Feline hypertrophic cardiomyopathy: phenotypical and functional changes in cardiomyocytes

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Introduction

Feline hypertrophic cardiomyopathy (fHCM) is the most frequently diagnosed cardiomyopathy in domestic cats. While we know that fHCM is associated with myocardial remodelling, the relevant underlying pathogenic processes are still poorly understood. The present study, based on results from our recent RNA sequencing study[1], aimed to determine molecular phenotypic changes in cardiomyocytes that could highlight pathogenic mechanism in fHCM.

Material & Methods

The hearts of 15 cats with confirmed fHCM (11 male, 4 female; mean age 8.93 years) and 31 control cats (16 young control cats (8 male, 8 female; mean age 1.5 years) and 15 adult controls (10 males, 5 females)) were subjected to RT-PCRs for a range of markers indicative of cardiomyocyte functional adaptations and alterations, the upregulation of which was detected in a recent RNA sequencing study of our group[1]. For each gene, a TaqMan-based two-step RT-qPCR protocol was established. Statistical analysis included the Shapiro-Wilk test to check for normality and log transformation to meet normality assumptions. An ANOVA including the main effect of group and sex as well as their interaction was modelled. In case of significant F-test for interaction of group and sex, Tukey post-hoc test for pairwise comparisons was performed.

Growth factor receptors

Insulin growth factor 1 receptor (IGF1R) and insulin receptor substrate 1 (IRS1). IRS1 is phosphorylated by IGF1R and activates key signalling pathways in cardiomyocytes (PI3K/Akt and MAPK), leading to proliferation, differentiation, and hypertrophy.[2–4]

Both markers show highest expression in young cats which decreases with age, and increased expression in HCM hearts. For IGF1R, a significant increase is found in HCM hearts of male cats.

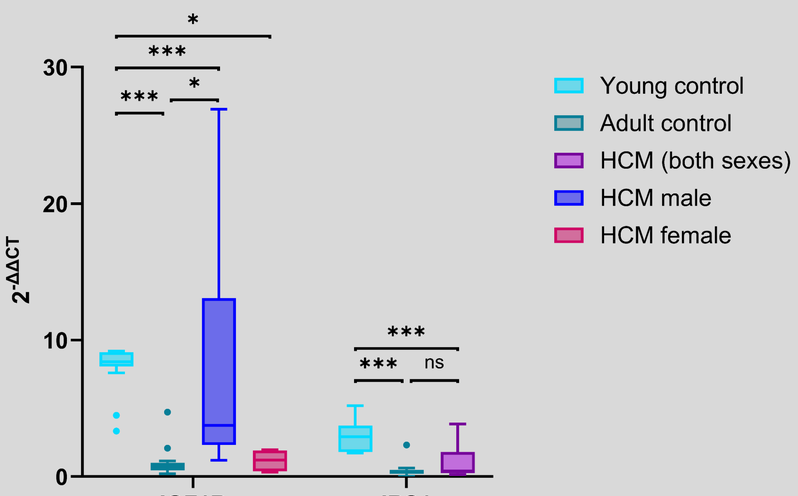
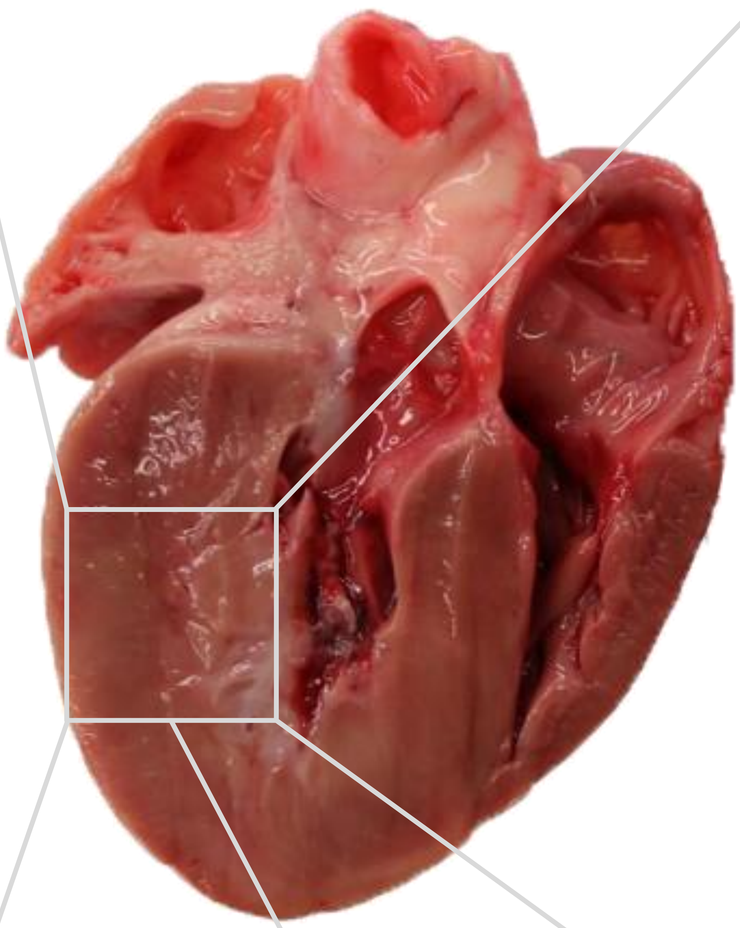


Figure 1. *Boxplots of relative mRNA levels of IGF1R and IRS1 in the myocardium of the control groups and cats with HCM. Asterisks indicate the significance: P ≤0.05 (*), P ≤0.01 (**), p ≤0.001 (***), ns: non-significant.*



Intercellular signalling

Integrin subunit alpha M (ITGAM) and integrin subunit alpha 10 (ITGA10) were investigated. Integrin-mediated signalling may lead to changes in cell shape, differentiation, growth, and survival by activation of relevant signalling pathways like PI3K/Akt/ERK.[5-6]

The highest expression is found in young hearts, decreasing with age, and increased in HCM hearts. ITGAM additionally shows a significant increase in HCM hearts of male cats.

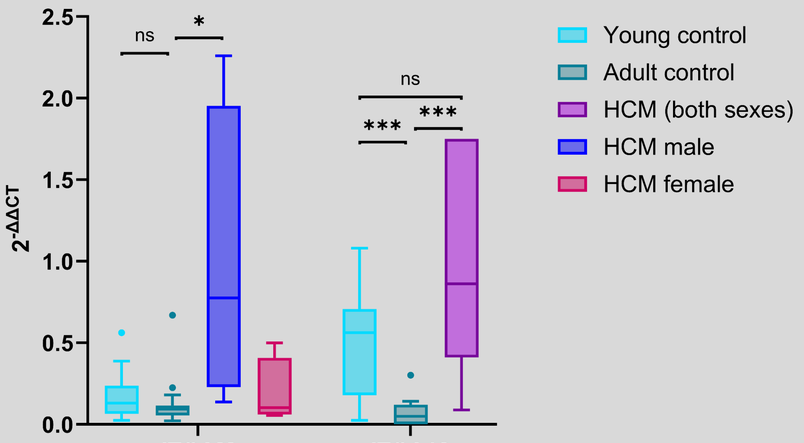


Figure 2. *Boxplots of relative mRNA levels of ITGAM and ITGA10 in the myocardium of the control groups and cats with HCM. Asterisks indicate the significance: P ≤0.05 (*), P ≤0.01 (**), p ≤0.001 (***), ns: non-significant.*

Vascularisation

Vascular endothelial growth factor A (VEGFA) is a key regulator of angiogenesis and plays an important role in cardiac morphogenesis, cardiac contractility, and myocardial repair.[7] Thrombospondin 1 (TSP1), in contrast, has antiangiogenic activity and is upregulated in cardiac remodelling and hypertrophy.[8]

Both markers show highest expression in HCM hearts and decreasing expression with age. No significant sex differences are detected in HCM hearts.

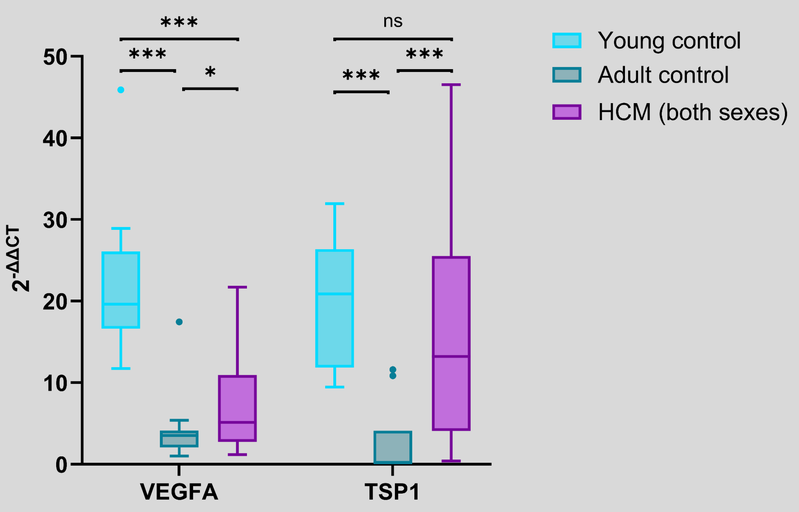


Figure 3. *Boxplots of relative mRNA levels of VEGFA and TSP1 in the myocardium of the control groups and cats with HCM. Asterisks indicate the significance: P ≤0.05 (*), P ≤0.01 (**), p ≤0.001 (***), ns: non-significant.*

Foetal genes

GATA binding protein 4 and 6 (GATA4, GATA6) and Myocyte enhancer factor 2c (MEF2C) regulate transcription of cardiac proteins during development and are involved in cardiac hypertrophy.[9-11]

All markers show highest expression in HCM hearts and decreasing expression with age. For MEF2C and GATA6, significant increases are found in HCM hearts of male cats.

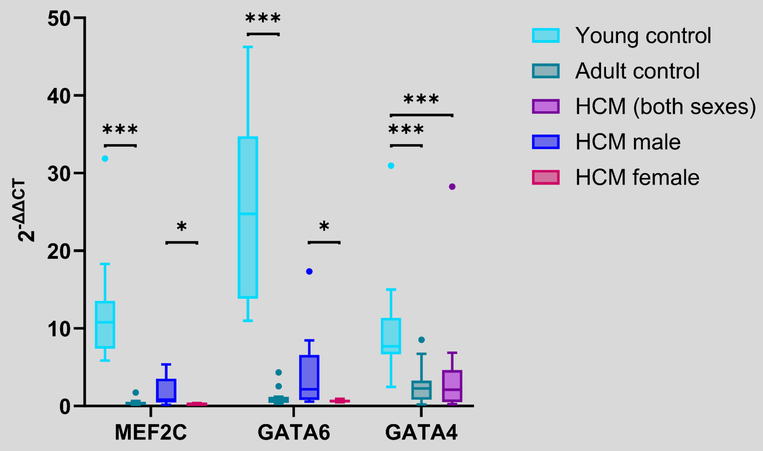


Figure 4. *Boxplots of relative mRNA levels of GATA4, GATA6 and MEF2C in the myocardium of the control groups and cats with HCM. Asterisks indicate the significance: P ≤0.05 (*), P ≤0.01 (**), p ≤0.001 (***), ns: non-significant.*

Hypertrophy

Phosphoinositide 3-kinase (PI3K), Muscle RAS oncogene homolog (MRAS), Serine-threonine-protein kinase B-raf (BRAF) and Mitofusin 2 (MFN2) were the genes of interest. Alterations of their levels and activities may enhance RAS/RAF/MEK/MAPK signalling and activate upregulation of molecular signalling towards cardiac hypertrophy.[9]

All investigated markers show higher expression in young hearts and HCM hearts, with sex differences identified for PI3K and MRAS (higher in male cats).

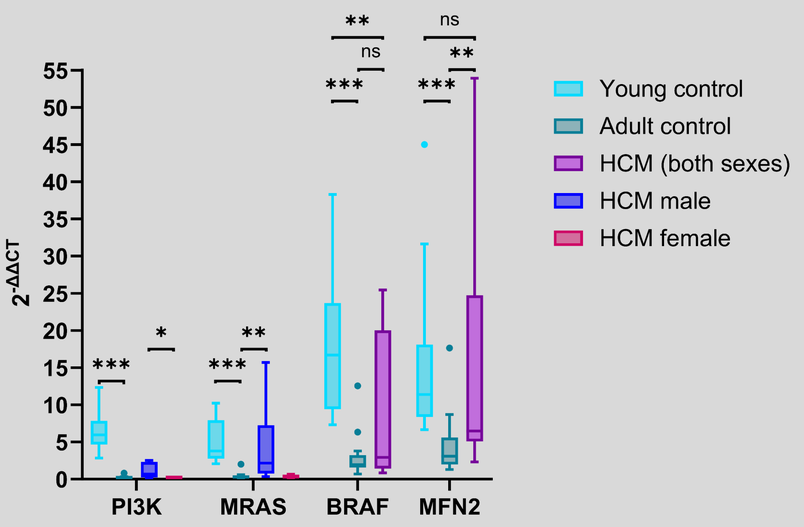


Figure 5. *Boxplots of relative mRNA levels of PI3K, MRAS, BRAF and MFN2 in the myocardium of the control groups and cats with HCM. Asterisks indicate the significance: P ≤0.05 (*), P ≤0.01 (**), p ≤0.001 (***), ns: non-significant.*

Conclusion

The results show age-related differences in the transcription of most markers. They confirm upregulation of a range of markers in fHCM compared to healthy hearts, with certain sex-related differences in fHCM. They provide evidence that adaptive attempts (hypertrophy and dedifferentiation) and increased signal transduction as well as intercellular signalling occur in cats with HCM. Moreover, for several markers, expression was most pronounced in male cats, which are known to be predisposed to fHCM.

References

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