B THYMOSIN β 4 – A NOVEL REGULATOR OF LOW DENSITY LIPOPROTEIN RECEPTOR RELATED PROTEIN 1 (LRP1) IN VASCULAR DISEASE

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Vascular stability and tone are maintained by contractile smooth muscle cells (VSMCs). Injury-induced growth factors stimulate a phenotypic switching of VSMCs, from their quiescent contractile state to a more active synthetic phenotype that proliferate and migrate. Chronic VSMC dedifferentiation leads to vascular thickening and stiffness, exacerbates inflammation and promotes atherosclerotic lesion development and susceptibility to abdominal aortic aneurysm (AAA). Inhibiting VSMC phenotypic transformation has thus been shown to attenuate progression of vascular disease. We previously identified Thymosin β 4 (T β 4) as a key regulator of embryonic VSMC differentiation. TMSB4X, encoding T β 4, is the most abundant transcript in healthy and AAA aorta, therefore we hypothesised that T β 4 may additionally function to maintain vasculature and protect against disease throughout postnatal life. We identified an interaction between T β 4 and Low density lipoprotein receptor related protein 1 (LRP1), an endocytic regulator of PDGFR β signalling which controls VSMC proliferation. LRP1 variants have been identified by GWAS as major risk loci for AAA and coronary artery disease. Adult T β 4-null mice displayed aortic VSMC and elastin defects, phenocopying LRP1 mutants and suggesting compromised vascular integrity. During development, T β 4 functions in a paracrine manner, secreted from endothelial cells (ECs) to induce mesoderm to VSMC differentiation. To distinguish between cell-autonomous and paracrine roles for T β 4, and simultaneously discern adult maintenance versus developmental requirements, we selectively induced deletion of T β 4 from VSMCs or ECs at 3 weeks of age. Histological assessment of aortas at 12 weeks demonstrated that VSMC-specific T β 4 knockdown recapitulated the global KO phenotype, revealing a postnatal requirement for T β 4 to maintain healthy vasculature. In keeping with this, we confirmed predisposition of these mice to disease in models of atherosclerosis and AAA. Aneurysmal aorta and plaques of T β 4KO were characterised by accelerated contractile-synthetic VSMC switching, elastin degradation and augmented PDGFR β signalling. *In vitro*, enhanced sensitivity to PDGF-BB, upon loss of T β 4, coincided with increased cell surface recycling of LRP1-PDGFR β complexes and reduced lysosomal targeting, suggesting that dysregulated endocytosis underlies enhanced phenotypic switching and proliferation. Given the VSMC differentiation, anti-inflammatory and anti-apoptotic roles of T β 4, we sought to determine the vasculoprotective potential of exogenous T β 4. In the AAA model, T β 4 treatment significantly reduced aortic dilatation and rupture, and preserved VSMC phenotype and elastin integrity, associated with normalisation of PDGFR β signalling. Our study identifies T β 4 as a key regulator of LRP1 for maintaining vascular health, providing insight that may

reveal useful therapeutic targets for modulation of VSMC phenotypic switching and disease progression.

C IDENTIFICATION OF THE MAJOR GENETIC CONTRIBUTORS TO TETRALOGY OF FALLOT

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There is strong evidence from familial recurrence studies for a genetic predisposition to sporadic, non-syndromic Tetralogy of Fallot (TOF). TOF is the most common, cyanotic congenital heart disease (CHD) phenotype yet the cause for the majority of cases remains elusive. Rare genetic variants have been identified as important contributors to the risk of CHD, but relatively small numbers of TOF cases have been studied to date. 829 TOF patients underwent whole exome sequencing (WES), the largest cohort of non-syndromic TOF patients reported to date. The prevalence of unique, deleterious variants was determined; defined by their absence in the Genome Aggregation Database (gnomAD) and a scaled combined annotation-dependent depletion (CADD) score of ≥ 20 . Clustering analysis of variants revealed that two genes, *NOTCH1* and *FLT4*, surpassed thresholds for genome-wide significance (assigned as $P < 5 \times 10^{-8}$), after correction for multiple comparisons. *NOTCH1* was most frequently found to harbour unique, deleterious variants. 31 variants were observed in 37 probands (4.5%; 95% confidence interval [CI]: 3.2–6.1%) and included

seven loss-of-function variants, 22 missense variants and two in-frame indels. Sanger sequencing of the unaffected parents of seven cases identified five *de novo* variants. Three *NOTCH1* variants (p.G200R, p.C607Y and p.N1875S) were subjected to functional evaluation and two showed a reduction in Jagged1-induced *NOTCH* signalling. *FLT4* variants were found in 2.4% (95% CI:1.6–3.8%) of TOF patients, with 21 patients harbouring 22 unique, deleterious variants. The variants identified were distinct to those that cause the congenital lymphoedema syndrome Milroy disease. In addition to *NOTCH1*, *FLT4* and the well-established TOF gene, *TBX1*, we identified potential association with variants in several other biologically plausible candidate genes. In summary, the *NOTCH1* locus is the most frequent site of genetic variants predisposing to non-syndromic TOF, followed by *FLT4*. Together, variants in these genes are found in almost 7% of TOF patients.

D STRESS MYOCARDIAL OXYGENATION AND NOT PERFUSION RESERVE DETERMINES ARRHYTHMIC RISK IN HYPERTROPHIC CARDIOMYOPATHY: INSIGHTS FROM A NOVEL OXYGEN-SENSITIVE CMR APPROACH

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Myocardial ischemia has long been implicated in promoting arrhythmic events and triggering sudden cardiac death in hypertrophic cardiomyopathy (HCM). However, the evidence for this is scarce due to challenges presented in direct ischemia assessment which generally requires an invasive approach. Blood oxygen level dependent cardiac magnetic resonance (BOLD CMR) permits the non-invasive assessment of tissue oxygenation, without gadolinium contrast, overcoming many limitations suffered by traditional methods. From a clinical

perspective, T2-prepared steady-state free precession (T2-SSFP) BOLD is promising, but suffers from reduced diagnostic accuracy, owing to imprecisions in BOLD measurements secondary to heart-rate (HR) dependence. To resolve this, we developed a novel oxygen-sensitive CMR approach, Fast Low Angle Shot (FLASH) interleaved T2-SSFP BOLD, which was designed to eliminate both HR dependence and spatial variations seen with standard T2-SSFP BOLD. A comparison of both standard and novel approaches in 20 healthy subjects confirmed that FLASH-normalised T2-SSFP BOLD is highly reproducible, HR independent and more precise than standard T2-SSFP BOLD. In addition, the mean BOLD effect did not differ between the two methods. Importantly, using this novel approach, one could visualise changes in oxygen-sensitive signal from rest to stress qualitatively, making it feasible for direct incorporation into a clinical work flow. We then set out to test the hypothesis that stress oxygenation (as assessed on FLASH normalised T2-SSFP BOLD) is more powerful than myocardial perfusion reserve (MPRI) at determining arrhythmic risk in HCM patients. Adenosine stress BOLD, first pass perfusion imaging and late gadolinium enhancement CMR were undertaken in 103 genotyped-HCM patients. All patients underwent 24-hour Holter to monitor for evidence of ventricular tachycardia (≥ 3 beats, ≥ 120 beats per minute). Thirty-two age- and sex-matched healthy subjects served as controls. Although both stress oxygenation and MPRI were impaired in HCM, only stress oxygenation, but not MPRI, associated with ventricular tachycardia on univariate analysis. There was a step-wise increase in ventricular tachycardia prevalence with decreasing quartiles of stress oxygenation. HCM patients with the lowest quartile of oxygenation were at a three-fold increased risk of ventricular tachycardia (OR 3.04, $p=0.04$) after adjusting for LGE mass, age and baseline risk of sudden cardiac death. Sarcomeric mutation status was an independent determinant of stress oxygenation, irrespective of the extent of hypertrophy, MPRI or LGE burden (univariate predictors). In conclusion, we have successfully developed and implemented a novel oxygen-sensitive CMR method which has provided important insights into the role of stress oxygenation as a promising biomarker of arrhythmic risk and potential therapeutic target for drug discovery in HCM.