

# Cat Welfare Trust Annual Report

Rosemary Fisher - Secretary

In 2016, the Trust was approached by Veterinary Cardiologist David Connolly, Senior Lecturer in Cardiology at the Royal Veterinary College. Their Cardiology group has a particular interest in hypertrophic cardiomyopathy (HCM) and has published widely on this subject. The Trust was asked for funding to help with an exciting project: To identify the genetic architecture promoting the development of hypertrophic cardiomyopathy in British Short Hair Cats to enable assessment of a novel therapeutic agent.

## Background:

HCM has an exceedingly high prevalence in cats: it affects up to 1 in 7 cats and is the most commonly diagnosed feline myocardial disease. The prognosis for cats with HCM is very variable, with average survival reported as being between 596 and 1,297 days. Affected cats with severe HCM frequently present with congestive heart failure (CHF), thromboembolic disease or may experience sudden cardiac death (SCD). Therefore, in many cats HCM is a devastating and distressing disease which results in significant morbidity and mortality.

HCM is also diagnosed in about 1 in 500 people. It is the leading cause of sudden death in young adults and results in significant disability in survivors. In both cats and humans, HCM is inherited as an autosomal dominant trait with variable penetrance. In humans, alterations in two genes,  $\beta$ -myosin heavy chain and myosin-binding protein C (MYBPC) account for approximately 75% of cases where an underlying mutation has been identified. In Maine Coon and Ragdoll cats the HCM causing mutations were also found in the MYBPC gene reflecting the close similarity of the disease between the two species.

## The Study:

### ***Report for the Cat Welfare Trust on exploring the genetic architecture of Hypertrophic Cardiomyopathy in British Short Hair Cats.***

*David J Connolly 12/10/2020*

First, I would like to express my gratitude to the Cat Welfare Trust for the generous support they have given me and my team to conduct this important work on characterising the genetic architecture of hypertrophic cardiomyopathy in British Short Hair cats.

### ***The original Aims of our proposal to the Cat Welfare Trust were:***

1. To phenotypically characterise 24 British Short Hair Cats (BSH) with severe hypertrophic cardiomyopathy (HCM) using echocardiography.
2. To identify 24 geriatric (12 year or older) BSH cats without evidence of HCM using echocardiography.
3. Using DNA extracted from residual blood we will use a genome-wide association (GWA) analysis to detect loci affecting HCM occurrence.

***We have updated these Aims as a result of obtaining further funding from a variety of sources enabling the scope of the project to be significantly increased. The updated aims of our proposal are:***

1. Map and evaluate genomic regions affecting HCM susceptibility in BSH cats – This will be done via GWAS to detect loci affecting HCM susceptibility using 100 BSH blood samples (50

HCM cases and 50 healthy, age-controlled cats controls). This work will analyse samples in a binary (case v control) manner as well as using the continuous variables from echocardiographic measurements and histopathological findings.

2. Identify the gene expression profile for HCM in cats – This will be identified using RNA-sequencing of ~20 myocardial samples (10 HCM, 10 healthy) from cases and controls (age and sex controlled as much as possible) followed by differential expression analysis and pathway enrichment analysis. We will have an emphasis on BSH cats and 5 of the HCM and 5 of the control samples will be from BSH cats. This type of work has not yet been published for feline HCM
3. Integrate results and identify genetic markers and biomarkers for prediction of HCM susceptibility.

### ***Experimental Design and Methods:***

#### ***Phenotyping:***

All cats used in the study underwent an echocardiogram by diplomates in cardiology or cardiology residents under their supervision using standardised equipment (GE Vivid E9 echo machine; GE systems, Hatfield, Hertfordshire, UK); heart measurements, medical history and clinical data obtained during these examinations were accessible for research via the EchoPAC PC (Version 110.0.2; GE Medical Systems, Hatfield, Hertfordshire, UK) clinical software at the QMHA. Diagnosis of HCM was based on maxLVWT (max left ventricular wall thickness) calculated from the maximum value of a series of 2D end-diastolic measurements in different right parasternal views of the interventricular septum (IVSd 2D max) and the left ventricular free wall (LVFWd 2D max) with one or more value >6mm. Control cats were elderly (>9 years old) and defined as LVWT<5mm.

#### ***Genotyping:***

DNA will be extracted from EDTA samples of HCM and control BSH cats using the Qiagen BioSprint 15 DNA Blood Kit using standard methods. We will analyse the disease as a binary trait (case vs. control) as well as using continuous variables based on echocardiography measurements in order to increase the resolution of the phenotype and power of the analysis and reduce the effect of variable disease expression. The study will include GWA and regional heritability analyses within BSH cat combined with a selective sweep approach to identify genomic regions under selection. Finally, whole-genome sequencing (WGS) of HCM-resistant and susceptible animals will be performed to identify putative causative mutations directly affecting HCM in the genomic regions of interest.

#### ***RNA sequencing:***

20 myocardial samples (mid left ventricular free wall) described above will be harvested for RNA extraction. The samples will be homogenised, and RNA will be extracted using the commercially available GenElute RNA extraction kit provided by Sigma-Aldrich, which we have used previously to extract high quality RNA. RNA will then be subject to genomic DNA removal using the TURBO DNase (Thermo Fisher Scientific).

Edinburgh Genomics UK (the leading company in the UK) will perform the library preparation of the samples and RNA-sequencing. RNA (1.2 µg with a RIN (RNA integrity number) of >8) will be extracted and sent to Edinburgh for Illumina library preparation. Illumina sequencing will be performed on a NovaSeq6000 with 50 paired-end reads (at least 375M + 375M reads).

The initial data analysis will be performed at Edinburgh Genomics, using their differential gene analysis pipeline, which includes:

- Mapping of reads to annotated genome using BWA/STAR, return of alignment file in BAM format.
- Assignment of reads to gene features and generation of matrix with read count per sample per gene using featureCounts; filtering and normalisation to produce a matrix suitable for exploratory purposes; return of raw and filtered/normalised matrices in .csv format with gene annotations.
- Exploratory analysis filtered/normalised matrices, return of expression distributions, principal components analysis and heat maps as image files showing up and down regulated transcripts
- Analysis of a differential gene set analysis between groups (with the statistically robust ROAST method), using gene sets from the Gene Ontology and Reactome.

With help from our experienced bioinformatics group here at the Royal Veterinary College, we will then perform in-house analysis to identify genes associated with pathways of particular biological interest in the setting of HCM by employing hierarchical clustering of the differentially-expressed genes followed by functional annotation using FuncAssociate<sup>17</sup>.

**Verification step:** Quantitative reverse transcription PCR (qRT-PCR) will be used to verify the mRNA-sequencing results of genes more strongly expressed in affected cats versus healthy controls and preference will be given to genes that affect biological pathways of interest. Furthermore, we will quantify the activity of key genes at the translational level by Western blot and immunocytochemistry. These techniques are routinely performed in our laboratory.

**Results to date:** We continue to phenotype BSH cats using echocardiography which are obtained primarily from cats referred into The Queen Mother Hospital for Animals at the Royal Veterinary College (RVC) and these comprise both HCM affected and control animals. Residual EDTA blood samples are collected (with all ethical approval in place) and stored in a -80°C Freezer. Additional cases (HCM and control) are obtained from BSH breeders and owners by ourselves and a number of collaborators across the UK. Recently due to the Covid-19 pandemic phenotyping and sample collection has been significantly delayed but fortunately our hospital case load is back up to normal and unless a second lockdown occurs we hope to be back on track with our sample collection.

As of June 1<sup>st</sup> 2020 we have carefully phenotyped and collected blood samples from 68 HCM affected BSH cats and 27 BSH control cats. Our emphasis is now to increase our number of control geriatric cats – these represent the most difficult samples to collect as we need normal cat >10 years old given the late onset of the phenotype seen in many cases of HCM.

Furthermore, to date we have collected left ventricular free-wall myocardial samples from 4 BSH cats with HCM and 6 control cases (with full ethical approval) as detailed above. We rely on the generosity of owners whose cats are euthanised for cardiac and non-cardiac reasons for this vital material. We will continue to collect myocardial samples along with blood samples and attempt to exceed our initial sample size.

Due to the Covid -19 pandemic access to the RVC laboratories has been severely restricted and therefore we have concentrated on sample collection at this stage of the work. Once we have reached our desired sample numbers our dedicated PhD student Tom Smedley will perform the DNA and RNA extraction and purification steps prior to sequencing. Then with input from Dr Androniki Psifidi (our veterinary geneticist and bioinformatics specialist), Tom will perform the secondary bioinformatic analysis to gain a greater understanding of not only potential causative genes but also modifier genes and transcriptional pathways underlying this severe disease in BSH cats.

Since we have significantly expanded the number of cats we are going to include in this work together with the delays caused by the Covid 19 pandemic we have not yet sent samples for genomic analysis or RNA sequencing and have therefore not yet used the generous contribution from the Cats Welfare Trust. Please be assured that these funds will be used for the work detailed above and we will keep you informed of progress.