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

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10.1136/heartjnl-2019-BCS.225

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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10.1136/heartjnl-2019-BCS.226

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