Knock-in mouse model of PRKAG2 cardiomyopathy (R299Q) exhibits altered Ca\textsuperscript{2+}-dependent cardiac contractility and reduced protein kinase A activity

C. Turtle; P. Robinson; A. Yavari; S. Ghaffari; K. Pinter; H. Watkins; C. Redwood
University of Oxford, Radcliffe Department of Medicine, Oxford, United Kingdom

Familial hypertrophic cardiomyopathy (HCM) is most commonly caused by mutations in sarcomeric proteins; however, a unifying mechanism of disease pathogenesis has yet to be identified. Beyond the sarcomere, mutations in the gene (PRKAG2) encoding the \(\gamma_2\) subunit of AMP-activated protein kinase (AMPK), an enzyme involved in energy balance regulation and cell signalling, cause ventricular hypertrophy and contractile dysfunction mimicking HCM. These AMPK \(\gamma_2\) mutations also result in glycogen accumulation and aberrant electrical conduction, obfuscating the cause of hypertrophy and dysfunction. Given our recent finding that cardiac troponin I (cTnI) is phosphorylated by AMPK at S150 and this phosphorylation enhances Ca\textsuperscript{2+} sensitivity of activation, we hypothesise that alterations at the myofilament level may contribute to disease pathogenesis in PRKAG2 cardiomyopathy as they do in HCM.

To test this, we have used a mouse model possessing an AMPK \(\gamma_2\) mutation (R299Q knock-in) and measured demembranated trabeculae mechanics and cardiomyocyte contractility using heterozygous and homozygous male mice, along with littermate wild type controls, between 6-8 weeks of age (prior to hypertrophy and significant glycogen accumulation). In trabeculae, we observed increased Ca\textsuperscript{2+} sensitivity of force production in fibres from mutant mice (\(\Delta p\text{Ca}_{50} = +0.05\) (Het) and +0.06 (Homo), \(p < 0.01\)). After treating fibres with protein kinase A (PKA), no differences in Ca\textsuperscript{2+} sensitivity of force production were observed between WT and mutant groups. Intact cardiomyocytes isolated from mutant hearts exhibited little difference in shortening or Ca\textsuperscript{2+} transient magnitudes but significantly slowed kinetics of both contraction and relaxation. Cardiomyocytes from mutant mice exhibited a greater response to isoproterenol. Biochemical analyses of untreated tissues indicated reduced phosphorylation of cTnI S23/S24 (\(\Delta\)mol P/mol TnI = -0.17 (Het) and -0.28 (Homo), \(p < 0.05\)) and phospholamban S16, indicating reduced basal PKA activity in mutant animals.

This work provides evidence that the R299Q PRKAG2 mutation causes altered cardiac contractile function irrespective of glycogen accumulation and therefore supports the hypothesis that impaired contractility contributes to disease pathogenesis. Further, the observed Ca\textsuperscript{2+} sensitization and reduction in phosphorylation of PKA target sites mimics similar alterations found in HCM caused by sarcomeric mutations. The reduction in PKA activity in the mutant hearts may be caused by novel crosstalk between the AMPK and PKA pathways, in particular as this occurs prior to hypertrophy.