The Genetics of Feline Familial Hypertrophic Cardiomyopathy in the Siberian Cat

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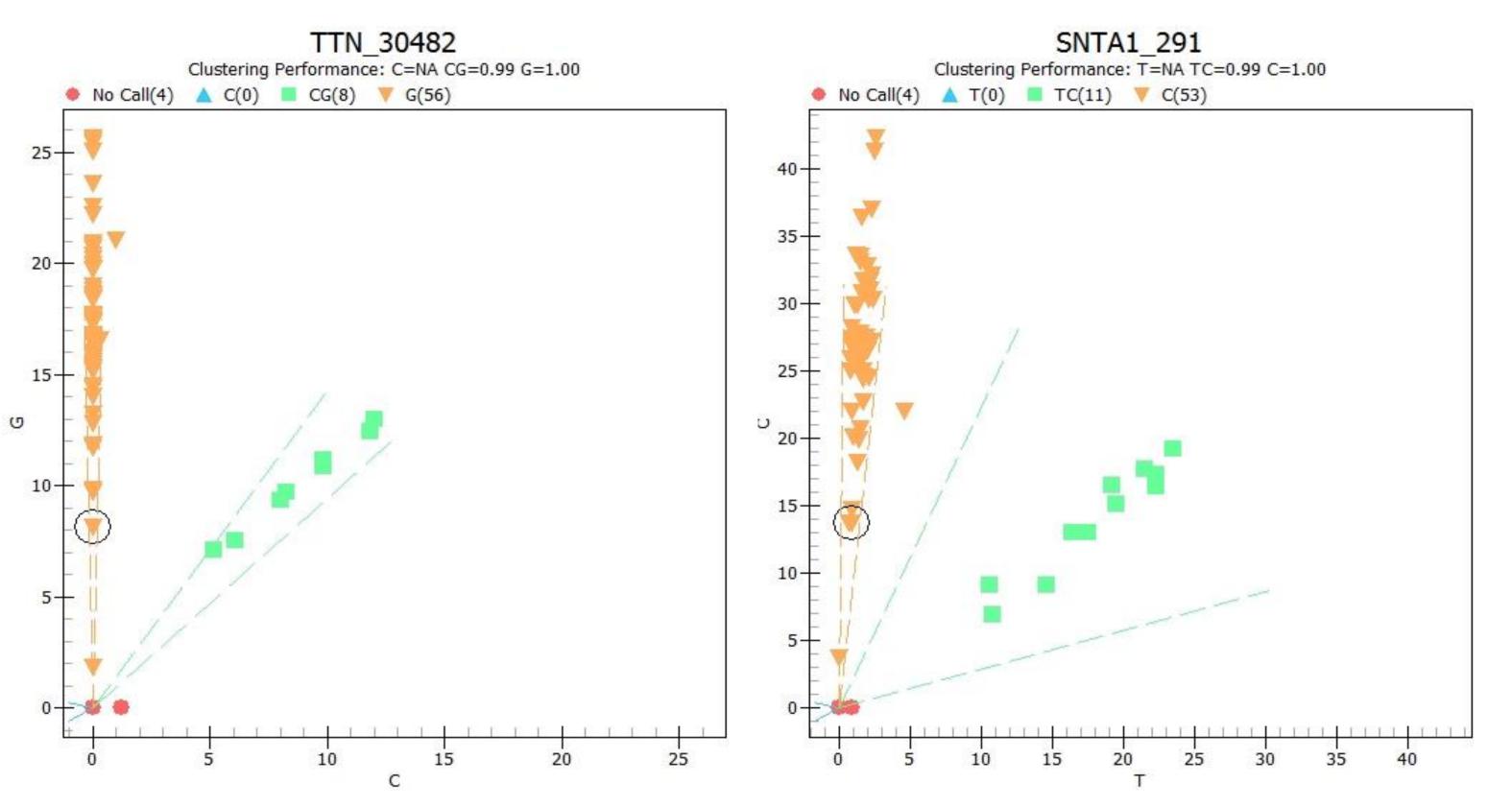
Abstract: Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disease in humans and cats. To date, a large number of genetic studies have established that HCM is caused by mutations in at least eleven genes encoding the contractile components of the sarcomere or adjacent Z-disc. Despite the identification of numerous causative mutations, the pathogenic and genetic processes are still poorly understood, making a large animal model of familial HCM especially useful. Causative mutations have been identified in Maine Coon and Ragdoll cats, however, HCM is thought to be inherited in other breeds, including the Siberian cat, where the novel causative element remains unknown. Utilizing the candidate gene approach to focus on associations between genetic variation within multiple important sarcomeric and cardiac genes, this study aims to identify the causative element of HCM within the Siberian cat breed. Genes implicated in eight cardiac diseases, including HCM, were selected based on their known biological, physiological, and/or functional relevance to cardiomyopathies. Non-synonymous single nucleotide polymorphisms were identified in genes encoding Ankyrin 2 (*ANK2*), Syntrophin Alpha 1 (*SNTA1*), and Titin (*TTN*) in an affected Siberian cat, all absent from nine unaffected genomes and 73 of unknown HCM status. Variants within these genes were compared between affected cats, cats with an affected family member, and cats diagnosed as normal upon ultrasound. By identifying the causative element in Siberian HCM, the development of a genetic test for the disease could help eradicate the disease in future Siberian populations and provide an additional large animal biomedical model of HCM.
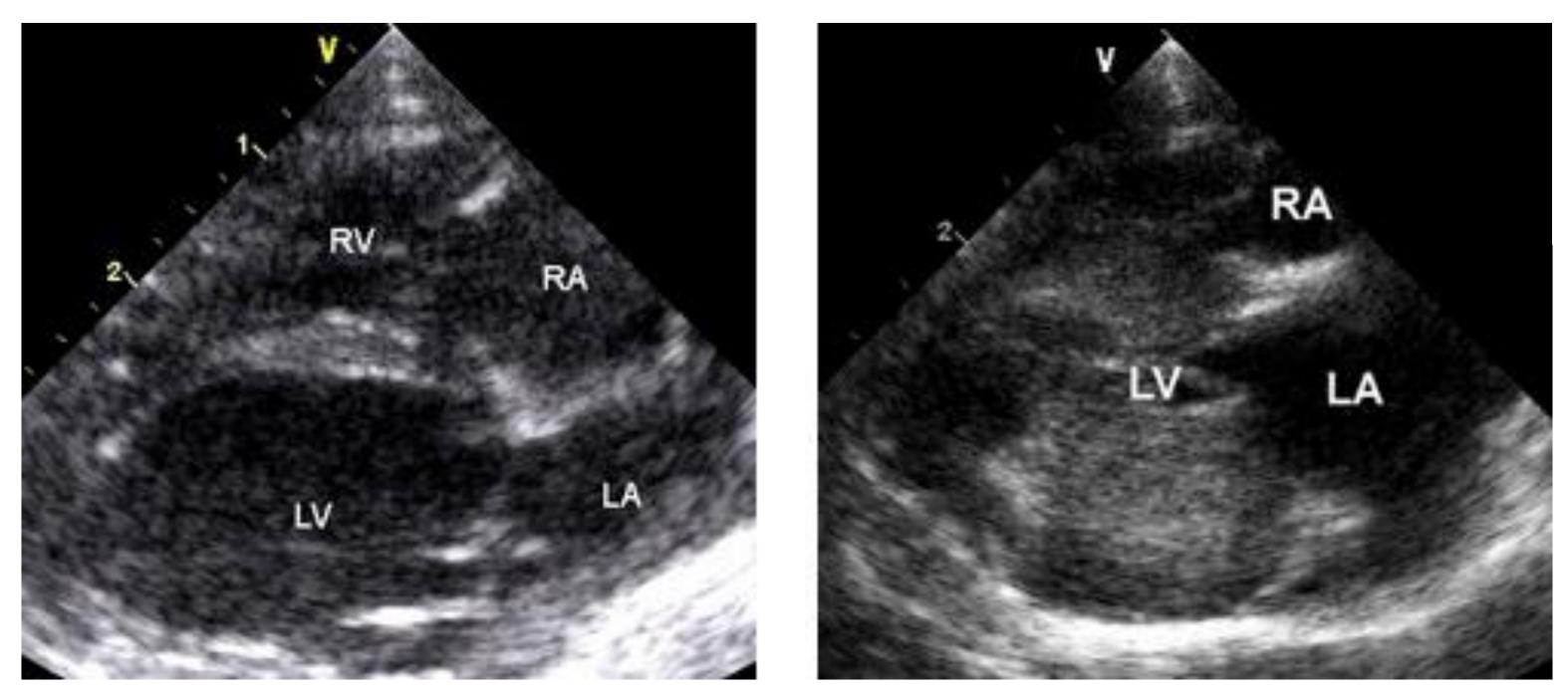
Introduction:

Hypertrophic cardiomyopathy (HCM) has been recognized as a common cause of heart failure, sudden death, and systemic thromboembolism in domestic cats since the 1970s (1), and is a relatively common genetic cardiac disease that is heterogeneous with respect to disease-causing mutations, presentation, prognosis, and treatment strategies. HCM is defined as an increase in volume of the myocardium due to an increase in the size of cardiomyocytes themselves, primarily in the left ventricle and ventricular septum. In addition, the normal alignment of muscle cells may be disrupted, a phenomenon known as myocardial disarray, causing disruptions of the electrical and contractile functions of the heart (2). Clinical diagnosis of HCM is via echocardiographic identification of unexplained left ventricular wall thickening, in the absence of ventricular dilation (Figure 1). Despite the identification of numerous causative mutations in humans, as stated above, the pathogenic and genetic processes are still poorly understood, making a large animal model of familial HCM especially useful for investigators to evaluate pathophysiologic processes and therapies.

While causative mutations in *cardiac myosin binding protein C* (*MYBPC3*) have been identified in Maine Coon (A31P) and Ragdoll (R820W) cats, HCM has also been documented as a hereditary trait in other breeds, including the Siberian cat, where the novel causative element remains unknown. Mutations in the cardiac genes most associated with HCM (*cardiac troponin I, troponin T, MYBPC3, cardiac essential myosin light chain, cardiac regulatory myosin light chain, alpha tropomyosin, actin, and beta-myosin heavy chain*) do not appear to be the primary cause of the disease in the Siberian (3).

Figure 1: Echocardiograms of feline hearts showing normal heart *(left)* and heart affected by HCM *(right)*. Note thickened wall of left ventricle *(LV - Left Ventricle, RV - Right Ventricle, LA - Left Atrium, RA - Right Atrium)* – Royal Veterinary College – University of London

Materials and Methods:

- HCM status was determined by a licensed veterinarian via echocardiography in 64 Siberian cats, with a LV free wall thickness cutoff of >6mm.
- Whole-genome sequencing (WGS) was performed on one affected Siberian cat and sequence was compared to a database containing 82 cats.
- 55 candidate genes implicated in HCM or other cardiac disease were selected, and variants in exonic or splice site regions were visually inspected in the affected cat's sequence.
- Only variants associated with a change in the protein (missense mutation, frame shift, stop codon) were considered potentially causative if present in the affected cat but not controls.
- Custom assay was designed for the Agena MassARRAY system to genotype the previously selected variants within 12 affected and 52 control Siberian cats.

Results:

- 12 affected and 52 unaffected Siberian samples were confirmed by a licensed veterinarian and included in the study.
- A 30x whole genome coverage was obtained in the affected Siberian cat.
- 15 of the 55 candidate genes contained variants unique to the affected Siberian cat; three of these genes (*TTN, ANK2, SNTA1*) contained four variants associated with a change in the protein.
- Variants were genotyped on the Agena MassARRAY system with a final coverage rate of 94.12%.
- Prevalence of each variant within the sample set is presented in Table 1.
- None of the SNPs previously identified in the affected Siberian WGS cat segregated concordantly with the disease phenotype in the 64 cats with known phenotype.

Figure 2: Cartesian plot denoting heterozygosity in TTN and SNTA1 SNPs. Triangles represent homozygous wild type while squares represent heterozygous samples. X and Y axes represent the normalized intensity of the genotyping reaction of each detected allele.

Gene	Variant	Effect	Prev. in HCM	Prev. in Control
TTN	G to A substitution	Arg to Cys	0/12	0/52
TTN	G to C substitution	Arg to Gly	1/12	7/52
ANK2	A to G substitution	Ile to Thr	1/12	1/52
SNTA1	C to T substitution	Arg to Cys	3/12	8/52

Table 1: Single nucleotide polymorphisms within the *TTN*, *ANK2*, and *SNTA1* genes, and their prevalence within affected and control cats.

Conclusions:

<u>**A causative mutation for HCM was not identified, although several non-synonymous single nucleotide polymorphisms were detected in *TTN, ANK2,* and *SNTA1***</u>. Mutations within these cardiac genes, or those most commonly associated with HCM, do not appear to be the only cause of HCM in the Siberian cat. Evaluation of additional cardiac genes is warranted to identify the molecular causes of feline HCM, as is further evaluation of regulatory elements (such as promoters or untranslated regions) of typical HCM genes.

References:
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2 Maron B.J. : Hypertrophic Cardiomyopathy: A Systematic Review. *JAMA.* 287:1308-1320, 2002.
3 Meurs, K.M., Norgard, M.M., Kuan, M., Haggstrom, J. and Kittleson, M. (2009), Analysis of 8 Sarcomeric Candidate Genes for Feline Hypertrophic Cardiomyopathy Mutations in Cats with Hypertrophic Cardiomyopathy. *Journal of Veterinary Internal Medicine,* 23:840–843, 2009.