compounds such as endothelin-1 (ET-1) and NO are required to maintain vascular tine. An imbalance, resulting in increased ET-1 and reduced NO levels promotes vascular stiffening and consequently diseases such as diabetic retinopathy, nephropathy and atherosclerosis. Diseases caused by an increased atherosclerosis burden including coronary heart disease and stroke are major causes of death in obese and diabetic populations.

Aims Determine **(1)** whether enhanced Aβ levels are sufficient to induce vascular dysfunction and **(2)** if reducing Aβ production can reverse diet-induced vascular dysfunction.

Methods Measurements of vascular function was determined in vivo by the vascular response to Acetylcholine (endothelial dependent or Sodium Nitroprusside (endothelial independent) using laser Doppler imaging in two studies; (i) Wild-type (C57BL/6) mice fed a regular chow diet were infused with murine αβ42 or scrambled peptide (ScP; 3.36μg/kg) in aCSF for 6 weeks or (ii) a BACE1 inhibitor (M-3; 10mg/kg) or vehicle (DMSO/PBS) into diet-induced obese (DIO) C57BL/6 mice. Western blotting and ELISAs were used to measure vascular NO signalling and Aβ production.

Results Circulating levels of Aβ42, not the more prevalent Aβ40 isoform, are increased in both high fat fed mice and obese/diabetic human patients. Infusion of M-3 into DIO mice rescued endothelial dependent reactivity (M-3 27.1 ± 5.9, vehicle 1.3 ± 2.9; $P<0.01$). In contrast, infusion of Aβ42 promoted impaired vascular responses Aβ 14.1±3.7, ScP 38.3±3.2; $P<0.001$) on regular chow with no change in body weight. In line with our hypothesis infusion of Aβ42 increases the ET-1/NO ratio (ScP 1.12β± 0.05, Aβ42 5.35 ± 0.89; $P<0.001$), while DIO mice treated with a BACE1 inhibitor, thus with reduced plasma Aβ42 levels, have a low ET-1/NO ratio (M-3 1.29 ± 0.9, DIO 4.6 ± 1.3; $P<0.05$).

Conclusions We suggest that amyloid processing has a role in normal vascular function with aberrant processing leading to endothelial dysfunction and hypertension. Here we show that pharmacological inhibition of BACE1 can reverse diet-induced endothelial dysfunction, via modulation of plasma Aβ42 levels, as infusion of Aβ42 can promote the dysfunction independent of a high fat diet.

214 A HISTONE DEACETYLASE 7-DERIVED 7-AMINO ACID PEPTIDE ACTS AS A PHOSPHORYLATION CARRIER

Junyao Yang, Ana Moraga*, Yanhua Hu, Lingfang Zeng. *King's College London*

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Histone deacetylase 7 (HDAC7) belongs to the class II HDAC family and plays a pivotal role in the maintenance of endothelium integrity. There are 8 splicing variants in mouse HDAC7 mRNAs. Within the 5' terminal non-coding area of some variants, there exist some short open reading frames (sORFs). Whether these sORFs can be translated and whether the resulting peptides play roles in cellular physiology remain unclear. In this study, we demonstrated that one sORF encoding a 7-amino-acid (7-aa) peptide could be translated in vascular progenitor cells (VPCs). Importantly, this 7-aa peptide (7A) could transfer the phosphate group from the phosphorylated Ser393 site of MEKK1 to the Thr145 site of 14-3-3γ protein. The phosphorylated 7A (7Ap) could then directly phosphorylate 14-3-3γ protein in a cell-free, in-gel buffer system. The adjacent histidine and proline residues are essential for the phosphate group reception and transfer. In vitro functional analyses revealed that 7A and 7Ap increased VPC self-renewal and migration and enhanced vascular endothelial growth factor (VEGF)-induced VPC migration and differentiation toward the endothelial cell (EC) lineage, in which MEKK1 and 14-3-3γ served as the upstream kinase and downstream effector, respectively. Knockdown of either MEKK1 or 14-3-3γ attenuated VEGF-induced VPC migration and differentiation. Exogenous 7Ap could rescue the effect of VEGF on the MEKK1 siRNA-transfected VPCs but not on the 14-3-3γ siRNA-transfected VPCs. In vivo studies revealed that 7A, especially 7Ap, induced capillary vessel formation in Matrigel plug assays, increased re endothelialization and suppressed neointima formation in the femoral artery injury model, and promoted foot blood perfusion recovery in the hindlimb ischemia model by increasing Sca1+ cell niche formation. These results indicate that the sORFs within the non-coding area can be translated and that 7A may play an important role in cellular processes, such as proliferation, migration and differentiation, by acting as a phosphorylation carrier.

215 CARDIAC MACROPHAGE INFILTRATION DURING CHRONIC KIDNEY DISEASE ACCELERATES CARDIOVASCULAR DISEASE

[1]Ana Isabel Garcia Diaz*, [2]Eleni Vloumidi, [2]Alex Sardini, [1]Charles Pusey, [1]James Tomlinson, [1]Kevin Woollard. *[1]Imperial College London; [2]MRC London Institute of Medical Sciences*

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The relationship between chronic kidney disease (CKD) and increasing rates of cardiovascular disease and mortality is complex, but is important due to the massive increased risk of cardiovascular events noted in CKD patients. Macrophages have critical roles in kidney and cardiovascular disease. However, since a deeper understanding of macrophage ontogeny and heterogeneity, we wanted to revisit the role of cardiovascular macrophages during CKD.

Using the folate induced nephropathy and 5/6 nephrectomy mouse models of CKD, we have new exciting data showing that during CKD, independent of atherosclerosis, inflammatory macrophages are infiltrating cardiac tissue. Using flow cytometry, RNA profiling, histology and ultrasound, we analysed the phenotype and function of the heart and immune cell infiltrate during CKD.

After 12 weeks of CKD, $CD11b^{pos}F480^{pos}CD169^{neg}$ monocyte derived macrophages infiltrate heart tissue in large numbers. This is only evident in cardiovascular tissue, with no systemic infiltrate in lungs, spleen, kidney or liver. Interestingly, we also noted an increase in cardiac $CD19^{pos}$ B-cell and $Gr1^{pos}$ neutrophil infiltrate over the course of CKD. The cellular infiltrate was associated with an increase in cardiac fibrosis markers and decrease in heart function, as shown by decreased ejection fraction. Measuring specific chemokine expression in heart and plasma identified a unique chemokine axis, which may be regulating macrophage and other immune cell recruitment to heart tissue during CKD. Moreover, we confirmed the ontogeny of these cardiac macrophages through $Ly6C^{high}$ monocyte lineages using CCR2 deficient mice, which improved cardiac function.

This work uncovers a unique pathway that mediates inflammatory monocyte derived macrophage infiltration in the heart during CKD. More work is now being performed to confirm mechanisms and cardiovascular phenotype in our CKD

models. Ultimately these studies may identify new therapeutic targets for CKD patients, to reduce their cardiovascular risk.

216 IDENTIFYING A NOVEL ROLE FOR PMCA1 (ATP2B1) IN HEART RHYTHM INSTABILITY

Claire Wilson*, Elizabeth Cartwright, Min Zi, Yatong Li. *University of Manchester*

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Arrhythmias continue to be a leading cause of death and disability across the world, and genetics are one of the mechanisms that are known to increase susceptibility. By identifying new genetic influences and further understanding the pathways involved in heart rhythm control we can begin to tackle some of the main challenges facing treatment development.

Here we aim to identify a new role for a gene linked to several features of heart failure *Atp2b1* (Plasma membrane calcium ATPase 1, PMCA1). Along with its role in hypertension and other aspects of cardiac physiology, we believe PMCA1 may also influence heart rhythm stability and consequently the development of arrhythmias.

To investigate the role of PMCA1 in cardiac rhythm, cardiomyocyte-specific knockout mice (PMCA1^{CKOÃ,Â}) were generated. *In vivo* electrocardiography showed PMCA1^{CKO} displayed signs of cardiac repolarisation dysfunction related to prolonged QT and JT intervals. Supplementary analysis using Langendorff-perfused hearts revealed PMCA1^{CKO} hearts have prolonged action potential duration compared to controls. Additionally using the methods highlighted above, PMCA1^{CKO} mice were shown to have an increase arrhythmia susceptibility to both *in vivo* and *ex vivo* programmed electrical stimulation. Further echocardiography and histological analysis showed these heart rhythm abnormalities occur in the absence of detectable structural heart disease with PMCA1^{CKO} cardiac structure and function being comparable to controls.

Our findings suggest a novel role for PMCA1 in heart rhythm stability, distinct from other cardiac disease. Furthermore, alterations in expression of *Atp2b1* could influence an individuals susceptibility to developing arrhythmias.

217 GENETIC ABLATION OF MICROTUBULE-ASSOCIATED PROTEIN 1S (MAP1S) PROTECTS THE HEART FROM PATHOLOGICAL HYPERTROPHY VIA REGULATION OF AUTOPHAGY

Yulia Suciati Kohar*, Mohammed Najai, Nicholas Stafford, Min Zi, Sukhpal Prehar, Delvac Oceandy. *University of Manchester*

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Background Autophagy is a process essential in maintaining cellular homeostasis, by degrading and recycling unwanted materials such as misfolded proteins and dysfunctional organelles. In the heart, autophagy is key in mediating pathological processes such as hypertrophy and remodelling. Autophagy is tightly regulated by a number of proteins and defective autophagy in response to pathological stimuli may lead to the development of adverse remodelling and eventually heart failure. In this study we investigated the role of microtubule-associated protein 1S (MAP1S) in regulating autophagy during a number of cardiac pathological conditions. MAP1S has previously been identified as an interacting partner of the major autophagy regulator LC3; however, its role in the heart is unknown.

Results We used siRNA gene silencing to knockdown MAP1S in neonatal rat cardiomyocytes (NRCM) and detected the autophagic flux using GFP-LC3 expressing adenovirus. Following stimulation with rapamycin (5 uM) and chloroquine (3 uM) for 2 hours, NRCM lacking MAP1S exhibited an increase in autophagy as indicated by a significant elevation in GFP-LC3 puncta formation. To confirm this finding we cultured fibroblasts from MAP1S knock out (MAP1S-/-) mice and induced autophagy using the same stimulus. Consistently, MAP1S-/- fibroblasts also showed increased autophagy after rapamycin/chloroquine treatment. Interestingly, the expression of autophagic modulators LC3II, Beclin and p62 did not differ between cells lacking MAP1S and controls, suggesting that MAP1S might affect autophagosome elongation and not the initiation process. *In vivo*, we confirmed higher autophagy in MAP1S-/- mice following rapamycin/chloroquine intraperitoneal injection as indicated by the number of amphisomes detected by electron microscopy. Next, to test the effects of pathological stimuli we subjected MAP1S-/- mice to transverse aortic constriction (TAC, 2 weeks) or myocardial infarction (MI, 4 weeks). Following TAC, MAP1S-/- mice displayed less hypertrophy as indicated by heart weight/body weight ratio, cardiomyocyte surface area (histology) and the expression of hypertrophic markers. Consistently, after MI MAP1S-/- mice also showed a reduction in hypertrophy.

Conclusions Our findings suggest that genetic inhibition of MAP1S induces autophagy in cardiomyocytes, whereas *in vivo* ablation of this gene in mice reduces cardiac hypertrophy in response to pathological stimuli such as TAC and MI.

218 KNOCKOUT P47PHOX REDUCES ANGIOTENSIN II-INDUCED CARDIAC OXIDATIVE STRESS AND HYPERTROPHY

Fangfei Liu*, Junjie Du, Jian-Mei Li. *School of Biological Sciences, University of Reading*

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Oxidative stress due to the activation of a Nox2-containing NADPH oxidase is involved in Angiotensin II (AngII)-induced cardiovascular dysfunction. p47^{phox} is a key regulatory subunit of Nox2. However, the role of p47^{phox} in AngII-induced cardiac damage remains unclear. In this study, we used littermates of C57BL/6 wild-type (WT) and p47^{phox} knockout (KO) mice (n=7) at the age of 10~12 months to investigate the effect of p47^{phox} knockout on AngII-induced cardiac oxidative stress and hypertrophy. In WT mice, AngII infusion (1 mg/kg/day for 14 days) increased significantly the systolic blood pressure (SBP) from 127±13 to 172±11 mmHg, and this was accompanied with significant indication of cardiac hypertrophy (heart/body weight ratio increased ~17.9±0.1%) as compared to vehicle infused controls ($p<0.05$). However, in p47^{phox} KO mice, AngII infusion caused a mild increase in SBP (from 119 ±9 to 149±10 mmHg, $p<0.05$) without significant increase in heart/body weight ratio. Cardiac production of reactive oxygen species (ROS) was examined by both lucigenin-chemiluminescence and DHE fluorescence. Compared to vehicle controls,