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SHORT COMMUNICATION

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The *TNNT2*:c.95-108G>A variant is common in Maine Coons and shows no association with hypertrophic cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is a common and potentially fatal heart disease in many cat breeds. An intronic variant in *TNNT2*, c.95-108G>A, was recently reported as the cause of HCM in the Maine Coon. The aim of this study was to determine this variant's allele frequency in different populations and its possible association with HCM. Based on 160 Maine Coon samples collected in Belgium, Italy, Sweden and the USA, the variant's allele frequency was estimated to be 0.32. Analysis of the 99 Lives feline whole genome sequencing database showed that the *TNNT2* variant also occurs in other breeds, as well as mixed-breed cats. Comparison of 31 affected and 58 healthy cats did not reveal significantly increased odds for HCM in homozygotes. Based on the combined evidence and in agreement with the standards and guidelines for the interpretation of sequence variants, this variant is currently classified as a variant of unknown significance and should not be used for breeding decisions regarding HCM.

KEYWORDS
allele frequency, *Felis catus*, hypertrophic cardiomyopathy, intronic variant, troponin

Hypertrophic cardiomyopathy (HCM; OMIA id 000515-9685) is the most common heart disease in cats (Payne et al., 2015) and is associated with an increased risk of life-threatening complications (Fox et al., 2018). HCM is known as a genetic disease in humans and several variants have been reported as HCM-causing in cats (Kittleson et al., 2015; Meurs et al., 2005; Meurs et al., 2007). The first feline HCM-causing variant, *MYBPC3*:c.91G>C, was identified in the Myosin Binding Protein C3 gene in Maine Coons (Meurs et al., 2005) and its HCM-causing nature has been supported by several studies (Longeri et al., 2013). This is currently not yet the case for a recently reported intronic variant in the cardiac troponin T2 (*TNNT2*) gene. This variant, *TNNT2*:c.95-108G>A, was deemed to be the cause of cardiomyopathy in a homozygous Maine Coon, based on a one case–parent trio and in silico predictions of aberrant splicing (McNamara et al., 2020).

The aims of this study were to investigate the *TNNT2*:c.95-108G>A variant further by (i) estimating its allele frequency in different Maine Coon populations and (ii) examining its association with HCM, followed by an evaluation of its pathogenicity according to the American College of Medical Genetics (ACMG) standards and guidelines for the interpretation of sequence variants (Richards et al., 2015).

One-hundred and sixty Maine Coon samples, originating from Belgium, Italy, Sweden and the USA, were genotyped for the *TNNT2*:c.95-108G>A variant (Table S1). The country-specific allele frequencies ranged from 0.26 to 0.35 and the overall allele frequency in the Maine Coon was estimated to be 0.32. The alleles were not significantly associated with the country of origin ($p = 0.5$) or year of birth ($p = 0.41$).

The 99 Lives database contains 296 cats from other breeds whose genotype for this variant was known (Lyons et al., 2021). The variant was detected in the British Shorthair, Devon Rex, Persian, Ragdoll, Siamese, Tennessee Rex and Thai breeds, as well as in random-bred cats (Table S2).

To analyse the association between the variant and HCM, cats with an unknown or equivocal phenotype, healthy controls younger than the originally described proband (8 months) and cats homozygous for the *MYBPC3*:c.91G>C variant were excluded. Overall, 31 affected cats (median age, 4.6 years; range, 0.7–15.2) and 58 healthy controls (median age, 7.2 years; range, 2.0–16.5) were considered. The phenotypes and genotypes are cross-tabulated in Table 1. The odds ratio for developing HCM in *TNNT2*:c. 95-108G>A homozygous cats compared with other genotypes was estimated to be 1.41, with a 95% confidence interval from 0.37 to 5.37 and a *p*-value of 0.61. A second analysis that used the 35 oldest healthy controls (median age, 9.17; minimum, 6.75), thereby minimizing the potential effect of the age in the control group while still retaining 80% power (for power calculations: see Appendix S1), yielded a non-significant ($p = 0.30$) odds ratio of 2.43. As this *TNNT2* variant itself had no significant effect, a possible interaction with the *MYBPC3*:c.91G>C variant was investigated. No significant effect of (i) heterozygosity for the *MYBPC3*:c.91G>C variant ($p = 0.51$) or (ii) the interaction between this variant and the *TNNT2*:c.95-108G>A variant ($p = 0.80$) studied here on HCM status was found.

TABLE 1 Distribution of the HCM phenotypes over the genotypes for the *TNNT2*:c.95-108G>A variant

	G/G	G/A	A/A
HCM	12	14	5
Healthy	25	26	7
HCM prevalence	0.32	0.35	0.42

The effect of the variant on the splicing of *TNNT2* transcripts was predicted by five computational tools: GENSCAN, ASSP, SSPNN, ESEfinder and Netgene2. Only two out of the five tools, ASSP (used by McNamara et al., 2020) and SSPNN, predicted the creation of a new splice acceptor site. This predicted splice site would add an intronic sequence of 106 bases to the 5′-end of exon 5 (exon numbered according to ENSFCAT00000052073.2) and create a premature stop codon. Sanger sequencing of myocardial cDNA from two Maine Coons carrying the variant (a healthy heterozygote and an HCM-affected homozygote) did not show the incorporation of the intronic sequence and therefore did not confirm the utilization of the predicted new splice site (Figure S1).

Combined, the commonality of the variant, the lack of association with HCM, the additional in silico predictions and the cDNA sequences do not support the *TNNT2* variant as causal for HCM. Therefore, a check based on the five relevant criteria from the ACMG consensus guidelines was performed to weigh the evidence supporting the classification of this *TNNT2* variant as pathogenic or benign (Richards et al., 2015). A summary of these criteria is provided in Table 2.

An allele frequency higher than 5% is considered stand-alone evidence for a benign classification of a variant in the context of human Mendelian diseases. Considering the high prevalence of HCM in cats and the different population structure of purebred cats compared with humans, this cut-off is probably too strict. For example, the allele frequency of the *MYBPC3*:c.91G>C variant in Maine Coons was reported to be 18% (Fries et al., 2008). Apart from the 5% cut-off, an allele frequency higher than expected for the disorder is also considered strong evidence for a benign classification in humans. However, the non-random selection of the samples and the lack of knowledge on the genetic heterogeneity of feline HCM impeded the calculation of a reliable cut-off (Broeckx et al., 2017). To avoid overinterpretation of a possibly unreliable figure, an alternative to the cut-off of 5% was not calculated.

Statistical association with the disease is considered strong evidence for pathogenicity if the odds ratio is higher

TABLE 2 Summary of relevant variant classification criteria

Criterion	Result	Remark	Conclusion
Allele frequency >5% (BA1) or higher than expected (BS1)	Allele frequency = 32%	No suitable cut-off for the allele frequency available	Validity of criterion questionable
Significant OR >5.0 (PS4)	OR = 1.41, $p = 0.61$		Not fulfilled
Observed in healthy adult with full penetrance expected at early age (BS2)	11-year-old healthy homozygote	Full penetrance of feline HCM cannot be assumed	Not fulfilled
Null variant in a gene where LOF is a known mechanism (PVS1)	LOF possible in *TNNT2*, LOF not confirmed on mRNA or protein level		Not fulfilled
Multiple lines of computational evidence (PP3/BP4)	2/5 predicted harmful effects, 3/5 did not	Should not be used with contradicting results	Not fulfilled

Note. The original criterion name as mentioned in Richards et al. (2015) has been added in parentheses.
Abbreviations: HCM, hypertrophic cardiomyopathy; LOF, loss of function, OR, odds ratio.

than 5.0 and statistically significant, but neither of these conditions was met in this study. The estimated odds ratio of 1.41 was far lower, compared with both the 5.0 threshold and the odds ratio of 19.4 for the *MYBPC3*:c.91G>C variant according to Longeri et al. (2013).

Another strong criterion for a benign classification is the detection of a variant in a homozygous state in a healthy adult, if full penetrance is expected at an early age. The oldest cat homozygous for the variant allele in the present study was still negative on echocardiography at the age of 11 years. However, incomplete and age-dependent penetrance is a well-known characteristic of HCM in humans (Lorenzini et al., 2020) and has also been described in cats (Longeri et al., 2013). Whereas 11 years of age already corresponds to the median longevity of Maine Coons (Egenvall et al., 2009; O'Neill et al., 2015) and HCM is thought to generally develop before this age, this cat may still develop HCM as the age of diagnosis ranges from 6 months to 21 years (Fox et al., 2018; Kittleson & Côté, 2021). This criterion is therefore also insufficiently supported.

The complete disruption of gene function by a null variant is considered the strongest evidence for pathogenicity. The predicted splice site would cause the loss of over 80% of the protein's amino acids (McNamara et al., 2020), and truncating variants in *TNNT2* can cause HCM in humans (Walsh et al., 2017). To meet this criterion for splice site variants, functional mRNA or protein evidence is required (Richards et al., 2015), but this was not presented in the report by McNamara et al. (2020). The cDNA sequencing in this study did not show the predicted incorporation of an intronic sequence, so this criterion is not met. In addition, the in silico support for the splice site effect of the variant showed inconsistencies in predictions of different programs. When the results of prediction programs vary, the ACMG criteria state that these results should not be used to classify a variant as benign or pathogenic (Richards et al., 2015).

Altogether, none of the five different criteria that support either a pathogenic or a benign role for the variant are met, which implies this variant has to be classified as a 'variant of unknown significance'. As such, this variant should not be used in clinical decision making, according to the ACMG guidelines (Richards et al., 2015). Extrapolating these guidelines to companion animals, we advise against the use of this variant in breeding decisions, especially as the variant's high allele frequency means that such use could impact the genetic diversity and thus the welfare of the breed.

This study has some potential limitations. The obtained allele frequency estimates could be biased as the samples used in this study were collected in the context of breed screening and/or scientific investigation and therefore do not represent a random sample. The control group, although generally older than the affected group, included some young animals, which may develop HCM at a later age. However, all controls were older than the proband described by McNamara et al. (2020) at the age of diagnosis, and removing the 23 youngest control cats did not substantially change the odds ratio or its p-value. If this proband showed a typical manifestation of the cardiomyopathy caused by homozygosity of the *TNNT2*:c.95-108G>A variant, expectations are that the older homozygotes in this study would be affected. Finally, cardiac cDNA was available for only two cats carrying the variant, a heterozygote and a homozygote. Sequencing of cardiac cDNA from more cats carrying the variant would allow a more confident assessment of the variant's effect on splicing.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study can be found in Supplementary Tables S1 and S2 and Supplementary Figure S1 of this publication.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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