# Altered spatio-specific CaMKII activation in autophagy deficient mice

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**Background:** Autophagy is linked to preventing the development of cardiac hypertrophy and failure. While aberrant activation of Ca2+/calmodulin-dependent kinase II (CaMKII) promotes myocardial remodeling, the role of autophagy in maintaining cardiac Ca2+ homeostasis and regulating CaMKII signaling is unknown.

**Objective:** To test whether loss of autophagy promotes subcellular alterations in CaMKII activation in early myocardial remodelling, and whether compromised in vivo cardiac function parallels those changes.

**Methods:** Young (10–15 weeks) cardiomyocyte-specific autophagy protein 5-deficient mice (Atg5–/–) mice and their littermate controls (Atg5+/+) underwent comprehensive in vivo phenotyping using echocardiography, exercise tolerance and hemodynamic stress testing. In vitro assessment included gravimetry, qPCR of hypertrophy marker genes and cellular and nuclear dimensions of isolated ventricular myocytes. CaMKII activation was studied by immunocytochemistry in cardiomyocytes upon exposure to basal (1Hz) or high (4Hz) pacing frequency. Autophosphorylated CaMKII (pT286) signal was evaluated in different subcellular spaces (i.e. cytoplasm, nucleoplasm and nuclear envelope).

**Results:** Before symptomatic cardiac dysfunction occurred, Atg5–/– mice showed compromised cardiac reserve in response to β-adrenergic stimulation (dp/dt max: 9475±126 vs 7364±496 mmHg/s, N=4–5; p=0.041), despite similar maximum heart rate. Consequently, effort intolerance (distance run: 251±22 vs 152±13 m, N=8; p=0.03) and maximal oxygen consumption (2093±66 vs 1763±131 ml/h/kg, N=8; p=0.04) were reduced during treadmill exercise tolerance testing. Increased heart-to-body weight ratio (8.1±0.5 vs 10.2±0.8 N=9; p=0.017) was associated with elevated mRNA expression of hypertrophy marker NppB (278% of Atg5+/+, N=5; p=0.016) in Atg5–/– mice, which showed enlarged cardiomyocytes and nuclei, as width-to-length ratio.

Because Atg5–/– cardiomyocytes exhibit elevated nuclear Ca2+ levels at high pacing frequency, we now measured subcellular CaMKII activation under the same experimental conditions. Interestingly, at 1Hz, p-CaMKII was increased specifically at the nuclear envelope (154% of Atg5+/+, N=5 mice, 153–159 cells; p=0.029), but not in the cytoplasm or nucleoplasm. Increasing pacing frequency to 4Hz did not alter p-CaMKII levels in Atg5+/+ cells. However, p-CaMKII was increased by ∼30% and ∼20% in the cytoplasm and nucleoplasm of Atg5–/– cells respectively (N=5 mice, 153–155 cells).

**Conclusion:** Loss of ATG5-dependent autophagy causes cardiac hypertrophy and impaired cardiac reserve upon acute stress, which involves CaMKII activation, likely through the imbalance of nuclear Ca2+ load. Although, selective increase in p-CaMKII at the nuclear envelope in Atg5–/– mice may temporarily protect from nuclear Ca2+ overload, excessive CaMKII activation in the cytoplasm and the nucleoplasm upon increased workload, likely drives hypertrophic signalling toward heart failure in autophagy-defective mice.

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