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Involvement of Wnt signaling in remodeling of the heart

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The Dishevelled (Dvl) protein plays a crucial role in activation of Wnt signaling pathway. Increased expression of Dvl was observed in the stable phase of cardiac hypertrophy following aortic banding; however its pathophysiological relevance is not known. In order to identify the role of Dvl in mediating cardiac hypertrophy we have used genetically engineered mice with cardiac specific over expression of Dvl (Dvl-Tg). Severe cardiomyopathy with gross pathology of the transgenic hearts was noted in Dvl-Tg mice at 3 months of age. Premature death occurred in all Dvl-Tg mice before the age of 6 months. As the animal ages, increase in fibrosis and apoptosis was noted.

Echocardiographic analysis of animals aged 3 months revealed increase in end-diastolic diameter and end-systolic diameter in Dvl-Tg mice as compared to wild-type animals. Consecutively, a drastic reduction in the ejection fraction in Dvl-Tg mice was observed. Hemodynamics of the left ventricle was further assessed by pressure-volume loop analysis. Increase in end-diastolic and end-systolic volume as well as a decrease in the maximal rise of left ventricular pressure (dp/dt max) was noted in Dvl-Tg mice which indicate impairment in contractility. Reduction in dp/dt min levels and the increase in the time constant of isovolumic relaxation (Tau-Glantz) levels demonstrate diastolic dysfunction in the Dvl-Tg mice. Taken together, echocardiography and invasive hemodynamics consistently demonstrated a significant impairment of cardiac function in Dvl-Tg mice. Consistent with reduced myocardial function morphological analysis of the heart revealed further evidence for adverse myocardial remodeling. Heart weight / body weight ratio (mg/g) of fresh tissue and heart weight / tibia length ratio (mg/mm) were significantly higher in Dvl-Tg mice. Histopathological analysis revealed the prevalence of cardiac hypertrophy with an in increase in cardiomyocyte size, fibrosis and apoptosis of the hearts in Dvl-Tg mice as compared to their WT animals. Supplementary *in vitro* experiments in cardiomyocytes using siRNA mediated depletion of Dvl indicated no baseline alterations in unstimulated cells where as, isoproterenol challenge failed to induce a hypertrophic response in cardiac myocytes. Thus Dvl is necessary for β-adrenergic cardiac hypertrophy.

Western blot analysis suggested the activation of both the canonical and non-canonical Wnt signaling pathways in Dvl-Tg mice. Activation of these pathways was found in 6 weeks old animals when the cardiac phenotype was still identical to WT animals indicating a causal link of non-canonical Wnt-signaling to cardiomyopathy. *In vitro* experiments with cardiomyocytes using a mutant Dvl lacking the DEP domain (which mediates non-canonical Wnt-signaling) did not induce cardiac hypertrophy. Similarly, pharmacological inhibition of CAMKII dependent signaling using KN93

prevented full length Dvl from executing its pro-hypertrophic effects on cardiac myocytes indicating that non canonical Wnt-signaling mediated by CAMKII is essential for Wnt-mediated cardiac hypertrophy. Even though both branches of Wnt-signaling are upregulated, our data demonstrate that non-canonical Wnt-signaling is indispensable for hypertrophic cardiomyocyte growth.