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Prospective Echocardiographic and Tissue Doppler Imaging Screening of a Population of Maine Coon Cats Tested for the A31P Mutation in the Myosin-Binding Protein C Gene: A Specific Analysis of the Heterozygous Status

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Background: A mutation in the sarcomeric gene coding for the myosin-binding protein C gene has been identified in a colony of Maine Coon cats with hypertrophic cardiomyopathy (MyBPC3-A31P mutation). However, the close correlation between genotype and phenotype (left ventricular hypertrophy [LVH] and dysfunction) has never been assessed in a large population, particularly in heterozygous (Hetero) cats.

Objectives: To investigate LV morphology and function with echocardiography and tissue Doppler imaging (TDI) in a population of Maine Coon cats tested for the MyBPC3-A31P mutation with focus on Hetero animals.

Animals: Ninety-six Maine Coon cats.

Methods: Prospective observational study. Cats were screened for the MyBPC3-A31P mutation and examined with both echocardiography and 2-dimensional color TDI.

Results: Fifty-two out of 96 cats did not have the mutation (wild-type genotype, Homo WT), 38/96 and 6/96 were Hetero- and homozygous-mutated (Homo M) cats, respectively. Only 11% of Hetero cats (4/38) had LVH and 29% (10/34) of Hetero cats without LVH were >4 years old (4.1–11.5 years). LVH was also detected in 2 Homo WT cats (4%). A significantly decreased ($P < .05$) longitudinal E/A (ratio between early and late diastolic myocardial velocities) in the basal segment of the interventricular septum was observed in Hetero cats without LVH (n = 34) compared with Homo WT cats without LVH (n = 50), thus confirming that the Hetero status is associated with regional diastolic dysfunction ($P < .05$).

Conclusions: The heterozygous status is not consistently associated with LVH and major myocardial dysfunction. Moreover, Homo WT cats can also develop LVH, suggesting that other genetic causes might be implicated.

Key words: Cardiomyopathy; Echocardiography; Feline; Genotype.

Hypertrophic cardiomyopathy (HCM) is the most common feline heart disease and remains a major cause of morbidity and mortality associated with risk of sudden death, congestive heart failure, and aortic thromboembolism.[1,2] This primary myocardial disorder is phenotypically characterized by increased cardiac mass with a hypertrophied nondilated left ventricle (LV)[3–5] in the absence of an obvious secondary cause of left ventricular hypertrophy (LVH) such as systemic arterial hypertension or hyperthyroidism.[3,4]

In humans, HCM is inherited, usually as an autosomal dominant trait, in at least 60% of cases,[6] and to date 450 independent causative mutations in 13 myofilament-related genes have been identified.[7–10]

A causative mutation in the cardiac myosin-binding protein C (MyBPC3) sarcomeric gene for inherited HCM has been identified in Maine Coon cats.[11] MyBPC3 gene was shown to be mutated in the exon 3 (Guanine to Cytosine) inducing the production of an aberrant protein by changing a conserved amino acid from the alanine (A) of the 31st codon to proline (P) (MyBPC3-A31P mutation). In this study, the phenotypes of carrier and homozygous-mutated (Homo M) cats varied from mild to severe myocardial hypertrophy with some developing congestive heart failure or sudden death. However, according to the authors the number of affected cats was too small, ie, 10 heterozygous (Hetero) and 6 Homo M, to suggest that disease outcome was related to the homozygosity or heterozygosity of the mutation.[11]

Tissue Doppler imaging (TDI) is a relatively recent ultrasound technique[12] that offers a regional noninvasive and quantitative analysis of myocardial radial[13] and longitudinal motions in the awake cat.[14–21] Two-dimensional (2D) color TDI affords satisfactory repeatability and reproducibility in cats[15] and this mode is more sensitive than conventional echocardiography in detecting myocardial dysfunction in both spontaneous feline HCM and in a feline model of the disease (the dystrophin-deficient hypertrophic muscular dystrophy or HFMD model) before occurrence of left ventricular free wall (LVFW) hypertrophy.[14,22] In a recent study, TDI-assessed early diastolic (Em) or summated early and late diastolic (EAsum) mitral annular velocities were shown to be incrementally reduced from normal cats to Maine Coon cats with only an abnormal genotype to Maine Coon cats with both the abnormal genotype and LVH, although Em or EAsum was insensitive for identifying genotypically affected cats with a normal phenotype.[21] However, in the latter report the pulsed-wave TDI mode

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(and not the 2D color mode) was used, and measurements were only performed in a single segment (lateral mitral annulus) for a single motion (longitudinal). Moreover, only a small number of Maine Coon cats with an abnormal genotype but no LVH were involved (n = 6), and the control group consisted of normal Domestic Shorthair cats (and not genetically unaffected Maine Coon cats). Thus, to the best of our knowledge, regional radial and longitudinal LVFW and interventricular septal (IVS) functions have never been assessed together in a large population of genetically affected (Hetero and Homo M) Maine Coon cats compared with genetically unaffected (homozygous wild type, Homo WT) cats of the same breed with 2D color TDI.

The aims of this prospective study were therefore (1) to investigate the close correlation between genotype and phenotype with both conventional echocardiography and 2D color TDI in a large population of Maine Coon cats screened for the MyBPC3-A31P mutation with focus on Hetero animals; (2) to compare LVFW and IVS TDI parameters between Homo WT and Hetero cats without LVH in order to determine if myocardial dysfunction could be present in nonhypertrophied wall segments of Hetero cats.

Material and Methods

Animals

Maine Coon cats screened for the MyBPC3-A31P mutation were prospectively recruited at the Cardiology Unit of Alfort (France) between February 2006 and December 2007. Owner's or breeder's consent was obtained before enrollment in the present study. Cats that were receiving cardiac medication (ie, angiotensin-converting enzyme inhibitor, diltiazem, furosemide) were not included in the study. A complete physical examination, ECG, blood pressure measurement, conventional echocardiography, and 2D color TDI examinations were performed on all animals.

Classification of the Maine Coon Cat Study Population. The study population was divided into 3 groups according to genotype (presence or absence of the MyBPC3-A31P mutation described by Meurs et al[11] based on direct DNA sequence analysis). Thus, Maine Coon cats were considered as Homo WT if the MyBPC3 mutation was absent, or as homozygous mutated and heterozygous if the mutation was present (Homo M and Hetero, respectively). These 3 groups of cats were further divided according to phenotype, ie, cats with or without HCM assessed by conventional echocardiographic examination (see details below).

Confirmation of the HCM Phenotype. A LVH pattern was determined by conventional echocardiography if the M-mode exam revealed LVFW or IVS end-diastolic thicknesses >6 mm[5] or if a subaortic IVS diastolic hypertrophy (>6 mm) was detected by the 2D right-side parasternal long-axis view of the heart.[23] In cats with a LVH pattern, HCM was diagnosed after excluding both systemic arterial hypertension (systolic arterial blood pressure [SABP] $>$ 160 mmHg in unstressed animals)[24] and hyperthyroidism in cats >4 years old (total thyroxin plasma levels [T4], reference interval: 10–50 nmol/L).

Blood Pressure Measurements

SABP was indirectly measured in awake cats, and by the same trained observers (CCS, VG, FS), by the standard Doppler method.[a] Several measurements were taken over 5–10 minutes to obtain the average of 5 values from a stable set of readings and in order to exclude a "white coat effect."[25]

Conventional Echocardiography and Doppler

Standard M-mode, 2D and Doppler blood flow measurements were performed with continuous ECG monitoring by 2 trained observers (VC and CCS) in awake standing cats by use of 2 ultrasonographic units[b] equipped with 7.5–10 MHz phased-array transducers, as previously described and validated.[26] LV end-diastolic and end-systolic diameters, LVFW thickness and IVS thickness in diastole and in systole were measured by use of the 2D-guided M-mode[27] in accordance with the recommendations of the American Society of Echocardiography,[28] and the LV fractional shortening (%FS) was calculated.

Pathologic hypertrophy was defined as a diastolic LVFW (LVFWd) or IVS (IVSd) thickness >6 mm.[5] Hypertrophy was considered as symmetric if the ratio IVSd/LVFWd was 0.7–1.3, or asymmetric with predominant septal thickening or predominant LVFW thickening, if IVSd/LVFWd was >1.3 or ≤ 0.7, respectively.[29]

The subaortic IVS thickness was also measured in end-diastole by 2D mode from the right parasternal 5-chamber view at the level of the attachments of the left *chordae* to the mitral valve leaflets.[23] The diameters of the aorta (Ao) and left atrium (LA) were measured by a 2D method, involving a short-axis right-sided parasternal view obtained at the level of the aortic valve.[30] Pulsed-wave Doppler parameters included maximal systolic aortic and diastolic mitral flow velocities and the isovolumic relaxation time (IVRT) (time interval between end of aortic flow velocity and onset of transmitral flow).

Tissue Doppler Study

All 2D color TDI examinations were performed in awake standing cats with continuous ECG monitoring by the same trained observers (VC, CCS) as for conventional echocardiography and with the same ultrasound units,[b] as described previously and validated by our group.[15,16] Real-time color Doppler was superimposed on the gray scale with a high frame rate (mostly around 200 frames/s, up to 280). Doppler receive gain was adjusted to maintain optimal coloring of the myocardium (ie, without any black spots), and Doppler velocity range was set as low as possible to avoid aliasing. All digital images were stored and analyzed by a specific software.[c] A 2×2 mm sample was used and a tissue velocity profile displayed in each sample location. Myocardial velocities resulting from radial LVFW motion were measured by the right parasternal ventricular short-axis view and measurements were made between the 2 papillary muscles in subendocardial and subepicardial segments of the LVFW. Longitudinal velocities were measured by the standard left apical 4-chamber view in 4 locations: the lateral mitral annulus and 3 myocardial segments, ie, 2 from the LVFW (at the base and the apex) and 1 from the IVS (at the base). Radial myocardial velocity gradients (MVG, cm/s, defined as the difference between subendocardial and subepicardial velocities) and longitudinal MVGs (defined as the difference between basal and apical velocities) were also calculated for each phase of the cardiac cycle. Time indices: TDI IVRT and isovolumic contraction time (IVCT) were assessed on the same 3 consecutive cardiac cycles, with IVRT defined as the time interval between end of the TDI systolic wave (S) to onset of the TDI early diastolic wave (E) and IVCT as the time interval between end of the TDI late diastolic wave (A) to onset of the TDI S wave. The heart rate (HR) was calculated by ECG monitoring during each radial and longitudinal TDI examination by averaging the same 3 cardiac cycles used for the velocity measurements.

Mutational Analysis

Mutational analysis was performed in a single laboratory.[d] A double independent analysis was performed for each animal.

DNA Extraction. Genomic DNA was extracted from ethanol-conserved buccal swab samples with the NucleoSpin 96 Tissue DNA Kit[e] according to the manufacturer's instructions.

Mutation Testing. Cats were screened for the MyBPC3-A31P mutation.[11] PCRs were processed in 30 μL containing 20 ng genomic DNA with the Eurobluetaq DNA polymerase[f] according to the manufacturer's instructions. The PCR products were sequenced with the BigDye Terminator v 3.1 Cycle Sequencing Kit.[g] Products of the sequencing reactions were analyzed on an Applied Biosystems 3100 sequencer.

Statistical Analysis

All data are expressed as mean ± SD. Age, body weight, SABP, conventional echocardiographic, and TDI parameters were compared between groups (Hetero, Homo WT, and Homo M Maine Coon cats with and without HCM) by a 1-way ANOVA followed if necessary by a Student's *t*-test with Bonferroni correction. *P*-values $<.05$ were considered as statistically significant.

Results

Study Feline Population

Ninety-six Maine Coon cats, 31 males (2 neutered) and 65 females (5 sterilized [ovariectomy] and 1 pregnant) were enrolled in the study. The feline population (mean ± SD age: 2.6 ± 2.1 years [0.3–11.5] and mean ± SD body weight: 5.1 ± 1.4 kg [2.4–9.8]) consisted of 52 Homo WT cats (52/96, 54%), 6 Homo M cats (6/96, 6%), and 38 Hetero cats (38/96, 40%) (Fig 1). Age and body weight were comparable between the 3 groups (Table 1). Conversely, SABP was significantly lower ($P < .05$) in the Homo M group than in the Hetero and Homo WT groups (Table1).

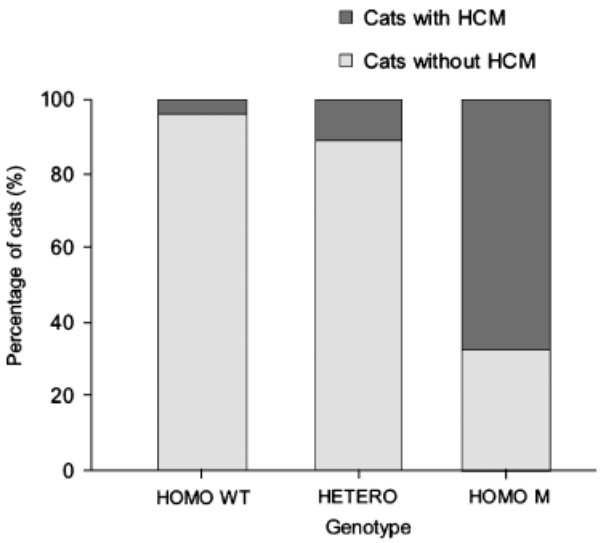

Fig 1. Distribution of the Maine Coon cats (n = 96) according to the genotype (Homo WT, Hetero, Homo M) and phenotype (presence or absence of hypertrophic cardiomyopathy, HCM). Homo WT group: cats without the MyBPC3-A31P mutation (n = 52). Hetero and Homo M groups, respectively, heterozygous (n = 38) or homozygous (n = 6) mutated cats.

Conventional Echocardiographic and Doppler Findings—Correlation with the Genotype (Homo WT, Homo M, and Hetero), and with Epidemiologic and Clinical Characteristics

When the 3 genotype groups of Maine Coon cats were compared, no differences were found between the Hetero and Homo WT groups for any of the conventional echocardiographic parameters assessed in the LVFW and the IVS (Table 2). However, and as expected, significant differences were found between Homo M and both Homo WT and Hetero cats (Table 2), including higher LA/Ao ratio, LVFWd, IVSd, subaortic IVSd, systolic IVS, and systolic maximal aortic velocity in Homo M cats ($P < .05$). Conversely, the Homo M group and the 2 other feline groups indicated comparable diastolic mitral E and A waves, IVRT, and HR (Table 2).

HCM was diagnosed in only 10 (7 males and 3 females) out of the 96 cats in the whole study population. This feline HCM group (mean ± SD age: 2.6 ± 1.5 years [0.8–4.6] and mean ± SD body weight: 6.4 ± 1.4 kg [5.0–9.8]) consisted of 2 cats (2 males) from the Homo WT group (2/10), 4 cats (2 males and 2 females) from the Homo M group (4/10) and 4 cats (3 males and 1 female) from the Hetero group (4/10) (Fig 1). Only 3 HCM cats (2 from the Homo M group and 1 cat from the Hetero group) had signs of congestive heart failure (dyspnea, cough) or presented syncope. At the time of writing, 1 of these 3 symptomatic cats is still alive 26 months after the initial HCM diagnosis, and the 2 others died either from acute congestive heart failure (Homo M) or sudden death

Table 1. Sex, age, body weight, and systolic arterial blood pressure in the whole population of 96 genotype-confirmed Maine Coon cats divided in 3 groups, ie, Homo WT group (homozygous wild type, cats without the MyBPC3-A31P mutation), Hetero and Homo M groups (respectively, cats heterozygous or homozygous for the MyBPC3-A31P mutation).

	Homozygous wild-type cats (Homo WT group) (n = 52)	Heterozygous cats (Hetero group) (n = 38)	Homozygous-mutated cats (Homo M group) (n = 6)
Sex			
Male (n)	18	11 (including 2 neutered)	2
Female (n)	34 (including 1 pregnant)	27 (including 5 sterilized)	4
Age (years)	2.2 ± 1.3	3.1 ± 2.9	2.9 ± 1.3
Range	0.8–6.4	0.3–11.5	1.3–4.6
Body weight (kg)	5.1 ± 1.3	5.0 ± 1.7	5.9 ± 0.6
Systolic arterial blood pressure (mmHg)	143 ± 13	146 ± 12	126 ± 15*†

Data are expressed as number (n) or mean ± SD.
*$P < .05$ versus Homo WT cats.
†$P < .05$ versus Hetero cats.

Table 2. Conventional echocardiographic and Doppler parameters in the whole population of 96 genotype-confirmed Maine Coon cats divided in 3 groups, ie, Homo WT group (homozygous wild type, cats without the MyBPC3-A31P mutation), Hetero and Homo M groups (respectively, heterozygous and homozygous cats for the MyBPC3-A31P mutation).

	Homozygous Wild-Type Cats (Homo WT group) (n = 52)	Heterozygous Cats (Hetero group) (n = 38)	Homozygous-Mutated Cats (Homo M group) (n = 6)
Morphologic parameters			
Left atrium/aorta	0.9 ± 0.1	0.9 ± 0.2	1.26 ± 0.8*†
Left ventricular diastolic diameter (mm)	16.0 ± 2.2	15.8 ± 2.2	14.3 ± 3.1
Left ventricular systolic diameter (mm)	8.3 ± 2.1	7.7 ± 2.0	7.7 ± 3.1
Left ventricular diastolic free wall (mm)	4.4 ± 0.8	4.3 ± 1.0	6.2 ± 2.8*†
Left ventricular systolic free wall (mm)	7.8 ± 1.0	7.7 ± 1.3	8.9 ± 2.3
Interventricular diastolic septum (mm)	4.6 ± 0.7	4.5 ± 0.9	6.2 ± 2.6*†
Interventricular systolic septum (mm)	7.7 ± 1.0	7.8 ± 1.3	9.3 ± 2.3*†
Subaortic interventricular septum in diastole (mm)	4.3 ± 0.9	4.3 ± 0.8	5.6 ± 2.3*†
Systolic functional parameter			
Fractional shortening (%)	48 ± 9	51 ± 9	52 ± 9
Systolic maximal ejection velocity			
Systolic maximal aortic velocity (m/s)	1.1 ± 0.2	1.1 ± 0.2	2.0 ± 1.5*†
Systolic maximal pulmonary velocity (m/s)	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.3
Diastolic Doppler parameters			
Mitral E wave assessed by pulsed-wave Doppler (m/s)	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.2
Mitral A wave assessed by pulsed-wave Doppler (m/s)	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.1
Mitral E/A ratio	1.5 ± 0.4	1.5 ± 0.4	1.3 ± 0.6
Time parameters			
Isovolumic relaxation time (ms)	54 ± 11	54 ± 11	54 ± 9
Heart rate (beats/min)	180 ± 23	188 ± 18	200 ± 31

E and A, mitral early and late diastolic waves assessed by pulsed-wave Doppler mode (m/s). Data are expressed as mean ± SD.
*$P < .05$ versus Homo WT cats.
†$P < .05$ versus Hetero cats.

(Hetero) after 15 and 17 months, respectively. A systolic apical or sternal LV heart murmur (grades I–V/VI) was detected in all HCM cats and the lowest grades were found in the 4 cats from the Hetero group (2 cats with grade I/VI and 2 others with grades II and III/VI). A gallop rhythm was detected in only 1 cat from the Homo M group. All HCM cats showed a regular sinus rhythm except 1 from the Homo WT group, which presented several isolated LV premature beats (< 10/minute).

Based upon M-mode echocardiographic findings, all cats with HCM exhibited a concentric hypertrophic pattern, which was symmetric in 80% of cases (8/10) and asymmetric with predominant septal thickening in only 20% of cases. In addition, a subaortic localized septal hypertrophy was diagnosed in 4 HCM cats (1 Homo WT, 1 Hetero, and 2 Homo M) and 2 of them (Homo M) presented a dynamic outflow tract obstruction with a systolic anterior motion of the anterior mitral valve leaflet and increased maximal systolic aortic flow velocities (2.32 and 4.96 m/s, reference range in Maine Coon cats[30]: 0.7–1.6 m/s).

Lastly, the age of Hetero cats with normal conventional echocardiography (34/38, 89%) ranged from 0.3 to 11.5 years, with 29% of animals >4 years (10/34, age range: 4.1–11.5 years).

Myocardial Function Assessed by 2D Color TDI in the Whole Feline Population (n = 96)

Radial and longitudinal TDI variables were firstly compared between the 3 genotype groups, ie, the Hetero (n = 38), Homo WT (n = 52), and Homo M (n = 6) groups independently of the phenotype (presence or absence of LVH). All tested radial TDI variables were comparable between the 3 genotype groups. However, significant differences (Fig 2) were found between the Homo M group ($P < .05$) and both the Homo WT and Hetero groups (Fig 2) for several longitudinal diastolic TDI parameters including E/A ratio at the lateral mitral annulus and E/A ratio at the basal and apical segments of the LVFW. The E/A ratio at the basal segment of the IVS was only available for 2 Homo M cats (without HCM) and was <1 in both of them (0.41 and 0.44). Therefore, statistical analysis regarding this TDI variable could only be performed for the Hetero and Homo WT groups (Fig 3A), and a significant decrease in the E/A ratio was observed at the basal segment of the IVS in the Hetero group compared with the Homo WT group ($P < .05$).

Myocardial Function Assessed by 2D Color TDI in Maine Coon Cats without HCM: Hetero Group (n = 34) Compared with the Homo WT Group (n = 50)

A 2nd statistical analysis was performed in which only the negative phenotype for HCM was considered in the 2 most represented feline groups, ie, the Homo WT (n = 50) and Hetero (n = 34) groups, which were comparable regarding age and body weight, ie, 2.2 ± 1.3 years old [1.0–6.4] and 3.1 ± 3.1 years old [0.3–11.5], 5.1 ± 1.3 kg [3.1–9.0] and 4.7 ± 1.4 kg [2.4–9.3], respectively.

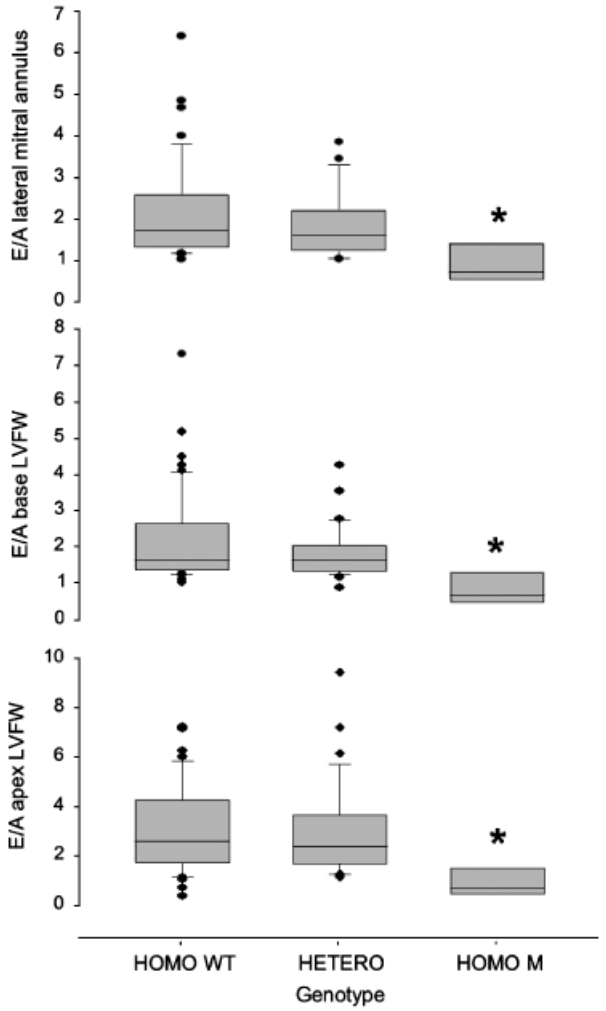

Fig 2. Longitudinal E/A ratio assessed in different locations (lateral mitral annulus, basal and apical segments of the left ventricular free wall) by 2-dimensional color tissue Doppler imaging on Maine Coon cats (n = 96) from the 3 genotype groups (Homo WT, Hetero, Homo M). Homo WT group, cats without the MyBPC3-A31P mutation; Hetero and Homo M groups, respectively, heterozygous or homozygous mutated cats; E/A ratio, ratio between early and late diastolic velocities; LVFW, left ventricular free wall. $^{*}P < .05$ versus corresponding Homo WT.

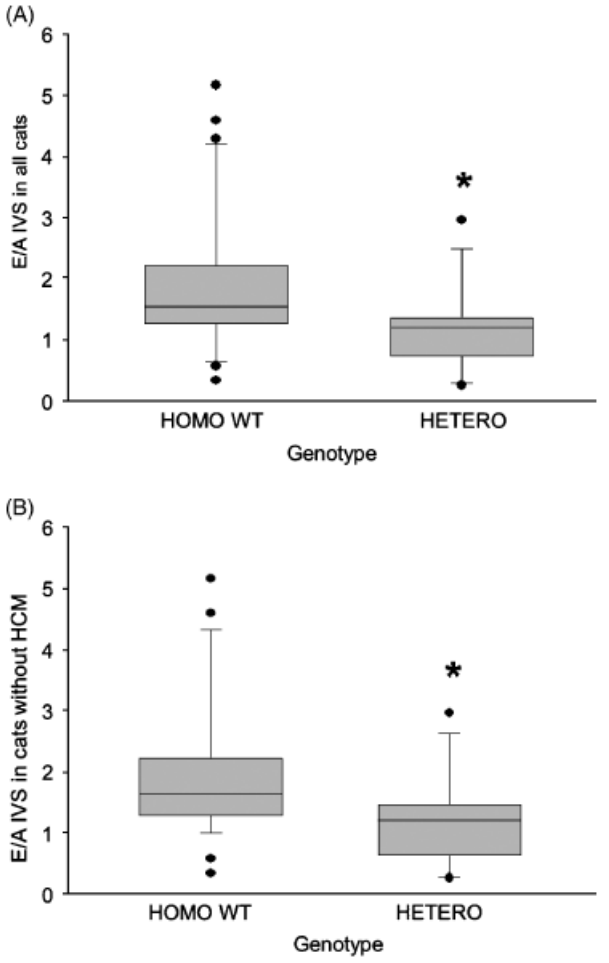

Fig 3. Longitudinal E/A ratio assessed in the basal segment of the interventricular septum by 2-dimensional color tissue Doppler imaging on Maine Coon cats from the 2 most represented genotype groups (Homo WT, Hetero): (**A**) all the cats (ie, with or without HCM, n = 90) (**B**) cats without HCM (n = 84). Homo WT group: cats without the MyBPC3-A31P mutation. Hetero group, heterozygous cats; E/A ratio, ratio between early and late diastolic velocities; IVS, interventricular septum wall; HCM, hypertrophic cardiomyopathy. $^{*}P < .05$ versus corresponding Homo WT.

Radial LVFW Motion. As shown in Table 3, the 2 groups indicated comparable systolic radial myocardial velocities and MVGs. Similarly, no differences between the 2 groups were found for TDI isovolumic time indices (IVRT and IVCT) or subendocardial and subepicardial E/A ratios. Diastolic dysfunction as assessed by an E/A ratio <1 was detected in 4/34 cats (12%) from the Hetero group in the subendocardial segment in 1 cat (0.8) and in the subepicardial segment in 3 cats (0.4, 0.6, and 0.9 respectively).

Longitudinal Motion of the LVFW and the IVS. As shown in Table 4, all longitudinal TDI parameters (including systolic and diastolic velocities and gradients as well as time indices) were comparable between the 2 groups except for E/A ratio in the IVS at the base, which was significantly ($P < .05$) lower in the Hetero group than in the Homo WT group (Figs 3B and 4). Diastolic dysfunction as assessed by an E/A ratio <1 was detected in the basal segment of the LVFW in 1/34 cat (3%) from the Hetero group (0.9) and in 2/50 cats (4%) from the Homo WT at the apical segment of the LVFW (0.4 and 0.7, respectively).

Discussion

The worldwide prevalence of the MyBPC3-A31P mutation estimated from 3,238 DNA samples has recently been shown to be high in Maine Coon cats (34%).[31] The present study provides for the 1st time an accurate analysis of the genotype-cardiac phenotype correlation in a large cohort of pure breed Maine Coon cats screened for this mutation. In this report, LV morphology as well as regional radial and longitudinal LVFW

Table 3. Radial tissue Doppler imaging (TDI) variables assessed in the left ventricular free wall (LVFW) of Maine Coon cats without hypertrophic cardiomyopathy (HCM) belonging either to the Homo WT group (homozygous wild type, cats without the MyBPC3-A31P mutation, n = 50) or the Hetero group (cats heterozygous for the MyBPC3-A31P mutation, n = 34).

	Homozygous Wild-Type Cats (Homo WT group) without HCM (n = 50)	Heterozygous Cats (Hetero group) without HCM (n = 34)
Systolic radial LVFW velocities		
S wave in the subendocardium (cm/s)	5.0 ± 1.1	5.2 ± 1.3
S wave in the subepicardium (cm/s)	2.9 ± 0.9	3.1 ± 1.2
Systolic myocardial velocity gradient of the LVFW		
Between endocardium and epicardium (cm/s)	2.1 ± 0.9	2.1 ± 0.8
Diastolic radial LVFW ratios		
E/A waves ratio in endocardium	2.2 ± 1.0	2.1 ± 1.1
E/A waves ratio in epicardium	2.1 ± 1.0	1.8 ± 0.9
TDI time parameters assessed in the LVFW		
IVCT in the subendocardium and subepicardium (ms)	46 ± 14	45 ± 12
IVRT in the subendocardium and subepicardium (ms)	51 ± 17	47 ± 14
Heart rate (beats/min)	179 ± 27	188 ± 17

Data are expressed as mean ± SD.

S, E, and A, peak velocity of the LVFW during systole, early diastole, and late diastole, respectively; IVCT, isovolumic contraction time (time interval between the end of the A wave to the beginning of the S wave); IVRT, isovolumic relaxation time (time interval between the end of the S wave to the beginning of the E wave).

Table 4. Longitudinal tissue Doppler imaging (TDI) variables assessed in the left ventricular free wall (LVFW) and the interventricular septum (IVS) of Maine Coon cats without hypertrophic cardiomyopathy belonging either to the Homo WT group (homozygous wild type, cats without the MyBPC3-A31P mutation, n = 50) or the Hetero group (cats heterozygous for the MyBPC3-A31P mutation, n = 34).

	Homozygous Wild-Type Cats (Homo WT group) without HCM (n = 50)	Heterozygous Cats (Hetero group) without HCM (n = 34)
Systolic longitudinal velocities		
S wave in the lateral mitral annulus (cm/s)	4.9 ± 1.6	4.8 ± 1.3
S wave in the basal LVFW segment (cm/s)	4.8 ± 1.3	5.0 ± 1.4
S wave in the apical LVFW segment (cm/s)	2.3 ± 1.2	2.6 ± 1.3
S wave in the IVS at the base (cm/s)	5.4 ± 1.7	5.7 ± 2.1
Myocardial systolic velocity gradient of the LVFW		
Between basal and apical segments (cm/s)	2.5 ± 1.0	2.4 ± 1.0
Diastolic longitudinal ratios		
Em/E mitral lateral annulus	13.7 ± 4.0	14.3 ± 6.2
E/A wave in mitral lateral annulus	2.1 ± 1.2	1.9 ± 0.8
E/A wave in the basal LVFW segment	2.2 ± 1.3	1.8 ± 0.7
E/A wave in the apical LVFW segment	3.0 ± 1.8	2.8 ± 1.9
E/A wave in the IVS at the base	2.0 ± 1.1	1.2 ± 0.8†
TDI time parameters assessed in the LVFW and IVS		
IVCT in the basal and apical LVFW segments (ms)	41 ± 17	44 ± 21
IVRT in the basal and apical LVFW segments (ms)	63 ± 19	57 ± 16
Heart rate during LVFW TDI examination (beats/min)	182 ± 20	179 ± 28
Heart rate during IVS TDI examination (beats/min)	183 ± 18	178 ± 15

Data are expressed as mean ± SD.

†$P < .05$ versus Homo WT cats.

S, E, and A: peak velocity of the LVFW or the IVS during systole, early diastole, and late diastole, respectively; Em, mitral wave velocity assessed during early diastole by pulsed-wave Doppler mode; IVCT, isovolumic contraction time (time interval between the end of the A wave to the beginning of the S wave); IVRT, isovolumic relaxation time (time interval between the end of the S wave to the beginning of the E wave).

and IVS functions (assessed by conventional echocardiography and 2D color TDI, respectively) were compared between genetically affected (Hetero and Homo M) cats and genetically unaffected (Homo WT), with a specific focus on the heterozygous status.

In humans, the spectrum of cardiovascular phenotype of patients with familial HCM is known to be broad even among individuals with the same mutation, ranging from the absence of LVH and a complete lack of cardiovascular clinical signs to severe HCM with dyspnea, chest pain, and sudden death most often owing to arrhythmias.[32,33] This heterogeneity of the cardiac phenotype is the result of complex and multiple factors including modifier genes, environmental influences, and also genotype.[33] Similarly to ours, several studies performed in humans have sought to accurately define the phenotype associated with mutations in the sarcomeric MyBPC3 gene, which represent 1 of the most frequent genetic causes of human HCM.[34] Again, a variability in the onset of the disease and prognosis have been observed with conflicting results according to authors. In some studies, patients with mutations in MYBPC3 did not differ significantly from patients with other HCM genotypes or genotype-negative HCM patients with respect to age at diagnosis, degree of LVH, and incidence of sudden death.[34] Conversely, in other studies[35–37] MyBPC3-HCM was

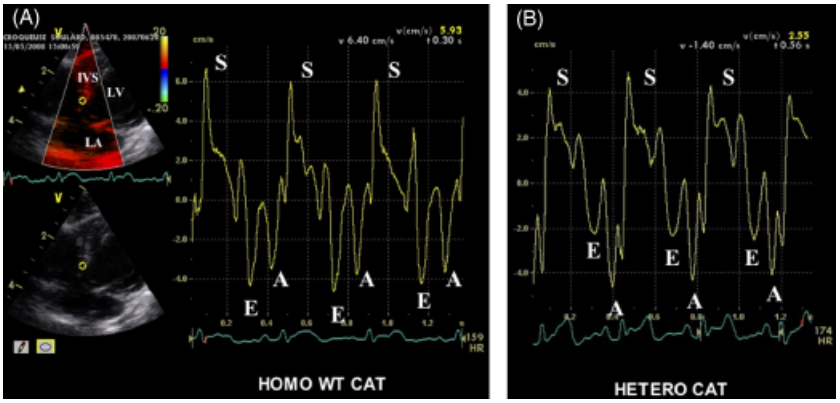

Fig 4. Longitudinal myocardial velocity profile obtained by 2-dimensional color tissue Doppler imaging at the basal segment of the interventricular septum in a Homo WT cat without HCM (**A**) compared with a Hetero cat without HCM (**B**). Note that E is lower than A for the Hetero cat and not for the Homo WT cat. Homo WT cat, cat without the MyBPC3-A31P mutation; Hetero cat, heterozygous cat; S, E, and A, peak myocardial velocity during systole, early diastole, and late diastole, respectively.

associated with later onset, less LVH, lower penetrance and finally, a better prognosis than HCM caused by other mutations such as that in the β-myosin heavy chain gene. In our study, although many of the recruited Hetero animals were young and therefore may not have fully expressed their phenotype, >25% of Hetero cats with normal echocardiography were >4 years old, with ages ranging from 4.1 to 11.5 years. This suggests that the MyBPC3-A31P mutation may be associated with a benign course of the disease at least in some Hetero Maine Coon cats.

Lastly, only 10 out of the 96 cats in the whole population (10%) presented HCM and only 3 (2 Homo M and 1 Hetero, 3%) had clinical signs related to the heart disease (dyspnea, cough, or syncope). However, the actual prevalence of HCM may have been underestimated, because most ultrasound examinations were screening tests requested by breeders who usually exclude Homo M cats from breeding programs. This represents a selection bias and therefore a limitation of the present study. Another limitation is that nearly half of our recruited cats (45/96 cats, 47%) were young (ie, < 2 years old). Therefore, long-term follow-up of these patients will be necessary to accurately establish both the onset of myocardial dysfunction and LVH, and then the disease course.

Whatever the genotype (Hetero, Homo M, or Homo WT), all cats with HCM were young (< 5 years old) with the age of the youngest and oldest Hetero HCM cat being 2.5 and 4.5 years, respectively. These results are in accordance with those of Kittleson et al[38] who found that in their Maine Coon colony, most cats had definitive evidence of HCM between 8 and 24 months of age, with a mean age of death (sudden or heart failure) of 32 months. Based upon 2D and M-mode echocardiographic findings, LV geometric patterns associated with HCM indicated little variability because all HCM cats exhibited concentric LVH, which was most often symmetric (80% of cases) with dynamic outflow tract obstruction in only 2 cats.

In our study, the correlation between features of myocardial dysfunction and the MyBPC3 genotype was analyzed by 1st comparing the 3 genotype groups (Hetero, Homo WT, and Homo M groups, n = 96) independently of the phenotype (presence or absence of LVH) as regards TDI variables. No differences were found between the 3 groups for all tested radial TDI variables. Conversely, a significant longitudinal diastolic alteration was observed in the LVFW of Homo M cats compared with both Homo WT and Hetero cats. This LVFW diastolic dysfunction was characterized by a decrease in E/A ratio at the lateral mitral annulus and a decrease in E/A ratio at the basal and apical segments. Our group has already shown that longitudinal LVFW diastolic function is altered in cats with HCM and that this myocardial dysfunction occurs independently of the presence of myocardial hypertrophy.[14] A similar longitudinal alteration was observed in the IVS at the base for 2 HCM cats for which IVS TDI was performed. This diastolic IVS alteration was the only significant TDI abnormality observed in the Hetero group compared with the Homo WT one.

In the 2nd part of the TDI study, we decided to compare LVFW and IVS TDI parameters between Homo WT (n = 50) and Hetero cats (n = 34) without LVH in order to determine if myocardial dysfunction was present in nonhypertrophied wall segments of Hetero cats. The accuracy of TDI in early detection of myocardial alterations has already been demonstrated in humans with HCM.[39,40] Similar findings were obtained in several animal models of the disease such as a transgenic rabbit model[41] and the feline HFMD model.[22] In the latter study, TDI appeared more sensitive than conventional echocardiography in detecting regional myocardial abnormalities as LVFW dysfunction was detected on all genetically affected HFMD cats despite the absence of LVH. These diastolic TDI alterations were more pronounced for the longitudinal than the radial motion. Similarly, in the present study, Hetero cats without LVH (compared with Homo WT cats without LVH) indicated several (but few) TDI alterations, with longitudinal E/A ratio at the basal segment in the IVS significantly lower in the Hetero group than in the Homo

WT group. No significant differences were found between the 2 feline groups for all radial and longitudinal LVFW TDI variables except that the radial and the longitudinal basal E/A ratio was <1 in a few Hetero cats (12 and 3%, respectively). These results confirm the accuracy of TDI to detect functional changes in nonhypertrophied wall segments. On the other hand, this dysfunction only refers to a segmental (and not diffuse) myocardial alteration, thus showing that, similarly to humans,[34] the heterozygous status is not systematically associated with LVH or major myocardial dysfunction.

The last result obtained from our study was that 2 cats from the Homo WT group (4%) had a positive phenotype for HCM, characterized by a mild form of symmetric hypertrophy with subaortic localized septal hypertrophy in 1 of them. This suggests that other causes than the A31P mutation may be implicated in the development of HCM in the Maine Coon breed. The presence of another mutation responsible for HCM in this breed cannot therefore be excluded.

In conclusion, this study demonstrates that the heterozygous status for the MyBPC3-A31P mutation is not systematically associated with occurrence of LVH and major myocardial dysfunction in the Maine Coon breed, as some Hetero cats may live years without overt signs of HCM and with only minor regional diastolic myocardial dysfunction. Further studies with long-term longitudinal follow-ups of Hetero cats are however required to better establish the MyBPC3 genotype-phenotype correlations in older animals.

Footnotes

[a] 811-BL, Parks Medical Electronics Inc, Aloha, OR
[b] Vivid 7 dimension and Vivid 7 BT03, General Electric Medical System, Waukesha, WI
[c] Echo Pac 5.4 software for Vivid 7, GE-Vingmed Ultrasound, Waukesha, WI
[d] Antagene, Veterinary Genetic Tests (http://www.antagene.com), Limonest, France
[e] NucleoSpin 96 Tissue DNA kit, Macherey-Nagel, Hoerdt, France
[f] Eurobio, Courtaboeuf, France
[g] Applied Biosystems, Foster City, CA

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