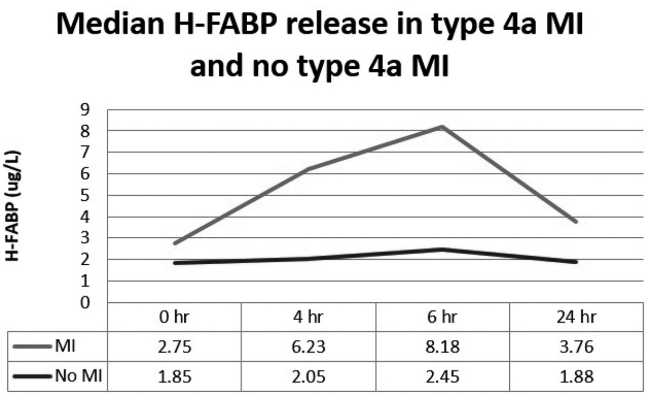

Abstract 219 Figure 2 H-FABP release between type 4a MI (n = 37) and no type 4a MI (n = 172)

major adverse clinical events (MACE); MI, target vessel revascularisation, heart failure, stroke and death.

Results We enrolled 241 patients of whom 32 were excluded due to withdrawal of consent or PCI being cancelled after angiography. A cohort of 209 patients was included in analysis, of whom 144 (68.9%) were male, mean age was 68.8 years, 28 (13.4%) were smokers, 31 (14.8%) were diabetic, 199 (95.2%) had hypercholesterolaemia and 138 (66.0%) had hypertension. Type 4a MI was observed in 37 (17.7%) patients. Comparing those with and without type 4a MI, there was no statistical difference in risk factors ($p > 0.05$) except for age, ($p = 0.015$). Median troponin at 6 h was 90.24 ng/L (95% CI 76.56 â€" 186.41) versus 14.43 ng/L (95% CI 16.37 â€" 21.26) in the type 4a / non type 4a groups respectively, p=<0.001, Figure 1. Median H-FABP at 4 h was most predictive of type 4a MI (followed by CKMB and myoglobin) with levels of 6.23 mg/L (95% CI 4.38–18.96), versus 2.05mg/L (95% CI 2.23–2.74), p=<0.001, AUC 0.91, Table 1, Figure 2. Results for TnI, CKMB, myoglobin, GBPP and CAIII are shown in Table 1. Multivariate logistic regression (stepwise elimination) showed H-FABP to be most predictive of type 4a MI, $p < 0.001$. Sensitivity of 4 h H-FABP (>6.32 mg/L) for type 4a MI was 51.5%, specificity 96.1%, positive predictive value (PPV) 73.9%, negative predictive value (NPV) 90.3%, odds ratio (OR) 26.39, relative risk (RR) 7.62. MACE was noted in 6 (2.9%) patients (three MI, two death and one stroke), 3 of which had type 4a MI at index PCI, $p = 0.036$. Table 2 compares median change in H-FABP and hsTnT from 0 â€" 6 h in patients who developed MACE at 1 year with CAIII performing best, $p = 0.02$.

Conclusions/implications Median 4 h H-FABP was most predictive of a 6 h hsTnT rise as a consequence of type 4a MI in elective PCI, followed by CKMB and myoglobin. H-FABP and CAIII were independently predictive of MACE at 1 year and MACE was associated with type 4a MI at index PCI.

Abstract 219 Table 1 Summary of biomarker results at 4 h

4 hr biomarker	Type 4a MI		No type 4a MI		AUC	p value
	Median	IQR	Median	IQR		
H-FABP (μg/L)	6.23	6.22	2.05	1.45	0.91	<0.001
TnI (μg/L)	<0.18	0.07	<0.18	0.01	0.62	0.004
CKMB (μg/L)	3.54	3.37	2.01	1.25	0.75	<0.001
Myoglobin (ng/ml)	72.75	55.68	35.82	38.91	0.72	<0.001
GPBB (pg/L)	3.37	3.50	3.45	2.48	0.50	0.889
CAIII (μg/L)	28.76	23.67	25.25	26.41	0.60	0.168

H-FABP: heart-type fatty acid-binding protein; hsTnT: highly sensitive troponin T; TnI: troponin I; CKMP: creatine kinase MB; GPBB: glycogen phosphorylase; CAIII: carbonic anhydrase III; IQR: interquartile range; AUC: area under curve

Abstract 219 Table 2 Summary of biomarker change from 0–6 h in 1 year MACE and non-MACE patients

Biomarker	MACE		No MACE		AUC	p value
	Median change	IQR	Median change	IQR		
hsTnT (ng/L)	38.32	135.61	7.07	23.49	0.94	0.06
H-FABP (μg/L)	3.73	21.36	0.56	2.45	0.91	0.04
TnI (μg/L)	0.47	0.01	0.09	0.22	0.97	0.11
CK-MB (μg/L)	0.45	4.01	0.18	1.14	0.89	0.29
Myoglobin (ng/ml)	15.47	185.49	2.80	36.55	0.94	0.07
GPBB (pg/L)	-0.86	8.08	1.75	5.13	0.71	0.31
CAIII (μg/L)	24.13	18.23	1.08	20.91	0.91	0.02

H-FABP: heart-type fatty acid-binding protein; hsTnT: highly sensitive troponin T; TnI: troponin I; CKMP: creatine kinase MB; GPBB: glycogen phosphorylase; CAIII: carbonic anhydrase III; IQR: interquartile range; AUC: area under curve

Young Investigators Prize 2016

A

THE CORONARY ARTERY DISEASE ASSOCIATED GENE HHIPL1 PROMOTES ATHEROSCLEROSIS

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Genome-wide association studies have identified chromosome 14q32 as a locus for coronary artery disease in humans. The disease associated variants fall in a gene called *Hedgehog interacting protein-like 1 (HHIPL1)*, which encodes an uncharacterised sequence homolog of a Hedgehog protein antagonist. Here, we present our investigation of HHIPL1 function and its role in atherosclerosis.

Epitope tagged HHIPL1 protein is present in the media of *HHIPL1* transfected cells and immunoprecipitates with GFP tagged Sonic Hedgehog (SHH) protein, demonstrating that HHIPL1 is a secreted interactor of SHH. We measured *HHIPL1* gene expression in different cardiovascular cell types and found that it is primarily expressed in aortic smooth muscle cells (AoSMCs). We performed siRNA knock-down of *HHIPL1* in AoSMCs and observed a reduction of 32% (+/-11%) in proliferation ($p = 0.002$ at 72 h) and a reduction of 27% (+/-4%) in migration ($p = 0.02$ at 12 h). We went on to examine the role of Hhipl1 in atherosclerosis *in vivo*. In atherosclerotic mouse aortas *Hhipl1* expression increased with disease progression (~2-fold increase at 6 weeks vs 32 weeks of age, $p = 0.001$). We crossed *Hhipl1*$^{-/-}$ mice onto *Apoe*$^{-/-}$and *Ldlr*$^{-/-}$ atherosclerosis prone backgrounds. *Hhipl1*$^{-/-}$ mice displayed a reduction of 57% (+/-28%) in lesion area compared with controls on an *Ldlr*$^{-/-}$ background *(en face* aorta, n = 10 *Hhipl1*$^{+/+}$*; Ldlr*$^{-/-}$vs n = 19 *Hhipl1*$^{-/-}$*; Ldlr*$^{-/-}$, t-test p = 0.00007*)* and 49% (+/-28%) reduction on an *Apoe*$^{-/-}$ background (*en face* aorta, n = 9 *Hhipl1*$^{+/+}$*; Apoe*$^{-/-}$ vs n = 17 *Hhipl1*$^{-/-}$*; Apoe*$^{-/-}$, t-test p = 0.0004).

Our data represent the first experimental investigation of HHIPL1. We find that HHIPL1 is a proatherogenic protein that regulates smooth muscle cell proliferation and migration,

presumably through its involvement in Hedgehog signalling. Ongoing analysis will further define the mechanisms through which HHIPL1 contributes to atherosclerosis.

B DKK3 STABILISES ATHEROSCLEROTIC PLAQUE VIA PROMOTING VASCULAR PROGENITOR AND FIBROBLAST DIFFERENTIATION TO SMOOTH MUSCLE CELLS

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Atherosclerosis, a chronic condition that can be converted into an acute clinical event caused by plaque rupture and thrombosis, has been the primary cause of mortality and morbidity worldwide. Dickkopf 3 (DKK3), a 36-kD secreted glycoprotein, has a role in cell differentiation, but little is known about its involvement in vascular disease. In the present study, we utilised a murine model of atherosclerosis ($ApoE^{-/-}$) in conjunction with $DKK3^{-/-}$ to assess the effects of DKK3 on plaque stability.

We found that the absence of DKK3 leads to vulnerable unstable atherosclerotic plaques, due to a reduced number of smooth muscle cells (SMCs) and reduced matrix protein deposition, as well as increased haemorrhage and macrophage infiltration. Using a cell linear tracing method, vascular progenitors and fibroblasts from SM22-LacZ transgenic mice were isolated and applied to the adventitial side of injured femoral arteries resulting in migration of both types of cells to the intima. Upon migration the cells displayed beta-gal positivity, indicating SMC differentiation.

Further *in vitro* studies revealed that DKK3 can induce differentiation of Sca1+ vascular progenitors and fibroblasts into SMCs via activation of the TGFβ/ATF6 and Wnt signalling pathways. Finally, we assessed the therapeutic potential of DKK3 in mouse and rabbit models and found that DKK3 increases atherosclerotic plaque stability via an increase in SMC numbers and reduced vascular inflammation. Cumulatively, we provide the first evidence that DKK3 is a potent SMC differentiation factor, which may have a therapeutic effect in reducing acute haemorrhagic conditions through promotion of atherosclerotic plaque stability.

C NOX4-DEPENDENT REPROGRAMMING OF GLUCOSE METABOLISM AND FATTY ACID OXIDATION FACILITATES CARDIAC ADAPTION TO CHRONIC PRESSURE-OVERLOAD

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Introduction Increased reactive oxygen species (ROS) production is involved in the pathophysiology of cardiac hypertrophy and failure. Interestingly, a specialised ROS-generating enzyme NADPH oxidase-4 (Nox4) was previously found to have beneficial effects by promoting adaptive remodelling during pressure-overload cardiac hypertrophy. Nox4 modulates intracellular signalling cascades but how it achieves beneficial effects in the chronically overloaded heart remains unclear.

Methods and results To obtain an unbiased global overview of putative Nox4-mediated changes, the proteome of cardiac-specific Nox4 transgenic (TG) and wild-type (WT) mouse hearts was first characterised through a 2D-DIGE approach. TG hearts had a significant over-representation of changes in protein levels of enzymes involved in glucose and fatty acid utilisation. We therefore analysed the metabolome using 1H-NMR and targeted LC-MS approaches. This identified a differential accumulation of glycolytic intermediates in the proximal part of glycolysis both in unstressed and pressure-overloaded TG hearts, as well as an increase in alanine levels (1.4 fold, $p = 0.05$), confirming significant alterations to metabolism. To specifically quantify glucose uptake, glycolysis, glucose oxidation and fatty acid oxidation rates, *ex vivo* working heart studies were conducted. TG hearts had a marked increase cf. WT in palmitate oxidation rate in the unstressed as well as pressure-overloaded heart (3.6 fold increase; $n = 6$/group; $p = 0.01$). Glucose uptake was unaltered but glycolysis and oxidation rates were decreased, suggesting diversion of glucose away from oxidation. Importantly, an increase in palmitate oxidation was not detrimental either for *in vivo* cardiac energetics (31P-NMR) or contractile function during pressure-overload hypertrophy. We found that activity of the hexosamine biosynthesis pathway (HBP), an alternative route for glucose metabolism, was increased in TG hearts as assessed by the O-GlcNAc post-translational modification of cardiac proteins by N-acetylglucosamine, the end-product of HBP. O-GlcNAc levels were 2.4 fold higher in TG cf. WT ($n = 4$/group; $p = 0.02$). In cultured cardiomyocytes, endogenous Nox4 induced similar changes in HBP and palmitate oxidation (extracellular flux analysis), and it was found that changes in O-GlcNAcylation regulated fatty acid oxidation.

Discussion These results show that Nox4 reprograms substrate utilisation in the heart by directing glucose towards the HBP and inducing a linked increase in fatty acid oxidation. These changes appear to enable the heart to better adapt to chronic pressure overload and may be important in the beneficial effects of Nox4 on cardiac remodelling. These data identify a novel redox mechanism that drives beneficial metabolic reprogramming in the heart and suggest potential new therapeutic approaches to promote adaptation to chronic overload stress.

D IMPACT OF HIGH-FLOW OXYGEN ON PERFUSION, MICROVASCULAR AND CAPILLIARY FUNCTION IN NORMAL VOLUNTEERS AND PATIENTS WITH CORONARY ARTERY DISEASE: A CARDIOVASCULAR MAGNETIC RESONANCE AND INVASIVE CORONARY PHYSIOLOGY STUDY

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