In vivo MRI Studies of Cardiac Morphology and Function in Transgenic Mice Overexpressing the Exon 22 Isoform of the Human $Ca_v1.2\alpha_{1C}$ Channel

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Introduction. Recently, we found that in human vascular smooth muscle cells, alternative splicing of the pore-forming Cav1.2α1C subunit of Cav1.2 calcium channels was affected by atherosclerosis.. The molecular signature of the effect is an appearance of the exon-22 isoform of the α1C subunit that is absent in cells from non-diseased arteries. The mechanism responsible for the pathogenic effect of mutation in vivo remains unknown. Magnetic resonance imaging (MRI) has been shown to offer quantitative measurements of in vivo cardiac morphology without relying on geometric assumptions while providing high temporal and spatial resolution, thus yielding accurate and reliable quantification of global and local myocardial function. We applied in vivo cardiac MRI to characterize the myocardial phenotype of transgenic mice overexpressing the mutated L-type calcium channel previously correlated with atherosclerosis in humans. Given the fact that these mutant mice have a normal lifespan and no obvious signs of cardiomyopathy, we used chronic β-adrenergic stimulation to induce dilated cardiomyopathy in transgenic and control animals, with the goal of amplifying underlying differences in susceptibility to cardiac stress and remodeling between these two groups.

Methods.

Animal preparation: To determine whether the human Cav1.2□1C22+ mutation is pathogenic, we generated transgenic mice (TG22+) overexpressing the Cav1.2α1C22+ splice variant. Alzet mini-osmotic pumps were implanted subcutaneously in both TG22+ and wild type (WT) adult mice and infused isoproterenol (IP) at a rate of 30 mg /day for 7 days. Control ("vehicle-only") mice were implanted with pumps containing saline only. All animal protocols were approved by the Animal Care and Use Committee of the NIH National Institute on Aging.

Cardiac MRI. MRI was performed on a 7 Tesla/ 30 cm Bruker Biospec MRI Scanner (Bruker Biospin, Ettlingen, Germany) equipped with an actively-shielded 20 cm gradient set, 72 mm birdcage transmit coil and 20 mm receive-only surface coil. The mice were anesthetized with 1.5 % v/v isoflurane:O_2 and loaded into the scanner in the prone position. Body temperature was maintained at 37°C using a MRI-compatible heater system with rectal thermometer feedback (SA Instruments, Stony Brook, NY). Cardio-respiratory triggering signals from the SA Instruments gating unit were used for synchronization of the image acquisition to physiological motion. A respiratory-gated, ECG-triggered fast gradient echo (FLASH) sequence was used, with TE = 2.3 ms, TR = 7 ms, FA = 40°, FOV = 30×30 mm, MTX = 128×128 and slice thickness 1 mm. After completion of MR studies, the mouse was sacrificed and the heart was dissected to determined total heart weight and LV weight. For LV mass and volume measurement, epicardial and endocardial borders were manually delineated at end-diastole and end-systole for each short axis slice and the area within these borders was tabulated. Myocardial volumes were calculated by summing delineated areas in consecutive, contiguous short-axis slices multiplying by the slice thickness.

Results. There was a good correlation of LV ex-vivo mass with LV mass measurements obtained by MRI (r =0.89; Fig.1). In both WT and TG22+ mice, IP-treated animals exhibited significantly increased LV mass and LV to body weight ratio relative to saline-infused controls. IP-induced stress resulted in a significantly greater LV weight for TG22+ mice than for WT mice (101.8 ± 8.1 vs 119.5 ±9.1 mg, P<0.05) as well as a significantly greater LV/BW ratio (3.5 ± 0.3 vs 4.0±0.3, P<0.05). TG22+ mice treated with IP also exhibited significantly lower LV ejection fraction relative to IP-treated WT mice (65.8± 10.8% vs 46.9± 16.0%, P<0.05). Similarly greater reductions in cardiac output (17±2.7 to 12.1±4.0, P<0.01) and increases in LV end-systolic epicardial diameter (from 4.96± 0.32 to 5.46± 0.41 mm, P<0.05) were observed in TG22+ versus WT mice. Mice treated with saline only exhibited no significant differences between WT and TG22+ groups in any quantity measured

Conclusions. Magnetic resonance imaging study revealed that chronic IP stress in TG22+ mice results in markedly contractile dysfunction greater than that seen in WT that leads to LV hypertrophy and the development of dilated cardiomyopathy. Thus, TG mice overexpressing the human atherosclerotic Cav1.2α1C22+ variant exhibit a pathological phenotype characterized by increased susceptibility to remodeling in response to β-adrenergic stress. This observation may provide insight into a potential pathogenic role of this channel.

References:. Tiwari, Yuwei Zhang, S., Heller, J., Abernethy, D.R., and Soldatov, N.M. PNAS, 2006:103, 17024-29.
Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, Demetris AJ, Feldman AM. Circ Res.1997:4 627-35.

Fig 1. Comparison of ex-vivo determined Weight vs. MRI

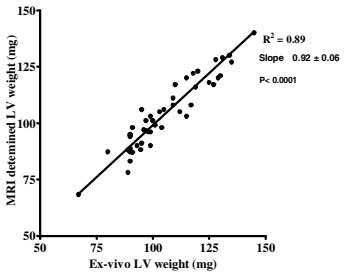

Fig 2. Cine mouse heart images at the end-systolic phase in the short axis view after 7 days of vehicle or LV chronic isoproterenol (30 mg) stress