our knowledge of their complexity in vivo is only partial. Here we wished to characterise plaque macrophages from two of the most common murine models; ApoE$^{-/-}$ (Apolipoprotein E null) and LDLR$^{-/-}$ (low density lipoprotein receptor null) and a relatively new and less characterised model; PCKS9-AAV8 (Adeno-associated virus serotype 8-proprotein convertase subtilisin kexin type 9) induced hyperlipidaemia.

Using immunohistochemistry, plaques from high-fat diet fed ApoE$^{-/-}$, LDLR$^{-/-}$ and PCSK9-AAV8 mice were simultaneously stained for the pan macrophage marker Mac-3 and the pro-/anti- inflammatory markers inducible nitric oxide synthase (iNOS) and Arginase I (ArgI). Plaques were imaged using fluorescence microscopy and analysed by ImageJ. Individual Mac-3 + cells were selected as the region of interest and corresponding iNOS and ArgI staining was quantified. The analysis allowed consideration for the spectrum of marker co-expression and characterisation of individual cells based on staining intensity. Using this approach, complex populations of plaque macrophagesincluding single+ ArgI, double positive, double negative and single+ iNOS were quantified. To understand the roles of these populations in atherosclerosis further, we correlated macrophage quality and quantities with lesion size and collagen content.

We show that ApoE$^{-/-}$ plaque macrophages are significantly more pro-inflammatory than LDLR$^{-/-}$ and PCSK9 plaque macrophages ($p < 0.05$ and $p < 0.0001$). The population responsible for the pro-inflammatory phenotype of ApoE$^{-/-}$ macrophages were single+ iNOS cells ($p < 0.0001$). We also show that the abundance of these cells significantly correlates (R^2=0.4791, $p = 0.0183$) with lesion size in the aortic sinus. In addition, the frequency of double negative macrophages correlated with lesion collagen content (R^2=0.4451, $p = 0.0178$).

For the first time, plaque macrophages from three murine atherosclerosis models have been comprehensively characterised using a multi-colour image analysis strategy and suggest that plaque macrophages from ApoE$^{-/-}$ mice are significantly more pro-inflammatory than LDLR$^{-/-}$ and PCSK9 macrophages. We show that single iNOS+ cells may have a role in promoting lesion formation and double negative cells may also have a role in lesion stability. We envisage our platform provides a novel tool to gain a further, in-depth understanding of macrophage phenotype in atherosclerosis and will use it to elucidate the action of modulators of macrophage polarisation *in vivo*.

166 HDAC3 UNCONVENTIONAL SPLICING MEDIATES ENDOTHELIAL-MESENCHYMAL TRANSITION IN CARDIAC FIBROSIS

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Introduction Endothelial-mesenchymal transition (EndMT) is a process where endothelial cells (ECs) undergo dramatic cytoskeletal remodelling and gene expression changes, leading to a more motile mesenchymal phenotype that can significantly contribute to cardiac fibrosis. Histone deacetylase 3 (HDAC3), a class I HDAC, is essential in the maintenance of endothelial homeostasis. Our previous study indicates that mouse HDAC3 mRNA can undergo unconventional splicing, and that the HDAC3-alpha (HDAC3a) spliced isoform promotes EndMT in human aortic endothelial cells (HAECs). This project aims to elucidate the role of HDAC3a in mediating EndMT and its underlying mechanisms in the development of pressure-overload-induced cardiac fibrosis.

Methods and results Our previous study reveals that HDAC3a promotes EndMT in HAECs via activation of transforming growth factor-beta 2, which could possibly be mediated through increased protease enzyme activity. Therefore HAECs overexpressed with HDAC3a via adenoviral gene transfer (Ad-HDAC3a) have undergone a protease array, which revealed that cells overexpressed with HDAC3a have an elevated level of a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1). This was further confirmed with increased mRNA and protein expression levels of ADAMTS1 in Ad-HDAC3a-infected HAECs using RT-qPCR and Western blotting analysis respectively (both $n = 4$, $P < 0.05$). A mouse model of pressure overload-induced cardiac fibrosis was established by transverse aortic constriction (TAC), and cardiac fibrosis was confirmed by increased Col1a1 expression seen using RT-qPCR and Picrosirius red staining in heart tissue sections from mice 7 d post-TAC. Immunofluorescent staining detected CD31$^+$/alpha-SMA$^+$ (indicative of EndMT) and HDAC3a$^+$/CD31$^+$ cells in heart tissues from TAC mice but not in those from sham-operated mice. There were significant increases in mRNA expression of HDAC3a and ADAMTS1, together with EndMT transcription factors Snai1, Snai2 and Twist1 in heart tissues from 7 d post-TAC mice compared to those from 7 d post-sham mice ($n = 7$–- 8, $P < 0.05$). Elevated HDAC3a and ADAMTS1 protein expression were also detected in the TAC heart tissues ($n = 5$, $P < 0.05$).

Conclusion These results indicate an association between the HDAC3 splicing isoform HDAC3a, ADAMTS1 and EndMT during TAC-induced cardiac fibrosis. Further investigation of the underlying mechanisms mediated by HDAC3a and ADAMTS1 in cardiac fibrosis is warranted.

167 THE MECHANISM OF P22PHOX C242T SNP INHIBITION OF TNF ALPHA-INDUCED NOX2 ACTIVATION IN HUMAN ENDOTHELIAL CELLS AND VESSELS

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NADPH oxidase, by generating reactive oxygen species, is involved in the pathophysiology of many cardiovascular diseases and represents a therapeutic target for the development of novel drugs. A single-nucleotide polymorphism (SNP) C242T of the p22^{phox} subunit of NADPH oxidase has been reported to be negatively associated with coronary heart disease (CHD) and may predict disease prevalence. However, the underlying mechanisms remain unknown.

Using computer molecular modelling we discovered that C242T SNP causes significant structural changes in the extracellular loop of p22^{phox} and reduces its interaction stability with the catalytic Nox2 subunit. Gene transfection of human pulmonary microvascular endothelial cells showed that C242T p22^{phox} reduced significantly Nox2 expression, but had no significant effect on basal endothelial superoxide ($O_2^{\cdot-}$) production, or the expression of Nox1 and Nox4. When cells were stimulated with TNFalpha (or high glucose), C242T p22^{phox} inhibited significantly TNFalpha-induced Nox2 maturation,