertrophy (E), and a dilated cardiomyopathy phenotype (F) were seen. A third ectopic papillary muscle was seen in some cats (white arrow, C).*

The results of the univariable analysis of LVWT, AMVL, and LA FS% are summarized in Table 6.3. Variables with $P < 0.25$ were selected for multivariable analysis with each echocardiographic predictor of HCM set as the dependent variable. The final general linear models are presented in Table 6.4. The independent explanatory variables were body weight, LA FS%, LVIDs, and AMVL for LVWT; maximal LA diameter and the presence of SAM for AMVL; and maximal LA diameter as the sole explanatory variable for LA FS%.

Table 6.3. The results of the univariable analysis to assess association between known predictors of hypertrophic cardiomyopathy in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats.

	LVWT	AMVL	LA FS%
Age (years)	0.270	0.828	0.867
Sex (Male : Female)	0.007*	0.012*	0.139*
Weight (kg)	0.035*	0.104*	0.200*
BCS (/9)	0.301	0.907	0.916
Murmur (%)	0.004*	0.370	0.489
Grade (1/2/3/4)	0.643	0.571	0.429
Heart rate (/min)	0.265	0.031*	0.181*
SABP (mmHg)	0.317	0.841	0.759
LA/Ao	0.030*	0.003*	0.027*
LA FS%	0.017*	0.044*	-
LAD Max (mm)	0.006*	<0.001*	<0.001*
LVWT (mm)	-	0.007*	0.017*
LVIDd (mm)	0.987	0.001*	0.015*
LVIDs (mm)	0.034*	0.553	0.490
LV FS%	0.044*	0.851	0.979
LVOT Vmax (m/s)	0.015*	0.119*	0.262
RVOT Vmax (m/s)	0.078*	0.616	0.411
AMVL (mm)	0.007*	-	0.044*
SAM	0.001*	0.037*	0.585
Fused papillary muscles	0.164*	0.649	0.352
ALMS1 (Positive/ Negative)	0.143*	0.237*	0.194*
ALMS1 (WT/Het/Hom)	0.151*	0.354	0.354

The association between the dependent variables (maximal left ventricular wall thickness in end-diastole [LVWT], anterior mitral valve leaflet [AMVL] length, and left atrial fractional shortening [LA FS%]) and other variables was tested by either Pearson's or Spearman's test for continuous variables; an independent t test or Mann-Whitney test for bivariate categorical variables, and a one-way ANOVA or Kruskal-Wallis test for >2 categorical variables. Significant variables with P value <0.25 (*) were selected for multivariable analysis.

Abbreviations: ALMS1, Alström syndrome protein 1; BCS, body condition score; Het, heterozygous; Hom, homozygous; LA/Ao, left atrium to aortic ratio; LAD Max, maximal left atrial diameter; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LV FS%, left ventricular fractional shortening; LVOT Vmax, maximal left ventricular outflow tract velocities; RVOT Vmax, maximal right ventricular outflow tract velocities; HCM, hypertrophic cardiomyopathy; SABP, systolic arterial blood pressure; SAM, systolic anterior motion of the mitral valve; WT, wild type.

Table 6.4. The results of the multivariable analysis with the predictors of hypertrophic cardiomyopathy (HCM) as dependent variables in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats.

1. LVWT, Adjusted R^2 = 0.583			
Explanatory variables	*B* coefficient (95% CI)	Standardized *B* coefficient	*P* value
Body weight (kg)	0.262 (.045-.479)	0.292	0.020
LA FS%	-0.040 (-.080--.001)	-0.248	0.046
LVIDs	-0.214 (-.300--.129)	-0.578	<0.001
AMVL (mm)	0.437 (.123-.751)	0.332	0.008
2. AMVL, Adjusted R^2 = 0.360			
Explanatory variables	*B* coefficient (95% CI)	Standardized *B* coefficient	*P* value
LAD Max	0.200 (.099-.309)	0.484	<0.001
SAM	1.034 (.111-1.958)	0.274	0.029
3. LA FS%, Adjusted R^2 = 0.262			
Explanatory variables	*B* coefficient (95% CI)	Standardized *B* coefficient	*P* value
LAD Max	-1.840 (-2.87--.872)	-0.530	<0.001

Abbreviations: AMVL, anterior mitral valve leaflet; LAD Max, left atrial maximal diameter; LA FS%, left atrial fractional shortening; LVWT, maximal left ventricular wall thickness; SAM, systolic anterior motion of the mitral valve.

The frequency of the *ALMS1* variant was 70.9%. Eleven cats were homozygous for the *ALMS1* variant, and 28 were heterozygous. Of the cats that were homozygous, 1 of 11 (9.1%) had HCM at the time of initial scan while 7 of the 28 (25.0%) cats that were heterozygous had HCM. Four (25.0%) of the 16 cats that did not have the *ALMS1* variant were diagnosed with HCM at the initial evaluation. Of the 11 cats that were found to have HCM at the time of rescan, 2 (18.2%) were homozygous, 8 (72.7%) were heterozygous and 1 (9.1%) did not carry the *ALMS1* variant. Therefore, of the 22 Sphynx cats that had HCM in this study, 13.6% were homozygous for the *ALMS1* variant, 63.6% were heterozygous and 22.7% did not have the *ALMS1* variant. There was no association between the presence of the *ALMS1* variant and the diagnosis of HCM at either time point (Table 6.1 and Table 6.3). The cat that developed the DCM phenotype at recheck examination was heterozygous for the ALMS variant.

In addition to the one cat that had HCM and CHF at the initial exam, two additional cats with HCM developed CHF during the study period. Each cat was managed as appropriate by emergency veterinarians and a cardiologist or an internist. The first cat (designated Cat1) was treated with furosemide, clopidogrel, spironolactone, and pimobendan. There was no evidence of dynamic LV outflow tract obstruction on serial echocardiography in this cat. The furosemide dose was escalated to 4 mg/kg PO q8h over 27 days until euthanasia was elected due to refractory CHF. The second cat (designated Cat2) presented in severe respiratory distress and was treated with oxygen and furosemide in addition to the clopidogrel that it was previously receiving. The cat was treated with 8.1 mg/kg of furosemide

continuous rate infusion over 7 hours but was euthanised due to the concern of impending respiratory arrest. The third cat (designated Cat3) developed unusual stiffening and staggering episodes that gradually increased in frequency over 1.5 years. The cat was treated with phenobarbitone for suspected epilepsy by a neurologist and this resolved the episodes. However, an implantable cardiac monitor (LINQ II, Medtronic) during this time showed frequent periods of ST segment elevation lasting hours to days (**Figure 6.3**). Not all episodes coincided with the periods of ST segment elevation. Due to the concern of myocardial ischemia, the cat was treated with diltiazem 4.4 mg/kg PO q12h and clopidogrel 18.75 mg PO q24h, which did not reduce the frequency or duration of ST-elevation. The cat eventually developed CHF and was treated with furosemide 2 mg/kg PO q12h, and spironolactone. However, the symptoms of CHF recurred in 16 days, and humane euthanasia was elected.

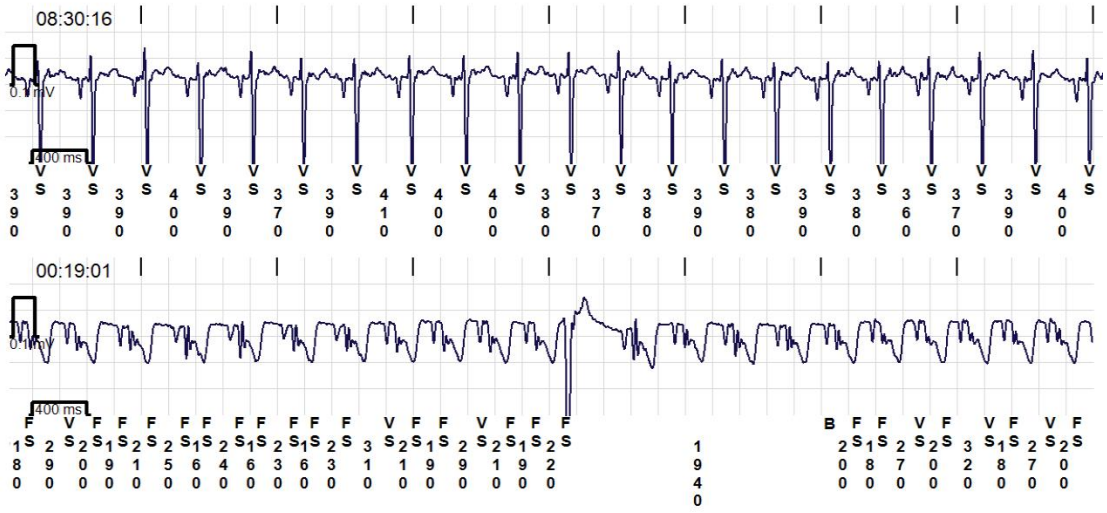

Figure 6.3. Two recordings from the implantable cardiac monitor in a Sphynx cat who later died of congestive heart failure. *The first recording shows normal sinus rhythm without ST-segment elevation. The second trace shows periods of ST-segment elevation. A single ventricular premature complex is also seen during the period of ST-segment elevation. Note the R-R interval is sensed inaccurately during the periods of ST elevation.*

The summary of the postmortem findings for all three cats that died due to CHF is presented in Figure 6.4. On gross examination, Cat1 had marked hypertrophy of the LVFW. The endocardium of the entire LVFW was pale, and this pallor extended into the myocardium. The epicardium appeared normal. Cat2 had severe generalized LVH without obvious discoloration of the LV endocardium, myocardium,

or epicardium. Cat3 had moderate thickening of the LVFW. Like Cat1, the entire LV endocardium and myocardium were pale in appearance. Additionally, a white 5 mm diameter, 1 mm raised area of thickening was seen in the epicardium of the apical region. Histologic examination showed myofibre disarray, myocardial hypertrophy, and interstitial fibrosis in each cat, consistent with the HCM diagnosis (Figure 6.4). Additionally, Cat1 had large areas of myofibre swelling and vacuolation, consistent with recent myocardial ischemia. Cat2 and Cat3 also had similar changes but also extensive areas of sub-endocardial fibrosis with small pale cells, consistent with chronic myocardial infarction. Cat3 had marked endocardial thickening of the LV anterior papillary muscle and nearby free wall segment. All 3 cats had abnormal coronary arteries characterised by asymmetric medial hypertrophy and intimal hyperplasia of the coronary arteries at ischemic regions (Figure 6.5).

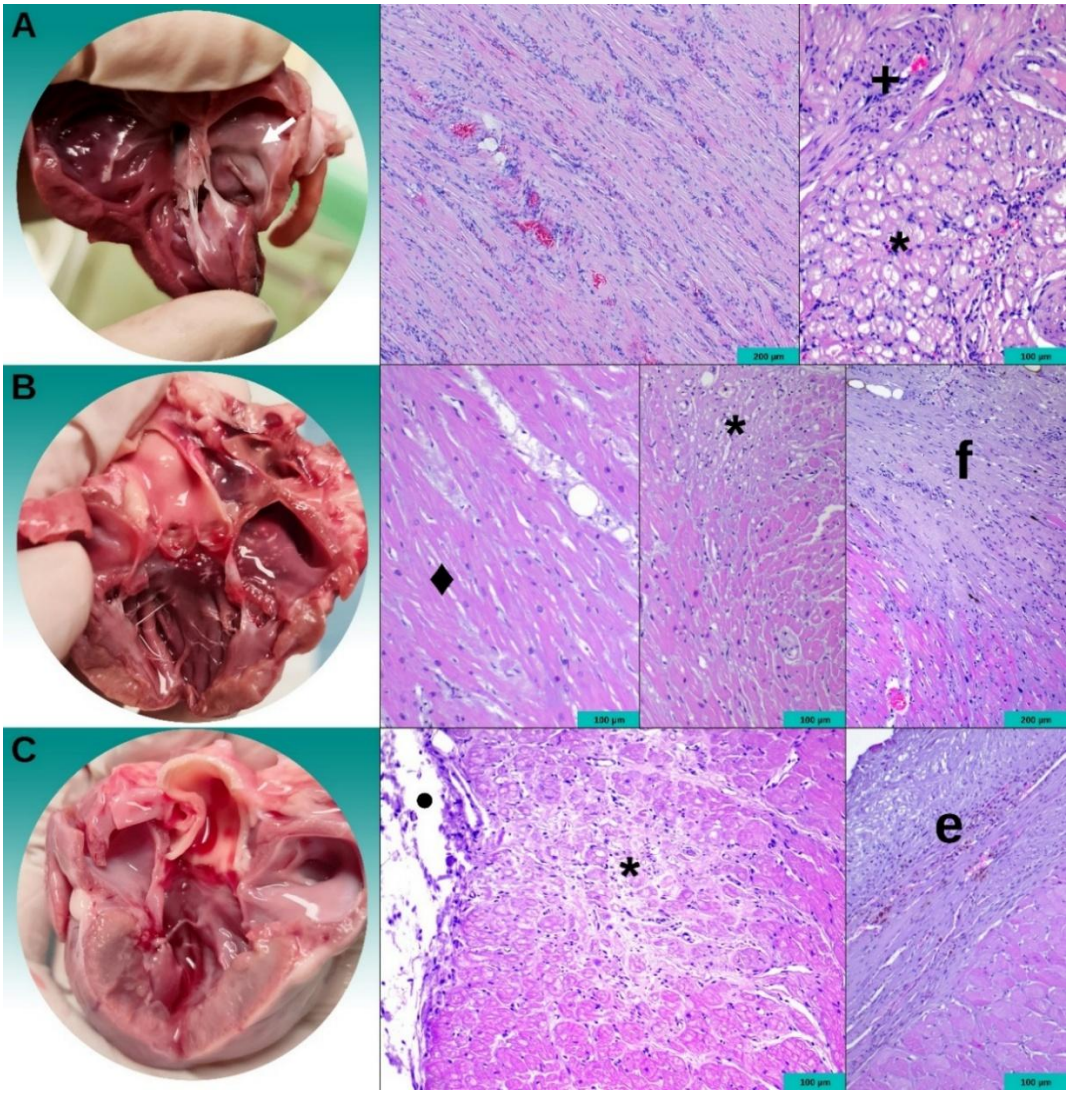

Figure 6.4. Postmortem images of 3 cats that were diagnosed with hypertrophic cardiomyopathy and died of congestive heart failure in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats.

Gross postmortem images are shown in the left, and the corresponding histologic images from the abnormal segments are shown in the right. (A) Cat-1 had left ventricular free wall hypertrophy and fused papillary muscles. The endocardial and myocardial layers of the entire left ventricular free wall segment were pale (white arrow). Histology showed myofibre disarray, myocardial hypertrophy, and patchy interstitial fibrosis, consistent with hypertrophic cardiomyopathy. Additionally, there were areas of cellular swelling and vacuolation (*) and abnormal coronary arteries (+) with thickening of the vessel wall and narrowing of the lumen, consistent with recent myocardial ischemia. (B) Cat-2 had septal hypertrophy with some freeze-thaw artifacts (small white areas of the endocardium and discoloration of the mitral and aortic valve leaflets). Histology showed myofibre disarray (◆), focal areas of myofibre atrophy and loss, vacuolation of myofibres, and fibrosis (*), suggestive of chronic myocardial infarction. The fibrosis extended into the sub-epicardial segment (f). (C) Cat-3 had marked left ventricular free wall hypertrophy. Histology showed multiple small pale foci with extensive sub-endocardial fibrosis (*), consistent with chronic myocardial infarction. There was a depressed area in the epicardium due to chronic infarction (●). This cat also had marked endocardial thickening of the left ventricular anterior papillary muscle and nearby free wall segment (e). There was mild endocardial thickening in the left ventricular outflow tract. No other left ventricular segments or the right ventricle had noticeable endocardial thickening. Hematoxylin and eosin staining.

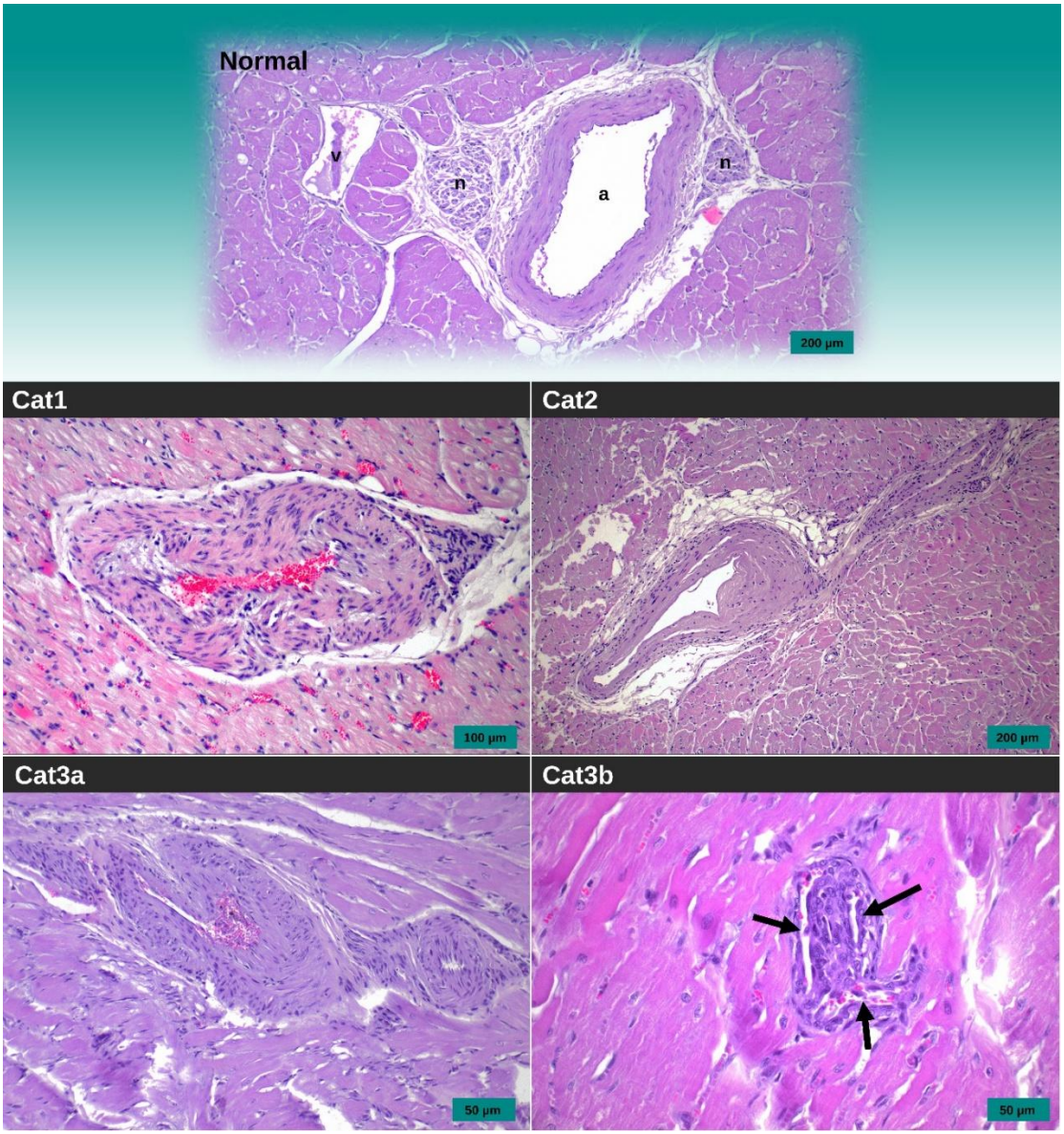

Figure 6.5. Coronary blood vessels in 3 cats that were diagnosed with hypertrophic cardiomyopathy and died of congestive heart failure in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats .

The normal coronary artery anatomy from a normal myocardial segment in Cat-3 is first shown as a reference (v, venule; n, nerve; a, artery). All cats had asymmetric medial hypertrophy and intimal hyperplasia of the coronary arteries, suggestive of coronary microvascular dysfunction as a part of hypertrophic cardiomyopathy disease spectrum. Additionally, 2 cats had disorganized vascular endothelium (Cat-1 and Cat-3a). Lastly, one cat had possible recanalization of coronary artery with 3 small lumens (Cat-3b, arrows). Hematoxylin and eosin staining

A pedigree line was produced using 42 related cats in the cohort, and 4 additional cats that were previously owned by the breeders (Figure 6.6). The founding members in this breeding line originated from the United States of America (2), Russia (1), United Kingdom (2), Australia (1) and New Zealand (2). The family line suggested an autosomal dominance pattern with incomplete penetrance for the mode of HCM inheritance in Sphynx cats.

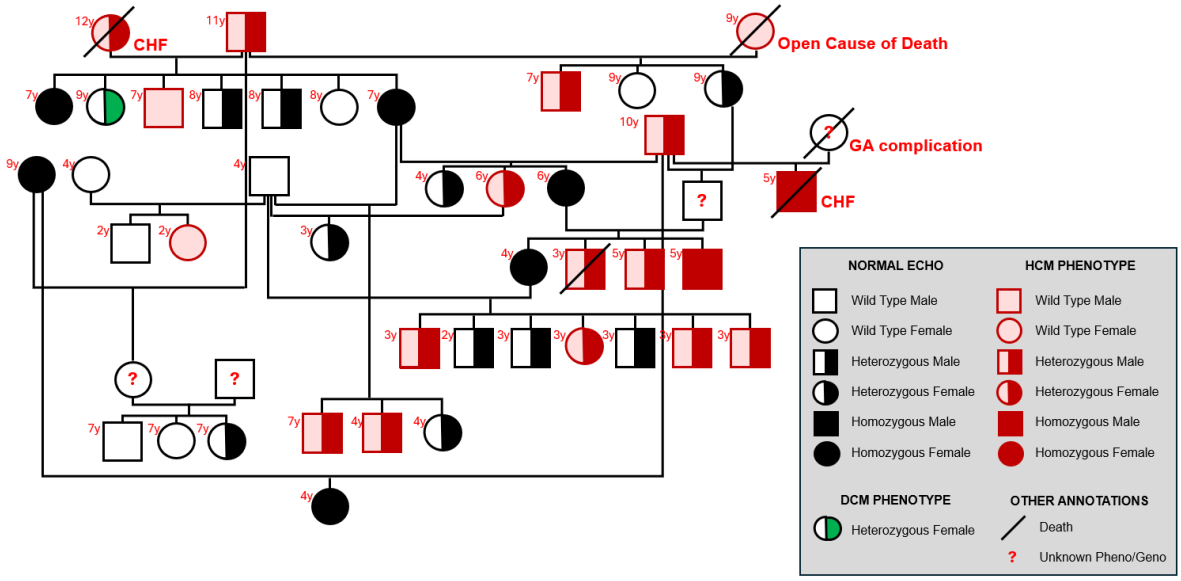

Figure 6.6. Pedigree line of 46 Sphynx cats, comprised of 42 studied cats and 4 historical cats, in a study to describe the prevalence of hypertrophic cardiomyopathy in Sphynx cats. *Historical cats with unknown phenotype (Pheno) and genotype (Geno) are marked with a red question mark. The presence of ALMS1 variant, hypertrophic cardiomyopathy (HCM) phenotype, and dilated cardiomyopathy (DCM) phenotype are annotated accordingly. Death is marked with a black cross line with the cause of death indicated next to the deceased cat. The age at the last follow-up is noted in left upper corner of each cat. Abbreviation: CHF, congestive heart failure; GA, general anaesthetic*

6.4 Discussion

The prevalence of HCM in the group of 55 Sphynx cats was 21.8% at the initial scan. One cat had reduced LVWT at recheck, which could be caused by early transmural fibrosis[23,24] or transient myocardial thickening.[25,26] This cat was removed from the HCM group as there was no further follow-up to characterise the phenotype more accurately. Following removal of this cat and introduction of 11 new cases of HCM from the second scan, the final prevalence of HCM was 40.0% (95% CI: 28.1-

53.2%) with a median age of 5.8 years. Previous studies of non-purebred cats have reported the overall prevalence of HCM to be 15% but this can increase up to 29.4% in cats older than 9 years old.[2,3] Similar to the study of French Sphynx cats, our results support the view that Sphynx cats are predisposed to HCM.[4] While only cats in New Zealand were investigated, the global genetic pool of Sphynx cats is small, and breeding cats are often shared internationally. As an example, 75% of the founding members of the studied family lines were imported from other countries. Therefore, the prevalence of HCM in Sphynx cats is potentially high across the world.

The frequency of *ALMS1* variant was 70.9%. This result is similar to the findings in North America and Europe,[11-13] and suggests that the *ALMS1* variant is commonly present in Sphynx cats throughout the world. The similarly high frequency of *ALMS1* variants in cats in New Zealand, North America, and Europe further illustrates how breeding cats are shared internationally.

The *ALMS1* variant was not associated with HCM in the present study. This finding is consistent with a recent study of European and American Sphynx cats that also did not detect any association between the *ALMS1* variant and HCM.[11] The original study that identified the *ALMS1* variant as a possible cause of HCM in Sphynx cats was case-control in design and compared Sphynx cats with HCM (case group) to healthy non-Sphynx cats (control group).[7] However, the high allelic frequency in Sphynx cats is now recognised[11-13] and this high allelic frequency likely resulted in the observed higher rate of detection in affected Sphynx cats when to compared to unaffected non-Sphynx cats. In this present study, a longer follow-up period and inclusion of older Sphynx cats would further aid assessment of the effect of *ALMS1* on the risk of HCM in Sphynx cats. However, echocardiographic variables that can precede LVH in cats with HCM were also assessed in the present study. The lack of association between *ALMS1* and these early features of HCM further suggests that the *ALMS1* variant is not associated with HCM in Sphynx cats.

In addition to increased risk of HCM, Sphynx cats have been suggested to develop HCM at an earlier age.[5,6] A previous retrospective study by Trehiou-Sechi et al. suggested that Sphynx cats with HCM have a similar lifespan compared to other breeds with HCM, although the outcome data was available in only 12 Sphynx cats.[5] In contrast, a recent study of 7936 cats, including 18 Sphynx cats, showed that Sphynx cats have the shortest lifespan compared to other breeds, although the cause of death was not investigated.[27] The results of the present study suggest that Sphynx cats may have a manifestation of disease that progresses more quickly to

death, although assessing the survival outcome was not the primary aim of our study. The incidence rate of cardiovascular death in Sphynx cats with HCM was 131.3 per 1000 cat-years at the time of writing. In contrast, only 57.7 cardiovascular deaths per 1000 cat years was reported in a study of similarly aged non-purebred cats.[28]

Histology of the hearts from all three cats that died with CHF revealed ischemic necrosis and evidence of coronary microvascular dysfunction (CMD), which is characterised by medial hypertrophy and intimal hyperplasia of the coronary arteries on histology.[29,30] One of these cats also had frequent periods of ST-elevation that appeared refractory to diltiazem. Myocardial ischemia and infarction can develop in HCM in both cats and humans.[24,30] In humans with HCM, CMD is the predominant cause of myocardial ischemia and infarction, and the aforementioned histologic changes to the coronary arteries, progressive LVH, and DLVOTO contribute to reducing coronary perfusion and/or increasing oxygen demand.[30] Medial hypertrophy and intimal hyperplasia of the coronary arteries have long been recognised in cats with HCM.[24,31,32] However, the frequency, risk, and clinical importance of myocardial ischemia and infarction in cats with HCM is largely unknown. Additional postmortem examinations of this breed would be required to determine whether the pathophysiology in this breed is different compared to non-purebred cats.

There are many variations in the distribution of fibrosis in both human and feline HCM.[33,34] However, significant endomyocardial thickening and fibrosis are often considered as a separate disease entity termed endomyocardial fibrosis, or a form of restrictive cardiomyopathy in both humans and cats, with exception being endomyocardial thickening in the LV outflow tract in patients with obstructive HCM.[14,35-38] In humans, endomyocardial fibrosis can develop concurrently with HCM.[39] In the present study, one cat had substantial endocardial thickening in addition to histologic and echocardiographic changes that are consistent with HCM. Given that this cat had characteristic features of HCM, it was included in the HCM group in the analysis.

Restrictive cardiomyopathy and dilated cardiomyopathy are possible outcomes of Alström syndrome in humans.[8] In the present study, the one cat that developed a DCM phenotype was a heterozygous carrier of the *ALMS1* variant. Due to the small sample size and high frequency of the *ALMS1* variant within this breed, the significance of this is uncertain and additional cases would be required to determine whether the same *ALMS1* variant could increase the risk of dilated cardiomyopathy in Sphynx cats.

Fused papillary muscles and third ectopic papillary muscles were found in several cats with no association with the diagnosis of HCM or echocardiographic variables that precede LV hypertrophy in feline HCM. The clinical relevance of atypical papillary muscle morphologies remains unknown.

There are several guidelines for HCM screening in breeding cats.[14,40] At minimum, these guidelines recommend the use of 2D, M-Mode, and Doppler imaging to identify cats with LV hypertrophy and outflow tract obstruction.[14,40] Additionally, subjective assessments are incorporated to assess the mitral valve apparatus and papillary muscle morphologies.[14,40] However, the effectiveness of these guidelines is currently unknown. For example, most cats in the present study had been screened by cardiac sonographers and boarded cardiologists prior to breeding. Furthermore, all imported breeding cats were determined clear of HCM by echocardiography prior to leaving their respective country. The high prevalence of HCM in the studied cohort despite having a history of vigorous screening raises concerns with the current approach to breed screening. A possible explanation for the high prevalence of HCM is that cats are bred at young adulthood after screening clear at an early age. However, delaying screening can be difficult as breeders would need to keep intact cats at home for additional years. A possible solution to this is carefully evaluating the family line and focusing on screening the older founding members of the breeding line. Another possible solution could be incorporating echocardiographic variables that precede LVH in feline HCM in the screening criteria. However, the accuracy, repeatability, and diagnostic cutoffs of these variables (LA FS%, AMVL length, and LVWT) in purebred cats have not been described. Ideally, determination of a genetic cause of HCM in this breed may allow development of a screening test prior to breeding.

There are some limitations in this study. Firstly, the median follow-up period of 1.8 years is relatively short for studies of HCM, as this disease can have a long pre-clinical period.[10,28] However, Sphynx cats develop HCM earlier than other breeds.[5,6] Additionally, echocardiographic variables that precede LVH in feline HCM were assessed to help overcome the short follow-up period, although the accuracy of these variables for predicting HCM in Sphynx cats is currently unknown. Secondly, the small sample size of 55 cats could have introduced a type II error. Lastly, only cats in New Zealand were studied. Arguably, the findings of this study may be limited to the cohort in New Zealand, but as Sphynx breeders have a global network and

routinely share their breeding cats internationally, the findings of this study might be relevant to other populations of Sphynx cats.

6.5 Conclusion

Hypertrophic cardiomyopathy is common in Sphynx cats. There is no association between the *ALMS1* variant and HCM in Sphynx cats. Sphynx cats appear to be predisposed to myocardial ischemia and infarction, which might be associated with early death in some cats with HCM.

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Chapter 7 Discussion

7.1 Summary of major findings

Five studies (Chapters 2 to 6) were conducted with aims to 1) describe the prevalence of feline HCM in New Zealand, 2) describe the pattern of HCM phenotypic development, 3) describe the HCM phenotypic progression with a specific focus on the SAM and obstructive blood flow, 4) describe the influence of psychologic or physiologic stress as an environmental contributor to the development of feline HCM, and 5) investigate the pattern of HCM disease progression and explore a potential genetic cause of HCM in Sphynx cats in New Zealand. The major findings of these five studies are summarised below.

The prevalence of subclinical HCM in non-purebred cats in New Zealand was 15.2% (95% confidence interval: 9.5-22.4%; Chapter 3). This is a similar prevalence to the United Kingdom and United States of America, which were 14.7% and 14.6%, respectively.[1,2] The comparable prevalence of subclinical HCM across three countries, in three different continents, further suggests that HCM is a global disease, and that practice guidelines from each country are likely applicable to other regions.

Despite the high prevalence of HCM in New Zealand, it is not the primary cause of death in many cats. In Chapter 3, a review of 10 years of mortality data from a colony of non-purebred cats (Centre of Feline Nutrition, Massey University) showed that only 3.8% (95% confidence interval: 1.2-8.6%) of deaths were likely caused by HCM. Similarly, a prospective follow-up of this colony of non-purebred cats over 3 years (Chapter 4) showed that 5.7% of deaths were likely caused by HCM during the study period. These observations support the findings of previous studies where cats with echocardiographic HCM may not progress to developing cardiac complications during the cats' life.[3,4]

In contrast, HCM was more common in Sphynx cats than non-purebred cats in New Zealand with a prevalence of 40% (95% confidence interval: 28.1-53.2%; Chapter 6). Furthermore, HCM progressed more rapidly in these cats, with 3 out of 5 deaths (60%) being caused by HCM during a median follow-up period of 1.8 years (minimum: 1.7 – maximum: 2.2). This observation suggests that the disease manifestation is different between cat breeds.

Prior to developing HCM, a gradual increase in the anterior mitral valve leaflet length was found in non-purebred cats (Chapter 4). In these cats, the length of the anterior mitral valve leaflet increased by median 0.5 mm per year (95% CI: 0.4-0.7 mm per year, *P* <0.001). Once the cats developed the echocardiographic criterion of HCM (LVWT ≥6 mm), the anterior mitral valve leaflet length stopped increasing. However, the LVWT continued to increase before and after the development of the echocardiographic criterion of HCM (LVWT ≥6 mm).

In cats with pre-existing HCM, the presence or absence of obstructive blood flow secondary to SAM was not permanent, and 8% of cats with HCM were found to either gain or lose SAM each year (Chapter 2). Interestingly, cats with SAM were also more likely to progress in disease severity compared to cats without SAM, as more cats with SAM at the initial echocardiographic exam developed left atrial enlargement, arrhythmias, and congestive heart failure during the median follow-up period of 2.1 years (minimum 1 year; maximum 5.9 years). In comparison, none of the cats without SAM developed left atrial enlargement, arrhythmias, or congestive heart failure during the same follow-up period. This result suggests that SAM was harmful in these cats.

Potential contributors to the development of HCM were investigated in Chapters 5 and 6. In Chapter 5, the effect of psychological stress on the diagnosis of subclinical HCM was assessed using the Cat-Stress-Score and hair cortisol concentration. In this study, neither marker of psychological stress was associated with the diagnosis of subclinical HCM. In Chapter 6, the effect of *ALMS1* gene variant on the diagnosis of HCM was investigated in Sphynx cats. In this study, almost three quarters of Sphynx cats in New Zealand carried the *ALMS1* variant. There was no evidence that the *ALMS1* variant contributed to the development of HCM in these cats.

7.2 Why is HCM so common in cats?

The prevalence of HCM in cats is already high at around 15%. Most cats without HCM will continue to increase in LVWT and some will eventually reach the diagnostic threshold of HCM. This observation is also demonstrated in the CATSCAN studies. In the CATSCAN I study, where 780 cats were prospectively screened from rehoming centres in the United Kingdom, the overall prevalence of subclinical HCM

was also around 15% but increased up to 29% in cats older than 9 years old.[1] In follow-up study (the CATSCAN II study), where 107 cats from the original CATSCAN I study were regularly examined over a median follow-up period of 5.6 years, a gradual increase in LVWT in most cats without HCM was also observed.[3] The high prevalence of subclinical HCM, especially in older cats, may be explained using major evolutionary theories of aging and longevity:[5]

1. *Mutation accumulation theory:* From the evolutionary perspective, there is less natural selection with older age. For example, HCM is a slowly progressive cardiac disease that typically develops in older age. Given that HCM typically develops past the reproductive stage, affected cats do not experience natural selection, and the genetic variants associated with HCM would have already been passed down the offspring by the age the parenting cats develop HCM. Over successful generations, the late-acting genetic variants associated with HCM would have accumulated and cause mortality later in life.

2. *Antagonistic pleiotropy theory (also known as 'pay later' theory):* Certain genetic variants that become expressed with later age (as hypothesised for feline HCM), may be a favoured trait at younger age. If this is true, these genetic variants may have been naturally selected and be actively accumulated in the feline population due to their beneficial effects early in life.

The mutation accumulation theory is a satisfactory explanation for cats, as most cats that are either wild or belong to breeders are bred before they are 3 years old but may develop HCM at a later age. Even in the wild, cats with subclinical HCM may still be reproductively successful due to the slowly progressive nature of HCM. Antagonistic pleiotropy theory is also plausible as some of the genetic variants associated with HCM may be associated with positive phenotypic trait, such as friendly temperament, softer coat, or better health at young age. Therefore, breeders may be inadvertently selecting cats with genetic variants associated with HCM to favour other positive features associated with the genetic variants.

When considering that HCM is likely caused by a genetic variant, the remarkably similar prevalence across New Zealand, United Kingdom, and United States of America is an unusual observation. One explanation for the stable prevalence across three countries is that the genetic variants associated with HCM

were introduced centuries ago and that these pathogenic genetic variants are now widely spread across the globe. The statistics on the number of imported and exported cats from New Zealand, United Kingdom, and United States of America are not readily available, but in 2023, 320,000 dogs, cats, and ferrets were reported to be non-commercially brought into the United Kingdom.[6] Some of these cats may be intact and reproduce after relocating to another country. Furthermore, the global movement of cats may dilute the prevalence if a significant number of cats are being imported and exported each year. To better understand the global burden of HCM, the genetic diversities of pet cats in New Zealand, United Kingdom, and United States of America, and the number of cats being imported and exported by these three countries should be assessed. Additionally, the prevalence of HCM in Asia and other European, American, and Australasian countries should be investigated.

An environmental factor may be essential to trigger the genetic variant associated with HCM. Furthermore, protective genes may exist in some cats, and these genes may be switched off by environmental factors at older age. If environmental factors indeed play an important role in the pathogenesis of HCM in older cats, such environmental factors must be common across the United Kingdom, United States of America and New Zealand to explain the stable prevalence of HCM between three countries. For example, the household products, pet foods, pet supplements, and veterinary medications are generally similar between the three countries, and these should be investigated in future studies to better understand the epigenetic component of HCM pathogenesis in cats. Additionally, the prevalence of HCM should be compared between wild versus pet cats, and indoor versus outdoor cats as wild cats are exposed to different environmental conditions to indoor pet cats.

7.3 What is the impact of feline HCM in New Zealand?

The estimated population of pet cats in New Zealand is 1.2 million,[7] Given that around 15% of cats have subclinical HCM, around 180,000 cats can be expected to have subclinical HCM. It is important to note that cats used to estimate the prevalence of HCM in this thesis (Chapter 3) were younger than the described general feline population in New Zealand in 2020.[7] Specifically, the data from 2020 suggested that the median age of pet cats in New Zealand is closer to 7 years old,[7] whereas the cats used in this thesis had a median age of 4.1 years (Chapter 3).

Furthermore, 20% of cats in New Zealand were estimated to be purebred.[7] As the prevalence of HCM increases with age of cats and in certain breeds, up to 250,000 cats may have HCM in New Zealand.

While up to 250,000 cats in New Zealand may have HCM, death due to HCM is uncommon. In fact, at most only 1 in 4 cats with HCM in the Centre of Feline Nutrition died due to HCM-related complications. Similarly, two studies (the REVEAL study and CATSCAN II study) showed that over half of cats with HCM did not develop HCM-related complications within a >5-year follow-up period.[3,4] The varying prognosis of HCM between cats may suggest different causes of HCM within the feline population. For example, a cat with benign HCM may have a different aetiologic complex compared to a cat with rapidly progressing HCM. It is also possible that certain cats have more than one cause of HCM and accumulation of causes of HCM will result in faster disease progression. Similarly, cats may be exposed to different magnitude and chronicity of epigenetic factors that may be trigger or modulate the gene associated with HCM pathogenesis. To date, the aetiologic complex of HCM is poorly understood in cats and further studies are needed to investigate the cause of HCM as previously discussed.

Even though only 1 in 4 cats with HCM will spontaneously progress into developing complications of HCM, veterinarians in New Zealand must be aware that common treatments can induce congestive heart failure in cats with previously stable subclinical HCM. For example, cats with pre-existing HCM may not tolerate supplemental fluid or corticosteroid therapy and develop congestive heart failure.[8-10] Ideally, all cats should be screened with echocardiography prior to commencing supplemental fluid or corticosteroid therapy given the high prevalence of subclinical HCM in New Zealand. However, such method is neither practical nor possible in the general population. For example, echocardiography by an experienced cardiac sonographer is expensive and usually ranges between $500 and $800 in New Zealand. Furthermore, some veterinary clinics in New Zealand may not have access to an experienced cardiac sonographer. These problems are not limited to New Zealand and several studies described alternative diagnostic methods to screen for HCM. In these studies, being male, older age, cardiac auscultatory findings of an audible gallop sound, loud heart murmur, and arrhythmias, as well as increased blood markers of myocardial wall tension (N-terminal pro-B-type natriuretic peptide) and injury (cardiac troponin I) all increased the likelihood of the cat having echocardiographic diagnosis of HCM.[1,11-13] Therefore, echocardiography can be

prioritised in older cats (i.e., > 6-year-olds) that are male and have a loud heart murmur and increased concentrations of N-terminal pro-B-type natriuretic peptide or cardiac troponin I.

Cats diagnosed with subclinical HCM should be monitored yearly using echocardiography. Options of monitoring HCM without echocardiography are limited at the current era. It may be tempting for veterinarians to monitor cats with HCM with cardiac auscultation and grading of a heart murmur. Indeed, the presence of a loud heart murmur suggests underlying HCM and obstructed blood flow secondary to SAM. However, it is now known that cats with HCM may spontaneously gain or lose SAM each year (Chapter 2). If the heart murmur was caused by SAM, the appearance and disappearance of a heart murmur can create false sense of concern or assurance to pet owners. Additionally, a loud heart murmur may be due to physiologic stimulation of being a veterinary clinic in some healthy cats and these cats can be mistakenly suspected to have HCM.[14] In comparison, measurements of blood markers of myocardial wall tension (N-terminal pro-B-type natriuretic peptide) and myocardial injury (cardiac troponin I) were previously shown to predict the progression of HCM into clinical stage; however, these blood markers are also influenced by the presence of SAM.[15,16] Therefore, physical exam findings and blood marker results should be interpreted with serial echocardiography.

Overall, subclinical HCM is common in New Zealand, and the use of echocardiography for screening and monitoring HCM can be unavailable or cost-inhibitive for some pet owners. If routine echocardiography is not possible, veterinarians should be cautious when using fluid or corticosteroid therapy. Furthermore, veterinarians should educate pet owners that around 1 in 4 cats with subclinical HCM will progress into developing congestive heart failure, arterial thromboembolism, and sudden cardiac death. Pet owners should be educated regarding the early clinical signs of congestive heart failure (increased sleeping respiratory rate) and arterial thromboembolism (sudden onset of pain or lameness) to promote early veterinary intervention in affected cats.

7.4 How can veterinarians help cat breeders in New Zealand?

The international guidelines for cardiac screening prior to breeding cats recommend the use of familial history, genetic tests for Maine Coons and Ragdolls,

and routine echocardiography prior to breeding.[17,18] However, there are several challenges even with these guidelines. First, a review of the familial history in a specific breeding line may not be straightforward due to the inheritance pattern of HCM. For example, cats in the breeding line may not develop HCM during their lifetime but produce progenies that later develop HCM. To identify these cats, information from multiple generations of cats must be combined with information provided by pet owners whom the kittens were rehomed to. Not all pet owners are willing to share the health information of their cats with the breeder. Second, genetic tests that are shown to predict the risk of HCM are only available for Maine Coons and Ragdolls.[19,20] Even in these breeds, cats that test negative on genetic tests can still develop HCM. [21] Third, screening echocardiography prior to breeding can successfully identify cats that develop HCM early but will not exclude cats that develop HCM at later age. Cats are typically bred at early age (e.g., less than 3 years old) and these cats may later develop HCM after already producing multiple litters of kittens. Lastly, echocardiography is expensive as previously mentioned, especially when performed by an experienced cardiac sonographer using a high-tech echocardiographic machine. The high cost of echocardiography may deter the breeders from performing appropriate cardiac screening or cause them to choose less experienced cardiac sonographers with older echocardiographic machine for cardiac screening. Diagnosis of mild heart disease in cats is generally inaccurate on echocardiography by less experienced veterinarians.[22]

If cardiomyopathy screening guidelines are to be developed specifically for cat breeders in New Zealand, they should ideally consider the limitations mentioned above. The guidelines should be practical for breeders while setting criteria that are neither too simple nor too extensive. Using extensive criteria set by cardiologists that exclude all cats with early features of cardiomyopathies, such as elongated anterior mitral valve leaflet length from the breeding line will likely reduce the genetic diversity and cause inbreeding. One possible guide is to set different criteria for different ages of cats. For example, if a cat is being bred before the age of one, the cat should be primarily screened for congenital heart defects. The cat should then be screened every one to two years throughout its life, and the onset of HCM should be recorded. Furthermore, pet owners should be made aware of the importance of sharing health information of the cats with the breeders, so the prevalence of HCM within the family line is recorded appropriately by the breeder. Breeders should record the cause of death in both the breeding line and rehomed cats to identify the incidence of cardiac

death within the population. Veterinarians should be given access to this information so they can support the breeder in interpreting its significance and making breeding decisions based on the data. Finding out the health information of the cats is emotionally distressing for breeders, but actively recording cats that show echocardiographic changes or clinical sign compatible with HCM from the breeding pool will allow identification of a line of cats at risk of HCM and likely the incidence of HCM related mortality in in future generation of cats.

7.5 How should we address phenotypic variations in future studies?

All genetic studies of feline HCM rely on an accurate phenotypic description of HCM using echocardiography.[19,20,23] However, not all cases of HCM progress into developing complications or death, and in affected cats, the geometry of the left ventricle and the presence of SAM can vary between cats or within a single cat over time (Chapters 2, 3, 4 and 6). Furthermore, some cats with a 'normal' echocardiogram may have early features of HCM such as an elongated AMVL.

These phenotypic variations pose an immense challenge for studies of feline HCM. For example, genetic studies may fail to associate a genetic variant to HCM when most of the control cats with 'normal' echocardiogram have early features of HCM that were not correctly identified. Similarly, investigating the association of echocardiographic or physical examination findings on the cumulative risk of HCM-associated deaths may not provide accurate information if the echocardiographic or physical examination findings were acquired at a single time-point and possibly years before the cat succumbed to death. This limitation is demonstrated in previous studies where the presence of SAM was not shown to be associated with earlier disease progression and death in cats with HCM.[4,24-27] However, cats with SAM were younger than those without in these studies,[4,24-27] and the work in this thesis (Chapter 2) highlighted that 8% of cats will either gain or lose SAM each year. That is, 40% of cats may have either gained or lost SAM if the echocardiographic data was collected 5 years ago. Due to the variations in cardiac phenotype in cats with HCM, serial echocardiography appears necessary when investigating features of HCM that change over time.

Overall, HCM is a heterogeneous disease from the perspective of the cardiac phenotype and possible progression to death. Some cats with a 'normal'

echocardiogram may have early features of HCM and confound the results of the study. Future studies should carefully consider this heterogeneous manifestation of HCM when designing a study.

7.6 How should we use pathology findings?

Chapter 3, 4 and 7 included pathology information but these were not compared with echocardiographic assessment of the heart. The major reason for not comparing the pathology information with echocardiographic data was because these two methods assess different aspect of the cardiac pathology. Echocardiography provides valuable information on the cardiac dimensions, geometry, and function, but does not provide a reliable assessment of the myocardial tissue. In comparison, postmortem examination of the heart provides an accurate assessment on the myocardial tissue, but limited information on the cardiac dimensions due to rigor mortis, and no information on the myocardial function prior to death. Overall, the echocardiographic and postmortem exam data should be used to harmonise the assessment of cardiomyopathy rather than replacing one another. An example for this approach is shown in Chapter 7, where combined information from clinical examination, echocardiography, and postmortem exam allowed identification of significant myocardial ischemia in cats that developed congestive heart failure due to HCM.

7.7 Conclusion and future directions

The work in this thesis enhanced the current understanding on the prevalence and phenotypic progression of HCM in domestic cats of New Zealand. However, many mysteries of HCM remain to be explored and several new and pre-existing hypotheses can be considered for future studies.

As previously discussed, the stable prevalence of HCM across the United Kingdom, United States of America and New Zealand warrants further investigation. If the cause of HCM is considered primarily genetic, common pathogenic variants must be shared across three countries. It is possible that the genotype associated with HCM in cats was present even from the early ages of domestication of cats or

simply spread across the globe by regular imports and exports of cats by people. Alternatively, environmental factors that may trigger the HCM pathogenesis may be shared between the three countries, and such environmental factors may include household products, veterinary medications, and pet foods that are commonly shared between three countries. Studies that explore the genetic diversity, common environmental factors shared between three countries, and number of cats being imported and exported out of each of these three countries will likely explain the reason behind the stable prevalence of HCM across the three countries. Additionally, comparing the prevalence of HCM in wild cats with that of pet cats in each country will likely provide additional information on possible influence of environmental factors on the pathogenesis of HCM in domestic cats.

As highlighted in the work in this thesis, cats with normal left ventricular wall thickness may have an elongated anterior mitral valve leaflet as an early feature of HCM. Carefully assessing and recognising the early features of HCM is important when characterising healthy controls in future studies. For example, cats with normal echocardiogram but with elongated anterior mitral valve leaflet should not be included as healthy controls when investigating the potential causes of HCM, as these cats may be in the process of developing HCM at the time of data collection. This approach may lead to a greater success of finding genetic variants associated with HCM in cats other than the Maine Coon and Ragdoll breeds. However, it is important to note that these early features of HCM are also subject to change over time and some young cats without elongated anterior mitral valve leaflet may later develop elongated anterior mitral valve leaflet as demonstrated in Chapter 4. Therefore, one approach would be to only consider old cats (i.e., > 10-year-olds) with normal echocardiographic findings as healthy controls. Another approach is to include microscopic and molecular criteria to define normal controls. For example, cats with normal echocardiogram but with myofibre disarray and features of hypercontractility on microscopic and molecular tests may be in the early stages of the HCM disease process and these cats should not be used as healthy controls in genetic studies. Albeit, introducing such criteria will be expensive, invasive, and heavily reduce the number of eligible healthy controls. However, this approach may be necessary to take a step forward in understanding the genetic cause of HCM.

It is important to note that the prevalence of HCM in the general feline population is only investigated in the United Kingdom, United States of America, and

New Zealand. Therefore, prevalence studies are needed in other countries to better understand the global burden of feline HCM.

This thesis did not investigate treatment options for HCM in cats, but the work in this thesis suggests that the obstructive blood flow secondary to SAM is considered harmful in cats. However, SAM is labile and cats with HCM can spontaneously gain or lose SAM over time. The treatment of SAM with beta-blockers can be considered when the obstructive blood flow is considered severe;[17] however, the definition of severity of obstructive blood flow is not agreed in the clinical setting. Furthermore, while treating SAM with beta-blockers show immediate improvement on echocardiography, the treatment may not have a long-term benefit. However, the lack of long-term benefit may be explained by the spontaneous loss of SAM in some cats over time. It is possible that only cats with persistently severe obstructive blood flow secondary to SAM will benefit from medical intervention. Further studies that investigate SAM as a potential therapeutic target in the management of HCM should ideally screen for the presence of SAM using serial echocardiography.

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