Multiple gene mutations altering cell fate and severity of heart disease

T. Tsoutsman,[1,2] M. Kelly,[1] E. Tu,[1] L. Lam,[1] C.E. Seidman,[3,4] J.G. Seidman[3] and C. Semsarian,[1,2,5] [1]Agnes Ginges Centre for Molecular Cardiology, Centenary Institute, Locked Bag No. 6, Newtown, NSW 2042, Australia, [2]Central Clinical School, University of Sydney, NSW 2006, Australia, [3]Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, USA, [4]Cardiovascular Division, Brigham and Women's Hospital, Boston, MA, USA and [5]Department of Cardiology, Royal Prince Alfred Hospital, Sydney, NSW 2050, Australia. (Introduced by L. Hool)

Introduction: Familial hypertrophic cardiomyopathy (FHC) is characterised by both genetic and clinical heterogeneity: more than 400 mutations in at least 15 genes have been identified leading to a diverse phenotype ranging from no symptoms to heart failure and sudden cardiac death. Although previously believed to be caused by a single gene defect, our recent study demonstrated that in an Australian FHC cohort up to 5% families carry two FHC-causing mutations (Ingles *et al.*, 2005). The presence of a second mutation results in earlier disease onset, increased cardiac hypertrophy, and higher incidence of sudden death events. This observation suggests that the severity of human FHC may relate to the number of disease-causing gene mutations.

Methods: A double mutant mouse model has been established to investigate the mechanisms involved in the development of the severe FHC phenotype. Two single mutant mouse models of FHC - Gly203Ser cardiac troponin I (TnI-203) and Arg403Gln α-myosin heavy chain (MHC-403) - were crossed to obtain offspring with a double mutation genotype (TnI-203/MHC-403). Four groups of littermates (non-transgenic (NTG), TnI-203, MHC-403, and TnI-203/MHC-403) were characterised by survival, heart:body weight (HW:BW) and lung:body weight (LW:BW) ratios, histopathology, ECG and qRT PCR mRNA expression of atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), cardiac L-type Ca^{2+} channel (LTCC), cardiac ryanodine receptor (RyR2), sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA2a) and phospholamban (PLB).

Results: Single mutant models have previously been shown to develop FHC by the age of 20-30 weeks with no effect on the life span (Semsarian *et al.*, 2002, Tsoutsman *et al.*, 2006). Unlike single mutant littermates, TnI-203/MHC-403 mice develop severe FHC phenotype resulting in 100% mortality by age 21 days. At age 14 days, TnI-203/MHC-403 mice developed significantly increased HW:BW ratio, marked interstitial myocardial fibrosis, and increased expression of the hypertrophy-related genes, ANF and BNP, compared to NTG and both single mutant littermates. This was associated with significant prolongation of PR, RR and corrected QT interval, consistent with the development of severe cardiomyopathy; marked down-regulation of mRNA levels of key regulators of intracellular Ca^{2+} homeostasis, RyR2, SERCA2a and PLB. At the age of 16-18 days LW:BW ratio in double mutant mice is significantly increased, mice rapidly developed a severe dilated cardiomyopathy leading to death from heart failure, most commonly between ages 16-20 days.

Conclusions: The presence of two disease-causing mutations may predispose individuals to higher risk of developing a severe cardiac phenotype, heart failure and early death compared to single-gene mutation. The TnI-203/MHC-403 model is an important molecular tool to facilitate the understanding of FHC pathogenesis and its complications, including the end-stage heart failure.

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