Primary effects of HCM mutations in humans and cats

A. E. Messer[1], M. Papadaki[1], P. Vikhorev[1], Y. Sebzali[1], M. El-Mezgueldi[2], A. Daley[3], D. Connolly[3] and S. B. Marston[1]

[1]Myocardial Function, Imperial College London, NHLI, London, W12 0NN, United Kingdom
[2]Department of Molecular and Cell Biology, University of Leicester, Leicester, LE1 9HN, United Kingdom
[3]Small Animal Medicine and Surgery Group, The Royal Veterinary College, Hatfield, Hertfordshire, AL9 7TA, United Kingdom

Hypertrophic cardiomyopathy (HCM) is the most commonly inherited cardiomyopathy. In cats it is even more common: 15% of a large population of unselected outbred cats has HCM. HCM-causing mutations in sarcomeric proteins have been proposed to increase myofilament Ca^{2+}-sensitivity but may have additional primary effects.

We have previously found that in dilated cardiomyopathy (DCM)-causing mutations in thin filament proteins the relationship between phosphorylation at Ser 22 and 23 of cardiac troponin I (TnI) and Ca^{2+}-sensitivity was abolished. This phenomenon has been termed 'uncoupling' and has been found for all thin-filament protein DCM-causing mutations investigated *in vitro* and in skinned myofibrils. It is associated with a blunted response to β1-adrenergic stimulation and reduced cardiac reserve that is potentially disease-causing.

Interestingly, uncoupling might also be a characteristic of HCM. Uncoupling has been demonstrated in a number of HCM mutants in TnI and troponin T (TnT). To determine whether either of these phenomena is common to HCM mutations we have studied them using the *in vitro* motility assay. We have looked at mutations in *TPM1* (E180G) and *TNNT2* (Δ14, Δ28+7, R92L, R92Q, ΔE160, S179F, K273E and K280N) using recombinant troponin. All of these mutations showed uncoupling. The ACTC E99K mutation showed uncoupling in both isolated thin filaments and single myofibrils.

Preliminary experiments were carried out with troponin from a Ragdoll cat heart with HCM due to the R820W mutation in *MYBPC3* and a non-affected cat as control. The mutant thin filaments showed an increase in Ca^{2+}-sensitivity and were also uncoupled. Thus, troponin from HCM cats was abnormal even though the mutation was in MyBP-C that was not present in the assay.
Overall these results suggest the Ca^{2+}-sensitising and uncoupling properties of HCM mutations may be more widely distributed than previously thought.