Ultrastructural myocardial changes in seven cats with spontaneous hypertrophic cardiomyopathy

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Abstract *Objectives:* Hypertrophic cardiomyopathy (HCM) is the most common heart disease in cats and shares clinical and pathological characteristics with human HCM. Little is known about the pathogenic mechanisms underlying development of spontaneous feline HCM.
Animals: The study population consisted of seven cats diagnosed with HCM and eight age-matched cats with no evidence of cardiac disease.
Methods: Fresh myocardial biopsies taken from the middle of the left ventricular posterior free wall were obtained and examined with transmission electron microscopy.
Results: Electron microscopic examination showed ultrastructural aberrations of the myocardial cytoarchitecture and of the interstitium in the seven cats with HCM. In the most severely affected cats the myofibrils were disorganized and subsarcolemmal mitochondria were depleted. In control cats, contraction band artifacts were commonly seen.
Conclusions: In this preliminary study we show that ultrastructural changes of the myocardium in seven cats with HCM involve the cytoskeleton and mitochondria. We suggest that our findings are important for future research aiming at elucidating the pathogenic mechanisms underlying the phenotypic expression of feline HCM.

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The results of this study prompt for a larger scale study, including quantitative measurements of mitochondrial distribution and cytoskeletal derangements in feline HCM.

Abbreviations

2-D	two-dimensional mode of echocardiography
Ao	aorta
ARVC	arrythmogenic right ventricular cardiomyopathy
ATP	adenosine triphosphate
BW	body weight
CON	control cats
CS	citrate synthase
ECG	electrocardiogram
ECM	extracellular matrix
HCM	hypertrophic cardiomyopathy
HW	heart weight
IF	interfibrillar
IVS	interventricular septum
IVSd	interventricular septum measured in diastole
LV	left ventricle
LVFW	left ventricular free wall
LVFWd	left ventricular free wall measured in diastole
MYBPC3	myosin binding protein C
MYH7	β-myosin heavy-chain
PN	perinuclear
SS	subsarcolemmal
TEM	transmission electron microscopy
T4	thyroxine

Introduction

Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left-ventricular hypertrophy and diastolic dysfunction. Hypertrophic cardiomyopathy is the most commonly diagnosed cardiac disease in domestic cats.[1,2] The clinical and pathological characteristics of feline HCM resemble those of HCM in humans,[3,4] in which the disease occurs with a prevalence of 0.2%.

Phenotypically, HCM exhibits marked heterogeneity. The disease can remain subclinical throughout life or can lead to the development of life-threatening complications at all ages, such as congestive heart failure, thromboembolic events and even sudden cardiac death.[5]

Two known genetic missense mutations in genes encoding the sarcomere protein myosin binding protein C (MYBPC3) are associated with development of HCM in certain cat breeds.[6,7] One is the A31P mutation in Maine Coon cats that, in homozygous carriers, is associated with development of HCM at an early age.[6,8,9] The other is the R820W that is associated with development of HCM in Ragdoll cats.[7] Mutations associated with HCM have not yet been identified in any other cat breed.

In humans, approximately 1,500 mutations residing in at least 11 genes are currently known to cause HCM.[10] The vast majority of mutations reside in genes encoding the sarcomere thick filament proteins β-myosin heavy-chain (MYH7) and MYBPC3.[11] Less frequent are mutations encoding thin filament proteins or nonsarcomere proteins, including Z-disc components and calcium-handling proteins recognized to be involved in the development of HCM.[10,12,13]

The pathogenic mechanisms responsible for the development of HCM remain largely unknown. Depletion of cardiac muscle energy reserves has been suggested as a unifying factor in HCM, regardless of the underlying genotype.[14,15] The heart relies on a high constant supply of adenosine triphosphate (ATP) for contractile work of the muscle and for ion-pumping. The main producers of ATP in the cells are mitochondria. These organelles take up 22%—35% of the volume density of the myocardial cells in mammals[16] to meet the continuous, high energetic demands.

The structural distribution of mitochondria in feline HCM has not previously been investigated. A previous study investigated myocyte ultrastructure in cats with HCM, but data regarding cat breed, clinical characteristics and genotype were not reported.[17]

The aim of the present observational study was comparison between the myocardial ultrastructure from seven cats of different breeds and with various phenotypic expressions of HCM with the myocardium from eight apparently healthy control

cats. According to the perception that the HCM heart is energy depleted, we hypothesized that alterations of the cardiac mitochondria would be present in cats with HCM.

Animals, materials and methods

The study was approved by the Danish Animal Experimental Inspectorate and conformed to The European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Client-owned cats were recruited through a national screening and breeding program for feline HCM, taking place at the University of Copenhagen, Hospital for Companion Animals. Written informed consent was obtained from owners of all the cats prior to their inclusion in the study.

Cats that had previously been diagnosed with feline HCM, based on echocardiographic findings and exclusion of other causes of ventricular hypertrophy, were recruited at the time the owners requested euthanasia of their animal. Pedigree-bred cats above 1 year of age that had not received any medical treatment other than cardiac medicine, contraception and deworming agents within the last 3 months and that did not have any apparent systemic, infectious or metabolic diseases were eligible for the study. A pedigree was provided by the owners of each cat.

The majority of cats recruited in the study were Maine Coon and Norwegian Forest cats. These breeds are frequently presented for echocardiographic examinations prior to breeding. Among the HCM cats were two British Shorthair cats and one Exotic Shorthair cat (Table 1).

Behavioral matters and gingivitis were the primary complaints by owners of control cats, but complaints also included abnormalities related to the eyelids, skin, coat and skeleton. Among the HCM cats, the reasons for euthanasia were progression of cardiac disease since the last visit, behavioral matters, gingivitis and nonspecific clinical signs of heart disease such as restlessness and anorexia.

Clinical characterization

Nine cats that had previously been diagnosed with HCM (one previously categorized as equivocal) and eight age-matched cats, eligible for inclusion in the control group, had physical examination, blood pressure measurement, electrocardiogram echocardiography, routine serum biochemistry, hematology, and total thyroxine measurements

Table 1 Anthropometric and echocardiographic characteristics of the cats.

Cat #	Breed	Genotype	Sex	Age, years	LVFWd	IVSd	LV hypertrophy	LA:Ao	Shortening fraction, %	HW/BW	Other
HCM											
HCM1	BSH	—	mc	5.7	7.05	6.79	Moderate	2.8	56	0.51	Arterial thromboembolism
HCM2	MCO	CC	mc	6.2	11.80	4.99	Severe	2.0	47	0.56	LVOTO
HCM3	MCO	CC	mc	2.2	8.17	7.95	Severe	3.4	51	0.57	Congestive heart failure, LVOTO
HCM4	ESH	—	mc	5.9	7.17	7.40	Moderate	1.1	48	0.51	LVOTO
HCM5	MCO	CC	fc	8.9	6.80	5.50	Mild	1.3	41	0.34	LVOTO
HCM6	BSH	—	mc	5.5	6.04	6.56	Moderate	2.3	38	0.47	LVOTO
HCM7	NFO	—	mc	8.7	7.87	7.14	Mild	1.8	50	0.42	Atrial fibrillation, LVOTO
Control											
CON1	MCO	GG	fc	7.1	4.00	4.10	—	1.2	44	0.35	
CON2	MCO	GG	mc	2.4	3.92	3.95	—	0.9	28	0.35	
CON3	MCO	GG	f	3.8	4.53	4.07	—	1.0	41	0.32	
CON4	MCO	GG	mc	2.9	4.40	4.46	—	1.1	57	0.31	
CON5	MCO	GG	f	3.3	3.97	3.93	—	1.3	45	0.28	
CON6	NFO	—	f	11.0	4.32	3.9	—	1.1	29	0.29	
CON7	NFO	—	mc	7.8	4.43	4.62		1.2	39	0.37	
CON8	NFO	—	mc	7.5	3.46	4.31		1.1	21	0.26	

MCO, Maine Coon cat; NFO, Norwegian Forest cat; ESH, Exotic Shorthair cat; BSH, British Shorthair cat; CC, homozygous for the A31P mutation in MYBPC3; GG, heterozygous (wild-type) for the A31P mutation in MYBPC3; f, female; fc, female castrate; mc, male castrate; LVFWd, left ventricular free wall in diastole; IVSd, interventricular septum in diastole, HW, heart weight; BW, body weight; LVOTO, left ventricular outflow tract obstruction.

performed. Cats of the breed Maine Coon were genetically screened for the A31P mutation in MYBPC3.[6]

A diagnosis of feline HCM was based on echocardiographic assessment of diastolic thickness of the left ventricular free wall (LVFWd) and the interventricular septum (IVSd), as previously described.[18] In brief, cats were classified as HCM-positive when the maximal diastolic thickness of the LVFWd, IVSd or both exceeded 5.5 mm and there were no indications of other causes of left ventricular hypertrophy on the clinical examination, blood tests and blood pressure measurements. Cats that were equivocal for HCM, i.e., a maximum LVFWd of 5.0—5.5 mm, were excluded from the study.

Tissue sampling and preparation

In HCM cats and control cats, euthanasia was induced with an intramuscular injection of dexmedetomidine, followed by an intravenous injection of pentobarbitone (150 mg/kg). Immediately after cardiac arrest, the heart was rapidly collected through a left lateral thoracotomy, and the wet weight recorded. One to three myocardial samples from the posterior LVFW, proximal to the papillary muscle, were obtained and cut into ≈ 1 mm^3 pieces. The remaining part of the LVFW was used in two other studies, investigating the myocardial cell bioenergetics in the normal feline heart[19] and in feline HCM.[20]

The biopsy samples were immediately transferred into freshly prepared 3% glutaraldehyde in 0.1 M Na-phosphate buffer (pH 7.2—7.4), fixed at 4 °C for 90 min and washed in phosphate buffer. Postfixation was performed in 1% osmium tetroxide for 60 min in the same buffer, followed by dehydration in a graded series of ethanol and embedding in Epon, as previously described.[21]

Semithin sections (1—2 μm) were cut and stained with toluidine blue. Regions of interest were selected via a light microscope and photographed on the basis of the presence of longitudinally oriented myofibers. The diameter of 3—5 longitudinally oriented, adjacent myofibers were measured on the light microscopic images.

The biopsy samples from two HCM cats contained only transverse fibers as evaluated by light microscopy and were excluded from further analysis.

Ultrathin sections (50—70 nm) of the myocardium were mounted on formvar-coated slot grids (2×1 mm) and contrasted, according to standard procedures.[21]

Transmission electron microscopy

Sections from two biopsies were analyzed from each cat. Transmission electron microscopy (TEM) was performed[c] at the University of Copenhagen, Core Facilities for Integrated Microscopy.

One person who was not blinded to the diagnosis of the cats performed the photographing and analyses of the myocardial biopsies from all the cats. Initially, the whole specimen, expanding approximately 0.1 mm^2, was inspected at 1870× magnification to get an overall impression of the sample quality and to evaluate the specific ultrastructural structures and abnormalities. Intracellular and extracellular structures were photographed at magnifications between 1870× and 46000×.

Then, from three different cardiac cells in each specimen, the following cellular compartments were photographed at 5800× and at 9700× magnifications, respectively, in a nonrandom fashion: (1) the lateral border of adjacent cardiomyocytes, including myofibrils, interfibrillar (IF) mitochondria, subsarcolemmal (SS) mitochondria, sarcolemma and extracellular space, (2) the intercalated disc, delineating two cardiomyocytes with surrounding myofibrillar strands and IF mitochondria and (3) the nucleus of a cardiomyocyte with its detached perinuclear (PN) mitochondria and surrounding tissue.

On the images captured at 5800× magnification, the maximum diameter of the cardiac cell nuclei was measured. Each of the mitochondrial subpopulations was investigated on the images. On electron micrographs at 5800× magnification, the minimum and maximum mitochondrial diameters of IF and PN mitochondria were measured. At 9700× magnification, the specific ultrastructure of the mitochondria was assessed.

Measurements of distances and image processing were performed using image processing software[d,e].

Statistics

For comparison of the continuous variables describing anthropometric and clinical data (age, body weight [BW], heart weight (HW), HW/BW ratio), an un-paired Student's *t*-test was used with

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[c] CM 100 BioTWIN, Philips, Eindhoven, The Netherlands.
[d] Image J.47 NIH, Bethesda, MD, USA.
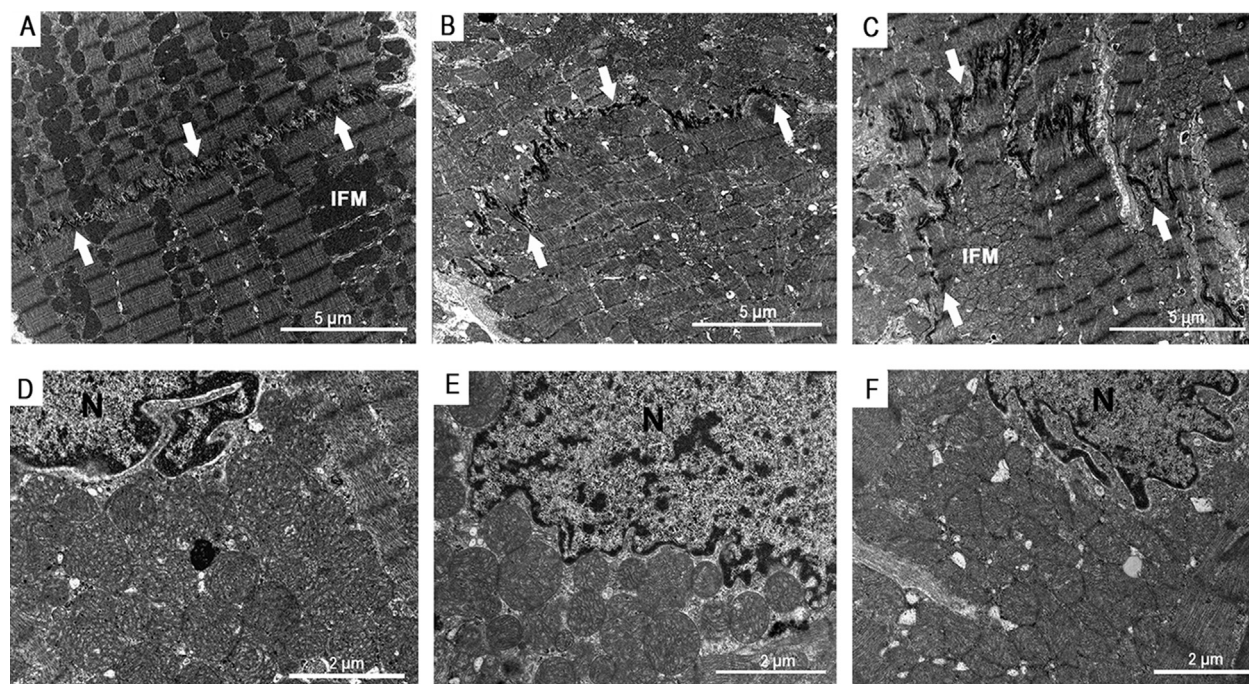[e] Adobe Photoshop CC 2014, Adobe Systems Software Ireland Ltd., Dublin, Ireland.

Fig. 1 Myocardial, intercalated disc and perinuclear mitochondrial ultrastructural organization. (A) In this control cat (CON3), the myofibrils are aligned in parallel rows. The intercalated disc is regularly arranged transversely across the cardiomyocyte (outlined by arrows). IFM, interfibrillar mitochondria. (B) In this cat with mild hypertrophy of the left ventricle (HCM7), the myofibrils are disorganized and the Z-lines are irregular. The ultrastructure of the intercalated disc is disrupted showing an irregular shape (outlined by arrows). (C) Myocardium from a cat with severe hypertrophy of the left ventricle (HCM2) showing myofibrillar disorganization, clumping of the IFM and marked disruption of the ultrastructure of the intercalated disc showing severe disorganization and interdigitation (outlined by arrows). (D) Perinuclear mitochondria at the pole of a cardiomyocyte nucleus (N) from the control cat (CON3) shown in panel A. The mitochondria are tightly packed and of varying sizes. (E) Perinuclear mitochondria from the same HCM cat (HCM7) as shown in panel B. The mitochondrial ultrastructure is preserved but the organelles are less densely packed and are of varying sizes. N, nucleus. (F) Perinuclear mitochondria from the same HCM cat (HCM2) as shown in panel C. There is variation in their size but mitochondrial ultrastructure is preserved.

statistical significance set at $p<0.05$. Data were analyzed using commercial statistics software[f]

Results

Baseline characteristics and echocardiographic measures of the seven HCM cats (six male/one female) and eight control cats (four male/four female) included in the study are presented in Table 1.

In the control group, two Maine Coon cats (CON 3 and CON 4) and two Norwegian Forest cats (CON6 and CON8), respectively, were parents and offspring. Among the HCM cats, none were close relatives i.e., brothers and sisters, parents and offspring, neither to each other nor to any of the control cats.

Hypertrophic cardiomyopathy cats and controls were not significantly different regarding age and body weight. The heart weight to BW ratio was significantly higher in HCM cats (4.5 ± 0.3) than in controls (3.3 ± 0.1; $p<0.001$).

All HCM cats had diffuse, asymmetrical hypertrophy of the left ventricle. The LVFW was hypertrophied in all the cats (Table 1). Five out of the seven cats had additional hypertrophy of the IVS (Table 1).

Left ventricular hypertrophy was arbitrarily categorized as mild, moderate or severe[22] and the left atrium was considered enlarged when the left-atrium-to-aorta ratio >1.5 (Table 1). None of the cats had decreased fractional shortening, indicating that the systolic function was preserved (Table 1). Four HCM cats received medical treatment consisting of an angiotensin converting enzyme inhibitor (benazepril, 0.4 mg/kg BW once daily or ramipril, 0.2 mg/kg BW once daily) as either monotherapy or in combination with a β-

adrenoceptor—blocking agent (atenolol, 6.25—12.5 mg/cat once daily).

Myocardial ultrastructure

The ultrastructural findings of the myocardium from control cats were consistent with existing literature of normal feline myocardial ultrastructure.[23] The IF, PN and SS mitochondria were identified in the specimens from all cats. Although PN mitochondria are often functionally regarded as IF mitochondria,[24—26] they are here described as a separate subpopulation because of their distinct localization adjacent to the nucleus.[23,27,28]

In the control cats, myofibrils were orderly organized in parallel rows with IF mitochondria arranged in rows between them (Fig. 1A). The intercalated discs had a regular structure, consisting of small pleats and with their transverse sections running perpendicular to the long axis of the myofibrils (Fig. 1A).

In HCM cats, disorganization of the myofibrils was evident. The ultrastructure of the intercalated discs was disrupted as seen by moderate and severe irregular folding and interdigitation (Fig. 1B and C).

Interfibrillar mitochondria were often organized into clusters (Fig. 1C) rather than being dispersed in regular rows between the myofibrils. In other areas, the mitochondria were replaced by fibrous tissue. There was no apparent change in the overall content of IF mitochondria.

In two cats with mild to moderate hypertrophy (HCM4 and HCM7), the normal ultrastructure of the cardiomyocytes was largely preserved. On the contrary, one cat with only mild hypertrophy on echocardiography (HCM5) had severe ultrastructural changes of the myocardium with marked myofibrillar disorganization. Noticeably, this cat was a Maine Coon cat, homozygous for the mutation in MYBPC3, and therefore at high risk of developing severe HCM.[4,8,9] In the remaining four HCM cats, diffuse myofibrillar disorganization and myofiber disarray were present.

In each of the seven cats with HCM, the diameters of IF mitochondria were similar to the length of the adjacent sarcomere, typically approximately 1.0 μm and always less than 2.0 μm.

Contraction band artifacts[29,30] were commonly found in the myofibers of control cats and less so in cats with HCM. In those cardiomyocytes where contraction band artifacts were abundant, mitochondria were packed tightly and were elongated in the transverse direction.

The PN mitochondria were characterized as pleomorphic in control cats and HCM cats. Similar diameters of PN were seen in the two groups (CON: 0.2—1.9 μm vs. HCM: 0.2—1.8 μm). In the control cats, PN mitochondria were placed in densely packed clusters at the nuclear poles (Fig. 1D). This structure was largely preserved in the HCM cats, although in some cats, cytoplasms surrounding the PN mitochondria seemed to be more abundant (Fig. 1E). The normal dense cristae, formed by the inner mitochondrial membranes, were preserved in the mitochondria of the HCM cats (Fig. 1E and F).

Ultrastructural organization of Z-discs

A common finding in cats with HCM was elongated, electron-dense rods extending from the Z-discs (Fig. 2). We did not find these structures in any of the control cats, although their presence has been
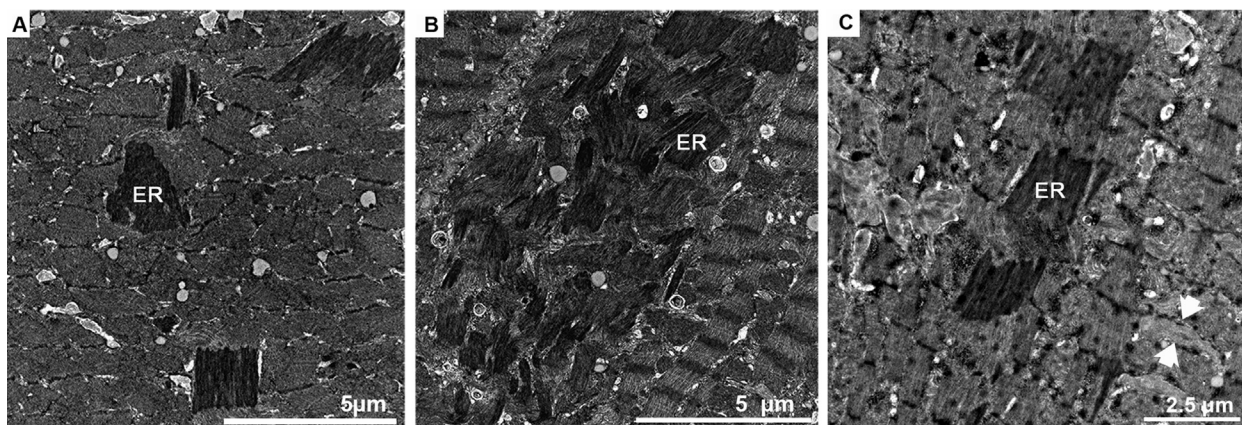

Fig. 2 Electron-dense rods in three HCM cats. (**A**) Electron-dense rods (ERs) in the myocardium from a cat with mild hypertrophy (HCM5). There is significant disorganization of the myofibrils and Z-discs are irregular. (**B**) HCM cat with moderate hypertrophy (HCM4) and significant accumulation of ERs. (**C**) HCM cat with severe hypertrophy (HCM3), showing presence of ERs, myofibrillar disorganization, irregular Z-discs and distended T-tubules (arrows).

reported in aging cats.[31] Electron-dense rods were abundant in cats regardless of the severity of hypertrophy (Fig. 2A—C).

Irregular and disrupted Z-discs were abundant in the disorganized myofibrils of cats with HCM (Fig. 2A and C).

Sarcoplasma ultrastructural alterations

In the normal feline myocardium, the sarcolemma displays a scalloping appearance, with deep invaginations located at the Z-discs.[23] Sub-sarcolemmal mitochondria are located beneath the sarcolemma, often with one mitochondrion for each sarcomere (Fig. 3A and B). Omega-shaped caveolae are present, randomly distributed along the sarcolemma and this was seen in the control cats (Fig. 3B).

In cats with mild hypertrophy, the scalloping appearance of the sarcolemma was preserved but clumping of SS mitochondria was observed in some cells. In cats with moderate to severe LV
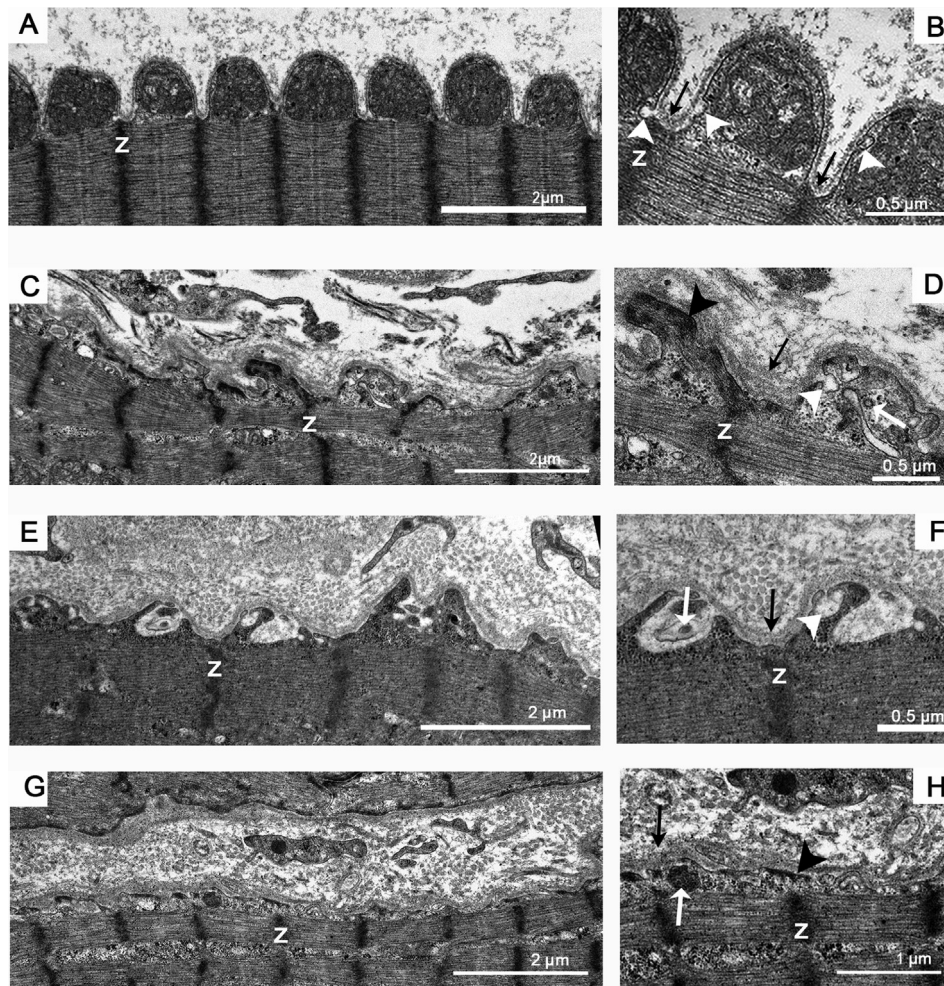

Fig. 3   Ultrastructural alterations of the myocardial sarcolemma with feline HCM. (**A**) Photomicrograph illustrating the normal hollow-crest structure of the sarcolemma and normal organization of subsarcolemmal mitochondria (SSM) with one mitochondria for each sarcomere in a control cat (CON3). Z, Z-disc. (**B**) High magnification of the sarcolemma from the same cat (CON3) as shown in panel A. The laminar coat is sparse and there is close proximity between the Z-discs and the sarcolemma at the costameres (black arrows). Omega-shaped caveolae are randomly distributed along the sarco-lemma (white arrowheads). (**C—H**) Sarcolemmal and subsarcolemmal structures aberrations in HCM cats. There is marked disruption of the sarcolemmal ultrastructure causing the caveolae to be distorted (white arrowheads). The thickness of the laminar coat is increased (black arrows) resulting in a loss of contact between the sarcolemma and the Z-discs (Z). Normal ultrastructure of SSM is absent and only remnants of mitochondria in the subsarcolemmal space are present (white arrows). Along the sarcolemma there are deposits of electron dense Z-disc material (black arrowheads). (**C and D**) HCM cat (HCM6) with moderate hypertrophy of the left ventricle. (**E and F**) HCM cat (HCM2) with severe hypertrophy of the left ventricle. (**G and H**) HCM cat (HCM3) with severe hypertrophy of the left ventricle. The right panels show high magnification of the left panels. Z marks the identical Z-disc in the left and right panels.

hypertrophy, there were areas with flattened sarcolemma, lining of the sarcolemma with electron-dense Z-disc material, and depletion and absence of SS mitochondria (Fig. 3C—H). In cats with severe hypertrophy, the sarcolemmal ultrastructure was observed to be severely altered compared to control cats. Alterations included loss of the normal hollow crest structure, presence of membrane bound vacuoles, and disruption of the caveolae.

Moreover, depletion and absence of the SS mitochondria became evident (Fig. 3C—H).

Other structures associated with HCM

The nuclei of cardiomyocytes were irregularly shaped and often contained prominent nucleoli (Fig. 4A and B), as previously described in hearts from both normal and HCM cats.[17,23] The maximum
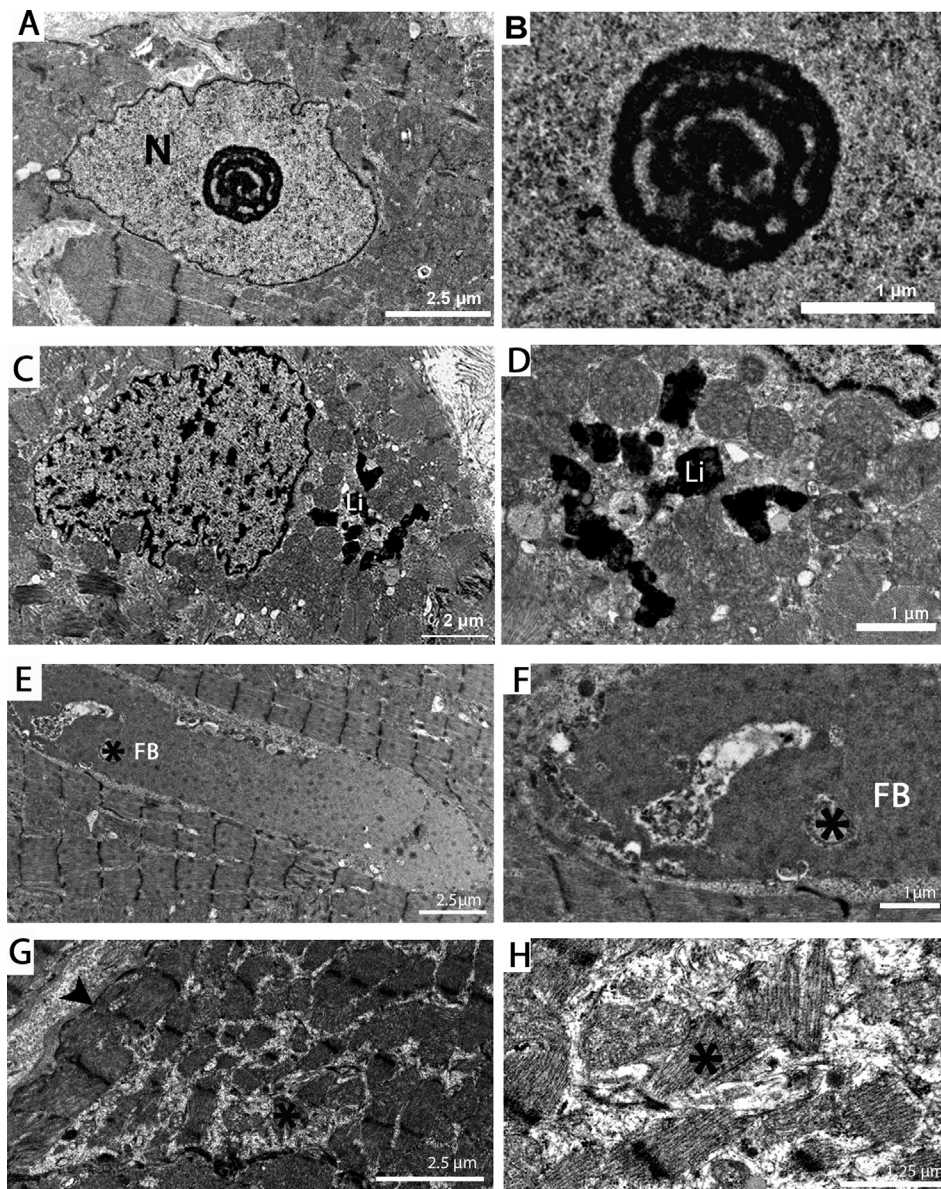

Fig. 4 Various ultrastructural findings in the myocardium from HCM cats. (**A**) Nucleus (N) with prominent nucleolus in an HCM cat (HCM2). (**B**) High magnification of the nucleolus from panel A. (**C**) Moderate amount of lipofuscin (Li) granules found at the nuclear pole in this HCM cat (HCM3). (**D**) High magnification of the lipofuscin granules (Li) shown in C. (**E**) A filamentous body (FB) was present in a cardiomyocyte from this cat (HCM7) with severe hypertrophy. (**F**) High magnification of the FB shown in F. (**G**) Myofibrillar degeneration in a cat (HCM 3) with severe hypertrophy. There is absence of SS mitochondria, a thickened laminar coat and deposition of electron-dense Z-disc material (arrowheads) in this cell. (**H**) High magnification of myofibrillar degenerative changes shown in panel G. Asterisks mark the same location in panels G and H.

diameter of the nucleus was similar in HCM cats (7.5 ± 0.5 μm) and control cats (7.0 ± 1.4 μm; $p=0.3$). This in accordance with a recent light microscopic study.[32]

Scattered among the PN mitochondria were lipofuscin granules, found in controls and in HCM cats in equal amounts (Fig. 4C and D). In a cat with severe hypertrophy, an inclusion body 21.0 μm long and 4.5 μm wide was present between two myofibrils. The content of this structure was filamentous amorphous material, consistent with degenerated myofibrils (Fig. 4E and F). Focal areas of myofiber degeneration were occasionally seen in cats with severe hypertrophy (Fig. 4G and H).

Interstitium ultrastructure

The laminar coat, consisting of a micro-threaded electron-dense extracellular matrix (ECM) was sparse in the control cats (Fig. 3A and B). Small amounts of type III collagen were recognized in the interstitium as characteristic electron-dense fibrils with a diameter of approximately 120 nm. Each of the seven HCM cats had some degree of interstitial remodeling, characterized by endomysial fibrosis (Figs. 3C–H and 5A and B). A common finding in cats with HCM was increased thickness of the laminar coat (Fig. 3C–H, 5E and F) and bundles of collagen fibrils in the intercellular space (Fig. 5A and B).

Extracellular matrix, identical to that forming the laminar coat, was found within tubular structures that were consistent with T-tubuli, causing them to be dilated and distorted (Fig. 5C and D). In four of the seven HCM cats (HCM2–HCM5), the ECM replaced major areas of the myocardium by forming interdigitating sheets between the myofibrils (Fig. 5E and F).

Discussion

We here present the findings of an electron microscopic study of fresh myocardial biopsies obtained from seven purebred cats with various phenotypic and genotypic expressions of spontaneously occurring HCM.

The following changes of myocardial ultrastructure were prominent among the HCM cats: remodeling of the myofibrils and IF mitochondria, changes of Z-disc morphology, sarcolemmal remodeling with depletion of the SS mitochondria, and endomysial fibrosis. In severely affected HCM cats, myofibrillar degeneration was present.
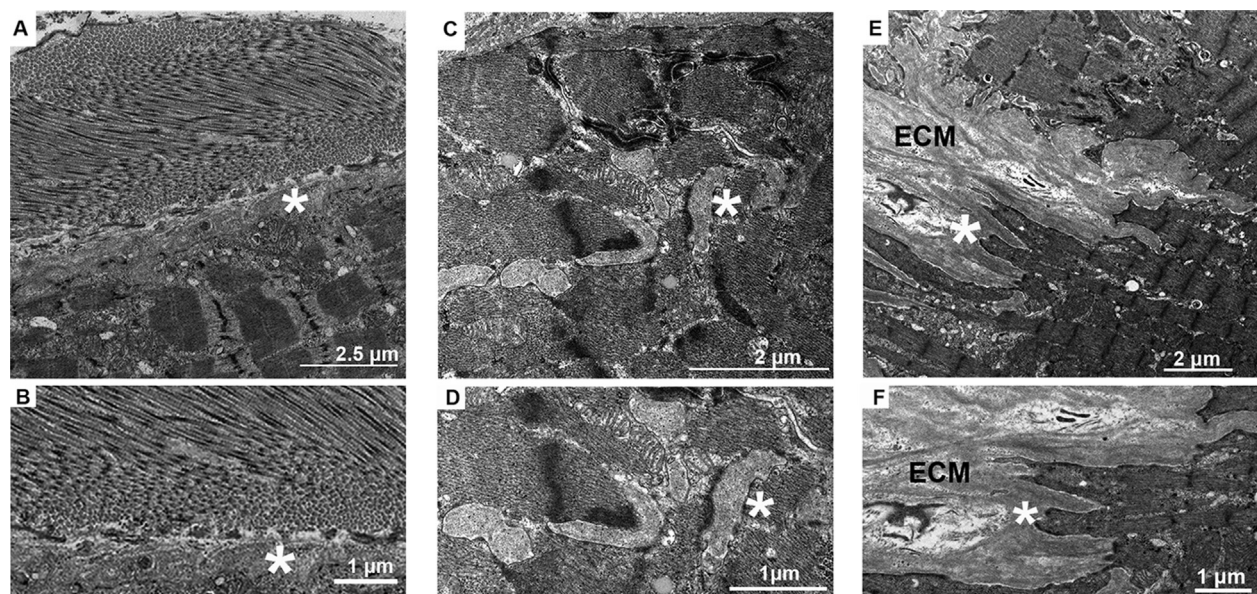

Fig. 5 Interstitium changes in HCM cats. (**A**) A thick bundle of collagen fibrils (CFs) is present in the extracellular space of this HCM cat (HCM6). The lower panel (**B**) shows high magnification of the CFs. (**C**) Oblique section of the myocardium in this HCM cat (HCM3), showing accumulation of electron-dense micro-thread matrix localized to structures consistent with T-tubuli. The lower panel (**D**) shows high magnification of tubular organization of matrix within the tubular structures. (**E**) Excessive amounts of the extracellular matrix (ECM) replace the normal myocardial ultrastructure in this homozygous Maine Coon cat with mild hypertrophy (HCM5). The lower panel (**F**) shows sheets of ECM interdigitating with projections of the cardiomyocyte. Asterisks mark the same location in the upper and lower panels.

To the best of our knowledge there are no previous reports on the mitochondrial ultrastructure in spontaneous feline HCM. We expected to find a decrease in the mitochondrial content in HCM hearts, following our recent finding[20] of decreased enzymatic activity of citrate synthase (CS), which is a marker of mitochondrial content.[33]

Previous studies assessing mitochondrial volume density in conditions causing cardiac hypertrophy have revealed controversial findings, showing either increased[34−36] or decreased[37−39] mitochondrial content. A transient increase in mitochondrial volume density followed by a decrease has also been reported with progression of cardiac hypertrophy.[40,41]

The presence of giant IF mitochondria expanding the length of several sarcomeres has been reported with various aquired and congenital forms of cardiomyopathy.[42−44]

In the seven cats with HCM, the overall density of cardiac IF and PN mitochondria was apparently preserved, although the spatial organization of the mitochondria was modified when myofibrils were disorganized. In cats with moderate and severe hypertrophy included in our study, it was a characteristic finding that SS mitochondria from the SS cellular compartment were depleted. Despite the lack of quantitative measurements of mitochondrial density in this preliminary study we suspect depletion of the SS mitochondria to be responsible for an overall decrease in mitochondrial content in feline HCM.

Controversy exists as to whether IF mitochondria and SS mitochondria are two spatially distinct subpopulations in muscle.[46] There is substantial evidence showing that SS mitochondria and IF mitochondria have different functional biochemical properties in the mammalian myocardium.[24,25,27] In animal models of cardiac disease, the SS mitochondria and IF mitochondria have been shown to be unequally susceptible to injury.[26,47,48] Our finding of SS mitochondrial depletion in late stage feline HCM supports this hypothesis. Future studies, using advanced imaging modalities to differentiate between mitochondrial subpopulations,[46] will be highly relevant to elucidate this matter.

Expansion of the ECM was profound in cats with HCM compared to control cats, regardless of the degree of hypertrophy. Excessive ECM formation in heart muscle can act as a substrate for the development of cardiac arrhythmias.[49] Functionally, T-tubules are responsible for calcium homeostasis and for controlling excitation-contraction coupling in muscle cells. Dysfunction

of the T-tubular system can compromise cardiac function and disturb calcium homeostasis.[50] While only speculative at this point, our findings of excessive ECM expansion in HCM hearts raise the suspicion that ECM accumulation may be involved in the development of arrhythmias that are known to occur in feline HCM.[51]

Our finding of electron-dense rods in seven HCM cats is in accordance with a previous study where similar structures were found in the hearts of cats with HCM.[17]

We did not find electron-dense rods in any of the control cats. This elicits the suspicion that these structures represent a pathological finding. One previous study reported electron-dense rods in three apparently healthy cats.[31] However, cardiac diseases were not excluded in these cats and their ages were unknown. It cannot therefore be excluded that these cats had cardiac disease and that electron-dense rods occur in the aging cat heart. Moreover, a recent study in dogs diagnosed with arrhythmogenic right-ventricular cardiomyopathy found electron-dense rods in diseased dogs but not in control dogs.[52]

The electron-dense rods that were visualized in HCM cats and boxer dogs with arrhythmogenic right-ventricular cardiomyopathy (ARVC) resemble nemaline rods found in human congenital skeletal myopathies and less commonly in cardiac muscle.[53−55] Nemaline myopathy is known to arise from mutations in thin filament actin proteins and in proteins related to alpha-actin, nebulin, tropomyosin and troponin-T.[55,56] Z-disc proteins are important for cardiac muscle cell structure[57] and play essential roles in cell signaling and stress sensing.[58,59]

At present, the significance of our finding of electron-dense rods in feline HCM remains elusive. While in humans, at least seven mutations in Z-disc proteins are related to development of human HCM[60] our findings may warrant further investigation of Z-disc protein changes in feline HCM.

Some limitations of our study should be considered. One limiting factor was the small study population. Feline HCM is characterized as a heterogeneous disease with a broad range of clinical and pathological presentations. Analyses of myocardial biopsies from seven HCM cats do not cover the morphological spectrum of feline HCM. It is likely that additional ultrastructural aberrations exist in this disease entity. There may even be breed specific ultrastructural characteristics which have been missed because of the inclusion of only four breeds and a small number of cats. Opposite, we did observe prominent unifying changes such as electron-dense rods in cats with various

phenotypic and genotypic expressions of HCM. These are important findings that may direct future research into exploring the role of structural protein disturbances in the development of feline HCM.

The observer performing the TEM was not blinded to the diagnosis of the cats. It must be underlined that each specimen was scanned in it whole area. Only morphological findings that were significant and widely distributed are described.

Finally, limitations of the sample quality (i.e., low contrast between IF mitochondria and myofibrils and presence of contraction band artifacts) prevented us from performing quantitative measurements of the mitochondrial density. Contraction bands are commonly found in freshly prepared biopsies and are not regarded as pathological changes.[45] They were more pronounced in the control cats than in cats with HCM and this difference would bias the myofibrillar-to-mitochondrial ratio. Quantification of the mitochondrial density and other ultrastructural changes would indeed strengthen the presentation. Future perspectives include investigation by the use of stereology in a larger number of cats from breeds that are predisposed to developing HCM.

Conclusions

The present study provides preliminary data of myocardial ultrastructural aberrations seen with TEM in seven cats with well-defined phenotypical expressions of HCM. Prominent changes of the myocardial ultrastructure include cytoarchitectural changes, excessive ECM formation and a presumed depletion of mitochondrial mass in the SS portion of the cardiomyocytes.

Mitochondrial structural depletion corresponds well with our previous findings of reduced mitochondrial function and overall mass in the feline HCM heart[20] and with the hypothesis that the HCM heart is energy depleted.[61,62]

Based on these preliminary findings, we propose cytoskeletal proteins and/or mitochondrial proteins to be considered of importance when searching for disease-causing genetic mutations or gene variants involved in feline HCM.

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Conflict of Interest Statement

The authors do not have any conflicts of interest to disclose.

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