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Myosin-binding protein C DNA variants in domestic cats (A31P, A74T, R820W) and their association with Hypertrophic Cardiomyopathy

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Abstract

Background—Two mutations in the *MYBPC3* gene have been identified in Maine Coon (MCO) and Ragdoll (RD) cats with hypertrophic cardiomyopathy (HCM).

Objective—The present study examines the frequency of these mutations and of the A74T polymorphism to describe their worldwide distribution and correlation with echocardiography.

Animals—1855 cats representing 28 breeds and random bred cats world-wide of which 446 underwent echocardiographic examination.

Methods—This is a prospective cross sectional study. Polymorphisms were genotyped using Illumina VeraCode GoldenGate or by direct sequencing. The disease status was defined by echocardiography according to established guidelines. Odds ratios for the joint probability of having HCM and the alleles were calculated by meta-analysis. Functional analysis was simulated.

Results—The *MYBPC3* A31P and R820W were restricted to MCO and RD respectively. Both purebred and random bred cats had HCM and the incidence increased with age. The A74T polymorphism was not associated with any phenotype. HCM was most prevalent in MCO homozygote for the A31P mutation and the penetrance increased with age. The penetrance of the heterozygote genotype was lower (0.08) compared to the P/P genotype (0.58) in MCO.

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Conclusions and Clinical Importance—A31P mutation occurs frequently in MCO cats. The high incidence of HCM in homozygotes for the mutation supports the causal nature of the A31P mutation. Penetrance is incomplete for heterozygotes at A31P locus, at least at a young age. The A74T variant does not appear to be correlated with HCM.

Keywords

HCM; domestic cat; SNP; mutations; meta-analysis

Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats. It also affects approximately one in 500 humans and exhibits an enormous phenotypic and genotypic heterogeneity.[1,2] In humans over 630 mutations in at least twelve different genes, ten of which are sarcomere genes, cause the "single" clinical entity of HCM.[3,4,5]

Animal models indicate that clinical disease is not the consequence of haplo-insufficiency or altered stoichiometry of sarcomere components, but occurs from the dominant effects of the mutant protein on sarcomeric function.[6] Presumably, sarcomere mutations alter the molecular process of muscle contraction and activate pathways for sarcomere replication that results in increased myocyte width and, consequently, wall thickness (hypertrophy).[7] However, many questions remain regarding the underlying disease mechanisms.

In cats, a breed prevalence, suggesting a heritable component for HCM, is described in Siberian, Sphynx, American Shorthair, Cornish Rex, Persian, European, British Shorthair, Bengal, Chartreux, Norwegian Forest cats.[7,8,9,10,11,12,13,14,15] Autosomal dominant inheritance and two causal mutations resulting in amino acid substitution - A31P and R820W - in *Cardiac Myosin Binding Protein C3* (*MYBPC3*) have been identified in Maine Coons and Ragdolls with HCM, respectively.[9,13] In humans, mutations in *MYBPC3* typically exhibit low and age-related penetrance in heterozygotes. A few modifying genes and their proteins have been identified that contribute to the penetrance of these mutations, including, the angiotensin-converting-enzyme gene and the gene that encodes for the angiotensin1-receptor.[16] These and other "modifying" genes, suggested by the incomplete penetrance and phenotypic heterogeneity, have not been identified in cats. Furthermore, no other major HCM causative mutations in other feline breeds have been identified.

Several studies have investigated the frequency of the HCM causative mutation in different countries and the correlation with HCM in Maine Coon cats.[17,18,19] However, these studies examined cats at only one time point and primarily younger cats that might not yet have begun to express a HCM phenotype. In March 2008, the "Osservatorio Italiano HCM Felina" (Italian Observatory on Feline Hypertrophic Cardiomyopathy) was formed (http://www.hcmfelina.com/) from a network of breeders, veterinary geneticists and clinicians to collectively evaluate feline HCM over time. This collaboration aims to monitor inherited diseases in cats, primarily HCM, in Italy. The Observatory provides services to breeders and the scientific community, including 1) breeding advice, 2) providing genetic consultation to breeders and clinicians, mainly regarding the proper application of new genetic diagnostic tools, 3) organizing a group of selected veterinarians at the national level who operate under controlled and shared guidelines, 4) creating a bio-bank and a database to share with the scientific community.

The present study examines the frequency of the specific HCM-associated *MYBPC3* mutations in purebred cats and worldwide random-bred cat populations. Besides the reported Maine Coon and Ragdoll mutations, another single nucleotide polymorphism (SNP) in *MYBPC3* (A74T) was suspected to cause HCM in Maine Coons, although later

disproven. [19,20] Therefore, the A74T polymorphism was genotyped by sequencing Italian cats that have been examined by echocardiography via the Observatory.

MATERIALS AND METHODS

Animals

Pedigree cats (n=1174) representing 28 breeds and 681 random-bred cats from 20 different geographic areas world-wide (n=1855) were genotyped for *MYBPC3* A31P and R820W mutations (Tab.1) using Illumina VeraCode GoldenGate technology or classic direct sequencing. Only pedigree and random-bred cats from Italy with results, and therefore used in the Odd Ratio (OR) analysis, were tested for A74T polymorphism by classic direct sequencing (Tab.3). Pedigree cats were provided by owners living in Italy and Italian-speaking Switzerland. Cats from the USA included cats from Northern and Southern California and Hawaii. No echocardiographic evaluations were performed on the majority of cats. However, a subset of 446 cats, including 232 Maine Coon, 37 Ragdoll, 37 random-bred from Italy, 55 Siberians, 35 Norwegian Forest cats, and seven cats of other breeds, were screened for HCM by echocardiography. The sequence from a subset of 203 cats (11 breeds) was closely examined in the flanking regions of the A31P, A74T and R820W sites in an attempt to identify additional polymorphisms.

HCM Phenotyping

Fifteen veterinarians, all of whom have practices limited to cardiology, were accredited as examiners by the Scientific Committee of the Observatory through a practical examination. The examinees submitted a complete study to the Scientific Committee. All echocardiographic images and measurements were digitally stored and analyzed to ensure that correct imaging planes, cursor placements, left ventricular and left atrial measurements were obtained. Measurements by the examinee had to be within 0.5mm (+/−0.25mm) of the measurements obtained by the Scientific Committee using dedicated softwares (MayLab-Desk vs 6.10 - Esaote and Sante DICOM Viewer vs 1.3.4). Veterinarians who successfully completed the examination were enrolled in the Observatory and performed all the echocardiographic examinations to define the disease status of each cat. The echocardiographic studies were performed following the guidelines published on the website of the Observatory and were based on the recommendations of the American College of Veterinary Internal Medicine.[17] The results were submitted by every examiner to an on-line database. Measurements of end-diastolic thickness of the left ventricular (LV) walls (both septal and posterior wall) were obtained from the right parasternal short-axis view at the papillary muscle level in B-mode. From the same view, M-mode measurements of the left ventricular chamber dimensions and LV walls thickness (both septal and posterior wall) were obtained and shortening fraction calculated. Every focal thickening of the left ventricle septal and posterior wall was measured as well, during diastole, with particular regards to the possible presence of subaortic interventricular septal thickness or abnormal morphology of papillary muscles. The diameter of the root of the aorta and the left atrium were also measured and the left atrium/aortic root ratio (LA/Ao) was calculated using B-mode imaging from the right parasternal short-axis view at the aortic valve level.

Criteria for the categorization of HCM are presented in Tab.2. HCM was classified as severe if LV wall was >7 mm, and where at least one of the following conditions were present: systolic LV chamber obliteration; presence of dynamic obstruction (systolic anterior motion of the mitral valve [SAM] or mid-ventricular dynamic obstruction); left atrial enlargement or presence of a thrombus in the left atrium along with spontaneous echo contrast.

Before final submission of the measurements and calculations in the national database, the data and images were analyzed using MayLab Desk vs 6.10 - Esaote and Sante DICOM Viewer v1.3.4 by two of three veterinarians comprising the Scientific Committee (PF and PK), to verify that the echocardiographic views and measurements were correctly obtained. In cases of doubt, the echocardiographic examination and measurements were analyzed by the third member of the Scientific Committee, (FP). Cats with severe papillary muscle hypertrophy (subjective evaluation) but no LV wall thickening were considered equivocal. In patients with a LV wall exceeding 6 mm, additional tests were performed to exclude cats with hyperthyroidism, renal failure, concurrent hypertension and diabetes mellitus (see Table 2).

Genotyping

Genomic DNA was extracted from either buccal swabs or peripheral blood using commercial kits (Illustra Blood Genomic Prep Mini-Spin Kit, GEHealthCareor QIAamp DNA blood mini kit, Qiagen) according to the manufacturers' instructions.

Samples from Italy were genotyped by DNA sequencing both strands, using the ABI Dye Terminator Sequencing Chemistry 3.1 and an ABI310 analyzer (Applied Biosystems), according to standard protocols. Template sequence for primer design and exon-intron boundary assignment was Ensemble ENSFCAG00000002530. Nomenclature for the description of sequence variations was according to denDunnen.[22] One amplicon of 209 bp included both the A31P mutation and A74T polymorphisms and a second 396 bp amplicon included the R820W mutation. These were obtained using the following primers: F1-5′GAAGCCAAGGTCAGTGGAAG, R1-5′CCTACGCAGTCATCGCTG and F2-5′CAGCAATGTGGGTGAGGAC, R2-5′CTGACCAGGGAGGGTGTG, respectively. An additional amplicon of 442 bp (intron1-2/exon2), partially overlapping with F1-R1 amplicon was produced to analyze the 3′ end of intron1-2, using the following primers: F3:5′TTCTGCCTACTGGCTGTGTG and R1:5′CCTACGCAGTCATCGCTG. Standard amplification with Flexi DNA Polymerase (Promega) was performed in 30 ul final volume, using 33 cycles with 58°C and 62°C annealing temperature for the A31P-A74T and R820W fragments, respectively, on a 9300ABI (Applied Biosytems). Big Dye Terminator v3.1 (Applied Biosystems) was used for sequencing and electrophoresis were performed on an Applied Biosystems 310 or 3100 DNA Analyzer. Multiple sequence traces were edited using BioEdit vs7.0.5, and aligned and compared using MEGA vs4.[23]

Samples from the other countries were genotyped using Illumina VeraCode GoldenGate Genotyping Assay (Illumina Inc.). Primers were designed with the VeraCode Assay Designer software. Golden Gate Assay amplification and BeadXpress reads were performed per the manufacturer's protocol on 50–500 ng of DNA or whole genome amplified product. BeadStudio software v3.1.3.0 with the Genotyping module vs3.2.23 (Illumina Inc.) was used to analyze the data. Any sample with a call rate<0.80 was removed from further clustering analysis. The single nucleotide polymorphisms (SNPs) had to have a Gen Train Score >0.55 to be included in the study.

Statistics

Allele and genotype frequencies were calculated by direct counting with the A31P wild-type allele being guanine (G) and the mutant allele being cytosine (C). The A74T wild-type allele was guanine (G) and the putative-mutant allele was adenine (A). The R820W wild-type allele was cytosine (C) and the mutant allele was thymine (T).

Cats that underwent echocardiography were grouped according to age-classes as follows: 0 = ≤12 months, 1 = 13–24 months; 2 = 25–36 months; 3 = 37–48 months; 4 = 49–60 months;

5 = ≥61 months. The presence of HCM in the breeds was calculated after excluding cats with an equivocal diagnosis (borderlines). When comparing genotype with phenotype in Maine Coons, the cats were classified as healthy or affected; the latter comprised all degrees of HCM severity: mild, moderate, and severe.

The OR for the probability of having HCM and the mutant alleles was calculated for both the A31P and A74T changes independently, considering four scenarios: Scenario 1: Number of Maine Coons (both affected and healthy) homozygous mutation P/P versus number of Maine Coons (both affected and healthy) homozygous wildtype A/A at A31P mutation; and number of Maine Coons (both affected and healthy) homozygous mutation T/T versus number of Maine Coons (both affected and healthy) homozygous wildtype A/A at A74T mutation.

Scenario 2: Number of Maine Coons (both affected and healthy) heterozygous A/P versus number of Maine Coons (both affected and healthy) A/A at A31P locus; and number of Maine Coons (both affected and healthy) heterozygous A/T versus number of Maine Coons (both affected and healthy) A/A at A74T mutation.

Scenario 3: Number of Maine Coons (both affected and healthy) versus number of Maine Coons (both affected and healthy) P/P at A31P mutation; and number of Maine Coons (both affected and healthy) A/T versus number of Maine Coons (both affected and healthy) T/T at A74T mutation.

Scenario 4: Number of Maine Coons (both affected and healthy) P/P and A/P versus A/A at A31P mutation; and number of Maine Coons (both affected and healthy) T/T and A/T versus A/A at A74T mutation.

A meta-analysis limited to A31P and A74T loci, pooling the present and previously published data[19,24] was performed using all four analysis scenarios.

The OR for the joint probability of having HCM and both mutant at A31P and A74T sites was calculated; the OR of having HCM and a specific gender was also calculated. The ability of the test for the A31P mutation to predict the echocardiographic phenotype was evaluated by calculating sensitivity and specificity.

The model of the first domain of the wild-type *MYBPC3* protein was made using Modeller 9v8[25] using the structure of human *MYBPC3* (PDB file: 2K1M) as the template. Stereochemical quality of the protein was evaluated with Procheck[26] and the energetics of the structure was evaluated with Prosa.[27] Mutant forms of the protein were created using the script “Mutate_model” of Modeller DSSP,[28] NACCESS,[29] HBPLUS.[30] An in-house Perl script searching for the existence of possible salt bridges on the basis of the criteria formulated by Kumar and Nussinov[31] and the server PoPMusIC[32] were used for functional analyses. The impact of mutations on protein structure, function, and stability was evaluated taking into account effects on secondary structures, variation of the residue’s solvent accessibility, disruption of H-bond and salt bridge patterns, and stability of the protein, similar to the evaluation of structural effects of mutations made on galactose-1-phosphate uridyltransferase, another protein involved in a genetic disease.[33]

RESULTS

Random-bred and pedigreed cats (N=1855) were genotyped for *MYBPC*-A31P, A74T, R820W or any combination of these polymorphisms (Tab.1) Only sequenced cats (column “Origin Italy” in Tab3) were genotyped for all the tree loci, while the cats analysed with the feline DNA array were typed for the A31P and R820W, as A74T polymorphism is not

included in the array (Tab.3). The A31P mutation was present only in Maine Coons. The allelic frequencies in Maine Coons from Italy and the USA was 0.23 and 0.145, respectively. All other cats of non Maine Coon and Ragdoll breeds were homozygous for the wild-type allele. The A74T SNP was present in random-bred cats and in all the pedigreed breeds, except the Devon Rex (n=1). The allele frequency was high in the British Shorthair (0.32) and random-bred cats (0.53), and ranged from a low 0.17 in Persian and Maine Coon cats to fixed in the four Bengal cats analyzed. The R820W mutant allele was recorded only in Ragdolls. The allelic frequencies were 0.17 in cats from Italy and 0.23 in cats from the USA. All other cats were homozygous for the wild-type allele (Tab.3).

The 446 cats were enrolled in Observatory at random, without any selection, that is why the sample is, in the authors opinion, very little and therefore, mostly unbiased. The cats were phenotyped using echocardiography (presented in Tab.4). HCM was identified in a majority of the cat breeds in the study. HCM was not identified in any of the Birman (n=8), Scottish Fold (n=2), or Sphynx (n=10) cats sampled. The one Devon Rex was considered equivocal (borderline). In affected breeds, prevalence ranged from 2.9% in Ragdolls (95% CI = 2.7–8.6%) to 16.7% in Bengals (95% CI = 13.2–46.5%). Random-bred cats from Italy had a prevalence of 15.4% (95% CI = 1.5–29.3%) and Maine Coon cats had a prevalence of 10.1% (95% CI = 5.8–14.3%). All three Persians enrolled were affected. Cats having equivocal examinations (n=46) were excluded from the following analysis.

When echocardiographic results were associated with age and genotype at A31P site (Maine Coon cats; Fig. 1A), a progressive and rapid increase in incidence of HCM in the homozygous mutants (n=12) was identified and all four cats older than 36 months of age were affected (Fig. 1A). The penetrance in heterozygous Maine Coons was low (0.08) and lower than in homozygous mutants (0.58), but higher than in wild-type homozygotes (0.05). Meta-analysis identified a similar penetrance in C/C cats (0.59) and in homozygous wild-type cats (0.058), but doubled the risk of HCM in C/G cats (0.10). and the average age of onset of HCM is still not determined for these heterozygotes. In our sample more than 80% of the heterozygous cats remained healthy at least until four years of age and the percentage of healthy heterozygous cats remained higher than 50% after 5 years. However, only a few cats in our study exceeded four years of age and the average age of onset of HCM could not be determined.

Two male wild-type cats (G/G) in age-classes 1 and 2 (Fig. 1A) had severe HCM. There were also three wild-type cats, one in age-class=4 and two in age-class=5 that were mildly affected.

Echocardiographic findings were not associated with age or genotype for the A74T polymorphism (Fig. 1B).

Analysis of the association between affected cats and genotype at A31P, A74T and both loci were performed only in Maine Coons, since this was the largest breed sampled and since A31P is exclusive to this breed. We evaluated 208 Maine Coons - 189 healthy and 19 affected; seven homozygous mutants, five heterozygotes and seven homozygous wild types (Tab.5A). Significantly different effects were identified between C/C and G/G genotypes for the echocardiographic phenotype (affected vs. unaffected). The OR for a cat homozygous for the A31P mutation to have HCM was 26.4 (95% CI 6.7–104; Fisher <.0001). The sensitivity was low (50%), but specificity was high 96% (CI .0.91–98) for cats of all ages. When a meta-analysis was done using 335 cats (151 cats from the present study population and 184 cats from previously published studies[19,24]), this significant effect was confirmed (OR 23.7; 95% CI=8.9–62.7; Fisher≤.00019), Tab.5A. However, Maine Coon heterozygotes for the A31P mutation had a much lower OR for developing HCM (OR 1.81; 95% CI 0.55–

5.97), as supported by the meta-analysis on 433 cats (196 cats from the present study population and 237 cats from previous published studies (OR 1.82; 95% CI 0.84–3.90, Tab. 5A).[21,25] When comparing homozygous mutants to pooled heterozygous and wild-type cats, the OR remained high (OR 21.46; 95% CI 5.93–77.8; Fisher ≤0.0001) (Tab.5A). Also, gender showed a significant effect (OR 5.95; 95% CI 1.93–18.65; Fisher ≤0.0007) with males more frequently affected (Tab.5C).

No statistically significant association between genotype and HCM was identified for the A74T putative-mutation in either the samples in the present study, or by meta-analysis (Tab. 5B), in part because the number of homozygous cats was low. The co-variability of A31P and A74T was considered, however, not all combinations of genotypes were available in the sample: only wildtype-wildtype and heterozygous-heterozygous or heterozygous-homozygous cats at A31P and A74T respectively were within the dataset, but no significant effects were identified (data not shown).

The R820W mutant allele was exclusive to Ragdolls (Tab.2). Statistical analysis of the association between HCM and genotype at this locus was not possible as only 37 cats were examined (33 healthy, 3 equivocal (borderline) and 1 mildly affected, Tab.4).

A smaller cohort of 203 cats, examined echocardiographically, was evaluated in the two larger amplicons (442 and 396 bp) for additional polymorphisms (Tab.6). Variability was mainly in the exon 2 region flanking A74T (positions 175, 186, 222), suggesting a less conserved domain (Fig. 6). All additional polymorphisms had no breed specificity and an OR was not calculated due to low sample size.

A functional analysis of each mutation was performed using a different approach than previous studies. The experimental structure of the feline protein is not available, but the wild-type forms of the first domain and of the human *MYBPC3* share more than 90% sequence identity. Therefore the structure of the human protein is a reliable starting point to model the structure of the feline protein using a comparative modeling protocol. Ten models were created and the best one in terms of stereochemical quality of the protein and energetics of the structure was chosen for the following steps. Mutations A74T and A31P were modeled using the “Mutate_model” script since this procedure gave the best results for side chain placement.[34] It appears that the A31P mutation affects a residue located in the middle of a beta-strand forming the central beta-sheet of the protein. The replacement of alanine with proline causes no predicted effects on solvent accessibility and potential salt bridge networks in this zone. However, the mutation is predicted to alter the secondary structures causing the interruption, and then the shortening, of the beta-strand in the core of the protein (Fig. 2, blue). In addition, this mutation impairs the formation of an H-bond involving residue 31 and His64. These two structural effects are potentially able to disrupt the overall fold and stability of the protein. This is in agreement with the decreased stability predicted for this mutant by the server PoPMusIC. On the contrary, A74T SNP seems not to alter any structural feature, such as H-bonds or salt bridge patterns, secondary structures or solvent accessibility, or overall stability of the protein. However, because it is located on the external part of the protein (Fig. 2, magenta), it could disrupt the interaction that *MYBPC3* protein might have with other proteins or structures.

DISCUSSION

This study focused on elucidating the effect of the A31P and A74T genotypic variants in cat *MYBPC3* on HCM development using a wide sample of pedigree and random-bred domestic cats. Humans carrying mutations in *MYBPC3* develop a broad spectrum of HCM phenotypes, and heterozygotes frequently do not have echocardiographic evidence of HCM

until they are in their fifth or sixth decade of life. This reduced and age-related penetrance has been speculated to be the result of the function of the protein itself in the sarcomere as well as complex and multiple factors such as modifier genes, environmental effects, and/or epigenetic factors.[35] Similarly, our study confirms that Maine Coon heterozygotes for the *MYBPC3*-A31P mutation usually lack echocardiographic evidence of HCM when young to middle-aged.[18,19] However, the present study also demonstrated that Maine Coons homozygous for the A31P mutation usually develop HCM and almost all do by the time they are middle-aged. These results were strengthened and corroborated by performing a meta-analysis using data from previous studies.[17,19,24]

In the past few years, the prevalence of the A31P mutation in Maine Coons has been recorded in different countries and its frequency varies by geographic area.[17,18,19,24] The prevalence ranges between moderately high in Germany (22%[19]) Asia (30.9%[17]) and North America (22.5% present work and 31.7%[17]), to high in Italy and France, (38.2% present work and 41.5%[24]) to even higher in Australia -New Zealand (46.3%[17]). No data are available on the prevalence of R820W in Ragdolls. The A74T was confirmed as a multi-breed polymorphism and was found in eight out of the ten analyzed breeds, even in some breeds where the number of cats was extremely low,[19] and in random-bred cats. The A74T genotypic frequency (35%) in Italian Maine Coons is in accordance with another previous study in Germany.[21]

In the present study, a large sample of cats from different breeds was genotyped to determine the allelic and genotypic frequencies at *MYBPC3* loci A31P, A74T and R820W. A31P and R820W were confirmed as exclusive mutations in the Maine Coons and Ragdolls, respectively, in this study. Since the two mutations are exclusive to these two breeds, they likely occurred after breed development, which is recent for the Ragdoll, but substantially longer ago for the Maine Coon, which have existed since the beginning of cat breeding in the USA in the early 1900s. The A74T was found in a large number of purebred and random-bred cats and so appears to be a non-specific polymorphism in cats.

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

The cats from Italy in this study (n=446) underwent echocardiographic evaluation and were recruited by interacting with breed associations that encouraged all their members to enroll their cats. Cats were enrolled in the Observatory randomly, without any preselection – suggesting, in the authors' opinion, the sampling is largely unbiased. However the assessments was likely slightly biased toward the evaluation of affected cats and cats at risk because they were chosen by breeders. This would inflate the calculated allele frequency in the populations. The same bias likely exists in other studies as breeders generally only screen their important breeding cats and cats known to be at risk to save costs. However the encouragement of participation by the Observatory may have alleviated some bias as the majority of the participating owners volunteered to test their cats without any priority criterion.

There was a high and statistically significant correlation between the *MYBPC3*-A31P mutation and the phenotype of disease in homozygous mutant Maine Coons. This strongly

supports the hypothesis that the mutation confers a significant risk of disease. The study also showed that penetrance was related to age in the homozygous mutant cats as some of the cats that were younger than 36 months of age did not have echocardiographic evidence of HCM, while all the recruited cats that were older than 36 months were affected.

The results in this study are similar to those of studies of humans with *MYBPC3* mutations, with regards to onset of detectable disease and disease severity. Humans with HCM due to a *MYBPC3* mutation (approximately 30% of the total known HCM mutations) have a milder phenotype with less hypertrophy and fewer T-wave abnormalities, have a later onset of HCM and have a lower penetrance compared with those due to beta myosin heavy chain and troponin T gene mutations.[36] Also prognosis and life expectancy are generally better than that observed among cats with HCM caused by mutations in other sarcomeric genes.[37,38]

MYBPC3-A74T genotype-phenotype correlation in Maine Coons was not statistically significant, strongly suggesting that it is a polymorphism, not a risk-associated mutation, consistent with previous studies.[19] However, the number of homozygotes for this SNP in this study was too few to make any definitive conclusions regarding its ability to cause HCM. The co-variability of A31P and A74T was examined, however, not all combinations of genotypes were available in the sampled cats. Particularly cats showing genotypes P/P at A31P and T/T at A74T were missing in the sampling (data not shown). An evaluation of the potential impact of the amino acid substitutions caused by A31P and A74T by the computer program PolyPhen did not suggest damaging effects on the protein, according to Wess.[19] The two polymorphisms, located within the 0–2 motif, are in a very external position and one could interact with Titin and the other with actin.[39] At the protein level, our analysis suggest that the A31P is able to perturb the overall fold and stability of the protein and so the mutation can cause HCM. To the contrary, A74T, also located externally, seems not to alter any structural feature of cMYBPC. However, it could potentially disturb the interaction that the protein might have with other proteins or interactors. Similar to previous reports,[19] we found Maine Coons with HCM that did not have the A31P mutation, suggesting at least one other cause of HCM in this breed.

Cat breeders and some clinicians debate whether to call HCM in Maine Coons due to the A31P mutation an autosomal dominant or autosomal recessive trait. In the original description of HCM in Maine Coons, the disease appeared to behave as an autosomal dominant trait. This, however, in the opinion of the authors, was in a colony of research cats that were likely to have been even more inbred than any particular line of Maine Coons from a breeder.[7] This inbreeding likely produced a high percentage of homozygous mutant genotypes. An alternative explanation (unpublished data) is that this group of cats had a higher prevalence of a modifying gene or possibly even another HCM-causing gene, since it is known that cats without the A31P mutation also developed HCM in this colony. Although calling a heritable disease autosomal recessive or dominant with decreased penetrance may be largely semantics, the mutation is in a gene that encodes for a structural protein and this form of mutation usually causes an autosomal dominant mode of transmission. When structural proteins are affected, often only half the protein must be dysfunctional for disease to occur.[40, 41] However in the case of *MYBPC3*, although it is a structural protein, the sarcomere apparently can function without any normal protein, since A31P and R820W homozygous mutant cats do not die *in utero*. Therefore, it is possible for a mutation in a gene that encodes for a structural protein to behave more like an autosomal recessive trait. However, autosomal recessive traits more commonly show up in young animals and are less likely to show variable expression. Therefore HCM in Maine Coon cats due to the A31P mutation more likely should be theoretically considered an autosomal dominant trait, with decreased penetrance.

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ABBREVIATIONS

A	adenine
BUN	blood urea nitrogen
C	cytosine
HCM	hypertrophic cardiomyopathy
G	guanine
IVS	interventricular septum
LA/Ao	left atrium/aortic root ratio
LV	left ventricle
LVW	left ventricular wall
MYBPC3	cardiac myosin binding protein c 3
OR	odds ratio
RMC	restrictive cardiomyopathy
SAM	systolic anterior motion of the mitral valve
T-4	l-thyroxine
T	thymine
SNP	single nucleotide polymorphism
US	Ultrasounds

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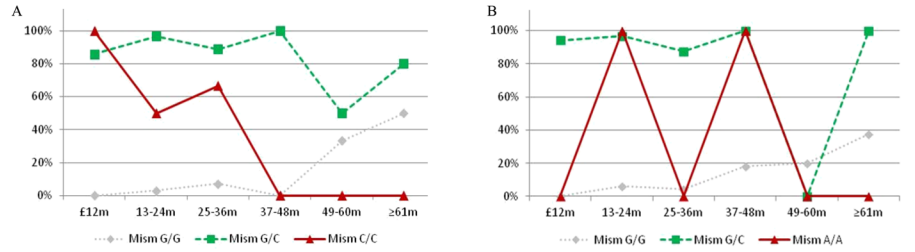

A31P	≤12m	13-24m	25-36m	37-48m	49-60m	≥61m
G/G	28	67	28	9	3	4
G/C	7	29	9	5	2	5
C/C	1	4	3	2	1	1

A74T	≤12m	13-24m	25-36m	37-48m	49-60m	≥61m
G/G	18	50	24	11	5	8
G/A	17	32	8	2	1	1
A/A	0	1	0	1	0	1

Fig. 1.

Fig. 1A Percentage of mismatches between genotypes and expected Ultrasounds results by age class. Mismatch occurs when Ultrasound result is not consistent with genotype under the hypothesis of a dominant causative effect on HCM of *MYBPC3* allele C at A31P locus. Below the total number by genotypes at each age class.

Fig. 1B Percentage of mismatches between genotypes and expected Ultrasounds results by age class. Mismatch occurs when Ultrasound result is not consistent with genotype under the hypothesis of a dominant causative effect on HCM of *MYBPC3* allele A at A74T locus. Below the total number by genotypes at each age class.

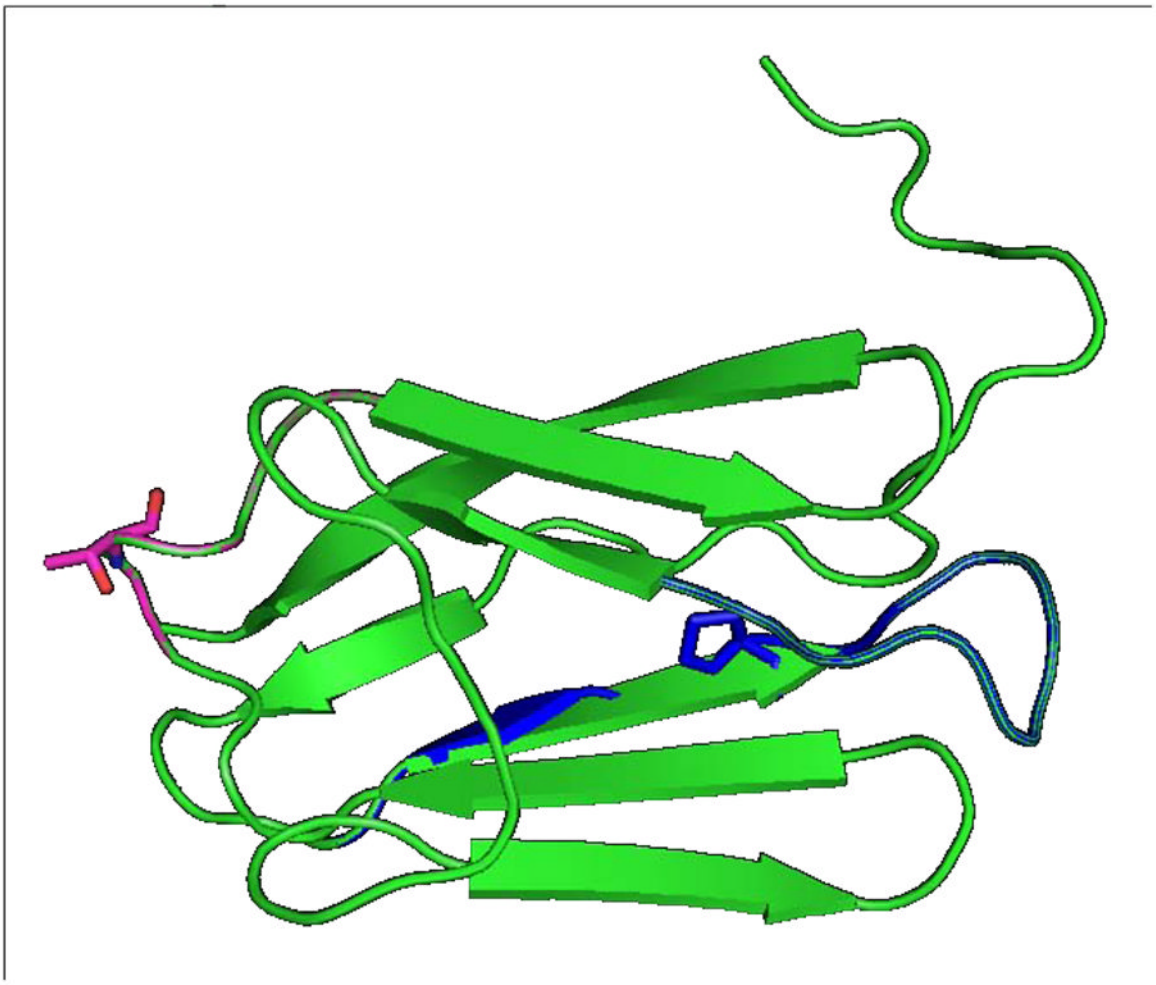

Fig. 2.

Model of the structure of the first domain of protein *MYBPC3* in wild type form (green) and of the polymorphic forms, A31P (blue) and A74T (magenta). The protein backbone is represented as ribbon, and the beta strands as flat arrows. Residues 31 and 74 of polymorphic forms are shown in stick representation. The picture was made using PyMOL Molecular Graphics System.

Tab 1

Breed and number of pedigreed and random bred cats genotyped at MYBPC3 HCM polymorphisms. Cats from Italy were genotyped for A31P, R820W and A74T. Cats from USA were typed for the A31P and R820W loci

Pedigreed Breed Populations						Random Bred Cats	
Breed	**Origin**	**n.**	**Breed**	**Origin**	**n.**	**Origin**	**n.**
Abyssinian (ABY)	USA	26	Norwegian Forest Cat (NFC)	USA	18	BRAZIL	30
American SH (ASH)	USA	13		Italy	23	CHINA	20
Bengal	Italy	4	Persian (PER)	USA	94	CYPRUS	30
Birman (SBI)	USA	29		Italy	3	DUBAI	9
British Shorthair (BSH)	USA	23	Ragdoll (RAG)	USA	25	EGYPT	46
	Italy	11		Italy	141	GERMANY	29
Burmese (BUR)	USA	24	Russian Blue (RUS)	USA	16	INDIA	27
Chartreuse (CHA)	USA	21	Scottish Fold (SFS)	USA	15	IRAQ	4
Cornish rex (CRX)	USA	15	Siamese (SIA)	USA	16	IRAN	112
Devon Rex (DRX)	Italy	1	Siberian (SIB)	USA	15	ISRAEL	46
Egyptian Mau (MAU)	USA	15		Italy	31	ITALY	59
Exotic (EXO)	USA	27	Singapura (SIN)	USA	17	KOREA	39
Havana Brown	USA	10	Sokoke (SOK)	USA	8	KENYA	36
Japanese Bobtail (JBT)	USA	19	Sphynx (SPH)	USA	23	PATE-LAMU	30
Korat (KOR)	USA	25		Italy	3	SINGAPORE	29
Maine Coon (MCO)	USA	31	Turkish Angora (TUA)	USA	13	SRI LANKA	24
	Italy	502	Turkish Van (TUV)	USA	17	TURKEY	62
Manx (MAN)	USA	15				USA	29
						VIETNAM	20
			Total Pedigreed	**1174**		**Total random bred**	**681**

Table 2

Echocardiographic diagnostic criteria: Measurements of end-diastolic of the left ventricular wall (LVW) and interventricular septum (IVS) obtained from the right parastrenal short axis view in B/M mode. Clinical results and diagnosis. Scheduled re-check times and further laboratory tests required to confirm the diagnosis. t4 = L-Thyroxine, BUN = Blood urea nitrogen

LVWed and/or IVSed thickness (mm)	**Results**	**HCM**	***Re-check time and laboratory tests***
≤5.5	normal	**negative**	*12 months*
> 5.5 and ≤ 6.0	questionable	**borderline**	*6 months*
> 6.0 and ≤ 6.5	affected	**mild**	*6 months + t4 creatinine, BUN and systemic blood pressure*
> 6.5 and ≤ 7.0	affected	**moderate**	*6 months + t4 creatinine, BUN, and systemic blood pressure*
> 7.0	affected	**severe**	*1–3 months + t4 creatinine, BUN, and systemic blood pressure*

Tab 3

Genotypic and allelic frequencies at *MYBPC3* polymorphisms in pedigreed and random bred cats. A=Adenine, T=Timine, G=Guanine, C=Cytosine.

Breed*	**Origin**	**A31P**					**A74T**					**R820W**				
		n.	**G/G**	**G/C**	**C/C**	**C**	**n.**	**G/G**	**G/A**	**A/A**	**A**	**n.**	**C/C**	**C/T**	**T/T**	**T**
Bengal	Italy	4	1	0	0	0	4	0.00	0.00	1.00	1.00	4	1	0	0	0
British Shorthair	Italy	11	1	0	0	0	11	0.64	0.09	0.27	0.32	10	1	0	0	0
Devon Rex	Italy	1	1	0	0	0	1	1.00	0.00	0.00	0.00	1	1	0	0	0
Maine Coon	Italy	502	0.62	0.30	0.08	0.23	359	0.68	0.29	0.03	0.17	95	1	0	0	0
	USA	31	0.774	0.161	0.064	0.145	·	·	·	·	·	31	1	0	0	0
Norwegian Forest	Italy	23	1	0	0	0	21	0.57	0.43	0.00	0.21	22	1	0	0	0
Persian	Italy	3	1	0	0	0	3	0.67	0.33	0.00	0.17	3	1	0	0	0
Ragdoll	Italy	27	1	0	0	0	26	0.50	0.46	0.04	0.27	141	0.67	0.32	0.01	0.17
	USA	25	1	0	0	0	·	·	·	·	·	24	0.583	0.375	0.041	0.229
Siberian	Italy	31	1	0	0	0	30	0.50	0.40	0.10	0.30	29	1	0	0	0
Sphynx	Italy	3	1	0	0	0	3	0.00	0.67	0.33	0.67	5	1	0	0	0
Random B.	Italy	20	1	0	0	0	18	0.17	0.61	0.22	0.53	18	1	0	0	0

*Only the breeds and populations positive for the mutant alleles at A31P (C) and R820W (T) or tested at A74T SNP are shown in the table. The remaining typed breeds are listed in Tab1.

Table 4

HCM ultrasound (US) results, cats classified by breed and by age*.

	Bengal N.7						Birman N.8						British Shorthair N.19						Devon Rex N.1						Italian Random Bred N.37						Maine Coon N.232					
	Age class						Age class						Age class						Age class						Age class						Age class					
US class	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	0	1	2	3	4	5	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**
Healthy	*3*	*2*					*1*	*1*	*4*		*1*	*1*		*4*	*10*	*3*										*3*	*9*	*1*	*1*	*8*	*35*	*95*	*36*	*14*	*3*	*6*
Border Line		***1***												*1*								***1***				***2***	***3***		***2***	***4***	***6***	***9***	***4***	***3***	***1***	***1***
Mild		**1**																										**1**		**1**		**2**	**2**	**1**	**2**	**3**
Moderate																														**1**	**1**	**1**	**1**			
Severe															**1**															**1**		**2**	**1**	**1**	**1**	**1**
	Norwegian Forest cat N.35						Persian N.3						Ragdoll N.37						Scottish Fold N.2						Siberian N.55						Sphynx N.10					
	Age class						Age class						Age class						Age class						Age class						Age class					
US class	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**
Healthy		*5*	*9*	*6*	*5*	*5*							***5***	***15***	***6***	***3***	***3***	***1***			***1***		***1***		*5*	*14*	*15*	*3*	*2*	*12*	*1*	*3*		*1*	*3*	*1*
Border Line			***1***	***1***										*1*	*2*										***1***					***1***				***1***		
Mild			**1**		**1**	**2**						**1**		**1**											**1**											
Moderate												**1**																								
Severe												**1**																		**1**						

*Age class 0 ≤12 months, class 1 = ranging 13 – 24 months; class 2 = group 25–36 months; class 3 = 37–48 months; class 4 = 49–60 months; class 5 ≥61 months.

Table 5

Odd Ratio (OR) between Hyprtrophic Cardiomyopathy (HCM), Ultra Sound (US) status (Border line cats excluded) and A31P - A74T variants (C=Cytosine; G=Guanine; A=Adenine) and gender in Maine Coons (MCO).

A

MCO	Pheno type	Geno type			C/C vs G/G			G/C vs G/G			C/C+ G/C vs G/G			C/C vs G/C+ G/G		
		C/C	G/C	G/G	Tot	OR	.95 CI	Tot	OR	.95 CI	Tot	OR	.95 CI OR	Tot	OR	.95 CI OR
A31P	*Healthy*	5	52	132	151	26.4	6.66 – 04.58	196	1.81	0.55 – 5.97	208	3.96	1.48 – 10.60	208	21.46	5.92 – 77.80
	HCM	7	5	7		Fisher exact t.	0.000007		Fisher exact t.	0.246		Fisher exact t.	0.0049		Fisher exact t.	0.00001
A31P*	*Healthy*	9	108	295	335	23.67	8.937–62.70	433	1.82	0.849–3.905	455	3.50	1.841–6.658	455	19.40	7.676–49.04
	HCM	13	12	18		Fisher exact t.	7.44e-10		Fisher exact t.	0.091		Fisher exact t.	0.0001		Fisher exact t.	2.084e

B

MCO	Pheno type	Geno type			A/A vs G/G			G/A vs G/G			A/A+ G/A vs G/G			G/G+ G/A vs A/A		
		A/A	G/A	G/G	Tot	OR	95 CI OR	Tot	OR	95 CI OR	Tot	OR	95 CI OR	Tot	OR	95 CI OR
A74T	*Healthy*	2	57	109	122	5.54	0.45 – 65.49	180	0.76	0.22 – 2.54	183	0.923	0.30 – 2.82	183	5.928	0.50 – 69.51
	HCM	1	4	10		Fisher exact t.	0.248		Fisher exact t.	0.453		Fisher exact t.	0.566		Fisher exact t.	0.227
A74T**	*Healthy*	6	75	154	179	4.812	1.09 – 21.10	253	1.026	0.42 – 2.50	262	1.307	0.57 – 2.94	262	4.770	1.12 – 20.30
	HCM	3	8	16		Fisher exact t.	0.056		Fisher exact t.	0.559		Fisher exact t.	0.327		Fisher exact t.	0.053

C

MCO	Pheno type	Gender		Males vs Females		
		M	F	Tot	OR	95 CI OR
	Healthy	73	116	208	5.95	1.93 – 18.65
	HCM	15	4		**Fisher exact t.**	0.0007

* Meta-analysis with data from Mary and Wess

** Meta-analysis with data from Wess. A31P; C mutant allele, G wild type allele.

Table 6

multiple nucleotide sequence alignment of cat *MYBPC3* including mutations implicated in HCM. Nucleotide positions refer to cat genome sequence at Ensambl # ENSFCAG00000002530. US = Ultrasounds

Breed	cat n./breed	cat n./profile	Intron 1-2	Exon 2 ENSFCAE000000025802 (26-267)										Intron 25-26	Exon 26 ENSFCAE000000025823 (2284-2572)	US results	
ENSFCAG00000002530			26-86 A>C	91 A31P	112	154	169	172	175	186	220 A74T	222	249	2283+28 T>C	2328	Affected	Border line
			A	G	G	A	C	G	G	G	G	C	T	T	C		
Bengal	4	4	-	·	·	·	·	·	·	·	**A**	·	·	C	·	0	1
British Shorthair	10	6	-	·	·	·	·	·	·	·	·	·	·	·	·	1	0
		2	-	·	·	·	·	·	·	·	**A**	·	·	C	·	0	0
		1	-	·	·	·	·	·	·	·	**R**	·	·	C	·	0	0
		1	-	·	·	·	·	·	·	R	·	·	·	C	·	0	0
Random bred from Italy	18	1	-	·	·	·	·	·	·	·	**R**	·	·	·	·	1	0
		2	-	-	·	·	·	·	·	·	·	·	·	Y	·	1	0
		1	-	-	·	·	G	·	·	·	·	·	·	Y	·	1	0
		4	-	-	·	·	·	·	·	·	**A**	·	·	C	·	0	0
		3	-	-	·	·	·	·	·	·	**R**	·	·	C	·	0	0
		1	-	-	·	·	·	·	R	·	**R**	·	·	C	·	0	0
		5	-	-	·	·	·	·	·	·	**R**	·	·	Y	·	1	1
		1	-	-	·	·	·	·	R	·	**R**	·	·	·	·	0	1
DevonRex	1	1	-	·	·	R	·	C	·	R	·	·	·	·	·	0	0
Persian	3	2	-	·	·	·	·	·	·	·	·	·	·	·	·	1	0
		1	-	·	·	·	·	·	·	R	·	·	·	·	·	1	0
Birman	7	2	-	·	·	·	·	·	·	·	·	·	·	·	·	0	0
		1	-	·	·	·	·	·	·	·	**A**	·	·	C	·	0	0
		1	-	·	·	·	·	·	·	·	**R**	·	·	C	·	0	0
		1	-	·	·	·	·	·	R	·	·	Y	·	·	·	0	0
		2	-	·	·	·	·	·	R	·	**R**	Y	·	C	·	0	0
Sphynx	3	1	-	·	·	·	·	·	·	·	**A**	·	·	C	·	0	0
		1	-	·	·	·	·	·	·	·	**R**	·	·	C	·	0	0
		1	-	·	·	·	·	·	R	·	**R**	Y	·	C	·	0	0
Norwegian Forest cat	21	10	-	·	·	·	·	·	·	·	·	·	·	·	·	0	0

Breed	cat n./breed	cat n./profile	Intron 1-2	Exon 2 ENSFCAE000000025802 (26-267)										Intron 25-26	Exon 26 ENSFCAE000000025823 (2284-2572)	US results	
ENSFCAG00000002530			26-86 A>C	91 A31P	112	154	169	172	175	186	220 A74T	222	249	2283+28 T>C	2328	Affected	Border line
			A	G	G	A	C	G	G	G	G	C	T	T	C		
		1	-	·	·	·	·	·	·	·	·	·	·	C	·	1	0
		4	-	·	·	·	·	·	·	·	**R**	·	·	·	·	0	0
		5	-	·	·	·	·	·	·	·	**R**	·	·	C	·	1	0
		1	-	·	·	·		·	R	·	·	Y	·	·	·	0	0
Siberian	28	9	-	·	·	·	·	·	·	·	·	·	·	·	·	0	0
		3	-	·	·	·	·	·	·	·	·	·	·	C	·	0	0
		5	-	·	·	·	·	·	·	·	**R**	·	·	·	·	1	0
		3	-	·	·	·	·	·	·	·	**A**	·	·	C	·	0	0
		5	-	·	·	·	·	·	·	·	**R**	·	·	C	·	1	0
		1	-	·	·	·	·	·	R	·	**R**	Y	·	C	·	0	0
		1	-	·	A	·	·	·	·	·	·	·	·	·	·	0	0
		1	-	·	·	·	·	·	·	·	·	·	K	·	·	0	0
Maine Coon	83	8	-	·	·	·	·	·	·	·	·	·	·	·	·	0	2
		2	C	·	·	·	·	·	·	·	·	·	·	·	·	0	0
		1	M	·	·	·	·	·	·	·	·	·	·	·	·	0	0
		1	M	·	·	·	·	·	·	·	·	·	·	Y	·	0	0
		1	-	·	·	·	·	S	·	·	·	·	·	·	·	0	0
		1	-	·	·	·	·	·	·	·	**A**	·	·	C	·	1	0
		2	A	·	·	·	·	·	·	·	**A**	·	·	C	·	0	0
		11	-	·	·	·	·	·	·	·	**R**	·	·	Y	·	1	0
		2	-	·	·	·	·	·	·	·	**R**	·	·	C	·	0	0
		1	A	·	·	·	·	·	·	·	**R**	·	·	C	·	0	0
		6	A	·	·	·	·	·	·	·	**R**	·	·	Y	·	0	0
		4	M	·	·	·	·	·	·	·	**R**	·	·	Y	·	0	1
		3	C	·	·	·	·	·	·	·	**R**	·	·	Y	·	0	1
		3	M	**S**	·	·	·	·	·	·	**R**	·	·	Y	·	1	0
		1	C	**S**	·	·	·	·	·	·	**R**	·	·	Y	·	0	0

Breed	cat n./breed	cat n./profile	Intron 1-2	Exon 2 ENSFCAE000000025802 (26-267)										Intron 25-26	Exon 26 ENSFCAE000000025823 (2284-2572)	US results	
ENSFCAG00000002530			26-86 A>C	91 A31P	112	154	169	172	175	186	220 A74T	222	249	2283+28 T>C	2328	Affected	Border line
			A	G	G	A	C	G	G	G	G	C	T	T	C		
		3	-	**S**	·	·	·	·	·	·	**R**	·	·	Y	·	0	0
		16	-	**S**	·	·	·	·	·	·	·	·	·	Y	·	2	2
		7	-	**C**	·	·	·	·	·	·	·	·	·	·	·	4	1
		3	M	**S**	·	·	·	-	-	·	-	-	·	·	·	0	0
		6	C	**S**	·	·	·	·	·	·	·	·	·	·	·	0	0
		1	C	**C**	·	·	·	-	-	·	-	-	·	·	·	1	0
Ragdoll	25	2	-	·	·	·	·	·	·	·	·	·	·	·	·	0	0
		1	-	·	·	·	·	·	·	·	·	·	·	·	**T**	1	0
		3	-	·	·	·	·	·	·	·	·	·	·	·	**Y**	0	1
		1	-	·	·	·	·	·	·	·	**A**	·	·	C	·	0	0
		3	-	·	·	·	·	·	·	·	**R**	·	·	Y	·	0	0
		6	-	·	·	·	·	·	·	·	**R**	·	·	C	·	0	0
		4	-	·	·	·	·	·	R	·	·	Y	·	C	·	0	0
		1	-	·	·	·	·	·	R	·	·	Y	·	Y	·	0	0
		3	-	·	·	·	·	·	R	·	**R**	Y	·	C	·	0	0
		1	-	·	·	R	·	S	·	·	·	·	·	·	·	0	0