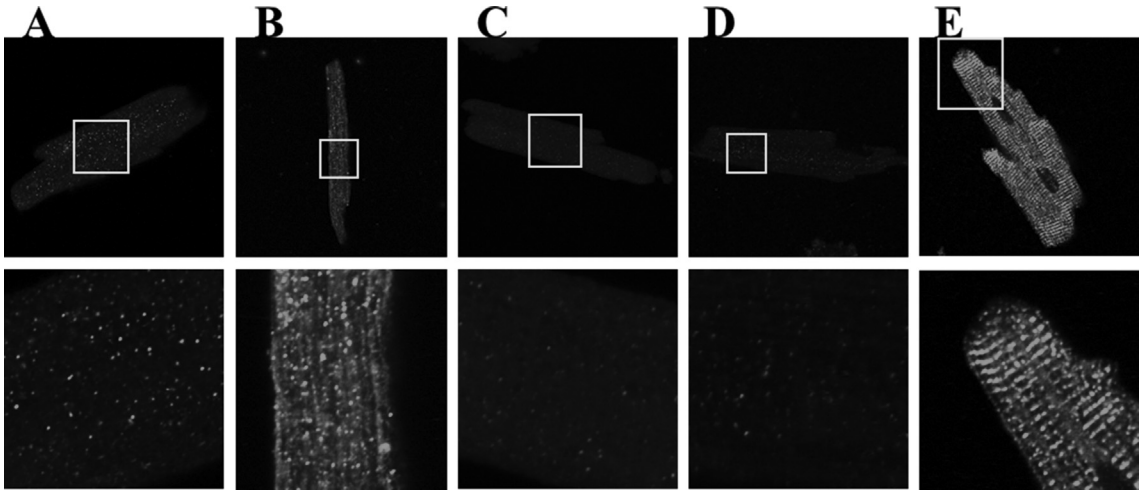


**Abstract 139 Figure 1** Effect of bafilomycin on the contractile response of cardiac ventricular myocytes to isoprenaline.

sarcoplasmic reticulum and has been identified as an important mediator of acute and chronic beta-adrenergic signalling in the heart. Genetic or pharmacological manipulation of NAADP signalling appears to be protective in pre-clinical models of cardiovascular diseases, such as ischaemia-reperfusion injury, arrhythmia and cardiac hypertrophy. Recent evidence has indicated that TPC2, rather than TPC1, mediates the effects of beta-adrenergic-evoked NAADP signalling, although whether this reflects differing subcellular distributions of TPC1 and TPC2 remains to be established.

**Methods** Single ventricular cells were isolated enzymatically from guinea pig hearts and superfused with physiological saline solution (36°C). Cells were field-stimulated (3 ms pulse duration) to fire action potentials. Cell shortening was measured with an edge-detection system. To confirm the involvement of a lysosome-related acidic compartment in beta-adrenergic signalling, the effect of isoprenaline (a beta-adrenoceptor agonist) on cell contraction was investigated in the presence and absence of bafilomycin (a vacuolar $H^+$-ATPase inhibitor that depletes acidic calcium stores). Data are presented as mean±SEM; comparisons were made using one-way ANOVA. In additional experiments, standard immunocytochemical techniques were employed to investigate the cellular distributions of TPC1, TPC2, transient receptor potential channel mucolipin-1 (TRP-ML1), lysosome-associated membrane protein-2 (LAMP2) and ryanodine receptor-2 (RyR2). Immunofluorescence was visualised using a Zeiss LSM 510 confocal microscope.

**Results** Isoprenaline (3 nM, 3 min) increased myocyte contraction (relative to control) by 86±9% (n=8) in the absence of bafilomycin, 62±13% (n=6) in the presence of 100 nM bafilomycin, and 44±12% (n=8) in the presence of 300 nM bafilomycin (Figure 1). The effect of bafilomycin tended closely toward statistical significance ($p<0.06$, one-way ANOVA). TPC1 and TPC2 displayed qualitatively similar patterns of punctate intracellular labelling. This punctate pattern resembled that for the lysosomal markers TRP-ML1 and LAMP2. In contrast, RyR2 labelling showed a striated appearance, consistent with the known organisation of the sarcoplasmic reticulum in cardiac myocytes (Figure 2).

**Conclusions/implications** This is the first direct immunocytochemical evidence in cardiac cells describing the localisation of TPC1 and TPC2. The subcellular distribution of TPC1 and TPC2 does not appear to explain their differing contributions to beta-adrenergic signalling. Our data are also consistent with a role for lysosomal calcium stores in the inotropic effect of beta-adrenoceptor agonists.

## 140 HETEROZYGOUS DELETION OF PMCA1 MIGHT SERVE A PROTECTIVE ROLE IN THE HEART FOLLOWING MYOCARDIAL INFARCTION

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**Introduction** Coronary artery disease (CAD) and its main consequence-myocardial infarction (MI) are the UK's biggest killers. Most of these deaths occur either acutely post-MI or chronically as a result of pathological cardiac remodelling eventually leading to heart failure. Recent genome-wide association studies (GWAS) elucidated a potential link between the PMCA1 gene, *atp2b1*, and these diseases.

**Objective** PMCA1 has been shown to associate with many of the key features predisposing to the development of heart failure. Given the GWAS findings this study aims to investigate the potential role of PMCA1 in the post-MI remodelling process.

**Methods** Moderate and severe MI via permanent ligation of the LAD coronary artery, alongside a sham procedure was induced in either wild type or mice expressing a heterozygous mutation of the PMCA1 gene ($PMCA1^{Ht}$). Occurrence of ischemia was confirmed by evaluation of plasma levels of cardiac troponin I (cTnI) and histologically. Post-surgery the animals were kept for either 1 or 4 weeks. Electrocardiography (ECG) and echocardiography were performed *in vivo* to assess cardiac function. To further characterise the cardiac response *in vitro*, histological analysis was performed at both time points.

**Results** 24 hours post LAD coronary artery ligation a significant increase in the plasma cTnI levels was recorded in both WT and $PMCA1^{Ht}$ mice. However, overall survival 4 weeks post-surgery was significantly lower in WT-MI mice when compared to $PMCA1^{Ht}$-MI mice and both WT and $PMCA1^{Ht}$ sham control groups. In addition, a significant difference in

infarct size was observed when WT-MI hearts were compared to their PMCA1$^{Ht}$ counterparts 1 week post-surgery. Interestingly, whilst both WT and PMCA1$^{Ht}$ MI-treated mice showed a significant deterioration in cardiac function 1 week post-surgery there were differences seen in cardiac structure between the two groups. For example, echocardiography revealed that the WT-MI hearts were significantly more hypertrophic when compared to their PMCA1$^{Ht}$ counterparts shown by a significant increase in the LV diameters alongside a significant difference in the normalised heart weight to tibia length ratios. ECG revealed significantly longer QT intervals and considerably more extra-systolic events among the WT-MI mice. Whilst histological analysis of cardiomyocyte size showed a significantly exacerbated hypertrophic response in the remote regions of WT-MI mice compared to PMCA1$^{Ht}$-MI mice.

**Conclusion** Heterozygous deletion of PMCA1 might serve a protective role in the heart following both moderate and severe MI. The protective mechanism most likely develops in the early post-operative phase, as an MI of similar extent is associated with higher mortality rates in WT mice. Future work will aim to elucidate the mechanism producing this phenotype mainly focusing on the potential role that PMCA1 might serve in the process.

## 141 HERITABILITY AND FAMILY-BASED GWAS ANALYSES TO DISCOVER NOVEL LIPIDOMIC BIOMARKERS OF CARDIOVASCULAR DISEASE

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**Introduction** Lipids have key roles in CVD but heritability studies focus on few species bar lipoproteins. Lipids play key roles in cell signalling, immunity, inflammation, vasodilation and cell death. Although not encoded, their activities are tightly linked to DNA-encoded entities (e.g. enzymes and other proteins) and those with a strong genetic influence (high heritability) may identify novel pathways in CVD. Purpose: Analysing eicosanoid, endocannabinoid and sphingolipid profiles in 250 British Caucasian families with GWAS data will identify particularly heritable lipid biomarkers for the discovery of causative genetic variation of CVD.

**Methods** An array of 79 eicosanoids and related species, 33 endocannabinoids and congeners, 63 ceramides and related species, from 204 plasma samples (31 families of 1+ individuals with hypertension) were extracted and analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Quality control was accessed and estimates of heritability of each lipid species was estimated using QTDT software. The concentration of each lipid species were assessed for normal distribution, outliers, and adjusted for age and sex. The resulting lipid concentrations will be analysed using FaST-LMM software for associations with 557,124 SNPs.

**Results** 19 species of eicosanoids were identified (mean concentrations 19 pg/ml – 7600 pg/ml); the species at highest abundance in plasma (HODEs) are derivatives of the omega-6 fatty acid linoleic acid. 8 species passed quality control assessments and 3 species were estimated to be significantly heritable (9-OxoODE 24%, 12-HETE 44%, 12(13)-EPOME 78%). 15 congeners of endocannabinoids were identified (mean concentrations 20 pg/ml – 4000 pg/ml); the species at highest abundance in plasma are glycerol derivatives. 9 species passed quality control assessments and all were estimated to be significantly heritable (27%–73%)%). 57 species of ceramides were identified (mean concentrations 0.01 pmol/ml – 190 pmol/ml); the species at highest abundance in plasma are NS ceramides involved in apoptosis. 36 species passed quality control assessments and all species were estimated to be significantly heritable (10%–63%)%).

**Conclusions** We demonstrate for the first time estimates of heritability for an array of bioactive lipids. Although found at concentrations million times lower than cholesterol, many of the heritability estimates are similar to plasma genetic biomarkers of CVD (ACE h2>45%). The association between heritable biomarkers, cardiovascular phenotypes and risk will be determined. Particularly heritable species and those with highly significant hits at GWAS will be profiled in the extended cohort (1400 samples, 250 families) and replication will take place in the UK biobank, or other. A subset of the most heritable species and those with hits at GWAS resulting from the full lipidomic profile of 3 lipidomic classes will be used to identify causal metabolic pathways, novel diagnostics and drug targets for CVD intervention.

## 142 INTRAVITAL IMAGING OF LEUKOCYTE, PLATELET AND STEM CELL TRAFFICKING IN VIVO IN THE CARDIAC MICROCIRCULATION FOLLOWING MYOCARDIAL ISCHAEMIA-REPERFUSION INJURY

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**Background** The clinical success of stem cell (SC) therapy for myocardial infarction is compromised by poor cardiac homing following systemic delivery. As such therapy may depend on beneficial paracrine effects, it is a further hindrance that little is known about the inflammatory response dynamics within myocardial microcirculation *in vivo*.

**Methods** 3D-printed stabilisers were bonded to the beating heart of anaesthetised (ketamine/xylazine) mice to enable confocal intravital imaging of ventricular microcirculation. PE-anti-Gr-1 and APC-anti-CD41 antibodies were injected to label neutrophils and platelets respectively with FITC-BSA enabling blood flow visualisation. In some mice, haematopoietic SCs (HSCs; HPC-7s) were introduced intra-arterially. IR injury was induced by 45 min (reversible) ligation of the LAD artery.

**Results** Neutrophil adhesion and platelet accumulation were both significantly ($p<0.001$) and rapidly increased in injured microvessels with platelet accumulation increasing with time. No difference in number or velocity of free-flowing neutrophils was observed. A significant ($p<0.05$) decrease in functional capillary density was also observed in injured hearts. Although HSC adhesion was not significantly enhanced following injury, a time-dependent increase in adhesion was observed in sham and injured hearts. No significant change in number or velocity of free-flowing HSCs was observed following injury. Interestingly, despite reduced capillary perfusion, approximately 10–20 HSCs were observed trafficking though the heart at each time point throughout reperfusion.

**Discussion** Intravital microscopy has allowed successful visualisation of the microvascular inflammatory response and HSC homing events in the beating mouse heart post-reperfusion.