

# CSANZ Abstracts 2011

## Ralph Reader Finalists – Basic

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### A Regulatable Model of Mutant $\alpha$ -Myosin Heavy Chain Overexpression to Study the Structural and Functional Consequences of Hypertrophy Regression

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Left ventricular hypertrophy (LVH) is a significant cause of morbidity and mortality, but whether its prevention or regression is feasible and/or beneficial is unclear. We generated an  $\alpha$ MHC<sup>403/+</sup>-tTA mouse model in which expression of the familial hypertrophic cardiomyopathy (FHC)-causing Arg403Gln  $\alpha$ -myosin heavy chain ( $\alpha$ MHC<sup>403</sup>) mutation is regulatable by oral doxycycline. Serial echocardiography and histology showed that mice expressing  $\alpha$ MHC<sup>403</sup> throughout life developed progressively increased left ventricular mass (LVM), LV diastolic diameter (LVDD), left atrial diameter (LAD), and reduced fractional shortening (LVFS) (Table 1), and myofibrillar disarray and fibrosis. Transgene inhibition from 6 or 20 weeks did not ameliorate the changes in LVM, LVFS or histology seen at 40 weeks (Table 1). To determine the importance of early transgene expression, additional mice underwent transgenic inhibition from conception to 6 weeks, and were then allowed to express  $\alpha$ MHC<sup>403</sup> from 6 to 40 weeks (W0-6), which improved LVDD, LAD and LVFS compared with untreated  $\alpha$ MHC<sup>403/+</sup>-tTA mice. Thus, early developmental defects associated with  $\alpha$ MHC<sup>403</sup> expression provide a structural template for the manifestations of hypertrophy in adult life.

**Table 1. Echocardiography at 40 Weeks.**

Parameter	WT	$\alpha$ MHC <sup>403/+</sup> -tTA	W0-6	W6-40	W20-40
LVM (mg)	83.4 ± 9.4	128.8 ± 23.3*	105.4 ± 21.7*	122.3 ± 28.2*	114.9 ± 17.7*
LVDD (mm)	3.4 ± 0.1	3.9 ± 0.2*	3.4 ± 0.2#	3.60 ± 0.12#	3.8 ± 0.3*
LVFS (%)	55 ± 2	41 ± 7*	52 ± 8*,#	45 ± 4*	43 ± 2*
LAD (mm)	1.7 ± 0.1	2.3 ± 0.2*	2.0 ± 0.2*,#	2.4 ± 0.2*	2.4 ± 0.2*

*n* = 5-9; W0-6, W6-40, W20-40 = transgenic inhibition until 6 weeks, from 6 to 40 weeks, or from 20 to 40 weeks, respectively. Statistical analysis by ANOVA and *t* test.

\* *P* < 0.05 vs WT.

# *P* < 0.05 vs untreated  $\alpha$ MHC<sup>403/+</sup>-tTA.

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### Molecular Imaging of Acute Thrombosis and Thrombolysis by Contrast Enhanced Ultrasound with Novel Platelet-targeted Microbubbles

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**Background:** Molecular imaging is a rapidly emerging enabling technology allowing non-invasive detection of vascular pathologies. However, imaging technologies offering a high resolution are currently not inherently real-time applications. We hypothesised that contrast enhanced ultrasound (CEU) with microbubbles selectively targeted to activated platelets would offer real-time molecular imaging of evolving arterial thrombosis.

**Methods and results:** Lipid-shell based air-filled microbubbles were conjugated to either a single-chain antibody (scFv) specific for activated GPIIb/IIIa (LIBS-MB), or a non-specific scFv (control-MB). Flow-chamber experiments demonstrated strong adhesion of LIBS-MB to immobilised activated platelets at 50 s<sup>-1</sup> compared with control-MB (84 ± 10 vs 15 ± 2; *p* < 0.001). Increasing the shear rate to 1000 s<sup>-1</sup> and 6000 s<sup>-1</sup> dislodged most control-MB while LIBS-MB remained strongly attached (*p* < 0.001). Platelet-rich thrombi were induced in carotid arteries of C57Bl6-mice *in vivo* by ferric chloride injury. Thrombi were then assessed with CEU-imaging before and 20 minutes after microbubble injection. Thrombosis was detected via the greyscale area, which was strongly increased after LIBS-MB but not after control-MB injection (214.25 ± 33.5 vs 9.96 ± 10.38; *p* < 0.001). After thrombolysis with urokinase, CEU-imaging showed a significant reduction in thrombus size (*p* < 0.001).

**Conclusions:** We are able to demonstrate that our targeted microbubbles specifically bind to activated platelets *in vitro* and allow real-time molecular imaging of acute arterial thrombosis as well as monitoring pharmacological thrombolysis *in vivo*. This non-invasive and cost effective imaging modality provides a unique opportunity to detect arterial (micro)thrombi at an early stage allowing for early diagnosis and therapy.

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