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# THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE AS A TARGET FOR CARDIOPROTECTION IN VENTRICULAR CARDIOMYOCYTES HARVESTED FROM PATIENTS WITH OBSTRUCTIVE HYPERTROPHIC CARDIOMYOPATHY

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**Rationale** The opening of the mitochondrial permeability transition pore (mPTP) is a critical determinant of ischaemia-reperfusion injury, and preventing its opening confers powerful cardioprotection in non-diseased myocardium. Whether this cardioprotective effect is present in the setting of hypertrophic cardiomyopathy (HCM) is unknown and is investigated in this study.

**Methodology** Local UCLH/UCL ethical committee approval had been granted for this study. Human adult ventricular cardiomyocytes were isolated from left ventricular septal tissue, harvested from consenting patients undergoing surgical myectomy for obstructive HCM. The cells were loaded with the fluorescent dye TMRM which localises to the mitochondria, generates oxidative stress within the mitochondria on confocal imaging, resulting in mPTP opening, as indicated by the collapse of the mitochondrial membrane potential. The time taken to induce the loss of mitochondrial membrane potential was used as a measure of mPTP opening sensitivity. Cells were randomised to the following: (1) DMSO 0.01% vehicle control (N=8/group); (2) cells pre-treated with ciclosporin-A (CsA) (0.2 µM) for 15 min (N=7/group); (3) cells pre-treated with atorvastatin (25 µM) (N=7/group).

**Results** In the control group, mPTP opening was induced after  $188.7\% \pm 22.7$  s of oxidative stress, providing evidence for a functional mPTP in the setting of HCM. Furthermore, pretreatment with the known mPTP inhibitor, CsA, and atorvastatin, delayed the onset of mPTP opening by  $51\% \pm 10\%$  ( $p < 0.001$ ) and  $35\% \pm 7\%$  ( $p < 0.05$ ), respectively.

**Conclusions** For the first time in diseased human ventricular myocytes, we have demonstrated that the mPTP is functional and that its opening can be inhibited by cardioprotective agents such as CsA and atorvastatin.

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# X-BOX BINDING PROTEIN 1 SPLICING TRIGGERS AN AUTOPHAGIC SURVIVAL PATHWAY IN ENDOTHELIAL CELLS

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An initial step in the development of atherosclerosis is endothelial cell dysfunction. Our previous study has shown that sustained activation of X-box binding protein 1 (XBP1) splicing, a key signal transducer in endoplasmic reticulum stress response, leads to endothelial apoptosis and atherosclerosis development (Zeng *et al*, PNAS 2009). Autophagy is characterised as a survival response as well as a pathway culminating in cell death. In this study, we aimed to investigate whether XBP1 splicing could also activate autophagy in endothelial cells, and its role in the cell survival and apoptosis. XBP1 splicing was introduced to human umbilical vein endothelial cells by adenoviral gene transfer, and autophagic vesicle formation was observed 48 and 72 h postinfection. Autophagy gene expression such as Beclin 1, LC3-β, NIX, ATG12, BNIP2, BNIP3, ATG5 and ATG12 was induced by XBP1 splicing as detected at RNA and protein levels. Endothelial cells transfected with the LC3β-GFP construct showed a high GFP expression of autophagic vesicle formation only in the presence of spliced XBP1 compared

with the non-infected cells. Moreover, our results showed that endostatin, a well-characterised endogenous inhibitor of angiogenesis that induces apoptosis, activated autophagy genes 4 h after treatment, through XBP1 splicing in an IRE1-α-dependent manner. Knockdown of XBP1 or IRE1α by ShRNA in endothelial cells ablated endostatin-induced autophagy responsive genes expression and enhanced the apoptotic effect of endostatin. These results suggest that activation of XBP1 splicing can trigger an autophagic survival signal pathway, providing novel insights into the mechanisms involved in endothelial survival.

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# QUANTITATIVE METABOLIC PROFILING OF SUBCLINICAL ATHEROSCLEROSIS BY SERUM NMR METABONOMICS

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**Objective** To determine associations of systemic metabolites with subclinical atherosclerosis.

**Methods** 4407 serum samples were measured by nuclear magnetic resonance (NMR) spectroscopy from the Cardiovascular Risk in Young Finns Study, and carotid intima-media thickness (IMT) was assessed by ultrasound. Numerous lipoprotein lipids subclasses as well as low-molecular-weight metabolites were quantified from the NMR data.

**Results** In these young adults (aged 24–45 years) data-driven analysis using self-organising maps showed distinct metabolic phenotypes associated with elevated IMT. The phenotypes were characterised by varying combinations of metabolic disturbances including elevated very-low-density lipoprotein (VLDL) and LDL subclasses, but also several low-molecular-weight metabolites. Results for 6-year incidence of high carotid IMT and IMT progression as shown by discrimination and reclassification will also be discussed.

**Conclusion** The study showed different metabolic phenotypes inherently associated with subclinical atherosclerosis. Prediction of subclinical atherosclerosis was improved by comprehensive metabolic profiling. The findings substantiate developments towards the use of multi-metabolic risk phenotypes in cardiovascular risk assessment.

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# COMPUTER MOLECULAR MODELLING OF THE P22PHOX PROTEIN STRUCTURAL CHANGES LINKED TO C242T POLYMORPHISM

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The p22phox is a key component of the cytochrome b558 of the NADPH oxidase (Nox), which by generating reactive oxygen species (ROS) is involved in the pathogenesis of cardiovascular disease. A p22phox polymorphism (C242T) has been found to reduce oxidative stress in the cardiovascular system and is negatively associated with the incidence of coronary heart disease (CHD). However, the mechanism involved remains unknown. In this study we used computer molecular modelling and bioinformatics to investigate the potential effect of C242T polymorphism on the 3-D protein structure of the p22phox. Based on the published sequence data of p22phox and the principle of regulated prediction algorithms, we found that p22phox consists of two domains: an N-terminal transmembrane domain (124 a.a.) and a C-terminal cytoplasmic domain (71 a.a.). In its most stable form, it has three

transmembrane helices leading to an extracellular N-terminus and an extracellular loop between helices two and three. The C242T polymorphism causes a change of His72 to Tyr72. This change results in significant morphological changes of the extracellular loop of the p22phox, which is in the putative interactive region of the p22phox with the catalytic subunit (Nox2). This may interfere with their association, and subsequently result in a reduced cytochrome b function and a reduced ROS production by NADPH oxidase. These results give us insight into the molecular mechanism of this polymorphism in reducing vascular oxidative stress and may explain how this polymorphism is linked with reduced incidence of CHD.

**BAS/  
BSCR49** **HEPATOCTE GROWTH FACTOR/C-MET SIGNALLING  
ACTIVATES NOTCH TRANSLOCATION AND IS ASSOCIATED  
WITH SMOOTH MUSCLE CELL MINERALISATION IN VITRO**

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Vascular calcification is an established pathological process that contributes to several forms of cardiovascular morbidity—notably, atherosclerosis. The molecular mechanisms involved continue to be explored. Hepatocyte growth factor (HGF) signalling, via its receptor c-MET, has been identified in association with atherosclerotic plaque development. We have demonstrated that overexpression of HGF in human smooth muscle cells (hSMC) accelerates their mineralisation. Reports demonstrating upregulation of the notch ligand  $\delta$ , and the presence of a feedback loop linked to the c-MET pathway, raise the possibility that the effects of HGF on mineralisation may be mediated via notch signalling. We aim to test the hypothesis that notch signalling is involved in Ad-HGF-induced in vitro mineralisation of hSMCs. We demonstrate accelerated mineralisation in response to adenoviral-mediated HGF overexpression, confirmed by alizarin red staining, calcium incorporation and increased alkaline phosphatase activity. In addition, we show upregulation and phosphorylation of c-MET and reduction of the mineralisation inhibitor osteopontin. We identify upregulation of the notch-3 intracellular domain via western blot analysis and, using immunocytochemistry, show an altered distribution of notch-3 in Ad-HGF-infected cells. Finally, we show (i) an attenuation of mineralisation in hSMCs following overexpression of NK4, the c-MET antagonist and (ii) that treatment of hSMCs with DAPT, the notch inhibitor, also decreased the rate of mineralisation compared with Ad-HGF infected cells and controls. These findings suggest a link with the notch pathway as a possible downstream effector of HGF and further elucidate a novel mechanism underpinning vascular calcification.

**BAS/  
BSCR50** **EFFECT OF IMMUNISATION WITH CHLAMYDIA  
PNEUMONIAE RECOMBINANT MAJOR OUTER MEMBRANE  
PROTEIN ON ATHEROSCLEROSIS DEVELOPMENT**

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The major outer membrane protein (MOMP) of *Chlamydia pneumoniae* is a 40 kDa porin and represents approximately 60% of the outer membrane protein pool. Previous studies have shown that MOMP can dampen down T-cell-mediated immune responses. We decided to assess if this effect could have an impact on atherosclerotic plaque development. We used recombinant *Mycobacterium*

*vaccae* (*M vaccae*) vectors expressing MOMP (with and without signal sequence) to vaccinate ApoE<sup>-/-</sup> mice intranasally. Animals received one initial dose and two booster doses 3 weeks apart and continued with a standard Chow diet for another 4 weeks. Control mice received phosphate-buffered saline or were left untreated. Plasma collected before immunisation and at termination was used to measure levels of interferon  $\gamma$ , interleukin (IL)-4 and IL-10 and also IgG1 and IgG2b. Atherosclerotic plaque development was assessed using paraffin sections of the brachiocephalic artery. Our results show that intranasal administration of *M vaccae* vectors expressing MOMP, with or without signal sequence, promotes anti-atherogenic T-cell responses and halts the development and progression of atherosclerotic plaques in ApoE<sup>-/-</sup> mice.

**BAS/  
BSCR51** **CONTROL OF VASCULAR CELL INFLAMMATORY  
RESPONSES THROUGH TILRR, AN INTERLEUKIN  
1 CO-RECEPTOR**

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Inflammatory responses are induced by members of the Toll-Like and interleukin (IL)-1 receptor family and controlled by NF- $\kappa$ B.

We have identified an IL-1RI co-receptor, TILRR, which potentiates activation of NF- $\kappa$ B and inflammatory responses. We show that induction of amplification depends on formation of a TILRR-containing receptor complex, which imparts enhanced recruitment of the MyD88 adaptor to the signalling receptor IL-1RI, and induction of Ras-dependent amplification of NF- $\kappa$ B.<sup>1</sup>

We have confirmed expression of TILRR in vascular cells and have demonstrated a correlation of the level of TILRR expression with the level of NF- $\kappa$ B activity and inflammatory responses, induced by IL-1 stimulation and by mechanotransduction.

Our recent studies have demonstrated expression of TILRR in vascular endothelial cells using immunohistochemistry. Sections of perfusion-fixed, paraffin-embedded vascular tissue were stained using a specific rabbit polyclonal anti-TILRR antibody, followed by incubation with a biotinylated goat anti-rabbit antibody. Ongoing studies using wild type and ApoE<sup>-/-</sup> mice are designed to assess the impact of diet on TILRR expression and on its association with the signalling partner IL-1RI.

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**BAS/  
BSCR52** **INVESTIGATING THE IMPORTANCE OF HEPARAN SULPHATE  
IN DETERMINING ENDOTHELIAL PROGENITOR CELL  
FUNCTION**

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The increased risk of cardiovascular disease with age may, in part, be due to a decline in the function of endothelial progenitor cells (EPCs). Cell surface heparan sulphate proteoglycans (HSPGs) can