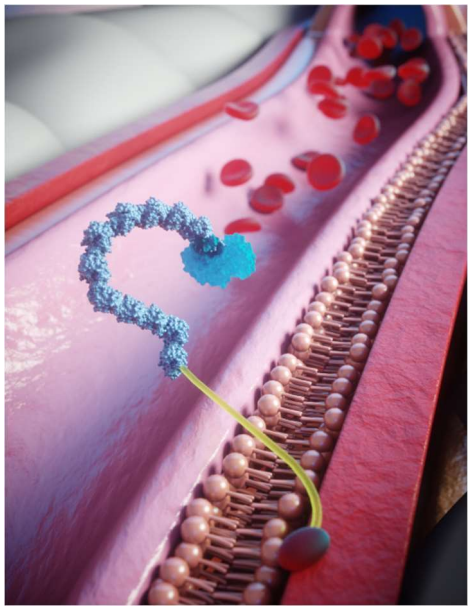
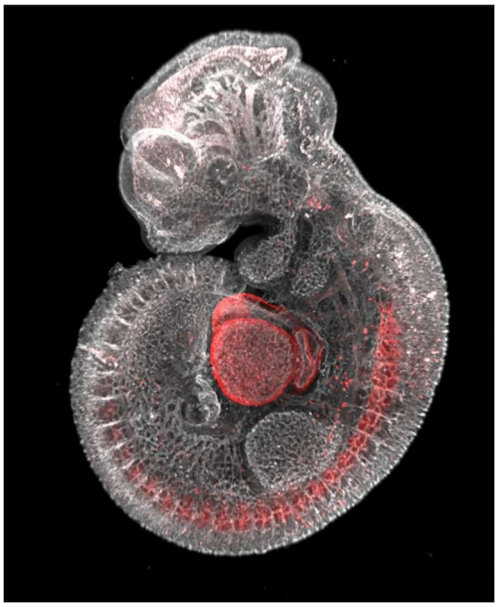
Oxford BHF Centre of Research Excellence

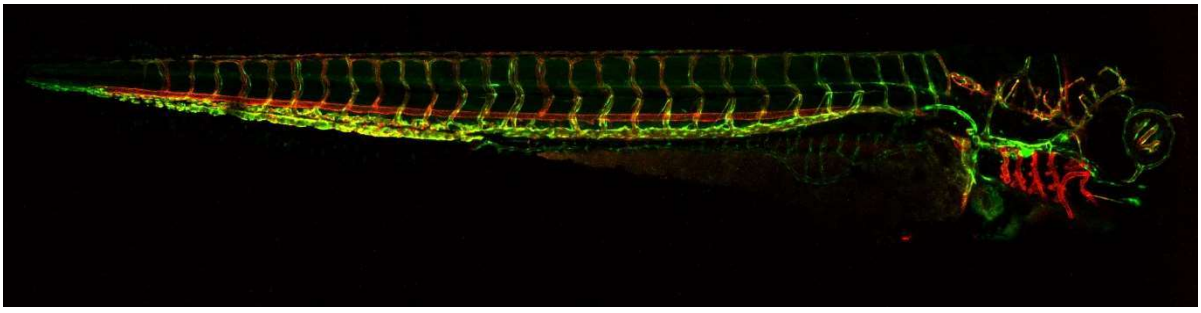
Annual Symposium

Wednesday 10th November 2021

Online via Zoom







Cover images contributed by (left to right and top to bottom):

1. Prof Ellie Tzima
2. Dr Richard Tyser
3. Dr Alice Neal

Oxford BHF Centre of Research Excellence
Research Symposium
Wednesday 10th November 2021
Virtual via Zoom

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10:00 - 10:10	**Professor Hugh Watkins** Director, Oxford BHF CRE. ***Welcome and Oxford BHF CRE updates***
Session 1 **Start Time 10:10**	**Chair: Professor Sir Rory Collins** **Oxford BHF CRE Transition Research Fellows**
10:10 – 10:25	**Dr Kerstin Timm**: *The crucial role of metabolism in cardio-oncology*
10:25 – 10:40	**Dr Peter Chan**: *Air pollution and cardiovascular health: what's more to know?*
10:40 – 10.55	**Dr Marion Mafham**: *Cardiovascular outcomes in clinical trials: definitions of truth*
10.55 – 11.10	**Dr Qiang Zhang**: '*Virtual contrast dye' to replace gadolinium and needles: How AI is advancing cardiac MRI*
11.10 – 11.30	*Refreshment Break*
Session 2 **11:30 – 12:15**	**Chair: Professor Sarah De Val** **Poster competition short-list talks**
	Eight four-minute talks from the short-listed poster competition entrants.
12.15-12.30	*Break for refreshments and time to enter Gather Town*
12.30 – 13.30	**Poster viewing and discussions in Gather Town**
13.30 – 13.45	**Image Competition – Viewing of entries and audience voting**
Session 3 **Start Time 13.45**	**Chair: Professor Manuela Zaccolo**
13.45 – 14.00	**Dr Chris Toepfer:** *Human stem cell models for defining cellular pathomechanisms in inherited cardiomyopathies*
14.00 – 14:15	**Dr Francesca Margara:** *Human-based Computational Investigations into Cardiac Electromechanical Alterations Caused by Drugs and Hypertrophic Cardiomyopathy*
14.15 – 14.30	**Prof Ellie Tzima:** *Mechano-pulling the strings on atherosclerosis*
14.30 – 14.45	*Refreshment Break*
Session 4 **Start time 14.45**	**Chair: Professor Cornelia van Duijn**
14.45 – 15.00	**Dr Jason Torres:** *Genetic insights from the Mexico City Prospective Study*
15.00 – 15.15	**Dr Alice Neal:** *Transcriptional regulation of arterial-venous patterning*
15.15 – 15.30	**Dr Richard Tyser** *Defining Cardiac Progenitors During Early Human Heart Development*
15:30 – 16:00	*Refreshment Break*
Session 5 **16:00 – 16:45**	**Keynote Speaker – Professor Eric Olson, UT Southwestern Medical Center** *Toward Genetic Therapies for Cardiovascular Disease*
16:45 – 17:00	**Professor Hugh Watkins – Presentation of poster and image competition prizes and closing remarks**

Speaker session 1 - 10:10 – 11:10

Oxford BHF CRE Transition Research Fellows

Chair: Professor Sir Rory Collins

Four of the current BHF CRE Transition Research Fellows give an overview of their research.

10:10 - 10:25

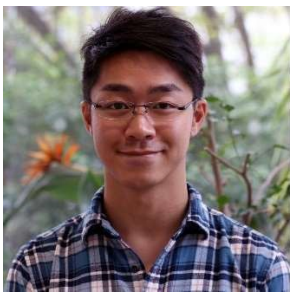
The crucial role of metabolism in cardio-oncology

Dr Kerstin Timm
Department of Pharmacology

Several chemotherapeutic agents, such as the anthracycline doxorubicin, have severe cardiotoxic side effects, which can lead to congestive heart failure in 5-10% of patients. There are currently no imaging techniques available to detect patients at risk of developing cardiotoxicity before the onset of functional decline and there are no specific cardio-protective drugs. My research focuses on both the early detection of cardiotoxicity using the clinically-translatable metabolic imaging technique, hyperpolarized magnetic resonance imaging (MRI), and the repurposing of existing drugs that target cardiac metabolism as potential cardio-protective therapy. I recently procured a high resolution respirometer, the Oroboros O2k oxygraphy to underpin metabolic imaging data with *ex vivo* mitochondrial function analysis. I am currently also establishing rodent models of cancer in which to assess new cancer therapies, the mitocans. These mitocans are based on inhibition of mitochondrial electron transport chain proteins, and I will test them for efficacy and cardiac safety using in *vivo* metabolic imaging of the heart and cancer and *ex vivo* respirometry of both tissues. These cancer models will furthermore allow me to test cardioprotective drugs in light of their potential benefit on the heart as well as additive or detrimental effects on tumour-treatment response.

10:25 - 10:40

Air pollution and cardiovascular health: what's more to know?



Dr Peter Ka Hung Chan
Nuffield Department of Population Health

Air pollution is widely considered as a leading risk factor of disease burden worldwide. The latest estimates from the Global Burden of Disease Study suggest that household air pollution from domestic use of solid fuels and ambient air pollution together contributed to over 7 million premature deaths in 2019, with cardiovascular disease being the predominant cause. Such estimates, however, have been based on epidemiological studies with longstanding limitations from the crude exposure assessment methods used and the lack of validation from actual personal exposure measurements, especially in low- and middle-income countries where most of the related disease burden lies. There is also inadequate evidence on the biological mechanisms underlying the cardiovascular effects of air pollution. In this talk, I shall explain the persistent knowledge gaps in this field, highlight the practical challenges in obtaining the necessary data, and present some preliminary findings from my current fellowship designed to address some of the limitations and advocate for better research in this field.

10:40 - 10:55

Cardiovascular outcomes in clinical trials: definitions of truth

Professor Marion Mafham
Nuffield Department of Population Health

Large clinical trials are essential to reliably assess the effects of cardiovascular interventions on important outcomes. Currently, clinical trials collect outcome data in a labour intensive way: Data collected from participant reports or extracted from the medical records is manually recorded by trained research staff, then checked by study monitors against the original source, and then copies of the original medical records are reviewed by a committee of experts aiming to establish a 'ground truth'. This process of ensuring an accurate chain of custody from one perfect source of truth, has produced reliable results but is prohibitively expensive, meaning that people turn to non-randomised 'real world' studies which can be biased and misleading. An alternative approach is to use a range of data sources in clinical trials, including routinely collected healthcare data, which can be integrated to produce a 'best estimate' of the truth that is sufficiently accurate to produce reliable trial results. This approach has been used to produce rapid, reliable results among patients admitted to hospital with COVID-19 in the 44,000 participant RECOVERY trial and data from existing 'gold-standard' clinical trials will be used to assess the utility of such methods in long-term cardiovascular disease trials.

10:55 - 11:10

Virtual contrast dye' to replace gadolinium and needles:
How AI is advancing cardiac MRI

Dr Qiang Zhang
Department of Cardiovascular Medicine

The current gold-standard for imaging heart muscle disease is CMR, using late gadolinium enhancement (LGE). However, this requires injection of a contrast agent into the patient, which prolongs the scan, increases the cost, and is cautioned in some patients. Clearly, having a faster scan that provides the same information, but without the need for needles and contrast agents, would be very attractive to patients and doctors who need these scans.

We have developed an AI solution called 'virtual native enhancement' (VNE). This combines MR images that do not normally need contrast injections, and uses AI to train machines to predict what a contrast-enhanced image would look like. This approach can produce images that are similar to traditional contrast-enhanced images, but without the need to inject the contrast agent.

Tested first on hypertrophic cardiomyopathy, it was shown that these AI-enhanced images can produce as clear or better quality images than the traditional LGE, providing doctors with the same information. VNE is much faster and significantly cheaper than the conventional scans. With further development on more heart conditions, it could lead to a next generation of CMR scans that are cheaper, safer, needle-free and more patient-friendly.

Speaker session 2 - 11:30 – 12:15

Chair: Professor Sarah De Val

A short-list of eight of the poster competition entrants present their posters.

Speaker session 3 – 13:45 – 14:30

Chair: Professor Manuela Zaccolo

13:45 – 14:00

Human stem cell models for defining cellular pathomechanisms in inherited cardiomyopathies

Dr Chris Toepfer
Sir Henry Dale Fellow, BHF CRE Intermediate Transition Fellow
Department of Cardiovascular Medicine

Human induced pluripotent stem cells (iPSCs) provide the opportunity to study human inherited cardiovascular diseases in a human model in the dish. Twinned with advances in CRISPR/Cas-9 and differentiation of iPSCs to cardiomyocytes we can begin to delve into mechanisms that define disease in patients that harbour hypertrophic (HCM) and dilated cardiomyopathy (DCM) variants. We use these cellular systems to phenotype variants and perform initial screens of potential therapies. In this session we describe the open access tools we have developed to move from genotype to phenotype and uncover fundamental disease mechanisms in HCM. Using this information we can begin to uncover therapeutic strategies by identifying potential druggable mechanisms of disease.

14:00 – 14:15

Human-based Computational Investigations into Cardiac Electromechanical Alterations Caused by Drugs and Hypertrophic Cardiomyopathy

Dr Francesca Margara
Research Associate
Department of Cardiovascular Medicine

The assessment of the cardiac safety and efficacy of therapeutic interventions remains a major challenge. Defining key disease- and patient-specific mechanisms is an important need for the development of pharmacological interventions that are safe and effective in the individual subject.

This work investigated the mechanisms explaining the cardiac safety and efficacy of pharmacological therapies in health and the genetic heart disease hypertrophic cardiomyopathy (HCM) through modelling and simulation of the human ventricular electromechanical function.

We constructed and evaluated a novel computational modelling and simulation framework of the human cardiomyocyte electromechanical function that enabled the simultaneous assessment and explanation of drug-induced effects on electrophysiology and contractility through mechanistic simulations informed by experimental data. Next, we developed a novel software tool for the automated analysis of calcium transients in cardiomyocytes enabling the phenotyping of primary and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). We used this to analyse hiPSC-CM data under three mutations that are known to cause HCM in patients and pharmacological action. Finally, we integrated these experimental findings with mechanistic modelling and simulation and defined computationally the key pathomechanisms of each HCM mutation that determine drug efficacy.

This work demonstrates the use of experimentally-informed human-based computational methodologies for precision cardiology, by identifying key mechanisms that determine the cardiac safety and efficacy of pharmacological therapies in health and HCM.

14:15 – 14:30

Mechano-pulling the strings on atherosclerosis

Professor Ellie Tzima
Wellcome Trust Senior Fellow, Professor of Cardiovascular Biology
Department of Cardiovascular Medicine

The Tzima lab investigates the role of mechanotransduction in regulating cardiovascular function in health and disease. Our group has made significant conceptual advances in our understanding of flow sensing and systematically characterised one of the most comprehensive models of endothelial mechanotransduction available to date. This talk will focus on the recent discovery of a new class of mechanosensors which determine the site-specific distribution of atherosclerosis. I will discuss our current understanding of the molecular mechanisms by which vascular endothelial cells sense fluid shear stress to ultimately promote inflammation and atherogenesis.

Speaker session 4 - 14:45 – 15:30

Chair: Professor Cornelia van Duijn

14:45 – 15:00

Genetic insights from the Mexico City Prospective Study

Dr Jason Torres
Senior Genetic Epidemiologist
Clinical Trial Service Unit and Epidemiological Studies Unit,
Nuffield Department of Population Health

As the largest blood-based prospective study in Latin America, the Mexico City Prospective Study (MCPS) presents unprecedented opportunities to discover and resolve risk factors for disease and premature death in a non-European, middle-income country. Between 1998 and 2004, 150K adults were recruited from two districts in Mexico City and questionnaire data, physical measurements and a blood sample were taken. Through a recent partnership with the Regeneron Genetics Center, genome-wide array and whole exome sequencing (WES) data has been obtained for the full cohort, and whole genome sequencing (WGS) has been completed on a subset of 10K participants. Genetic analysis has revealed complex patterns of relatedness, population structure, and Mesoamerican admixture in the cohort. The incorporation of MCPS into a transethnic exome-wide association study of BMI has uncovered novel rare variant associations, including protein-truncating variants in *GPR75* that correspond to 54% lower odds of obesity. Genome-wide association analysis of type 2 diabetes has corroborated associations at the *SLC16A11* locus involving variants common in Mexico but less frequent in Europe. The availability of NMR-based metabolomic assays in combination with genetic data offer additional opportunities to resolve risk factors for non-communicable disease.

15:00 – 13:15

Transcriptional regulation of arterial-venous patterning

Dr Alice Neal
BHF Senior Fellow
Department of Physiology, Anatomy & Genetics

Venous and arterial endothelial cells are molecularly and functionally distinct and the correct patterning of arterial-venous gene expression is essential for vascular development. We have discovered DNA enhancer elements that drive gene expression exclusively in endothelial cells of embryonic veins. Analysis of these enhancer elements has shown that venous endotheli cell specific gene expression requires the combination of ETS and SMAD1/5 transcription factor binding downstream of VEGF and BMP signalling. Using a combination of mouse and zebrafish transgenic

models we have recently uncovered additional transcription factors that are essential for venous gene expression. We are currently working towards an integrated model of how combinations of transcription factors can achieve specific patterns of gene expression in endothelial cell subtypes to drive venous endothelial cell fate acquisition. We are using these animal models to investigate the mechanisms that drive development of the coronary vasculature and it's regeneration after injury.

15:15 – 15:30

Defining Cardiac Progenitors During Early Human Heart Development
Dr Richard Tyser
BHF Immediate Research Fellow
Department of Physiology, Anatomy & Genetics

On average our hearts beat around 3.5 billion times during our lifetime, but how does it form during human development? The human heart starts to form at around 20 days post fertilisation with the largest morphological changes occurring by 7 weeks, coinciding with the period in which the heart is most vulnerable to congenital defects. Congenital heart defects are the most common type of birth defect, being diagnosed in at least 1 in 150 births: equating to around 13 babies each day in the United Kingdom. It is therefore important to understand the cellular composition of the heart and the transcriptional programs that regulate early human cardiac development. We have begun to characterise these early stages of human cardiac development, gaining a unique insight into the cell types which make up the forming heart at both an anatomical and transcriptional level. This has revealed a surprising diversity in the cardiomyocyte and endocardial cell types present within the early heart and defined the transcriptional profile of the emerging human epicardium *in vivo*. Understanding how this vital organ forms not only addresses questions of fundamental biological significance but also provides clinically relevant insight into the potential origins of congenital heart disease and reparative therapies to treat heart disease.

Speaker session 5 – 16:00 – 17:00

KEYNOTE LECTURE

Toward Genetic Therapies for Cardiovascular Disease

Professor Eric Olson
UT Midwestern Medical Center

Biography

Eric Olson is the founding Chair of the Department of Molecular Biology at UT Southwestern Medical Center. He also directs the Hamon Center for Regenerative Science and Medicine and the Wellstone Center for Muscular Dystrophy Research at UT Southwestern. He holds the Robert A. Welch Distinguished Chair, the Pogue Chair Distinguished Chair in Cardiac Birth Defects and the Annie and Willie Nelson Professorship in Stem Cell Research.

Eric Olson and his trainees discovered many of the key genes and mechanisms responsible for development and disease of the heart and other muscles. His most recent work has provided a new strategy for correction of Duchenne muscular dystrophy using CRISPR gene editing.

Dr. Olson is a member of the U.S. National Academy of Sciences, the Institute of Medicine, and the American Academy of Arts and Sciences. His work has been cited over 110,000 times in the scientific literature with an h index of 190.

Eric Olson has co-founded multiple biotechnology companies to design new therapies for heart and muscle disease.

Presentation abstract

We seek to delineate the mechanisms that govern development, disease and regeneration of the heart and other muscles and to build upon this knowledge to restore muscle function during disease and aging. In one approach, we are optimizing strategies for CRISPR-mediated gene editing to eliminate disease-causing mutations responsible for Duchenne muscular dystrophy (DMD) and various genetic cardiomyopathies. We refer to this approach as myoediting. We have optimized myoediting for DMD and cardiomyopathies in human cardiomyocytes derived from iPS cells generated from blood samples of affected patients and in animal models of these disorders. In another approach, we have discovered a collection of previously unrecognized micropeptides with key roles in many aspects of muscle and cardiac development, disease, and physiology. Among these is the cardiac-specific transmembrane peptide DWORF, which stimulates calcium cycling in cardiomyocytes by activating the SERCA pump. DWORF expression is downregulated in heart failure and viral delivery of DWORF in mice with heart failure is sufficient to enhance cardiac contractility. Opportunities and challenges in the path toward correction of genetic forms of heart disease through gene therapy will be discussed.

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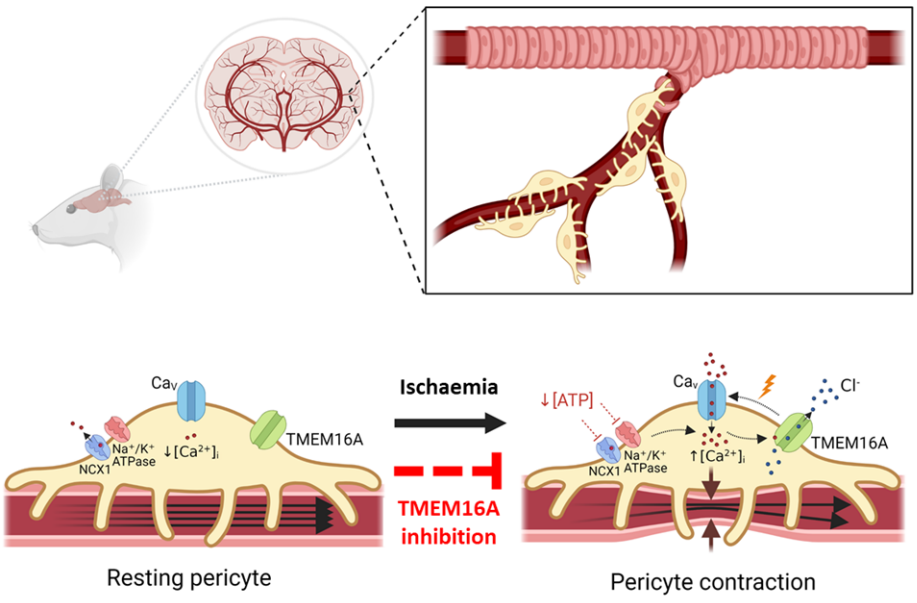
POSTER NO: 1

Title: TMEM16A Channel is a Key Regulator of Cerebral Blood Flow at the Capillary Level

Authors: Zeki Ilkan[†*1]; Nils Korte[†2]; Claire Pearson[1]; Thomas Pfeiffer[2]; Prabhav Singhal[2]; David Attwell[2]; Paolo Tammaro[1]

Departmental affiliations: [1]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, UK; [2]Department of Neuroscience, Physiology & Pharmacology, University College London, Gower St., London, WC1E 6BT, UK; *Presenting author - postdoctoral research fellow (non-clinical); [†]Equal contribution

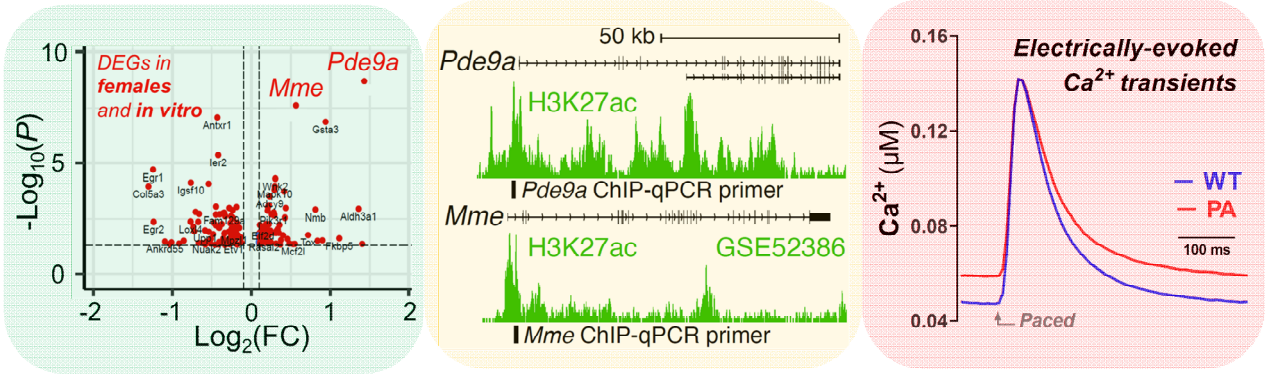
Research Rationale: Cerebral blood flow is increased by neuronal activity. This ensures an adequate energy supply to reverse the ion movements generating synaptic and action potentials. Cerebral blood flow is partly controlled at the capillary level, by pericytes contracting or relaxing to alter capillary diameter. Pericytes are also important in pathology: during ischaemia, pericytes contract and then die in rigor, hampering restoration of blood flow to capillaries. Thus, explaining pericyte contraction is key for understanding the regulation of energy supply to the brain and its pathology, and for developing new therapies. Here, we show that the Ca^{2+}-gated anion channel TMEM16A is expressed on pericytes, and plays an essential role in mediating the pericyte contraction evoked by $[Ca^{2+}]_i$ rises. **Methodology:** Whole-cell patch-clamp recordings of TMEM16A currents, capillary bright-field imaging, and confocal microscopy were used in this study. Cortical brain slices were obtained from P21 Sprague-Dawley rats or adult mice. **Results:** TMEM16A currents were reduced by the specific channel blocker Ani9 (2 μM) by 73% (p=0.0001; n=9-14 pericytes; N=7-9 rats). Capillary constriction evoked by a thromboxane analogue or endothelin-1 (ET-1) was significantly reduced during TMEM16A inhibition. Removing the depolarizing Cl^- gradient across the cell membrane by blocking NKCC1 with bumetanide abolished pericyte contraction, and this was restored by lowering $[Cl^-]_o$ to re-introduce a depolarizing gradient of chloride ions. Pericyte $[Ca^{2+}]_i$ was elevated by ET-1 in the absence but not in the presence of Ani9. ET-1 or Ani9 did not affect the whole-cell electrical activity of cortical neurons, suggesting that modulation of pericyte tone by these agents was not secondary to changes in neuronal firing patterns. Exposure of cortical slices to oxygen and glucose deprivation to simulate cerebral ischaemia caused significant capillary constriction and pericyte death, which were ameliorated by Ani9. **Conclusions:** These data reveal an essential role for TMEM16A in regulating cerebral blood flow, and offer novel therapeutic approaches for treating disorders of cerebral circulation, such as stroke and Alzheimer's disease.



POSTER NO: 2

Title: *Using a mouse model of an inborn error of metabolism to identify novel epigenetic responses in the heart caused by elevated propionyl-CoA, a ubiquitous metabolic intermediate*

Authors: Kyung Chan (KC) Park*[1], Alzbeta Hulikova[1], Nicholas T. Crump[2], Niamh Louwman[1], Kerrie L. Ford[1], Thomas A. Milne[2], Pawel Swietach[1]. **Presenter/Postdoctoral Research Scientist (non-clinical).*
Departmental affiliations: (1) DPAG; (2) MRC Molecular Haematology Unit, RDM.



***Research rationale*:** Propionyl-CoA is a three-carbon intermediate of metabolic pathways that process odd-number carbon chains. Like other acyls (e.g. acetate, butyrate), propionate is a substrate for protein post-translational modifications and can act as an inhibitor of deacetylase enzymes, for example resulting in increased histone propionylation and net acetylation. Histone marks induced by propionyl-CoA can have a profound and lasting effect on tissues, likely by perturbing epigenetic regulation of transcription. In most cells, levels of propionyl-CoA are kept low by propionyl-CoA carboxylase (PCC). However, build-up of this metabolite has been reported in common metabolic diseases and, most notably, in the rare disease propionic acidaemia (mutations in PCC). The aim of this study was to establish the effect of propionyl-CoA on cardiac gene expression and physiology using a mouse model of elevated propionyl-CoA signalling.

***Methodology*:** Experiments were performed using either wild-type neonatal ventricular myocytes treated with propionate *in vitro*, or the hypomorphic mouse model of PA (*Pcca*$^{-/-}$ A138T) at 8 weeks of age. IC-MS metabolomics was performed on methanol-extracted metabolites. RNA-sequencing (RNA-seq) was carried out on an Illumina HiSeq 4000. For chromatin immunoprecipitation (ChIP), chromatin was isolated from PFA-fixed ventricular tissue in Langendorff mode. Ca^{2+} transients were imaged in isolated ventricular myocytes using the Ca^{2+} reporter FuraRed. Cine-MRI was performed in the supine position under isoflurane.

***Results*:** PA mice had the metabolic signature of propionate accumulation in plasma and cardiac lysates (metabolomics). RNA-seq of ventricular lysates identified differentially expressed genes (DEGs), but the effect was more pronounced in females (743, vs 401 in males). Thus, subsequent experiments were performed in female mice. To determine which DEGs are likely a direct response to propionate, RNA-seq was performed on wild-type neonatal myocytes treated with exogenous propionate. The most significant DEGs common to both datasets were *Pde9a* (cGMP-selective phosphodiesterase 9A) and *Mme* (membrane metallo-endopeptidase; catalyses natriuretic peptide degradation). ChIP-qPCR for histone acylation in PA and WT hearts demonstrated a 1.7-fold increase in H3K27ac at *Pde9a*, and strikingly, increases in propionylation at *Pde9a* and *Mme* (2.0- and 2.3-fold, respectively), indicating a mechanism for this transcriptional induction. Changes to these genes are expected to affect cyclic nucleotide signalling, and thus impact multiple stages of excitation-contraction coupling. Indeed, ventricular myocytes isolated from PA mice had higher diastolic [Ca^{2+}] and reduced sarcoplasmic reticulum (SR) Ca^{2+} load, due to lower SERCA activity. Cine-MRI confirmed contractile dysfunction *in vivo*, with PA mice manifesting increased end-systolic and end-diastolic volumes.

***Conclusions*:** We demonstrate that cardiac elevations of the metabolic intermediate, propionyl-CoA, increases histone modifications that drive transcriptional changes in the heart, including the induction of genes involved in cyclic nucleotide signalling. We also present evidence for histone propionylation, which has not been described previously in the heart. Thus, using a mouse model of a rare metabolic disease, we show how propionyl-CoA signalling affects cardiac function through epigenetic changes.

POSTER NO: 3

Acidic calcium stores in the cardiac atria: Insights from health and disease

Rebecca A Capel[1]*, Thamali Ayagama[1], Samuel J Bose[1], Emily Akerman[1], Daniel Aston[1], David A Priestman[1], Georgina Berridge[2], Eva Rog-Zielinska[3], Ulrich Schotten[4], Roman Fischer[2], Lisa Heather[5], Frances Platt[1], Holger Kramer[5], Sander Verheule[4], Rebecca AB Burton[1]

* Presenting author, post-doctoral researcher, non-clinical. 1 Department of Pharmacology, University of Oxford, 2 Target Discovery Institute, University of Oxford, 3 Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg, 4 Fysiologie, School for Cardiovascular Diseases, Maastricht University, 5 Department of Physiology Anatomy and Genetics, University of Oxford, 6 Biological Mass Spectrometry and Proteomics Facility, MRC London Institute of Medical Sciences, Imperial College London

Research Rationale

Acidic calcium stores in the heart provide a significant contribution to basal calcium transient amplitude and β-adrenergic response in both atrial and ventricular myocytes[1]. In ventricular myocytes, lysosomes are positioned in close apposition to the sarcoplasmic reticulum (SR) and mitochondria, forming potential signalling microdomains[2]. Atrial myocytes express both lysosomes and atrial granules (AG), an acidic, calcium-containing secretory granule[3]. We set out to determine the functional role and structural position of acidic calcium stores in atrial tissue and how they are changed in atrial fibrillation (AF).

Methodology

For functional studies, we performed force transducer recordings on isolated murine atria. For Goat studies: Goats were maintained in AF for 6 months (study protocol approved by Maastricht University ethical board for animal experimentation and carried out in accordance with Basel Declaration and European directive 2010/63/EU). Following sacrifice experiments (n=4 persistent AF and n=4 sham controls), we performed electron tomography[2] (EM) and proteomic studies. For proteomic studies, we developed a centrifugation method to stratify endolysosomal and mitochondrial fractions from whole tissue lysate[4]. Selected changes were confirmed with Western blotting and, for Cathepsin D, further tested in human plasma samples from Discovery Life Sciences. Gene Ontology, KeGG, Panther and Cytoscape were used to study the major molecular networks altered in AF.

Results

Abolition of lysosomal calcium signalling in murine atria (1μM Bafilomycin A1 or 10μM Ned-19) significantly reduced spontaneous beating rate (P<0.05, ANOVA) and caused a rightward shift in EC50 of the β-adrenergic response in both beating rate and force (P<0.05, F test). EM of healthy goat samples showed lysosomes and AG formed membrane contact sites (MCS) with SR (mean distance to closest SR, 14.03 ± 1.49 nm, n=36, and 11.95 ± 1.18 nm, n=32, respectively). In contrast to ventricular myocytes, acidic calcium stores rarely formed MCS with mitochondria [median (IQR) distance 100.2 nm (31.09-306.9) for lysosomes and 89.69 nm (34.5-155.2) for AG]. After 6 months of persistent AF, lysosomal diameter increased from 286.2 ± 17.22 nm (n=37) to 481.3 ± 51.37 nm (n=19, P<0.05 ANOVA). AG diameter was unchanged. AG were observed significantly further away from SR (mean 21.50 ± 1.64 nm, n=43, P<0.05 by ANOVA) and closer to mitochondria (24.4 nm (14.8-90.65), P<0.05 by Kruskal-Wallis). Lysosomal positions tended in the same direction. KEGG pathway analysis of proteomic studies identified the largest increases in mitochondrial TCA, OXPHOS, glycolysis, the AMPK pathway, endocytosis and protein processing in the endoplasmic reticulum. Of particular note in individual protein changes, AF was associated with a significant increase in lysosomal protein cathepsin D which was confirmed in patient plasma samples (n=3 sinus rhythm vs n=3 AF).

Conclusions

Our study confirms that acidic calcium stores play a significant role in healthy atria and indicates that significant changes in acidic store position and signalling occur in AF. Future studies must determine the functional consequences of these changes on cellular physiology and AF disease progression.

References

1 :- Terrar (2020) *Adv Exp Med Biol* 1131, 395-443
2 :- Aston et al. (2017) *Sci Rep* 7, 40620
3 : - Somlyo et al. (1988) *Proc Natl Acad Sci U S A* 85, 6222-6226
4 :- Ayagama et al. (2021) *iScience* 24: 102949
5 :- Kottmeier and Wheat (1967) *Circ Res* 21, 17-24

POSTER NO: 4

Title: Development of Small Molecule PAK1 Activators for the Treatment of Heart Failure
Authors: James S.H. Bae*, Ming Lei
Departmental affiliations: Postdoctoral Researcher, Pharmacology

Research Rationale:

- *P21 activated kinase (PAK1) is a multi-functional protein involved in important physiological roles – regulating cardiac contractile function and maintaining Ca^{2+} homeostasis.*
- *The emerging evidence for PAK1's role in providing cardio protection suggest clinical significance and implications for therapeutic targeting.*
- *To develop a novel treatment for hypertrophic cardiomyopathy and ventricular arrhythmias, we have designed small molecule PAK1 activators to fulfill unmet medical needs.*

Methods:

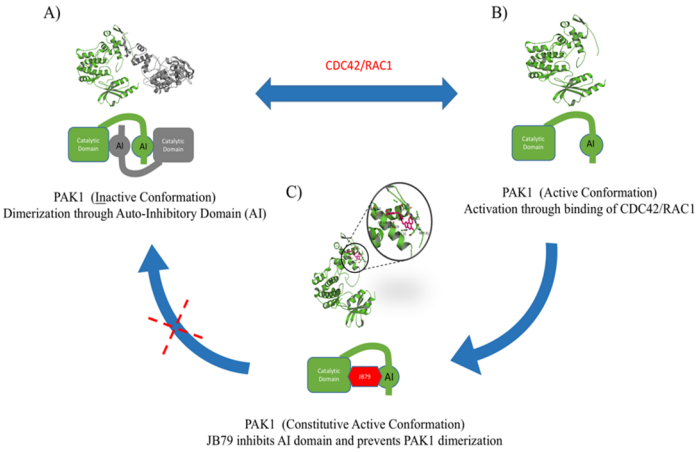
- *PAK1 activating peptide (PAP) – a proof of concept shows that PAK1 modulation can be achived.*
- *Virtual ligand screening was performed in order to search for small molecules that bind to the autoinhibitory domain (AID), which prevent the kinases from becoming inactive (Figure).*
- *Compounds with high affinity at the active site were then synthesized and tested using in-vitro kinase assays, specifically ADP-Glo Kinase assay and Rapid-fire mass spectrometry.*
- *Cardiac protective effects of PAK1 activators were measured and assessed for their therapeutic potential, using both in-vivo and in-vitro assays.*

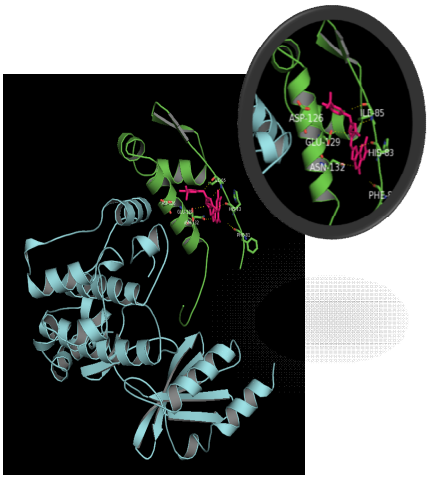
Results:

- *JB01 was amongst the first hit ligand that showed a significant increase in PAK1 activity.*
- *This compound was then further optimized to JB79 with higher affinity and better pharmacokinetic profile than those of JB01.*
- *Cardiac protective effects of JB79 shows reduced cardio hypertrophy and arrhythmias after myocardial infraction. In-vitro assays including calcium transients using induced pluripotent stem cells (IPSC) shows improved cardiac calcium handling during stress.*

***Conclusions**: This is a first report of a small molecule that could directly activate PAK1. It shows a promising novel strategy for developing other kinase activators as well as a starting point for further optimizations. This may be used for both as a research tool to study the mechanism of kinase activation as well as a novel approach in treating heart failure.*

Funding source: this project is supported by BHF and BHFCRE at Oxford





POSTER NO: 5

Title: The role of microRNA-31 in cardiac fibrosis associated with atrial fibrillation

Authors: K Aguilar-Agon [#] [*1], J Trompf [#1], L Moreira[1], N Mehta[1], S Tom[1], D Biggs[2], B Davies[2], D McAndrew[2], N Evans[1], R Hiram[3], M Mehdizadeh[3], S Nattel[3], C Lygate[2], S Reilly[1].

([#]) Authors equally contributed to this work. (*) Research Assistant

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Research Rationale - Atrial fibrillation (AF), the most common debilitating cardiac arrhythmia in man, is challenging to treat, as it causes electrical and structural (fibrotic) remodelling in the atria. This remodelling promotes AF maintenance and its mechanisms are not fully understood. We recently demonstrated that microRNA-31 (miR-31) facilitates electrical remodelling in AF [1]. Here we test whether an upregulation of miR-31 in fibroblasts (FBs) impacts on AF-associated structural remodelling and AF arrhythmogenesis.

Methods and Results - Using atrial fibroblasts (AFBs) isolated from the right atrial tissue biopsies of patients who had cardiac surgery (n=26), we showed that miR-31 expression (by qPCR) is increased in AFBs in the presence of persistent AF (persAF; ***Fig. A***). *In vitro* overexpression of miR-31 in human AFBs increased cell proliferation, collagen accumulation (scar-in-a-jar/Sirius Red assays) under basal and TGF-β1-stimulated conditions; these effects were partially reversed by miR-31 inhibition (***Fig. B-C***). *In vivo* studies in newly generated fibroblast-specific miR-31 overexpressing mice (n=106) demonstrated that higher abundance in miR-31 promotes atrial fibrosis (by hydroxyproline assay; ***Fig. D***) associated with changes in mRNA and protein levels of multiple fibrosis-related genes like fibronectin, α-SMA and collagens-1/-3. Atrial fibrosis was accompanied by increased AF-inducibility (by transesophageal electrostimulation; ***Fig. E***) in miR31-Tg mice. RNA-sequencing (RNAseq) of human AFBs revealed that miR-31 alters 12 pro-fibrotic pathways, with 167 significantly altered fibrosis-related genes, including previously reported miR-31 putative targets like STK40 and BAP1. Subsequent *in vivo* and *in vitro* experiments in the murine (***Fig. D-E***) and human AFBs revealed that calcitonin receptor (CTR), a recently discovered important player in atrial fibrosis and AF arrhythmogenesis [2], is a new direct target (by reporter assay/qPCR/immunoblot) of miR-31.

Conclusions - AF-associated upregulation of miR-31 in AFBs promotes, via inhibition of CTRs, cellular pro-fibrotic phenotype and *in vivo* atrial fibrogenesis and susceptibility to AF. Strategies to inhibit miR-31 in atrial myocardium may help to control atrial fibrogenesis and arrhythmia. Simultaneous upregulation of miR-31 in both atrial myocytes [1] and fibroblasts observed in AF indicates that miR-31, via a widespread impact on global transcriptome, and electrical and structural remodelling, may be a common driver of fibrosis and arrhythmia in AF.

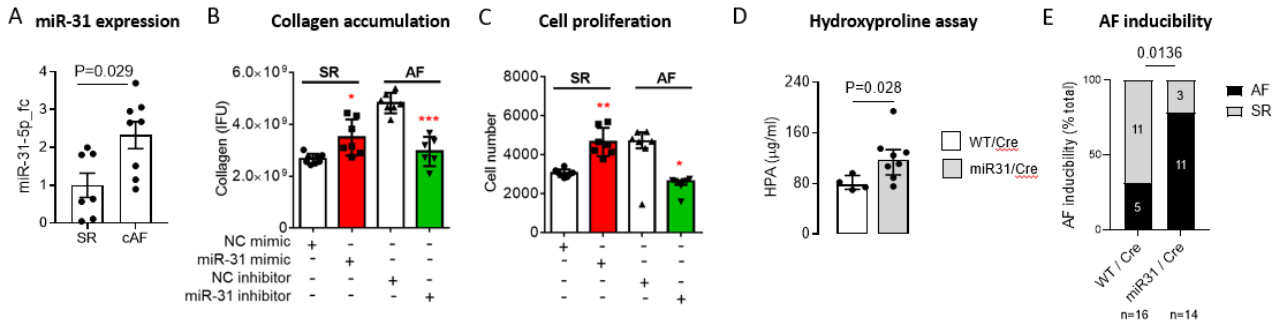

Figure. (**A**) miR-31-5p expression in human atrial fibroblasts in chronic AF (cAF) vs controls in sinus rhythm (SR). (**B-C**) Effect of miR-31 overexpression or inhibition on collagen accumulation and cell proliferation compared to negative controls (NC). (**D**) Fibrotic tissue content (by hydroxyproline (HPA) assay) in murine atria tissue (**E**) Atrial fibrillation (AF) inducibility (transoesophageal electrostimulation) in mice. Two-sided tests: unpaired t-test (A-B), Mann-Whitney U-test (C-D) and one-sided Fisher's exact test (E).

1. Reilly SN et al. *Science Translational Medicine*. (2016). doi: 10.1126/scitranslmed.aac4296.
2. Moreira LM et al. *Nature*. (2020). doi: 10.1038/s41586-020-2890-8.

POSTER NO: 6

Title: Mechanisms of homoarginine supplementation in the heart: β1 adrenergic receptor signalling and other targets.

Authors: Sevasti Zervou[1]* Hannah A Lake [1], Debra J McAndrew [1] James McCullagh[2], Elisabete Pires [2], Stefan Neubauer[1], Craig A Lygate[1]

Departmental affiliations: [1]Division of Cardiovascular Medicine, Radcliffe Department of Medicine and [2]Chemistry Research Laboratory, Department of Chemistry, University of Oxford.

Research rationale. L-homoarginine (hArg) is a non-proteinergic amino acid biosynthesised in the kidneys by the enzyme AGAT (arginine:glycine amidinotransferase). Epidemiological studies have shown that low plasma levels independently predict poor outcomes in cardiovascular disease, but the mechanisms of hArg action remain unknown. We previously reported that mice supplemented with hArg prior to the onset of heart failure had elevated myocardial levels of the amino acid and maintained better cardiac function *in vivo* in particular, maximal responses to β1 adrenergic receptor (β1-AR) stimulation (Atzler *et al.* 2017 *Circulation* 135:400-402). We therefore hypothesised that hArg treatment preserves β1-AR sensitivity and aimed to investigate the molecular pathways involved using candidate and multi-omics approaches. **Methodology.** Heart failure tissue from our published study was used to interrogate β1-AR desensitisation pathways. C57BL/6J mice were given either normal drinking water or water dosed with 14mg /L hArg for 4 weeks prior to myocardial infarction (MI) or sham-operation, with tissue harvested following haemodynamics assessment at 6-weeks post-surgery. **Results and Conclusions.** Classical desensitisation of the β1-AR pathway was observed in non-treated MI hearts, characterised by higher protein expression of G-protein receptor kinase 2 (GRK2) and β-arrestin compared to sham-operated mice. Contrary to expectations, hArg-treated hearts had similar expression levels post-MI and paradoxical up-regulation was observed in the hArg-treated sham-operated hearts ($P<0.01$). Since these differences were observed in the absence of MI, we sought to understand the effects of hArg in non-diseased hearts. C57BL/6J mice were given 14mg/L hArg in drinking water for 4 weeks and compared to untreated littermates (n=6-8 per group). Metabolomic analysis using LC-MS confirmed that myocardial hArg levels were almost 2-fold higher vs control ($P<0.001$) and strongly correlated with cyclic adenosine monophosphate (cAMP) levels, a second messenger downstream of β1AR/Gs_α ($r=0.61$, $P=0.01$). Unbiased gene expression analysis using RNA seq Poly-A library (Illumina) revealed significant increases in other components of the β1-AR signalling pathway, e.g. Adenylyl cyclase 7 and 4 (*Adcy7* 1.36-fold FDR=1.4E-05, *Adcy4* 1.15-fold, FDR=0.04), *Pde8* (phosphodiesterase 8; 1.20-fold, FDR=0.004) and Gs_α (1.41-fold, FDR=0.05), *β-arrestin* (1.14-fold, FDR=0.05). cAMP response element (*Creb5*) was elevated by 30% (1.3-fold FDR=0.04) supporting cAMP pathway stimulation by hArg. In agreement with *in vivo* observations, GRK2 protein expression increased in HL-1 cells in response to high-dose β1-AR agonist (isoproterenol), whereas in cells maintained in 10μM hArg, GRK2 levels were already high prior to isoproterenol exposure ($P=0.02$) and did not increase further when stimulated. Gene set enrichment analysis (GSEA) suggests a pleiotropic response to hArg in the heart with significant enrichment of 'TGFβ-signalling' and down-regulation of 'Fatty Acid Metabolism' amongst others. Reciprocal changes in glucose utilisation were observed, such as elevated glucose transporter 4 mRNA (*Glut4; P*=0.013), and reductions in pyruvate dehydrogenase kinase 4 (*Pdk4*; $P=0.01$), and in the rate-limiting glycolytic enzyme fructose bisphosphatase 2 (*Fbp2*; $P=0.01$). Furthermore, significant positive correlations with hArg levels were observed in the metabolomic data, for glutathione ($r=0.81$, $P<0.0001$) and NADH ($r=0.57$; $P=0.02$), suggesting a relationship with cellular redox status. **Conclusions.** Dietary supplementation with hArg up-regulates the β1-AR desensitisation pathway in normal hearts without affecting function, while paradoxically preserving β1-AR responses in chronic heart failure. Multi-omics analysis suggests not just a complex effect on β1-AR signalling, but multiple mechanisms whereby hArg could potentially influence function. * presenting, non-student.

POSTER NO: 7

Title: C16:0-ceramide as a potential modulator of redox signalling in human aortic endothelial cells

Authors: Kirsten Lee* (5^{th} year medical student), Nadia Akawi, Charalambos Antoniades

Departmental affiliations: University of Oxford, Division of Cardiovascular Medicine, Oxford UK

Research Rationale: Ceramides are the structural centres of sphingolipid metabolism and are significantly linked to cardiovascular disease. They have the ability to modulate the vascular redox state and have been shown to be related to endothelial dysfunction. However, it is yet to be determined if longer, endogenous ceramides behave with the same mechanism of action as shorter, well-studied ceramides. We therefore investigated the effect of C16:0-ceramide in an endothelial cell model, since high plasma levels of this ceramide was found in cardiovascular patient cohorts.

Methodology: The effect of C16:0-ceramide on the generation of superoxides ($O_2\bullet^-$) was studied on a human aortic endothelial cell line (teloHAEC) and in primary human aortic endothelial cells (primary HAEC). Cells were treated with C16:0-ceramide for 20 minutes, under incubation, and analysed using luminometric techniques and confocal microscopy.

Results: C16:0-ceramide was found to penetrate cells and generate $O_2\bullet^-$ intracellularly. Here, we show that uncoupled eNOS is responsible for the enhanced generation of $O_2\bullet^-$ by C16:0-ceramide, since it was observed that $O_2\bullet^-$ generation was reduced in the presence of L-NAME, an inhibitor of eNOS.

Conclusion: The findings of this study provide an insight into how C16:0-ceramide modulates vascular redox signalling which leads eventually to endothelial dysfunction, the hallmark of cardiovascular disease

POSTER NO: 8

Title: Independent effects of adiposity measures on risk of atrial fibrillation in men and women: A study of 0.5M individuals

Authors: C. Fielder Camm[1]*, Ben Lacey[1], M. Sofia Massa[1], Adam Von Ende[1], Parag Gajendragadkar[1], Alexander Stiby[1], Elsa Valdes-Marquez[1], Sarah Lewington[1,2], Rohan Wijesurendra[1,3], Sarah Parish[1,2], Barbara Casadei[3], Jemma C. Hopewell[1]

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1. Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom.
2. MRC Population Health Research Unit, NDPH, University of Oxford United Kingdom.
3. Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom
* Presenting author. Clinical DPhil student

Research Rationale: Atrial fibrillation (AF) is a common cardiac arrhythmia which is associated with higher risk of stroke. Atrial fibrillation (AF) has a higher prevalence in men than women and is associated with measures of adiposity and lean mass (LM). However, it remains uncertain if the risks of AF associated with these measures vary by sex.

Methodology: Among 477,904 UK Biobank participants aged 40-69 without prior AF, 23,134 incident AF cases were identified (14,400 men, 8,734 women; median follow-up 11.1 years). Cox proportional hazards models were used to estimate covariate adjusted hazard ratios (HR) describing the association of AF with weight, measures of adiposity (fat mass [FM], waist circumference [WC]), and LM and their independent relevance, by sex. Models were adjusted for age at risk, ethnicity, deprivation, alcohol, and smoking.

Results: Weight and WC were independently associated with risk of AF (HR:1.25 [1.23-1.27] per-10kg, HR:1.11 [1.09-1.14] per-10cm respectively), with comparable effects in both sexes. The association with weight was principally driven by LM which, per-5kg, conferred double the risk of AF compared to FM when mutually adjusted (HR:1.20 [1.19-1.21], HR:1.10 [1.09-1.11] respectively); however, the effect of LM was weaker in men than women (p-interaction=4.3×10^{-9}). Comparing the relative effects of LM, FM and WC identified different patterns within each sex; LM was the strongest predictor for both, whereas WC was stronger than FM in men but not women.

Conclusions: LM and FM (as constituents of weight), and WC are risk factors for AF. However, the independent relevance of general adiposity for AF was more limited in men than women. The relevance of both WC and LM suggest a potentially important role for visceral adiposity and muscle mass in AF development. The relative effects of LM and adiposity measures on risk of AF suggest that the relevance of body composition, particularly in the context of AF-focused weight loss programmes, merits further investigation. The independent association of WC on risk of AF suggests that further investigation into the causal effects of visceral fact is warranted.

POSTER NO: 9

Title: Myocardial *NOS1AP* overexpression increases arrhythmia inducibility and slows conduction velocity whilst shortening QT duration in mice

Authors: Parag Gajendragadkar[1]*, Jakub Tomek[2], Xing Liu[1], Jillian Simon[1≠], Barbara Casadei[1≠]
*presenting (DPhil student, clinical); ≠Joint supervisors

Departmental affiliations: [1]Department of Cardiovascular Medicine, Radcliffe Department of Medicine
[2]Department of Physiology, Anatomy and Genetics

Research Rationale

Of the parameters on a surface ECG, the QT interval, which represents ventricular repolarisation, has arguably been the most studied. Both QT interval shortening and prolongation have been linked with risk of lethal ventricular arrhythmias. Common noncoding variants that map within an enhancer of the Nitric Oxide Synthase 1 Adaptor Protein (*NOS1AP*) gene increase myocardial NOS1AP transcript expression and the susceptibility to long QT syndromes. However, the mechanism by which the NOS1AP protein affect the QT interval and the risk of arrhythmia remains unclear.

Methodology

We generated a transgenic mouse overexpressing human *NOS1AP* in the myocardium (NOS1AP-Tg). We investigated surface ECG parameters, cardiac structure by echocardiography, as well as propensity to *ex-vivo* ventricular arrhythmias and *in-vivo* atrial arrhythmias. Subsequent investigation focussed on action potential durations, presence of cardiac fibrosis, calcium handling and conduction velocity in the left ventricle as well as assessments of Nitric Oxide Synthase 1 (NOS1) mRNA, protein and activity.

Results

NOS1AP-Tg showed a modest increase in NOS1AP protein (~2.5-fold) in all cardiac chambers, with increased localisation to the sarcolemmal membrane and nuclear immunofluorescence compared to wild-type. Subsequent phenotyping revealed a longer P-wave duration, PR interval and QRS interval, but a shorter measured QT interval on surface ECG. The mice had an increased propensity to the induction of *ex-vivo* ventricular arrhythmias and *in-vivo* atrial arrhythmias in the absence of echocardiographic differences in cardiac structure or function. Investigation of the cardiac electrical substrate resulting from *NOS1AP* overexpression revealed no significant difference in action potential durations (APD) between genotypes at physiological heart rates, although a shorted APD was seen at slower heart rates in NOS1AP-Tg hearts. There was a significant reduction in conduction velocity in the left ventricles of transgenic mice in the absence of an increase in cardiac fibrosis. The slower conduction velocity in NOS1AP-Tg was not explained by changes in the rapid sodium current I_{Na} but was accompanied by lower connexin-43 protein content, despite a significant increase in connexin-43 expression. No differences in calcium handling, NOS1 content or NOS activity were detected between genotypes.

Conclusion

Our findings suggest that NOS1AP impairs cardiac electrical conductance and coupling by reducing Connexin-43 protein stability and highlight the need for investigations of the impact of gene variation on NOS1AP protein and subcellular localisation in the human myocardium.

POSTER NO: 10

Epicardial adipose tissue volume measured by an automated computed tomography deep learning network predicts mortality and cardiovascular events

Henry W. WEST[1*], Michelle C. WILLIAMS[2], Muhammad SIDDIQUE[1], Lucrezia VOLPE[1], Pete TOMLINS[3], Cheerag SHIRODARIA[3,4], Stefan NEUBAUER[1], Keith M. CHANNON[1], David E. NEWBY[2], Charalambos ANTONIADES[1]

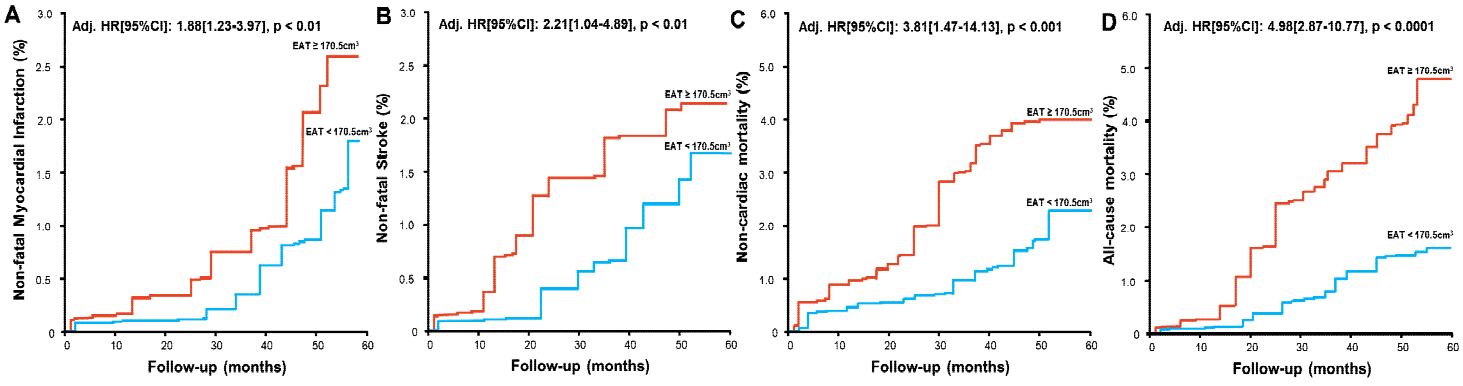
[1]Acute Vascular Imaging Centre, Radcliffe Department of Medicine, University of Oxford, UK, [2]University of Edinburgh, Scotland, UK, [3]Caristo Diagnostics Pty Ltd, Oxford, UK, [4]Oxford University Hospitals NHS Foundation Trust, Oxford, UK **DPhil student & Clinical Research Fellow*

Research Rationale: Epicardial adipose tissue (EAT) is a visceral fat deposit within the pericardial sac which surrounds the heart myocardium and coronary arteries. The automated quantification of EAT volume is possible from routine CCTA scans via a deep-learning approach. The use of automated EAT quantification for the assessment of cardiovascular risk in addition to standard measures of obesity like BMI has not been fully explored.

Methodology: A deep-learning automated EAT segmentation tool using a 3D Residual-U-Net neural network architecture for 3D volumetric segmentation of CCTA data was created and trained on over 2800 consecutive CCTA performed as part of clinical care in patients with stable chest pain from 2015 onwards within the European arm of the Oxford Risk Factors And Non Invasive Imaging (ORFAN) Study. External validation in 720 patients demonstrated excellent correlation between machine and human expert (CCC = 0.97). The prognostic value of deep-learning derived EAT volume was assessed against 5 years outcomes from the SCOTHEART trial (n=1588), with adjustment for cardiovascular disease (CVD) risk factors. A universal optimal EAT volume cut-off was selected by identifying the EAT value that maximized the Youden's J index (sum of sensitivity and specificity) for the three outcomes of interest – high risk was deemed to be EAT ≥ 170.5cm^3.

Results: There were 35 deaths (all-cause mortality), 35 non-fatal myocardial infarctions and 8 non-fatal strokes during the 5 years follow up period. By using multi-variable cox-regression, EAT volume was predictive of non-fatal myocardial infarction (Adj.HR[95%CI] 1.88[1.23-3.97], p<0.01; Figure A), non-fatal stroke (2.21[1.04-4.89], p<0.01]; Figure B), non-cardiac mortality (3.81[1.47-14.13], p<0.001; Figure C) and all-cause mortality (4.98[2.87-10.77], p <0.0001; Figure D) independently from CVD risk factors.

Conclusion: Automatically segmented EAT volume measured using a deep learning network, predicts 5-year all-cause mortality, heart attacks and stroke independently of BMI or common clinical risk profile of the patients. This suggests that measures of visceral obesity will be of value in the interpretation of cardiovascular computed tomography.



POSTER NO: 11

Title: Novel insights into abnormal haemodynamics in hypertrophic cardiomyopathy from 4D flow cardiac magnetic resonance.

Authors: Z. Ashkir* (clinical DPhil student)[1], S. Myerson[1], A.J. Lewandowski[2], M. Mahmod[1], R. Ariga[1], C. Carhall[3], T. Ebbers[3], H. Watkins[4], S. Neubauer[1], B. Raman[1] **Departmental affiliations**: [1]Oxford Centre for Clinical Magnetic Resonance Research (OCMR), Division of Cardiovascular Medicine

Research rationale

Hypertrophic cardiomyopathy (HCM) is characterised by multiple pathological and metabolic factors that mediate impaired ventricular relaxation and lead to derangements in blood flow haemodynamics. These changes have been linked to symptom onset and exercise intolerance in patients. Time-resolved three-dimensional phase contrast cardiac magnetic resonance imaging, or '4D flow CMR', is a novel technique that permits evaluation of ventricular blood flow and kinetic energy throughout the cardiac cycle. Recent studies suggest that 4D flow CMR is sensitive to early changes in diastolic and systolic function in health and disease (1–3). We aimed to characterise abnormalities in ventricular haemodynamics in HCM using 4D flow CMR and describe their relationship with phenotypic and metabolic perturbations that underpin HCM.

Methodology

38 patients with non-obstructive HCM and 20 age and sex matched controls underwent transthoracic echocardiography, 4D flow CMR and 31-Phosphorus magnetic resonance spectroscopy (MRS). Using a previously validated method, LV blood flow was separated into four functional components: Direct Flow - blood that transits the ventricle within one cardiac cycle; Retained Inflow - blood that enters the LV during diastole but is retained for at least one cycle; Delayed Ejection Flow - blood already in the LV during diastole and which leaves during systole; and Residual Volume - blood that remains in the LV for at least two cycles **(Figure 1)**. Flow component distribution at end-diastole and component end-diastolic kinetic energy/ml (ED KE/ml) were calculated. Myocardial energetics were assessed on MRS by measuring the phosphocreatine-to-ATP concentration ratio.

Results

As expected, the HCM group had significantly greater LV wall thickness (p= <0.001), LV mass index (p=0.003), LV ejection fraction (p=0.016), and reduced energetics (p=0.04). LV stroke volume index was similar to that in the control group. Diastolic function on echocardiography was not significantly different between groups. HCM patients had a greater proportion of Direct Flow (47±9% vs 38±5%, p<0.001) alongside significantly reduced Retained Inflow (18±3% vs 20±5%, p=0.016) and Residual Volume (21±8% vs 26.6%, p=0.03). **(Figure 1)**. Direct Flow proportion correlated significantly with LV wall thickness (rho=.548, p<0.001) and LV mass index (rho=.477, p=<0.001). There were no significant differences between the groups for any of the end-diastolic kinetic energy components. The phosphocreatine-to-ATP ratio correlated inversely with all components of end-diastolic kinetic energy for both groups. In the HCM patients, this was strongest for the ejection components (Direct Flow: rho -.362, p=0.04 and Delayed Ejection Flow: rho -.365, p=0.04).

Conclusions

HCM is characterised by greater Direct Flow at the expense of non-ejection components (Retained Inflow and Residual Volume) to maintain stroke volume. The significant correlation of Direct Flow with LV wall thickness and mass index suggests that this change is closely linked to disease severity in HCM. Finally, the significant inverse relationship between end-diastolic kinetic energy components and the phosphocreatine-to-ATP ratio in the HCM group suggests that, with greater myocardial energy depletion, less kinetic energy can be transferred into useful potential energy during active relaxation.

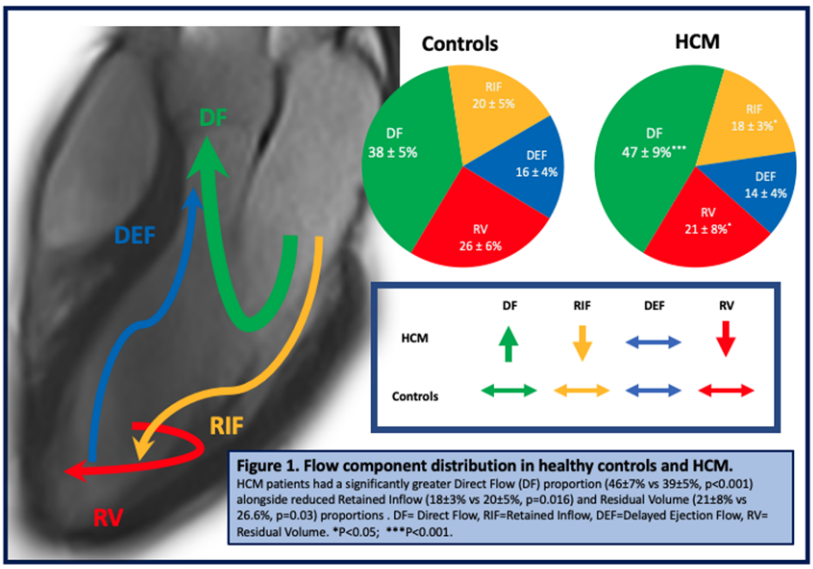


Figure 1. Flow component distribution in healthy controls and HCM. HCM patients had a significantly greater Direct Flow (DF) proportion (46±7% vs 39±5%, p<0.001) alongside reduced Retained Inflow (18±3% vs 20±5%, p=0.016) and Residual Volume (21±8% vs 26.6%, p=0.03) proportions . DF= Direct Flow, RIF=Retained Inflow, DEF=Delayed Ejection Flow, RV= Residual Volume. *P<0.05; ***P<0.001.

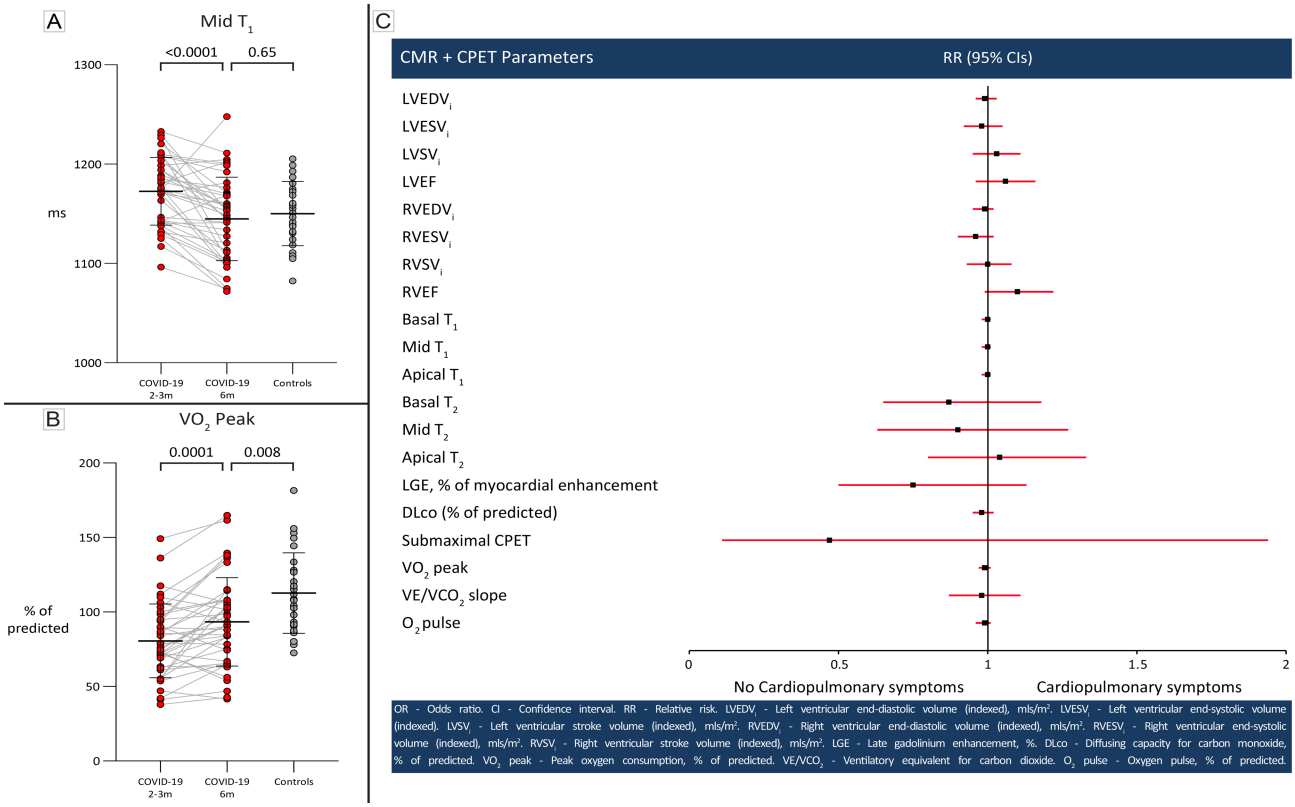
POSTER NO: 12

Title: Longitudinal assessment of cardiopulmonary health and symptoms in moderate to severe COVID-19.

Authors: Mark Philip Cassar[1*], Adam J. Lewandowski[1,2,3], Masliza Mahmod[1], Cheng Xie[3], Elizabeth M. Tunnicliffe[1], Azlan Helmy Abd Samat[1], Nayia Petousi[4], Nick P. Talbot[5], David Holdsworth[5], Ling-Pei Ho[6], Stefan K. Piechnik[1], Vanessa M. Ferreira[1], Stefan Neubauer[1,3], Betty Raman[1,3]. Departmental affiliations: (1) Oxford Centre for Clinical Magnetic Resonance Research, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, United Kingdom (2) Oxford Cardiovascular Clinical Research Facility, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, United Kingdom (3) Radcliffe Department of Medicine, British Heart Foundation Centre of Research Excellence, University of Oxford, United Kingdom (4) Nuffield Department of Medicine, University of Oxford, United Kingdom (5) Department of Physiology, Anatomy and Genetics, University of Oxford, United Kingdom (6) Weatherall Institute of Molecular Medicine, Nuffield Department of Medicine, University of Oxford, United Kingdom.
*Presenting author, DPhil student, Clinical research fellow

Research Rationale: The longitudinal trajectory of cardiopulmonary abnormalities following infection with COVID-19 is currently unknown. We aimed to describe the natural history of cardiopulmonary manifestations in post-acute COVID-19 and evaluate its association with symptom burden at 6 months from infection.
Methodology: 58 COVID-19 patients and 30 age, sex, BMI and comorbidity-matched controls underwent CMR, CPET and a symptom-based questionnaire at 2-3 months (2-3m) post-infection. Repeat assessments (including DLco) were performed in 46 patients 6 months (6m) post-infection.
Results: The mean age of patients was 55 years, and 59% were men. One third required admission to ITU.
CMR: At 2-3m, 83% of patients reported cardiopulmonary symptoms (chest pain, dyspnoea, palpitations, dizziness or syncope), compared to 33% in controls. Patients and controls had comparable cardiac volumes and function on CMR. Myocardial native T_1, a marker of fibroinflammation, was significantly higher in patients and focal fibrosis was mildly increased. Sixty percent of patients had lung parenchymal abnormalities on CMR. By 6m, 52% of patients reported cardiopulmonary symptoms. Native T_1 improved and was no longer different from controls. Lung parenchymal abnormalities were still more common in patients (P<0.001).
CPET: At 2-3m, patients had significantly lower peak oxygen consumption (pVO_2) versus controls. In addition, the VE/VCO_2 slope was higher in patients, suggestive of impaired lung efficiency when compared to controls. By 6m, despite improvements in pVO_2 and lung efficiency, both remained abnormal compared to controls. There was no association between CMR or CPET parameters and persistent cardiac symptoms at 6m.
Conclusions: More than half the patients previously hospitalised with COVID-19 report enduring symptoms by six months from infection. However, persistent symptoms did not associate with objective measures of cardiopulmonary health. Further research is needed to better understand the pathophysiological basis for enduring symptoms among patients.

Graphical abstract: A – B: Longitudinal change in cardiac MRI and CPET parameters in COVID-19 patients, compared to controls. C: Relationship of CMR and CPET parameters with cardiopulmonary symptoms in COVID-19 patients.



POSTER NO: 13

Title: Allocation of time between machine-learned movement behaviours and risk of incident cardiovascular disease

Authors: Rosemary Walmsley[*,a,b][Presenting author; DPhil student; non-clinical], Shing Chan[a,b], Derrick Bennett [†b,c], Aiden Doherty[†, a,b,c] [†Contributed equally]

Departmental affiliations: [a]Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford; [b]Nuffield Department of Population Health, University of Oxford; [c]National Institute of Health Research Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust

Research rationale: To classify movement behaviours in wrist-worn accelerometer data using machine-learning methods and to investigate the association between these behaviours and risk of incident cardiovascular disease (CVD) in the large UK Biobank (UKB) prospective cohort study.

Methodology: Just over 100,000 participants in UKB were asked to wear a wrist-worn accelerometer for 7 days. Using camera data from a separate study of 152 free-living participants to provide a 'ground truth', we developed a machine-learning model to classify movement behaviours (moderate-to-vigorous physical activity behaviours (MVPA), light physical activity behaviours, sedentary behaviour, sleep) in wrist-worn accelerometer data and applied this to the UKB accelerometer data. Compositional data analysis Cox regression was used to investigate how reallocating time between different movement behaviours was associated with CVD incidence.

Results: In leave-one-participant-out analysis, our machine-learning method classified free-living movement behaviours with mean accuracy 88% (95% CI 87% to 89%) and Cohen's kappa 0.80 (95% CI 0.79 to 0.82). After excluding participants with poor quality accelerometer data, prevalent CVD or missing covariate data, 87,498 UK Biobank participants were included in the final analysis, among whom there were 4,105 incident CVD events. Reallocating time from any behaviour to MVPA, or reallocating time from sedentary behaviour to any behaviour, was associated with lower CVD risk. For an average individual, reallocating 20 min/day to MVPA from all other behaviours proportionally was associated with 9% (95% CI 7% to 10%) lower risk, while reallocating 1 hour/day to sedentary behaviour from all other behaviours proportionally was associated with 5% (95% CI 3% to 7%) higher risk.

Conclusions: Reallocating time from other behaviours to MVPA, and from sedentary behaviour to other behaviours, was associated with lower risk of incident CVD, and should be promoted by interventions and guidelines. Machine-learning methods developed using camera data in free-living adults were able to accurately classify movement behaviours in wrist-worn accelerometer data.

Graphical abstract not included.

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- **I would like to be considered to give a short talk at the symposium.**

POSTER NO: 14

Title: Pro-thrombotic Left Atrial Flow characteristics are found in patients with stroke risk factors regardless of Atrial Fibrillation

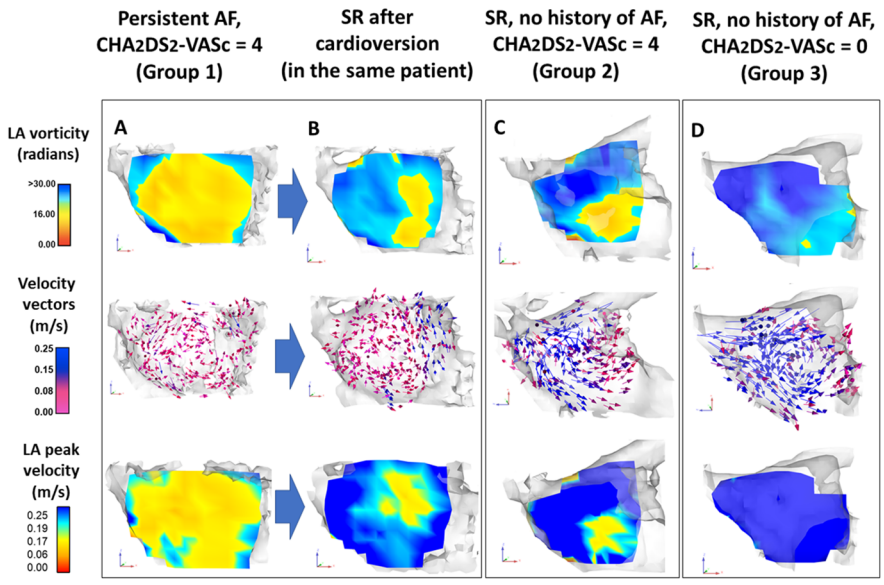
Authors: Marco Spartera* MD, DPhil (Clinical); Antonio Stracquadanio; Guilherme Pessoa-Amorim; Adam Von Ende; Alison Fletcher; Peter Manley; Vanessa M. Ferreira, FRCPC; Aaron T. Hess, PhD; Jemma C. Hopewell; Stefan Neubauer; Rohan S. Wijesurendra; Barbara Casadei.
Departmental affiliations: Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford

Research rationale: Altered left atrial (LA) blood flow characteristics account for clot formation and cardioembolic stroke risk in atrial fibrillation (AF). In up to 20% of ischaemic strokes, no embolic source is identified. We hypothesised that exposure to stroke risk factors is sufficient to alter LA blood flow (therefore increasing risks of clots) even in the presence of sinus rhythm (SR).

Methods: Cardiac function was assessed using cardiac magnetic resonance cine whilst LA flow characteristics were evaluated using 4D flow imaging. We investigated 95 individuals: 37 patients with persistent AF, who were studied before and after cardioversion [**Group 1**; median CHA2DS2-VASc = 2.0 (1.5-3.5)]; 35 individuals with no history of AF but similar stroke risk to Group 1 [**Group 2**; median CHA2DS2-VASc = 3.0 (2.0-4.0)]; and 23 low-risk individuals in SR [**Group 3**; median CHA2DS2-VASc = 0.0 (0.0-0.0)].

Results: Before cardioversion, Group 1 displayed impaired LV and LA function, reduced LA flow velocities and vorticity and a higher normalized vortex volume (all P<0.001 vs Group 2 and 3). After restoration of SR at ≥4 weeks post-cardioversion, LV systolic function and LA flow parameters improved significantly (all P<0.001 vs pre-cardioversion) and were no longer different from those in Group 2. However, in the presence of SR, LA flow peak and mean velocity, and vorticity were lower in Groups 1 and 2 vs Group 3 (all P<0.01), and were associated with impaired LA emptying fraction (LAEF) and LV diastolic dysfunction.

C**onclusions:** Pro-thrombotic LA flow characteristics are present in SR patients with higher stroke risk, regardless of a history of AF.



POSTER NO: 15

Title: **Selective regulation of cardiac Troponin I by PDE4D9**

Authors: Ying-Chi Chao* (postdoc) and Manuela Zaccolo
Departmental affiliations: Department of Physiology, Anatomy and Genetics, University of Oxford, UK

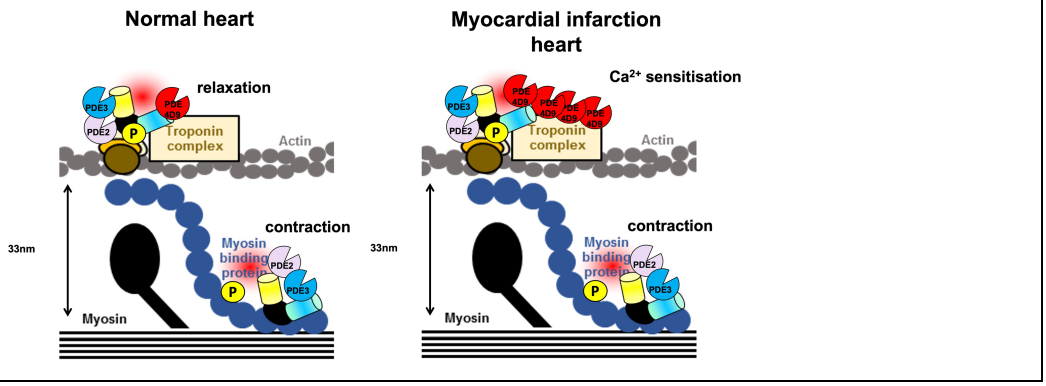
Rationale: Compartmentalisation of cAMP/PKA signalling underpins sympathetic regulation of the heart. Signalling compartmentalisation is achieved via spatial segregation of the molecular components of this pathway into distinct multiprotein complexes, or signalosomes, localised to different subcellular sites and in control of specific cellular functions. Typically, signalosomes include cAMP-hydrolysing phosphodiesterases (PDEs), a superfamily of enzymes that comprises multiple isoforms. At the myofilaments, both cardiac myosin binding protein C (MyBPC) and Troponin I (TPNI) are predominant targets of cAMP/PKA. PKA phosphorylation of TPNI and MyBPC leads to modulation of myofilament Ca^{2+} sensitivity and speed of cross-bridge formation, resulting in enhanced cardiac relaxation and contraction, respectively. How these opposite functional outcomes of the cAMP signal are locally coordinated is not known.

Methodology: We used targeted fluorescence resonance energy transfer (FRET)-based CUTie sensors and real-time imaging to directly monitor cAMP levels at TPNI and MyBPC in living cardiac myocytes. We screened different PDE inhibitors to reveal which PDEs may be responsible for selective regulation of TPNI phosphorylation. By using peptide arrays, we identified the potential binding sites between TPNI and its interacting PDE to generate disrupting peptides and TPNI-Mutant expression plasmid to interfere with this interaction. Furthermore, we explored the functional relevance of this regulation in a rat model of myocardial infarction.

Results: We found that TPNI and MyBPC are regulated by distinct cAMP pools and that PDE4D9 selectively regulates cAMP at the troponin complex but not at MyBPC. Immunoprecipitation experiments showed that PDE4D9 directly interacts with TPNI, and treatment with a disrupting peptides or overexpression of a TPNI-Mutant attenuate the PDE4D9-TPNI interaction. Detecting of TPNI phosphorylation level and contractility experiments demonstrated that interfering with the interaction between PDE4D9 and TPNI affects the β-ARs/cAMP/PKA signalling at TPNI and impacts on the myocyte contractile properties. In addition, we found that the regulation of TPNI phosphorylation by PDE4D9 is impaired in infarcted hearts.

Conclusion: Both MyBPC and TPNI are predominant targets of PKA at the myofilament. Notably, MyBPC and TPNI are spaced only about 30 nm apart on the myofilaments. Our results demonstrate that TPNI phosphorylation is selectively regulated by a local cAMP nanodomain under the control of PDE4D9 and that this cAMP nanodomain does not control MyBPC phosphorylation. Our findings indicate that interfering with the PDE4D9-TPNI interaction may be an effective way to re-establish the appropriate level of signalling at the myofilaments in pathological conditions associated with Ca^{2+} sensitisation.

Graphic Abstract:



POSTER NO: 16

Title: Nuclear PDE3A inhibits PKA phosphorylation of HDAC1 and decreases expression of the hypertrophic regulator GATA4

Authors: Gunasekaran Subramaniam*(Post doc-nonclinical), Katharina Schleicher, Duangnapa Kovanich, Anna Zerio, Milda Folkmanaite, Manuela Zaccolo

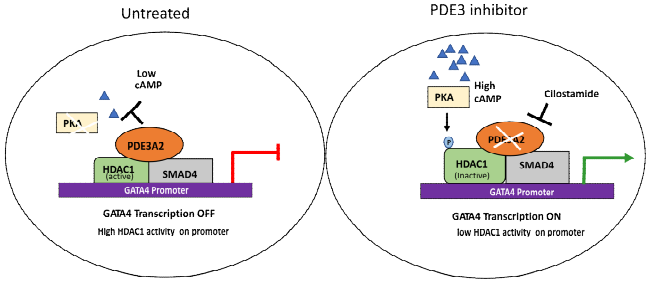
Departmental affiliations: Department of Physiology, Anatomy, and Genetics, University of Oxford

Research rationale: Cyclic nucleotide phosphodiesterases (PDEs) hydrolyze intracellular cAMP and cGMP to regulate cyclic nucleotide-dependent signaling. The PDE3 gene family consists of two sub-families, PDE3A and PDE3B, and at least three PDE3A splice variants, PDE3A1, PDE3A2, and PDE3A3. PDE3 inhibitors are used in the clinic as last resort positive inotropes to control acute heart failure that unresponsive to other treatments but they are associated with increased mortality in the long term, characterised by worsened cardiac remodelling and fatal arrythmic events. The mechanism responsible for the cardiac long term detrimental effects of PDE3 inhibition remains to be defined. We have previously shown that PDE3 inhibition or displacement of PDE3A2 has pro- hypertrophic effects and a large body of literature links upregulation of GATA4 expression with cardiac hypertrophy. We recently observed that inhibition of PDE3A enhances the expression of GATA4 in cardiomyocytes. Here we investigated the mechanistic link between PDE3A2 inhibition and GATA4 expression and the effects on cardiac myocyte hypertrophy.

Methodology: We performed LC-MS/MS analyses of PDE3 isoform-specific interactomes and of the phospho-proteome associated with selective PDE3 inhibition in rat cardiac myocytes to identify proteins that interact with and are phosphorylated on selective inhibition of PDE3 isoforms. Gene ontology (GO) analysis identified proteins that are known to be associated with enhanced GATA4 expression and hypertrophy. These were selected for further validation that was carried out using FRET-based real time imaging of cAMP levels, protein-protein interaction analysis, enzyme activity studies and hypertrophy assays.

Results: GO term analyses of the PDE3 phospho-proteome and PDE3 isoform-specific interactome identified several nuclear proteins. Among these, SMAD4, a mediator of Transforming growth factor beta (TGF-β) signalling, was found to be significantly enriched in the PDE3A1 and PDE3A2 interactomes. Interaction of endogenous PDE3A2 with SMAD4 was confirmed by co-immunoprecipitation experiments. FRET analysis of cAMP changes using a SMAD4-targeted reporter showed increased cAMP levels selectively on inhibition of PDE3, but not on inhibition of PDE2, confirming close proximity of PDE3 to SMAD4 in the nucleus. We also found that the SMAD4-PDE3A2 complex includes PKA catalytic and regulatory subunits and the epigenetic regulator Histone deacetylase 1 (HDAC1), a protein that results to be significantly enriched in the PDE3-specific phosphoproteome. Further analysis confirmed that inhibition of PDE3 increases the PKA-dependent phosphorylation on HDAC1, that this phosphorylation results in inhibition of HDAC1 deacetylase activity and that this phosphorylation leads to increased GATA4 expression and cardiac myocyte hypetrophic growth.

Conclusions: Our findings are compatible with a model whereby PDE3A2-SMAD4-HDAC1 constitute a nuclear nanodomain where local regulation of cAMP levels by PDE3A2 modulate PKA-dependent phosphorylation of HDAC1 and GATA4 expression, resulting in enhanced expression of pro-hypertrophic genes. This mechanism may underpin the long term cardiac detrimental effects observed in heart failure patients treated with PDE3 inhibitors.



POSTER NO: 17

Title: **Transcription factor WT1 mediates HSPG-dependent signalling by directly regulating endosulfatase expression in the embryonic epicardium.**

Authors: Andia N. Redpath*[1], Irina-Elena Lupu[1], Samuel Krasner[1], Joaquim Vieira[1] and Nicola Smart[1].
Departmental affiliations: [1]Department of Physiology, Anatomy and Genetics.
*Presenting author: Postdoctoral researcher (non-clinical)

Research rationale: The outermost layer of the heart consists of a single-cell layer of mesothelial epithelial tissue, described as the epicardium. During development, the epicardium contributes to the heart's cellular components and also guides and supports the forming vascular network [1]. Heparan sulfate proteoglycans (HSPG) are extracellular matrix (ECM) components which function as co-receptors for signalling pathways, such as BMP and TGF [2]. A multitude of coordinated HSPG-dependent pathways control epicardial cell activity, i.e. formation, proliferation, epiEMT, and maturation [1,3]. Endosulfatases Sulf1 and Sulf2 - enzymes that modify HSPGs – present another level of control to refine signalling separate to ligand presence [2]. WT1, a transiently expressed transcription factor, is required for heart development, and denotes the active epicardial state both in the embryonic and injured heart [1]. Wt1 and Sulf1 are enriched in the activated adult epicardium post-injury [1,4]. WT1 was identified to regulate Sulf expression in the kidney, and through their desulfation activity regulate essential VEGF-A signalling [5]. However, regulation of Sulfs and their specific roles in the epicardium are unknown.

Methodology: Here, we used microscopy and flow cytometry-based single molecule RNA in situ hybridisation techniques, and single-cell RNA-sequencing to investigate the expression of Sulfs, over the course of embryonic heart development. We used knock-down and knock-out models to investigate WT1 regulation of Sulf expression in the epicardium. In addition, we carried out ATAC-Seq to investigate open chromatin in the Sulf1 and Sulf2 gene, and to identify potential WT1 transcription factor occupancy. Finally, we ran Ligand-Receptor inference analysis to identify all putative HSPG-dependent signalling pathways occurring in the E13.5 epicardium, and to shortlist pathways that may be mediated by Sulfs downstream of WT1.

Results: Sulf1 is expressed in epicardial progenitors and the forming epicardium, whilst Sulf2 is expressed in cardiomyocytes during early cardiac development (E9.5 – E11.5). Sulf1 is enriched in the epicardium at E13.5 and strongly co-localises with Wt1 expression, with levels decreasing coincidently with loss of Wt1 as the epicardium quiesces or undergoes epiEMT. We found that WT1 differentially regulates Sulf1 and Sulf2 expression, in contrast to what has been reported in the kidney [5]. Knock-down of WT1 in epicardial explants and an epicardial cell line resulted in decreased Sulf1 but increased Sulf2 expression. Wt1 null embryonic hearts, which form an irregular epicardial layer at E11.5 and E13.5, similarly demonstrated lower levels of Sulf1 and upregulated Sulf2. We found that WT1 binds to the Sulf1 promoter, but no binding was detected within the Sulf2 gene in E13.5 epicardial cells. Thus, while WT1 directly regulates Sulf1 expression, upregulation of Sulf2 occurs independently of WT1. Changes in the cell state (Epi-Mes) following loss of the transcription factor is likely to be the cause, as Sulf2 marks the mesenchymal state in epicardium-derived cells.

Conclusion: Our findings show WT1 directly regulates expression of Sulf1, but not Sulf2, to mediate HS-dependent signalling in the embryonic epicardium. This project provides further understanding of how Sulfs mechanistically influence epicardial behaviour and potentially guide vascular development in the heart.

1. Redpath, A. N. & Smart, N. Recapturing embryonic potential in the adult epicardium: Prospects for cardiac repair. *Stem Cells Translational Medicine* **10**, 511–521 (2021).
2. Lamanna, W. C. *et al.* The heparanome-The enigma of encoding and decoding heparan sulfate sulfation. *Journal of Biotechnology* **129**, 290–307 (2007).
3. Kruithof, B. P. T. *et al.* BMP and FGF regulate the differentiation of multipotential pericardial mesoderm into the myocardial or epicardial lineage. *Developmental Biology* **295**, 507–522 (2006).
4. Bochmann, L. *et al.* Revealing New Mouse Epicardial Cell Markers through Transcriptomics. *PLoS ONE* **5**, e11429 (2010).
5. Schumacher, V. A. *et al.* WT1-Dependent Sulfatase Expression Maintains the Normal Glomerular Filtration Barrier. *Journal of the American Society of Nephrology* **22**, 1286–1296 (2011).

POSTER NO: 18

Title: "IP_3-mediated Ca^{2+} release regulates atrial Ca^{2+} transients through stimulation of adenylyl cyclase 1 and cAMP"

Authors: Samuel J. Bose[1], Matthew Read[1], Andreas Koschinski[2], Emily Ackerman[1], Rebecca A. Capel[1], Thamali Ayagama[1], Derek A. Terrar[1], Manuela Zaccolo[2], Rebecca A.B. Burton[1]
Departmental affiliations: [1]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT. [2]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, OX1 3PT.

Research rationale: Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and a major cause of stroke, accounting for 14% of all cases in the UK, yet the underlying mechanisms of AF are poorly understood, and existing treatment options have significant limitations. Excessive stimulation of the IP_3 signalling pathway has been linked to the initiation and maintenance of AF through abnormal calcium handling, however little is known about the mechanisms involved in this process. Our previous work has demonstrated a link between atrial IP_3 signalling and activation of calcium sensitive adenylyl cyclases AC1 and AC8 [1]. Here we demonstrate that stimulation of the IP_3 pathway in neonatal rat atrial myocytes (NRAMs) using the α-adrenoceptor agonist phenylephrine (PE) results in changes in cAMP as shown using fluorescence resonance energy transfer (FRET) biosensors EPAC-S^{H187} and AKAP79-CUTie. These changes were inhibited using pharmacological inhibition of either IP_3 receptors (IP_3R) or AC1.

Methodology: Experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986. Data are presented as mean ± SEM. NRAMS were isolated from 3-day old Sprague Dawley rats by enzymatic digestion. Cells were seeded onto coverslips and infected with either the cytosolic FRET sensor EPAC-S^{H187} or membrane localised sensor AKAP79-CUTie after 72 hours. FRET imaging was performed at room temperature 24 hours after infection using an inverted microscope attached to a coolSNAP HQ^2 camera and an optical beam splitter (Photometrics) for simultaneous recording of YFP and CFP emissions. PE (3 µM) was added to the bath in the presence or absence of 2-APB (2.5µM) or Xestospongin-C (0.3 µM) to inhibit IP_3R, or ST034307 (1 µM) to inhibit AC1. Sensor saturation was measured by addition of Forskolin (FSK, 10 µM) and 3-Isobutyl-1-methylxanthine (IBMX, 100 µM). FRET changes were measured as changes in the background-subtracted 480 nm/545 nm fluorescence emission intensity on excitation at 430 nm.

Results: Addition of 3 µM PE resulted in a bi-phasic FRET change with initial peak corresponding to 20.07 ± 1.71% of the EPAC-S^{H187} saturation level (n = 50). In the presence of 2.5 µM 2-APB or 0.3 µM Xestospongin-C, this peak was reduced to 5.32 ± 0.81% and 10.13 ± 1.69% respectively (n = 32-42, $P < 0.0001$, One-Way ANOVA). 1 µM ST034307 reduced the initial peak in response to PE to 11.98% ± 1.78 % EPAC-S^{H187} saturation (n = 20, $P < 0.01$, one-way ANOVA). Using the AKAP79-CUTie sensor, 3 µM PE resulted in a FRET change of 12.30 ± 1.50 % AKAP79-CUTie saturation, which was inhibited by both 2-APB (3.15 ± 1.46%) and Xestospongin-C (6.98 ± 0.70%) respectively ($P < 0.01$, one-way ANOVA).

Conclusions: These data show that activation of the IP_3 signalling pathway in atrial cells results in downstream activation of cAMP, and that these changes are in-part localised to the cell membrane within the region of AKAP79. Furthermore, these cAMP changes were inhibited by the AC1 inhibitor ST034307, indicating a role for calcium sensitive adenylyl cyclases in this pathway. Based on our previous observations linking activation of IP_3 signalling in to the activation of AC1 and AC8 [1], we hypothesise that the changes observed in cAMP in response to IP_3 are secondary to the activation of AC1 and AC8. These data support further investigation of cardiac ACs and the IP_3 signalling pathway as a potential pharmacological target for the treatment of atrial arrhythmias.

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References: 1. Capel, R.A., et al., Am J Physiol Heart Circ Physiol, 2020.

POSTER NO: 19

Title: **Smoking and COVID-19 outcomes: a Mendelian randomisation study using UK Biobank**

Authors: **Adam Von Ende*[3], Ash Kieran Clift[1,2], Pui San Tan[1], Hannah M. Sallis[4,5,6], Nicola Lindson[1], Carol A.C. Coupland[1,7], Marcus R. Munafò[4,5,6], Paul Aveyard[1], Julia Hippisley-Cox[1*] & Jemma C. Hopewell[3*]**

Departmental affiliations:

1: Nuffield Department of Primary Care Health Sciences, University of Oxford
2: Cancer Research UK Oxford Centre, Department of Oncology, University of Oxford
3: Clinical Trials Service Unit, Nuffield Department of Population Health, University of Oxford
4: MRC Integrative Epidemiology Unit at the University of Bristol
5: School of Psychological Science, University of Bristol
6: NIHR Bristol Biomedical Research Centre, University Hospitals Bristol NHS Foundation Trust and University of Bristol
7: Division of Primary Care, University of Nottingham

Research Rationale: Throughout the COVID-19 pandemic, conflicting evidence has emerged regarding the relevance of smoking for risk of COVID-19 and its severity, and uncertainty remains about the causal nature of this relationship.

Methodology: We undertook a two-sample Mendelian randomisation (MR) analysis using UK Biobank. COVID-19 outcomes in UK Biobank were derived from Public Health England SARS-CoV-2 testing data, hospital admissions, and death certificates. Genetic instruments for smoking initiation and smoking heaviness were constructed based on genetic variants identified in published meta-analyses of genome-wide association studies. The random-effects inverse-variance weighted (IVW) method was used to estimate causal effects of smoking behaviours on COVID-19 related outcomes, with estimates reported as odds ratios (OR) and 95% confidence intervals (CI) per standard deviation difference in the genetically proxied smoking behaviour. Methodologic sensitivity analyses (i.e. MR-Egger, weighted median, and MR-PRESSO) were conducted to assess the robustness of findings to violations of instrumental variables assumptions. Results were also considered alongside conventional observational analyses.

Results: In analyses of 281,105 White British participants, genetically predicted propensity to initiate smoking was associated with higher risks of confirmed infection (OR 1.45 [95% CI: 1.10-1.91]), hospitalisation (OR 1.60 [95% CI: 1.13-2.27]), and death (OR 1.35 [95% CI: 0.82-2.22]). In 114,080 White British ever-smokers, genetically predicted higher number of cigarettes smoked per day was associated with higher risks of all outcomes (infection OR 2.51 [95% CI: 1.20-5.24]; hospitalisation OR 5.08 [95% CI: 2.04-12.66] and death OR 10.02 [95% CI: 2.53-39.72]). Results were consistent across different MR methods and there was no evidence of heterogeneity or directional pleiotropy. Observational analyses also supported higher risks of hospitalisation and death with initiation and intensity of smoking.

Conclusions: Mendelian randomisation results for smoking initiation and heaviness support a causal effect of tobacco smoking on risks of a range of COVID-19 outcomes

POSTER NO: 20

Title: *In Silico* Human Induced Pluripotent Stem Cell Derived Cardiomyocyte Electro-Mechanical Modelling and Simulation

Authors: Milda Folkmanaite[1,2*], Xin Zhou[2], Francesca Margara[2], Manuela Zaccolo[1] & Blanca Rodriguez[2]
Departmental affiliations: [1]Department of Physiology, Anatomy and Genetics; [2]Department of Computer Science
*Presenting author is a non-clinical DPhil student.

Research Rationale: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) enable accessible human data-based cardiology studies. However, an immature hiPSC-CM electrophysiological and contractile phenotype hinders data translation to adult cardiomyocytes. *In silico* hiPSC-CM investigations could aid in hiPSC-CM data translation but most hiPSC-CM models do not feature a contractile element which limits their application for such studies. In the light of the growing use of hiPSC-CM, it is vital to enable investigations of hiPSC-CM-specific contractile features. To address the need of hiPSC-CM model with integrated contractile element, we aim to develop an electromechanical hiPSC-CM computer model.

Methodology: We coupled a published hiPSC-CM electrophysiological model with a model of human adult cardiomyocyte contractile machinery by linking intracellular calcium and calcium-bound troponin dynamics. The established electromechanical hiPSC-CM model was calibrated using experimental hiPSC-CM active tension data and its simulated electromechanical biomarkers were also evaluated against experimental action potential and calcium transient data.

Results: First, we test if the model successfully reproduces hiPSC-CM contractile phenotype. We compute active tension biomarkers and compare them with experimental data. Simulations show a peak twitch tension of 0.44 kPa which takes 201 ms to peak (TP) and 164 ms to achieve 50% relaxation (RT50), which all agree with the experimental hiPSC-CM values. Second, we demonstrate that calcium transient and action potential biomarkers with the electromechanical hiPSC-CM model remain within the experimentally established ranges. Finally, a comparison with human adult cardiomyocyte electromechanical models shows that TP is 14.9-25.6% and RT50 is 35.5-41% larger in simulations with hiPSC-CM model. This agrees with experimental data ranges demonstrating that models can capture relative differences between the cells which provides further confidence for their usability in future data translation.

Conclusions: Altogether, we present a new electromechanical hiPSC-CM model for comprehensive *in silico* hiPSC-CM-based studies, for mechanistic investigations and translation to adult cardiomyocytes behaviour.

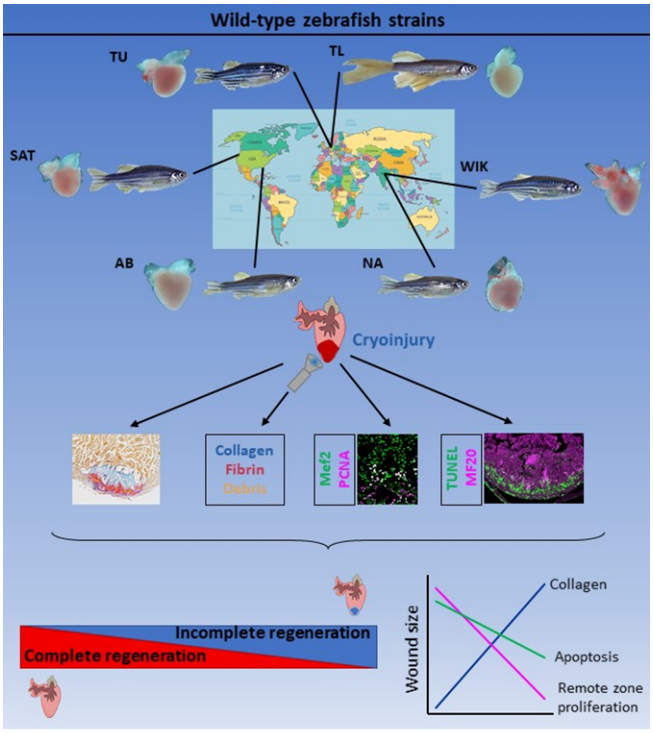
POSTER NO: 21

Title: Comparison of the regenerative capacity of six wild-type zebrafish strains reveals inter-strain variations in the wound healing process, cardiomyocyte proliferation and apoptosis levels following ventricular cryoinjury.

Authors: Konstantinos Lekkos[1]*, Zhilian Hu[1], Jana Koth[2], Katherine Banecki[1], Helen Potts[1], Gennaro Ruggiero[1], Mathilda Mommersteeg[1].

Departmental affiliations: [1]Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, OX1 3PT, UK. [2]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DS, UK.
*Presenting author, BSc/MSc student (non-clinical).

Research rationale: It is well established that teleost fish maintain the capacity to regenerate lost cardiac tissue throughout their adult life. However, recent reports suggest that variations exist both between and within species. Based on variations observed in published studies, we hypothesised that there is also strain-dependent variability in heart regeneration in zebrafish.
Methods: To test our hypothesis, we characterised the regenerative response of six commonly used wild-type zebrafish strains (AB, NA, SAT, TL, TU and WIK). In order to model myocardial infarction, we cryoinjured the ventricles of the hearts and collected hearts for analysis at 1-, 7-, 21- and 90-days post-cryoinjury (dpci).
Results: As heart regeneration is driven by cardiomyocyte proliferation, we analysed proliferation of these cells at 7dpci and found significantly more proliferation in the WIK compared to the other lines, both in cardiomyocytes bordering the wound as in the rest of the ventricle. Accordingly, analysis of wound size confirmed that the WIK line had the strongest reduction in wound between 7 and 21dpci. However, comparing 21 with 90dpci wound size, there was no further reduction in the WIK. This seems to be linked to the high percentage of collagen present in the WIK wound at 7 and 21dpci, as correlation analysis showed that the amount of collagen deposition at 7dpci is a predictor of the regenerative outcome at 90dpci. Intriguingly, it was cardiomyocyte proliferation in the remote area that correlated to wound length at 21dpci, not that of the border-zone. At 90dcpi, it was the NA line showing the highest percentage of scar-free regenerated hearts. On the other side of the spectrum was the TU line, in which 80% of hearts did not regenerate the compact wall with a large scar remaining. Further correlation analysis suggested a potentially pro-regenerative role for apoptosis at 1dpci.
Conclusions: In conclusion, comparing six wild-type zebrafish strains with different regenerative capacities has, for the first time, allowed us to identify correlations between different cellular processes occurring during the regenerative process.



Graphical abstract: Hearts from wild-type zebrafish strains were cryoinjured and allowed to regenerate. Measurements of the wound size and composition, cardiomyocyte proliferation and apoptosis revealed intraspecies differences in the regenerative response. A correlation analysis provided links between collagen deposition in the early wound, cardiomyocyte cycling at the remote zone of the ventricle and 1dpci apoptosis with the regenerative outcome.

POSTER NO: 22

Title: Effect of altered lipid trafficking on the modulation of vascular tone by the TMEM16A chloride channel

Authors: Lara F. Scofano*(DPhil student, non-clinical)[1], Rumaitha Al-Hosni[1], Kathryn E. Acheson[1], Zuzanna Borawska[1], Claire Smith[1], Zeki Ilkan[1], Frances M. Platt[1], and Paolo Tammaro[1]

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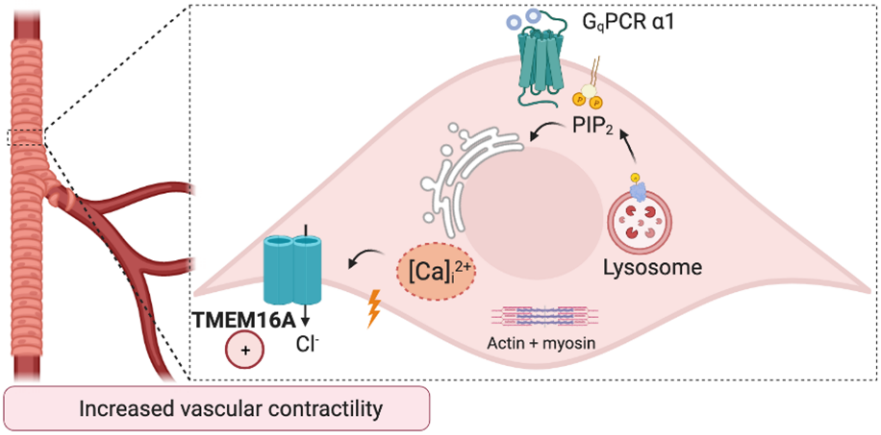
Research rationale. The TMEM16A Ca^{2+}-activated Cl^- channel play a key role in the control of vascular tone and blood flow. TMEM16A has a pore with sections exposed to plasmalemmal lipids[1]. This structural arrangement may confer the channel the capacity to respond to plasmalemmal lipids, including phosphatidylinositol 4,5-biphosphate (PIP_2)[2]. The lysosomal NPC1 protein regulates cellular distribution of lipids. Loss-of-function mutations in NPC1 lead to Niemann-Pick disease Type C (NPC), a prematurely fatal neurodegenerative disorder with a range of systemic alterations including vascular[3]. Here, we ask whether TMEM16A is modulated by NPC1 and examine the impact of this modulation on the tone of isolated systemic arteries and cerebral capillaries, where TMEM16A is highly expressed.

Methods. Whole-cell patch-clamp recordings of native and heterologous TMEM16A currents, isometric tension recordings and confocal and DIC imaging were used in this study. Acute brain slices and isolated arteries were obtained from mice carrying *Npc1* deletion (*Npc1*$^{-/-}$). Data are given as mean±SEM alongside the number of independent experiments.

Results. Heterologously expressed TMEM16A currents in mammalian cell lines were enhanced by 2.3±0.3 (n=14) fold during pharmacological inhibition of NPC1 or by 2.6±1.0 (n=15) fold as a consequence of genetic deletion of the *Npc1* gene. These increases were prevented by of treatment with β-cyclodextrin or re-introduction of *Npc1* in the gene in *Npc1* mutant cells. Depletion of plasmalemmal PIP_2 or an inactivating mutation in the channel PIP_2 binding site (TMEM16A-R482A), prevented TMEM16A activation during NPC1 inhibition. Exposure of cortical slices to oxygen-glucose deprivation to simulate cerebral ischaemia caused significant capillary constriction and pericyte death in *Npc1* mutant mice, which were ameliorated by Ani9, a selective TMEM16A inhibitor. Artery rings and pericytes in cerebral capillaries obtained from *Npc1* mutant mice showed increased contractility in response to phenylephrine, which was prevented by Ani9. The underlying mechanism involves augmented plasmalemmal PIP_2 levels during NPC1 inhibition, which was enhanced in live cells by 1.2 ±0.6 fold (n=67) during pharmacological inhibition of NPC1 and was rescued by treatment with β -cyclodextrin.

Conclusions. PIP_2-dependent changes in TMEM16A activity may form the basis of vascular overactivity during pathology caused by loss of NPC1 function and establish a role for the lysosome in the control of cell excitability and vascular tone.



References

[1] Dinsdale RL, Pipatpolkai T, Agostinelli E, Russell AJ, Stansfeld PJ, Tammaro P. An outer-pore gate modulates the pharmacology of the TMEM16A channel. *Proc Natl Acad Sci U S A*2021;118(34). **[2]** Ta CM, Acheson KE, Rorsman NJG, Jongkind RC, Tammaro P. Contrasting effects of phosphatidylinositol 4,5-bisphosphate on cloned TMEM16A and TMEM16B channels. *Br J Pharmacol.* 2017;174(18):2984-2999. **[3]** Platt FM. Emptying the stores: lysosomal diseases and therapeutic strategies. *Nat Rev Drug Discov.* 2018;17(2):133-150.

POSTER NO: 23

Title: **Calcitonin signalling system regulates function and arhythmogenicity of atrial cardiomyocytes.**

Authors: Lucia M Moreira[1]*, Paul Robinson[1], Alexander J Sparrow[1], Neelam Mehta[1], Kathryn Aguilar-Agon[1], Ioannis Akoumianakis[1], Laura Herdman[1], Rana Sayeed[2], George Krasopoulos[2], Hugh Watkins[1], Keith M Channon[1], Charalambos Antoniades[1], Stanley Nattel[3], Charles Redwood[1], Svetlana Reilly[1]

Departmental affiliations: [1]Radcliffe Department of Medicine (Cardiovascular Division) University of Oxford. [2]Cardiothoracic Surgery, Oxford Heart Centre, John Radcliffe Hospital, Oxford, UK. [3]Research Centre, Montreal Heart Institute, Department of Pharmacology and Therapeutics, McGill University.

Research rationale - Atrial fibrillation (AF), the commonest cardiac arrhythmia in man, is a major therapeutic challenge due to the structural and electrical myocardial remodelling. We recently discovered that atrial cardiomyocytes (CMs) secrete significant amounts of calcitonin (CT), whose impaired secretion in AF favours production of fibrotic tissue by atrial fibroblasts and AF arrhythmogenesis [1]. The direct effects of CT (and its precursor procalcitonin, PCT) signalling on CM function and arrhythmogenicity are unknown, and form the objectives of this study.

Methodology and Results – Using freshly isolated atrial guinea pig CMs, we first confirmed (by qPCR and immunofluorescence) that CMs express CT-receptors (CTR) to enable CT actions in the cell. Functional studies found that CT administration inhibits spontaneous calcium (Ca^{2+})-release events induced by pacing (at 2 Hz; IonOptix μstep system) in fura2-loaded CMs (n=22 cells) in a concentration-dependent manner. These effects were accompanied by a reduction in Ca^{2+} transient amplitude.

In a parallel clinical study (using serum samples from 20 patients who underwent cardiac surgery) we noted that higher pre-operative circulating levels of CT (by ELISA) are associated with a significant ~2.8-fold reduction in the incidence of post-operative AF (poAF), a major (in ~40% of cases) complication of cardiac surgery. Furthermore, patients with poAF also failed to recover supressed CT levels 3 days after the surgery and had ~10-fold higher levels of CT-precursor procalcitonin (PCT). Indicating that PCT may also potentially contribute to the arrhythmogenesis of AF. Experiments in guinea pig atrial CMs showed that unlike CT, PCT promoted spontaneous Ca^{2+} release pro-arrhythmic events in a concentration-dependent manner and increased Ca^{2+} transient amplitude in atrial CMs.

Evaluation of the expression and phosphorylation of selected Ca^{2+} handling proteins, which may potentially account for the observed changes in Ca^{2+} transients, showed no differences in total or phospho-(ser2808) ryanodine receptor, total or phospho-(Ser16) phospholamban, and SERCA2α in response to CT, while PCT increased phosphosphorylation of (ser2808)RyR with no changes in PLN or SERCA2α.

Conclusions – Our findings suggest that CT and PCT exert opposite effects on the function of atrial CMs. While, CT potently supresses cell arrhythmogeneity, PCT is pro-arrhythmic. Thus, strategies to maintain physiological levels of CT (for example, with CT-based agents) combined with PCT-neutralising agents (e.g., PCT-antibodies) may potentially benefit clinical management of AF.

[1] Moreira, L.M., et al. Paracrine signalling by cardiac calcitonin controls atrial fibrogenesis and arrhythmia. *Nature* 587, 460–465 (2020). https://doi.org/10.1038/s41586-020-2890-8

* = DPhil Student

POSTER NO: 24

Title: Simulation model for lifetime prediction of complications in people with diabetes without previous cardiovascular disease using ASCEND risk equations

Authors: Mi Jun Keng* (DPhil student; non-clinical), Jose Leal, Louise Bowman, Jane Armitage, Borislava Mihaylova
Departmental affiliations: Nuffield Department of Population Health (all authors)

Research rationale: Decision-analytic disease models are commonly used in healthcare to synthesize clinical and economic data and inform assessments of overall health effects and/or cost-effectiveness of interventions. In diabetes, which is associated with disabling and costly complications over time, such models are crucial to simulate the impact of treatments over a person's lifetime. The diabetes model recommended in the UK by NICE is the UKPDS Outcomes Model[1] developed using patient data from 1977-2007 and thus reflects historic patterns of care and event rates. The model has been shown to over-predict cardiovascular events and deaths in contemporary cohorts.[2] Using data from the ASCEND study (data from 2005-2017), we developed new risk equations to simulate complications in people with diabetes but without previous cardiovascular disease.

Methodology: Data from 15480 participants (mean follow-up 7.4 years) in the ASCEND study[3] was used to fit parametric risk equations for time to first occurrence of cardiovascular (myocardial infarction (MI), coronary revascularisation (CRV), transient ischaemic attack (TIA), ischaemic stroke, heart failure), bleeding (gastrointestinal (GI) bleed, intracranial haemorrhage, other major bleed), cancer (GI tract cancer, non-GI tract cancer), microvascular (end-stage renal disease (ESRD), amputation) and death (vascular, non-vascular) events, adjusting for participants' baseline socio-demographic characteristics, clinical risk factors, and occurrence of previous adverse events in the study. These equations were integrated in a stochastic patient-level simulation model, which predicts the occurrence of adverse events each year over a person's lifetime based on a person's baseline profile and adverse events as they occur over time. The simulation model was used to predict the life expectancies of ASCEND participants, as well as 18250 UK Biobank (UKB) participants matching the ASCEND eligibility criteria. The validity of the simulation model (i.e. comparison of model-simulated incidences of events with that observed) was assessed internally using the ASCEND participants' data, and externally using the UKB participants' data.

Results: In contrast to ASCEND, the UKB cohort was younger (mean age 59 vs 63 years) with greater proportion of participants with type 1 diabetes (13% vs 6%), poorer lipid and blood pressure profile (non-HDL cholesterol ≥3.5mmol/L, 37% vs 21%; SBP ≥140mmHg, 51% vs 41%), and poorer renal function (eGFR <90ml/min/1.73m^2, 61% vs 54%; microalbuminuria, 17% vs 13%). The simulation model performed well over 10 years of follow-up in ASCEND and also reasonably well in the UKB cohort. For the UKB, the predicted rates of MI, CRV, cancers, intracranial haemorrhage, and other major bleed were similar to those observed. However, the predicted rates were greater than those observed for TIA (absolute difference in 10-year cumulative incidence, 1.8%), ischaemic stroke (0.8%), heart failure (1.6%), GI bleed (1.3%), amputation (0.4%) and ESRD (0.4%). These discrepancies could be attributable to mis-recordings and/or events not recorded in routine hospital records which were used for identifying these events. The predicted rates were also greater than those observed for vascular (1.2%) and non-vascular deaths (2.7%), which suggest that there could be differences in death rates not captured by the ASCEND risk equations; these discrepancies are currently being investigated. The predicted life expectancies of participants with type 2 diabetes aged 60-70 was 17.7 years and 16.9 years in the ASCEND and UKB cohorts respectively, which is at least 5 years more than predicted for the UKPDS cohort[4], despite the over-prediction of mortality.

Conclusions: Temporal changes in event rates put into question the validity of existing diabetes models and new or updated risk equations are needed to reflect contemporary event rates. We propose a simulation model to predict outcomes in people with diabetes without previous cardiovascular disease using a large contemporary well-curated dataset.

1. Hayes AJ, Leal J, Gray AM, Holman RR, Clarke PM. UKPDS Outcomes Model 2: a new version of a model to simulate lifetime health outcomes of patients with type 2 diabetes mellitus using data from the 30 year United Kingdom Prospective Diabetes Study: UKPDS 82. *Diabetologia*. 2013;56(9):1925-1933. doi:10.1007/s00125-013-2940-y
2. Keng MJ, Leal J, Mafham M, Bowman L, Armitage J, Mihaylova B. Performance of the UKPDS Outcomes Model 2 in a contemporary UK type 2 diabetes trial cohort. *Value Health*. 2021;In press.
3. The ASCEND Study Collaborative Group. Effects of Aspirin for Primary Prevention in Persons with Diabetes Mellitus. *N Engl J Med*. 2018;379(16):1529-1539. doi:10.1056/NEJMoa1804988
4. Leal J, Gray AM, Clarke PM. Development of life-expectancy tables for people with type 2 diabetes. *Eur Heart J*. 2009;30(7):834-839. doi:10.1093/eurheartj/ehn567

POSTER NO: 25

Title: Characterising differences between the regenerative and non-regenerative immune response in *Astyanax mexicanus*

Authors: Helen G. Potts[1], Madeleine Lemiex[2], Robin P. Choudhury[3], Mathilda T.M. Mommersteeg[1]

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Research Rationale:

The human heart cannot regenerate following myocardial infarction and instead forms a fibrotic scar that impairs cardiac function. This often leads to heart failure, which is incurable and a major cause of morbidity. Research in the field of cardiac regeneration aims to address this unmet clinical need by stimulating the adult human heart to repair itself, as witnessed in other organisms, like the zebrafish. The *Astyanax mexicanus* is a uniquely suited model to study cardiac regeneration as it comprises two closely related populations: (1) 'regenerative' surface (SF) populations that efficiently replace lost cardiac tissue after injury and (2) the 'non-regenerative Pachón (PF) cave population that form a scar. The immune response to injury is known to be a key regulator of successful regeneration. However, how this response differs between successful regeneration and scarring is unknown.

Methodology:

To fully characterise the *A. mexicanus* immune response, single cell RNA-sequencing, differential gene expression analysis (DGE) and *in situ* hybridisation were used to determine the immune cell populations present in the heart at 1,3, 7, 14 and 30 days post-cryoinjury (dpci).

Results:

We found striking spatiotemporal differences in the dynamics of both myeloid and lymphoid populations in the PF and SF. Immediately after injury, the non-regenerative PF show a stronger response to injury with a significantly greater influx of neutrophils into the wound (1dpci $p=0.0156$; 3dpci $p=0.0075$). By 7dpci, this inflammatory response is resolved and DGE shows that PF neutrophils have returned to the uninjured state. In contrast, the regenerative SF show a greater immune response at late-stages of wound healing (7, 14 and 30dpci). Specifically, at 7 and 14dpci SF have a transcriptionally unique population of neutrophils that remain activated and upregulate TNFα-NFκB signalling. Furthermore, we observed a stark contrast in the B cell response with SF displaying a significant influx of B cells at 14 and 30dpci that is absent in the PF.

Conclusions:

Further studies will aim to determine the role of these late-stage neutrophils in successful regeneration and explore this previously unknown link between adaptive B cells and successful regeneration. Understanding how the immune response differs between successful regeneration (SF) and scarring (PF) would be a significant step towards identifying novel therapeutic targets for immunomodulation in heart attack patients.

POSTER NO: 26

Title: Investigation of the Possible Roles of Cardiomyocyte derived Extracellular Vesicles in Hypertrophic Cardiomyopathy

Authors: Anan Huang, Dphil[1]*, Yingjie Wang, Postdoc[1], Kamayni Singh, Postdoc[1], Adam Lokman, Postdoc[1], Julia Beglov, Postdoc[1], Houman Ashrafian[1], Charles Redwood[1], Hugh Watkins[1]

Departmental affiliations: 1. Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford OX3 9DU, UK

Rationale: Inherited hypertrophic cardiomyopathy (HCM) is principally caused by mutations to genes encoding cardiac contractile proteins with abnormal cardiomyocyte contractility and Ca^{2+} handling. For the non-myocyte compartment, our preliminary research and current literature reveal early and excessive cardiac fibrosis and over-activated immune response in this type of mouse model. In acute myocardial infarction or cardiac hypertrophy models, there is evidence that stress signals may be transmitted to non-myocytes through ejection of extracellular vesicles encapsulating certain cell component. We hypothesise that similar mechanisms play a role in HCM pathogenesis.

Methodology: $ACTC^{E99k}$ transgenic ($ACTC^{E99k}$) mice which carry a missense mutation in cardiac actin were used as a HCM model. Non-transgenic (NTG) mice were used as negative control. Heart tissue is stained by Sirius red to qualify cardiac fibrosis. Multiple color FACS was performed to differentiate the subtypes of immune cells. Heart samples of $ACTC^{E99k}$ and NTG mice in 10 wks, 24 wks and 60 wks were collected for Bulk RNA sequencing. Oxidative phosphorylation (OXPHOS) and mitophagy related protein expression was detected by Western Blot.

Results: Excessive cardiac fibrosis were detected in $ACTC^{E99k}$ mice since 10 wks. Increased proportion of immune cells were detected in $ACTC^{E99k}$ mice. Bulk RNA sequencing results identified decreased expression of OXPHOS and mitochondrial function related genes. The blot results showed that OXPHOS protein levels were significantly downregulated in the heart tissue of $ACTC^{E99k}$ mice. The FACS results indicated that significant increased TnnI3 could be detected in fibroblasts and cardiac $CD45^{+}$ immune cells.

Conclusions: $ACTC^{E99k}$ induced hypertrophic cardiomyopathy undergoes excessive cardiac fibrosis and activated immune response and Mitochondrial dysfunction. Cell components derived cardiomyocytes can be detected in non-myocytes. Further study may concentrate on whether dysfunctional mitochondria can be encapsulated into EVs and transmitted to non-myocytes to trigger activation of non-myocytes.