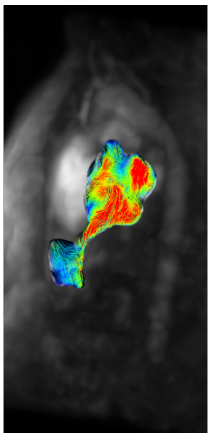
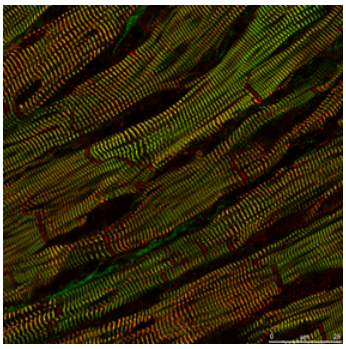
Oxford BHF Centre of Research Excellence

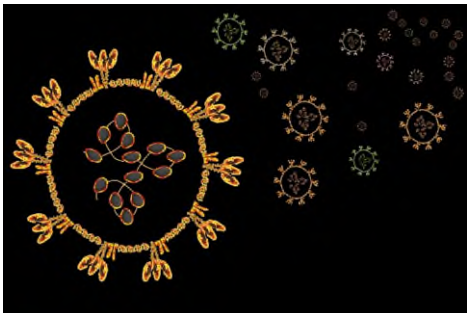
Annual Symposium

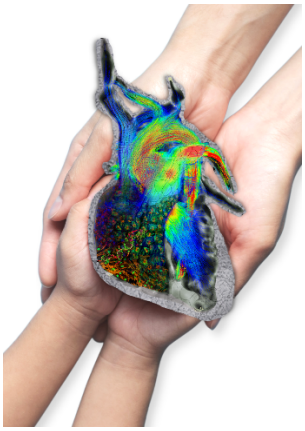
Wednesday 23rd November 2022

at Oxford Town Hall









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

1. *"Stripes"* - Dr Charlotte Hooper/Dr Katja Gehmlich
2. *"The Avatars of Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-CoV-2)* - Dr Hasan Mohiaddin (winner judges' vote 2021)
3. *"4D flow cardiac magnetic resonance in hypertrophic cardiomyopathy- novel insights from another dimension"* – Zakariye Ashkir
4. *"The Growing Heart"* - Cheryl Tan (winner, audience vote 2021)

Oxford BHF Centre of Research Excellence
Research Symposium
Wednesday 23rd November 2022
Oxford Town Hall

9:15	**Professor Hugh Watkins** Director, Oxford BHF CRE **Welcome and Oxford BHF CRE updates**
Session 1 **Start Time 9:30**	**Chair: Professor Paul Riley** **Oxford BHF CRE Themes**
	Professor Aiden Doherty: Big Data Theme **Professor Jemma Hopewell**: Human Genetics Theme **Professor Nicola Smart**: Development & Regeneration Theme **Professor Angela Russell**: Target Discovery Theme
10:30 – 11:00	*Refreshment Break & poster viewing*
Session 2 **11:00 – 12:30**	**Chair: Associate Professor Sarah de Val** **Poster competition short-list talks / technologies blitz /BHF comms**
	Four-minute talks from the short-listed poster competition entrants. Showcase of cardiovascular research tools and facilities Media and communications presentation by Dr Lisa Jones of the BHF
12.30 – 14.00	**Poster viewing and discussions** **Image Competition – Viewing of entries and audience voting** ***Lunch & networking***
Session 3 **Start Time 14:00**	**Chair: Professor Sir Rory Collins** **Keynote Speaker – Professor Ben Goldacre, Primary Care Health Sciences** *How we can all help to achieve better, broader, safer use of health data*
15.00 – 15.30	*Refreshment Break*
Session 4 **15.30– 17.00**	**Chair: Professor Barbara Casadei** **Talks from across the Oxford BHF CRE Themes**
15.30 – 15.45	**Dr Diego Aguilar-Ramirez** *NMR metabolomics in the Mexico City Prospective Study*
15.45 – 16.00	**Associate Professor Mathilda Mommersteeg** *Oxidative phosphorylation is required for fish heart regeneration*
16.00 – 16.15	**Dr Ying-Jie Wang** *Role of non-myocytes in hypertrophic cardiomyopathy, a common genetic heart muscle disorder*
16.15 – 16.30	**Associate Professor Svetlana Reilly** *Towards new therapeutics in atrial fibrillation and cardiac fibrosis*
16.30 – 16.45	**Associate Professor Adam Lewandowski** *Acute and Chronic Cardiovascular Adaptations in People Born Preterm*
16.45 – 17.15	**Professor Keith Channon – Presentation of poster and image competition prizes and closing remarks**
17:15 – 18:30	*Drinks reception*

Speaker session 1 – 9.30 – 10.30am

Chair: Professor Paul Riley

Talks from the Oxford BHF CRE Themes

- Professor Aiden Doherty: Big Data Theme
- Professor Jemma Hopewell: Human Genetics Theme
- Professor Nicola Smart: Development & Regeneration Theme
- Professor Angela Russell: Target Discovery Theme

Speaker session 2 – 11.00am – 12.30pm

Chair: Sarah de Val

- Four-minute talks from the short-listed poster competition entrants
- Showcase of cardiovascular research tools and facilities
- Media and communications presentation by Dr Lisa Jones of the BHF

Speaker session 3 – 2.00 – 3.00pm

Chair: Sir Rory Collins

Keynote speaker

Professor Ben Goldacre, Director, Bennett Institute for Applied Data Science

How we can all help to achieve better, broader, safer use of health data

The NHS has a phenomenal resource in its data: detailed records on the lives of a huge and diverse population, going back many decades. This data has tremendous power to save lives through research, innovation, and data-driven service improvement.

Sadly that power has never been fully tapped, due to a range of practical barriers. The health data that analysts need to access for their work also contains the confidential medical secrets of every citizen in the country. This data is also often very hard to work with, because it was created for practical purposes around direct care, rather than as a research resource.

This talk will discuss the opportunities and challenges in health data, but also how those challenges can be overcome. Specifically it will cover the Goldacre Review, commissioned by Secretary of State for Health and Social Care, and published in April 2022; the changes in the 2022 NHS Data Strategy; and the work of OpenSAFELY.org as an example of how research with NHS data can be made both open and secure.

Biography

Ben is a doctor, academic, writer, and broadcaster. He trained in medicine at Oxford and UCL, in psychiatry at the Maudsley, and in epidemiology at LSHTM. His academic and policy work is in informatics, epidemiology and evidence based medicine, where he works on various problems including variation in care, better uses of routinely collected electronic health data, evidence-based social policy, access to clinical trial data, efficient trial design, and retracted papers.

He runs the **Bennett Institute for Applied Data Science**. This is a multidisciplinary team of academics, clinicians and software developers, all pooling skills and knowledge to turn large datasets into tools and services as well as pure academic research papers.

- **OpenSAFELY** is a fully open source and highly secure analytics platform for NHS data created during the COVID-19 pandemic. It is currently executing code across an unprecedented scale of data: 58 million patients full raw GP records - 70 billion rows of information - linked onto various other sources including SGSS, SUS/HES, ECDS, ISARIC, ICNARC, ONS death, and more. All code for the platform, and for data management and analysis of each output, is shared under open licenses for review and re-use. OpenSAFELY has delivered a range of outputs in journals such as Nature, Lancet and the BMJ from a large national academic collaboration.
- **OpenPrescribing** is a live, freely accessible explorer for 8,000 individual NHS GP practices' prescribing data: it implements cutting edge data science techniques in a real working tool which serves over 150,000 unique users a year, and thousands of subscribers receiving regular context alerts on changes in their prescribing behaviour. Alongside this tool the **DataLab** have also rapidly delivered a substantial body of work describing variation in prescribing behaviour across the NHS, and the drivers of practice change.
- The **TrialsTracker** is a range of automated online tools monitoring the reporting status of all clinical trials, with papers in the BMJ and Lancet; its sister project **COMPare Trials** monitors detailed data on outcomes within reported clinical trials.

In policy work, he is currently **leading a review** into the Better, Broader, Safer Use of NHS Data, reporting to the Secretary of State for Health and Social Care. He is chair of the **HealthTech Advisory Board**, a member of the Data Science Advisory Board for the Joint Biosecurity Centre, and has previously served on various national committees including the Dept for Education Data and Evidence Board and the Ministry of Justice **Data, Evidence and Science Board**. He co-authored this **influential Cabinet Office paper**, advocating for randomised trials in government, and setting out mechanisms to drive this forwards; and conducted an independent external review for the Department for Education, on improving the creation and use of evidence in the teaching sector (the public component of this work is published **here**). He is the co-founder of the **AllTrials** campaign. He also **engages more broadly with policy makers** and has given evidence on numerous occasions to various parliamentary select committees including the Public Accounts Committee (withheld clinical trials and Tamiflu),Science and Technology (withheld clinical trials, homeopathy), Health (privacy and electronic patient data), and Culture Media & Sport (libel).

Alongside this he also works in public engagement, writing and broadcasting for a general audience on problems in evidence based medicine. His books have sold over 600,000 copies; his TED talks have had over 4 million views; while being accessible to a general audience, these lectures and books are also used in university teaching around the world.

Speaker session 4 – 3.30 – 5.00pm

Chair: Professor Barbara Casadei

Talks from across the BHF CRE Themes

NMR metabolomics in the Mexico City Prospective Study

Dr Diego Aguilar-Ramírez
Early-Career Research Fellow, Oxford Population Health

Blood lipids, particularly the cholesterol in low-density lipoproteins (LDL-C), are causally related to atherosclerotic cardiovascular disease. Key cardiovascular risk factors including adiposity, diabetes, and kidney disease, are also linked to perturbations in blood lipids. However, evidence around blood lipids has mostly relied on simple assays of these biomarkers and primarily comes from studies in high-income countries. The Mexico City Prospective Study (MCPS) is the largest blood-based prospective cohort study in Latin America, which recruited ~160,000 adults aged ≥35 years in 1998-2004. To date, about ~40,000 have been profiled using nuclear magnetic resonance (NMR) spectroscopy metabolomics in blood (which measures >100 biomarkers including lipids, lipoproteins, and other molecules), making it one of the largest metabolomics datasets in a non-European population. During this talk, I will highlight key initial findings involving the NMR metabolic profiles of adiposity and kidney disease in MCPS. I will then outline the path forward for the integration of genetics, metabolomics, and key phenotypes using genetic epidemiology methods, which will soon be possible as cohort-wide NMR data is expected by mid-2023.

Oxidative phosphorylation is required for fish heart regeneration

Mathilda Mommersteeg
Associate Professor of Developmental and Regenerative Medicine

In contrast to patients after myocardial infarction, certain fish can fully regenerate their hearts. However, not all fish are able to regenerate to the same extent, allowing comparative inter- and intra-species analysis to identify novel mechanism controlling successful heart regeneration. While work in our lab generally focusses on using the Mexican cavefish as a comparative model, we have now taken this intraspecies comparison to the zebrafish model and compared the regenerative response of seven different wild type zebrafish strains to cryo-injury. This showed that there is differential regeneration between the different strains and using RNAseq, we identified oxidative phosphorylation (OXPHOS) as a crucial positive regulator of this difference. While myocardial proliferation is important for the initial stages of regeneration, long term regenerative outcome is

determined by levels of OXPHOS and prolonged cardiomyocyte dedifferentiation state. These findings indicate that the current stance in the field, that OXPHOS is damaging the ability for heart regeneration, needs to be re-evaluated, opening up new possibilities for targeting pathways to enhance heart repair.

Role of non-myocytes in hypertrophic cardiomyopathy, a common genetic heart muscle disorder

Dr Ying-Jie Wang
Senior postdoctoral researcher

Hypertrophic cardiomyopathy (HCM) is a common, serious, genetic heart muscle disorder. The biophysical mechanisms by which HCM mutations in sarcomeric proteins disrupt cardiomyocyte function are largely understood, but the cellular and molecular pathways leading on to the complex and adverse remodeling of the non-myocyte compartment are unexplained. Here we report that cardiac tissue from people with HCM exhibit a prominent chronic infiltration of immune cells. We found a similar infiltration in a translationally faithful HCM mouse model (*Actc1*^{E99K}). RNA Seq data from human and mouse HCM hearts revealed a profound and active immune response. This immune reaction causatively contributes to pathogenesis of HCM as genetic depletion of lymphocytes (*Rag-1*$^{-/-}$) in these mice led to profound adverse cardiac remodeling. Detailed longitudinal characterization of HCM hearts revealed that regulatory T cells (Tregs) were enriched and functionally activated in HCM. Both Treg transfer and *in vivo* Treg expansion significantly ameliorated cardiac remodeling in HCM. These data contribute to our understanding of HCM and support Tregs as a clinically testable novel therapeutic strategy in cardiac fibrosis.

Towards new therapeutics in atrial fibrillation and cardiac fibrosis

Svetlana Reilly
Associate Professor of Cardiovascular Science, Senior Research Fellow.

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia in humans, and is associated with significant mortality and morbidity. Long-term treatment of AF is inadequate, as it is hampered by electrical and structural (hallmarked by fibrosis) remodelling of the atrial myocardium. Current pharmacological strategies in AF primarily target ion channels and electrical remodelling, and blood coagulation (to prevent stroke), but not atrial fibrosis. While many pathophysiological aspects of atrial fibrosis are understood, no clinically effective targets have yet been identified. Thus, there is a need to uncover new players in atrial remodelling that may lead to more effective therapeutics for patients with AF. Our recent work uncovered a new cardiac hormone produced by the atria, calcitonin, which has been recognized to be primarily secreted by the thyroid gland. Intriguingly, we found that calcitonin produced by the atrial myocaridium activates cell-surface calcitonin-receptors of the neighbouring atrial fibroblasts in a paracrine manner to inhibit production of profibrotic

extracellular matrix proteins. Thus, the calcitonin signalling cascade represents a potentially important endogenous regulatory mechanism to keep atrial fibrogenesis in check. As calcitonin signalling is impaired in patients with persistent AF, restoring the activity of this signalling cascade may open doors for new therapies in AF.

Acute and Chronic Cardiovascular Adaptations in People Born Preterm

Adam Lewandowski
Associate Professor of Cardiovascular Science and BHF Intermediate Research Fellow

Preterm birth (<37 weeks' gestation) is a common complication affecting around 10% of pregnancies worldwide. It results in an abrupt transition for the fetus from the intrauterine to extrauterine environment that has been associated with acute and chronic cardiovascular remodelling as early as infancy. Birth registry studies have shown that being born preterm is linked with long-term clinical risk including heart failure, ischaemic heart disease, and early cardiovascular related mortality. Our team was the first to identify that adults born preterm have unique morphological and functional cardiac changes that appear to emerge in early postnatal life and are independent of related cardiopulmonary alterations observed in this subgroup of the population. My talk will highlight some of our ongoing observational and clinical trials we are doing using multimodality imaging and physiological stress testing to better understand acute and chronic adaptations of the cardiovascular system of people born preterm.

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3	**Lauren Philiips**	The role of endothelial angiotensin II receptors in the release of nitric oxide
4	**JinHeng Lin**	Mechanism of neuropeptide-Y-stimulated vasoconstriction in rat mesenteric and coronary arteries
5	**Kyung Chan Park**	Differential metabolic processing of propionate into b-alanine underpins the sex-dependence of histone modifications and contractile dysfunction in a mouse model of elevated propionate/propionyl-CoA
6	**Rita Alonaizan**	Glucose metabolism regulates cardiomyocyte proliferation and heart regeneration in *Astyanax mexicanus* fish
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POSTER NO: 1

Title: **Epicardial growth factors promote spouting angiogenesis during heart development and offer new tools for heart regeneration**

Authors: Jacinta Kalisch-Smith* post-doctoral researcher, Andia Redpath, Nicola Smart
Institute for Developmental and Regenerative Medicine, DPAG

Research Rationale

Repairing the heart after a heart attack (myocardial infarction [MI]) requires forming new blood vessels. This allows the remaining cardiac tissue to survive, and aids the regeneration of new cells. Current attempts to form blood vessels (neovascularise) the heart following injury have been largely unsuccessful. By studying how blood vessels grow during development, and the pro-angiogenic signalling pathways they use, we hope find new factors to promote neovascularisation in the adult. One source of coronary blood vessels in development comes from the sinus venosus. This is a large vein on the dorsal side of the heart. Previous work from our lab has shown that the adult coronary sinus has some regenerative capacity, and is derived from the sinus venosus. During development, the epicardium (outer layer of the heart) contributes pro-angiogenic cues (e.g. VEGFC, CXCL12, ELA), and following MI can reactivate its signalling pathways. Understanding epicardial development may therefore be a valuable resource for finding new growth factor signalling pathways that can be repurposed for regeneration. We have taken the approach to find and validate new epicardial-derived signals and validate them in culture on sinus venosus-derived tissue.

Methodology

E12.5 and E13.5 single cell RNAseq datasets were assessed with CellTalk R programme to distinguish receptor-ligand inference networks, whereby growth factor ligands were located in epicardial tissue, and receptors for these pathways located on the sinus venosus endothelial cells. Signalling pathways were investigated for known roles in sprouting angiogenesis and cell migration. To validate candidate epicardial growth factors, a sinus venosus (SV) explant culture system was used. SV tissue was dissected at E11.5 and cultured for 5 days on a thin layer of matrix on culture plate inserts. Explants were serum starved on day 2, and on day 3 were applied with known or novel growth factors. Day 5 explants were collected and stained with CD31 marking endothelial cells, and ERG, marking their cell nuclei. Sprouting angiogenesis was assessed using the Angiotools programme. ERG + nuclei were counted in angiogenic sprouts and in the tissue piece.

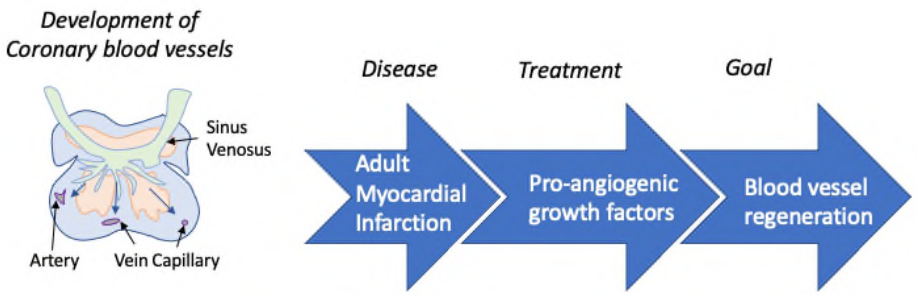
Results

Analysis of epicardial/SV receptor-ligand networks found >30 growth factor pathways. We have validated known positive (VEGFC) and negative (VEGFA) growth factors using SV explants, and started to validate novel growth factors. One novel factor, pleiotrophin (PTN), has shown dose-dependent positive impacts on proliferation and sprouting angiogenesis.

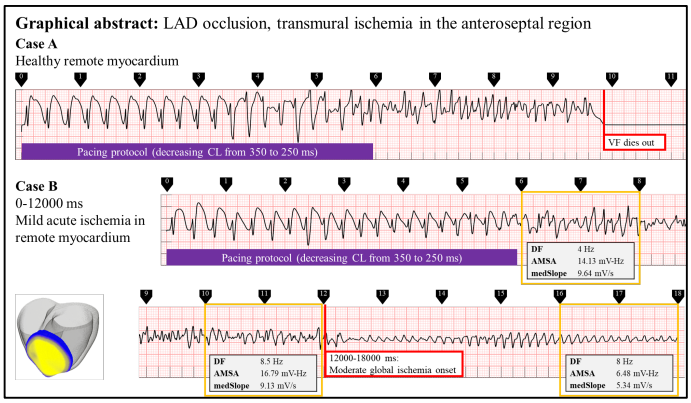
Conclusions

PTN is a promising candidate for neovascularisation following cardiac injury. Future work will examine how growth factors impact cell cycle regulation and SV differentiation into coronary arteries.

Graphical Abstract



POSTER NO: 2



Title: Progressive severity in global ischemia stabilizes ventricular fibrillation independently of co-existing regional heterogeneities

Authors: Hector Martinez-Navarro, Leto L Riebel, Blanca Rodriguez

Departmental affiliations: (postdoc, non-clinical) Computational Cardiovascular Science, Dept. of CompSci, Oxford

Research rationale

Ventricular fibrillation (VF) patients require urgent defibrillation to restore the coordinated activation of the heart. However, the efficacy of defibrillation is dependent on its timing. Acute myocardial ischemia is not only a precursor of ventricular tachyarrhythmias but also a consequence of VF. The dynamically changing ischemic substrate affects both, the inducibility and sustainability of VF. This interplay can be investigated by analysing electrocardiographic signals, which may be useful for predicting success in defibrillation. However, ethical limitations and data scarcity apply, as defibrillation success in fibrillatory patients is the highest priority. Modelling and simulation offer a powerful approach to investigate ventricular fibrillation dynamics and their manifestation on the ECG.

Methodology

Simulations of human ventricular electrophysiological activity from ionic currents to ECG were conducted using a novel GPU-accelerated software. 28 human 'virtual ventricles' were simulated affected by acute regional ischemia considering different locations, transmural extents, and severity of ischemia in the remote myocardium. Acceleration of sinus rhythm pacing was simulated to induce VF by applying progressively shorter cycle lengths. ECG signals were computed and analysed to quantify dominant frequency (DF), amplitude spectral analysis (AMSA), and median slope (medSlope).

Results

GPU-accelerated simulations enabled obtaining data for sustained arrhythmic events for longer than 10s and 28 ischemic scenarios. Ventricular tachycardia was inducible in all ischemic scenarios but not in the healthy ventricles. In cases with a healthy remote myocardium, the location of regional acute ischemia, namely LAD occlusion, determined VF inducibility: only 3/7 cases resulted in VF sustained for a maximum of 6s. Ischemia in the remote myocardium was key for tachycardia inducibility (DF=3-5Hz) and its posterior break up into stable VF (DF=7-8Hz) (see Figure); 6/7 cases presenting mild acute global ischemia yielded VF within 6s after the pacing protocol started. VF was indefinitely self-sustained only when ischemia affected the remote myocardium during and after the spiral wave break-up (14/16 cases). ECG analysis showed that the frequency spectrum became drastically homogenous when remote ischemic severity increased, and therefore AMSA was the most sensitive marker for describing VF. Moderate remote ischemia (9.95±2.22 mV-Hz; $p<0.001$) presented considerably lower AMSA values when compared with cases with no ischemia (28.2±3.41 mV-Hz) or mild ischemia (21.6±6.21 mV-Hz).

Conclusions

GPU-enabled simulations dissect the key factors driving ventricular fibrillation and its manifestation in the ECG in acute regional ischemia. Severity of ischemia in the remote myocardium is critical for inducibility of tachycardia and its transition to fibrillation. ECG markers do not necessarily reflect changes in fibrillatory dominant frequency. Human-based methodologies developed here offer a powerful test bed for the evaluation of novel therapeutic strategies.

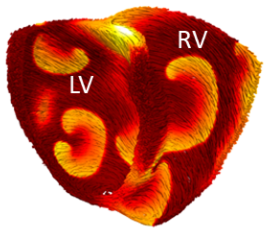


Figure: Visualization of the anterior myocardial wall (endocardial view) with sustained fibrillation.

References

[1] C. P. Bradley *et al.*, 2011, doi: 10.1161/CIRCEP.110.961284.

[2] G. Ristagno *et al.*, 2015, doi: 10.1161/CIRCULATIONAHA.114.010989.

POSTER NO: 3

Title: **The role of endothelial angiotensin II receptors in the release of nitric oxide**

Authors: L. C. Phillips[1], J. Lin[1], Z. Mohammadi[1, 2], C. J. Garland[1], K. A. Dora[1]
Departmental affiliations:
[1]University of Oxford, Department of Pharmacology, Oxford, United Kingdom.
[2] University of Bath, Department of Pharmacy and Pharmacology, Bath, United Kingdom

Research rationale. The renin-angiotensin-aldosterone system (RAAS) plays a major role in the cardiovascular system, including blood pressure regulation. The principal mediator of this system, angiotensin II (AngII), is known to act through two receptors, type 1 (AT1R) and type 2 (AT2R). AT1R has been thoroughly studied and acts to stimulate vasoconstriction, however, the role of AT2R in response to AngII remains undefined. We investigated whether AT2Rs and/or nitric oxide (NO) influence AngII-mediated vasoconstriction in rat small mesenteric arteries (RMA).

Methodology. Third order mesenteric arteries (200-300 µm) were isolated from male Wistar rats. Receptor expression was assessed using whole RMAs imaged *en face* for immunolabel. Vasoconstriction in response to AngII (10 pmol/L – 10 nmol/L) was measured using wire myography in the presence of either AT1R, AT2R or NO synthase (NOS) blockade with losartan (1 µmol/L), PD123319 (1 µmol/L) or L-NAME (100 µmol/L), respectively. Responses are presented as percentage of 45 mM K+ vasoconstriction. All data are shown as the mean±SEM, and subjected to two-way ANOVA with repeated measures (Greisser-Greenhouse correction) and Bonferroni multiple comparisons.

Results. Expression of AT1R was found within both vascular endothelial (VEC) and smooth muscle cells (VSMC), whereas AT2R was restricted to VECs. AngII stimulated concentration-dependent vasoconstriction in RMA (E_{max} 48.2±6.6%; EC_{50} 7.8 nM; n=28). Block of AT1R abolished vasoconstriction to AngII (E_{max} 1.0±0.3%, $p<0.05$; n=14). In contrast, AT2R block augmented vasoconstriction to AngII (E_{max} 102.6±23.6%; EC_{50} 1.7 nM; n=6). The influence of the endothelium was investigated in denuded arteries, where AngII vasoconstriction was markedly augmented (E_{max} 194.2±25.6% $p<0.05$; EC_{50} 1.7 nM; n=8), and was now not further increased during block of AT2Rs (E_{max}135.9±12.9%; EC_{50} 1.2 nM; n=5). Similarly, block of NOS with L-NAME augmented contraction to AngII (to that seen in denuded arteries; E_{max} 178.6 ± 23.2% $p<0.05$; EC_{50} 2.5 nM; n=21), and block with L-NAME was not further increased during block of AT2Rs (E_{max} 194.4 ± 29.2%; EC_{50} 0.8 nM; n=6), effectively removing the influence of the endothelium.

Conclusions. Together these results demonstrate that both VEC AT1R and AT2R suppress VSM AT1R-mediated vasoconstriction by releasing NO from the endothelium.

POSTER NO: 4

Title: Mechanism of neuropeptide-Y-stimulated vasoconstriction in rat mesenteric and coronary arteries
Authors: JinHeng Lin*, Lauren Scullion, Christopher Garland, Kim Dora
Departmental affiliations: Department of Pharmacology

*Presenting author; non-clinical postdoc.

Research rationale: The sympathetic co-transmitter neuropeptide Y (NPY) is a vasoconstrictor, and serum [NPY] is linked to a number of cardiovascular diseases. NPY receptors are $G_{i/o}$-coupled, but the downstream signalling pathways leading to vascular smooth muscle cell (VSMC) contraction remain poorly understood. Therefore, the present study aimed to investigate the mechanisms of NPY-induced vasoconstriction in rat mesenteric (RMA) and coronary (RCA) arteries.

Methodology: Third order mesenteric or septal arteries (100-360 μm diameter) from male Wistar rats were used for wire myography experiments. Myography data are presented as constriction to the maximal [NPY] (300 nM), E_{max}, normalised as a percentage of the constriction to 45mM K^+. For electrophysiology experiments, arteries were impaled with a microelectrode to measure VSMC membrane potential (V_m). Electrophysiology data are presented as change in V_m (ΔV_m) after stimulation with 100 nM NPY. For Ca^{2+} imaging experiments, arteries were loaded with Calbryte-520-AM to examine VSMC Ca^{2+} events, with data presented as events per second (Hz). Arteries were also fixed *in situ* of wire myograph chambers for immunohistochemistry. Values are expressed as the mean ± SEM.

Results: Under blockage of NO synthesis with L-NAME, NPY (0.1–300 nM) stimulated concentration-dependent vasoconstriction in both RMA (E_{max} 71.4 ± 13.5%, n=21) and RCA (E_{max} 74.4 ± 5.2%, n=6). This constriction was blocked by BIBO 3304 (1 μM), a NPY Y_1 receptor (Y_1R) selective antagonist (RMA 2.0 ± 1.1%, n=6; RCA 20.0 ± 1.1%, n=5) and selective agonists to Y_1R, but not Y_2R and Y_5R, constricted the arteries. Y_1R expression was detected in the endothelial cells and VSMCs of both RMA (n=6) and RCA (n=7) via immunohistochemistry. To investigate signalling downstream of Y_1R activation, gallein (100 μM), a selective Gβγ subunit inhibitor, attenuated NPY-induced vasoconstriction in RMA (5.8 ± 3.3%, n=5) and RCA (22.7 ± 6.8%, n=5). Furthermore, voltage-gated Ca^{2+} channels underlie NPY-mediated vasoconstriction, as nifedipine (1 μM) inhibited constriction of RMA (9.5 ± 1.6%, n=5) and RCA (6.6 ± 1.6%, n=5). In electrophysiology experiments, NPY treatment led to concentration-dependent depolarisation of RMA VSMC (ΔV_m 9.5 ± 1.2 mV; n=6), which was absent in the presence gallein (ΔV_m -3.0 ± 2.2 mV; n=5). Interestingly, at high [NPY] (10 – 100 nM), the arteries developed transient, action potential-like spikes (mean amplitude 18.0 ± 4.3 mV, at a frequency of 1.1 ± 0.2 Hz). This depolarisation could be linked to Ca^{2+} mobilisation, as 100 nM NPY stimulated RMA VSMC Ca^{2+} waves (0.31 ± 0.06 Hz, n=5) and whole-field synchronised Ca^{2+} flashes (1.03 ± 0.32 Hz, n=5), which were both completely abolished by pre-treatment with gallein (0 Hz for both waves and flashes; n=4).

Conclusions: Taken together, the present data suggest that in the VSMC of RMA and RCA, Y_1R activation dissociates the Gβγ subunit, which initiates Ca^{2+} mobilisation, membrane depolarisation, and raises the open probability of voltage-gated Ca^{2+} channels to cause vasoconstriction. This model could partially explain the development of microvascular vasospasm leading to cardiovascular dysfunction under sympathetic stress, especially in patients with poor endothelial function.

POSTER NO: 5

Title: Differential metabolic processing of propionate into β-alanine underpins the sex-dependence of histone modifications and contractile dysfunction in a mouse model of elevated propionate/propionyl-CoA

Authors: Kyung Chan (KC) Park***1**, Nicholas T. Crump[2], Niamh Louwman[1], Steve Krywawych[3], Kerrie L. Ford[1], David Hauton[4], Elisabete Pires[4], Andreas Koschinski[1], Ricardo Carnicer[5], Manuela Zaccolo[1], James McCullagh[4], Alzbeta Hulikova[1], Thomas A. Milne[2], Pawel Swietach[1]. **Presenter/Postdoc (non-clinical).*

Departmental affiliations: (1) DPAG (Oxford); (2) MRC WIMM (Oxford); (3) Great Ormond Street Hospital (London); (4) Department of Chemistry (Oxford); (5) Cardiovascular Medicine (Oxford).

BHF CRE Theme: Target Discovery.

***Research rationale*:** Propionate is a three-carbon metabolic intermediate produced from the breakdown of propiogenic substrates, such as branched-chain amino acids and odd-chain fatty acids. Under metabolic stress, propionate may accumulate to levels that can modify proteins post-translationally. If these chemical changes occur on histones, propionate may evoke a transcriptional response, resulting in profound and lasting effects on the heart. Here, we used a mouse model of propionic acidaemia (PA) to generate a stably raised level of propionate in tissues, including the heart, and investigate the consequences on cardiac gene expression and contractile function.

***Methodology*:** Sex-balanced experiments were performed on the hypomorphic mouse model of PA (*Pcca*$^{-/-}$ A138T) and their wildtype (WT) animals. Additional experiments used WT neonatal ventricular myocytes (NRVMs) treated with exogenous propionate. IC-MS and AQC derivatisation metabolomics was performed on MeOH-extracted metabolites. RNA-seq was carried out in heart tissue on an Illumina HiSeq 4000. Chromatin was isolated from PFA-fixed heart tissue for immunoprecipitation (ChIP) using acetylation (H3K27ac) and propionylation (pan-propionyl-lysine) antibodies. Ca^{2+} transients were imaged in freshly isolated myocytes (FuraRed). Contractile function *in vivo* was assessed by echocardiography and cine-MRI.

***Results*:** Mass spectrometry confirmed elevated plasma propionate in 8-week PA mice, reaching levels detected in PA patient serum. Metabolomic analyses confirmed a metabolic signature of PA that was largely sex-independent, with the exception of β-alanine (3-aminopropionate) and its dipeptides (carnosine, anserine) which were higher in male PA mice. Despite carrying the same mutation as males, female PA hearts had expanded end-diastolic and end-systolic volumes, weaker systolic contractions, and produced abnormal Ca^{2+} transients (raised diastolic Ca^{2+}), consistent with contractile dysfunction. Sex-balanced RNA-seq demonstrated an enrichment of the 'Cardiac Muscle Contraction' KEGG pathway in female hearts only. Among the differentially-expressed genes (DEGs), female PA hearts induced *Pde9a* and *Mme*, genes previously linked to cardiac dysfunction. These DEGs could also be upregulated by propionate *in vitro*, highlighting propionate as the chemical trigger in PA mice. Reference-normalised ChIP-seq showed genome-wide elevations in histone propionylation and acetylation, indicating two concurrent modes of action: direct transfer of a propionyl group from propionyl-CoA, and inhibition of histone deacetylases by propionate. Moreover, the increase in propionylation and H3K27ac were greater at the transcriptional start site of upregulated genes. Sex-balanced ChIP-qPCR experiments showed elevated histone acetylation and propionylation at the promoters of *Pde9a* and *Mme*, but only in female PA hearts.

***Conclusion*:** Perturbed propionate metabolism *in vivo* alters histone acylation and gene expression, which impacts cardiac contractile function. However, these effects were more profound in female PA mice, despite carrying the same genetic lesion as males. We speculate that augmented sequestration of excess propionate into the epigenetically-inert β-alanine pool, protects the cardiac genome from transcriptional changes in males. This mechanism may have a broader impact on how histones respond to short-chain acyls.

POSTER NO: 6

Title: Glucose metabolism regulates cardiomyocyte proliferation and heart regeneration in *Astyanax mexicanus* fish

Authors: R Alonaizan*[1], WT Stockdale[1], HG Potts[1], ME Lemieux[2], K Lekkos[1], J Walsby-Tickle[3], MTM Mommersteeg[1]
* Non-clinical postdoctoral fellow

Departmental affiliations: 1 Department of Physiology, Anatomy & Genetics, University of Oxford, Oxford, UK; 2 Bioinfo, Plantagenet, Canada; 3 Department of Chemistry, University of Oxford, Oxford, UK

Research rationale: Unlike humans, certain species possess a robust ability to regenerate their hearts. We identified the *Astyanax mexicanus* fish as a unique model for heart regeneration research. While *Astyanax* surface fish regenerate their heart after injury, their cave-dwelling counterparts cannot and, like humans, form a permanent scar. We found that there are similar levels of cardiomyocytes at the wound border zone that label for proliferation marker PCNA in both fish. However, BrdU pulse-chase revealed that these cells fail to contribute to new cardiomyocytes in the cavefish. This study examined the underlying mechanisms of successful versus defective cardiomyocyte proliferation in *Astyanax* hearts.

Methodology: Cardiomyocyte ploidy was quantified by measuring DAPI intensity in confocal images of thick heart sections. scRNA-seq, Seahorse and ATP metabolic assays were used to investigate regulators of cardiomyocyte proliferation.

Results: Examining cavefish cardiomyocytes showed failed cell cycle progression instead of successful division like in surface fish cardiomyocytes. We identified metabolism-related genes among those most differentially expressed. Specifically, glycolytic genes were highly upregulated in surface fish border zone cardiomyocytes, but not in cavefish. Consequently, there was a reduction in glycolysis in the cavefish heart after injury, resulting in reduced ATP available for regeneration in cavefish hearts compared to surface fish after injury. Inhibiting glucose metabolism in surface fish using 2-deoxy-d-glucose might recapitulate the defective cell cycle found in cavefish.

Conclusions: Glucose metabolism plays an important role in cardiomyocyte cell cycle progression. The inability to maintain sufficient ATP in the cavefish heart might be linked to their inability to support a regenerative response.

POSTER NO: 7

Title: Sodium-bicarbonate co-transport produces a milieu that is conducive for pro-hypertrophic signalling in cardiomyocytes.

Authors: Aminah Loonat[1], Friedrich Baark[2], Eleanor Gill[1*] (non-clinical postdoctoral scientist), Mark Richards[1], Alzbeta Hulikova[1], Kate Curtis[1], Stefania Monterisi[1], Bobby White[1], Abigail Wilson[1], James Clark[2], Pawel Swietach[1]

Departmental affiliations:
[1] Department of Physiology, Anatomy and Genetics, University of Oxford, Sherrington Building, Parks Road, OX1 3PT, Oxford, England
[2] School of Cardiovascular Medicine and Science, British Heart Foundation (BHF) Centre, King's College London, London, England

Research rationale: Maladaptive cardiac hypertrophy predisposes individuals to increased risk of heart failure and sudden death. However, current treatments are either inaccessible to drugs or are involved in several other pathways, and are therefore not suitable for slowing the progression of hypertrophy. Interestingly, one potential target would be the cell-surface SLC proteins NHE (*Slc9a1*), NBCe1 (*Slc4a4*) and NBCn1 (*Slc4a7*), which have been associated with growth of other cell types. The aim of this study was therefore to assess whether
Methodology: Cardiac hypertrophy was evoked in vivo by either chemical stressors or abdominal aortic banding (AAB), and changes in cardiac structure and function were assessed by echocardiography. Neonatal rat ventricular myocytes (NRVMs) also received chemical stressors before performing gene expression of *Nppa*/*Nppb*, protein phosphorylation of GATA4 and SRB staining, a previously established method of quantifying hypertrophy.
Results: NRVMs treated with 10μM PE exhibited increased SRB staining only in the presence of HCO_3^- and CO_2, and perinuclear/nuclear SRB ratio increased with increasing intracellular pH. Moreover, the PE-induced increase in SRB ratio and production of *Nppb* was attenuated following treatment with S0859, a drug that targets both NBCe1 and NBCn1. S0859 also inhibited the PE-induced phosphorylation of GATA4 and JNK in alkaline conditions, but not in the absence of HCO_3^-. Interestingly, *Slc4a7* but not *Slc4a4* expression was increased in hypertrophic hearts and NRVMs, and NBCn1 activity was higher in PE-treated NRVMs at more alkaline conditions, indicating that NBCn1 is likely the predominant HCO_3^- transporter involved in hypertrophy. Moreover, *Slc4a7* knockdown reduced PE-induced SRB ratio to a greater extent than knockdown of *Slc4a4*. To support this data, AAB increased diastolic LV interventricular diameter and anterior/posterior wall thickness, associated with reduced ejection fraction and fractional shortening; however, these differences were ablated following treatment with S0859.
Conclusions: NBCn1 is the predominant isoform involved in HCO_3^- dependent alkalinisation and consequent hypertrophy of cardiomyocytes, and its inhibition prevents cardiac hypertrophy and systolic dysfunction in vivo. Its cell surface expression therefore makes it an attractive therapeutic target for the prevention of cardiac hypertrophy and heart failure.
Abbreviations: AAB, abdominal aortic banding; NBCe1, electrogenic Na^+-$2HCO_3^-$ cotransporter; NBCn1, electroneutral Na^+-HCO_3^+; NHE1, Na^+/H^+-exchanger-1; NRVMs, neonatal rat ventricular myocytes; LV, left ventricle; PE, phenylephrine.
References: (1)

POSTER NO: 8

Title: Overcoming the Chemokine Network in Inflammation

Authors: Serena Vales*, Jhanna Kryukova, Megan Payne, Soumyanetra Chandra, Gintare Smagurauskaite, Katrin Hafner, Graham Davies and Shoumo Bhattacharya
*DPhil Student (non-clinical)
Departmental affiliations: RDM Cardiovascular Medicine

Research rationale: Monocytes and T-cells play a key role in the atherosclerotic plaque and in myocarditis. Multiple chemokines are known to be expressed in these diseases, resulting in a redundant network that cannot be targeted by drugs inhibiting a single chemokine or receptor. A perhaps unlikely solution we present are natural chemokine-binding proteins (CKBPs) derived from salivary proteins of ticks. Tick CKBPs (evasins) are categorised into two main classes, EVAA and EVAB, which bind to multiple CC- or CXC- chemokines respectively. The promiscuous binding of tick CKBPs overcomes chemokine redundancy and allows ticks to evade detection from the host at the bite site. CKBPs are effective in animal inflammatory disease models, but clinical translation has been limited by potential immunogenicity and production cost. Using structural biology approaches our lab made the key discovery of peptides from a tick class A CKBP, which overcome chemokine redundancy and the limitations imposed by immunogenicity and cost (1). However, they do not possess the broad range of chemokine-binding needed to overcome chemokine redundancy, requiring the identification of further candidate peptides.
Hypothesis: Phage-display can be used to identify peptides with broad range of chemokine binding activities from tick CKBPs.
Methodology: A phage display library consisting of hexadecapeptides at single amino acid resolution was generated from 21 tick class A CKBPs. The library was screened using 15 matrix-attached CC-class chemokines and bound phage analysed by next-generation sequencing (NGS) to calculate enrichment. Chemotaxis assays were performed using 96-well Boyden chambers using THP-1 monocyte or activated T-cells and migrated cells counted using flow cytometry. Peptide binding was assayed using biolayer interferometry using a HIS:SUMO:peptide fusion. Peptide potency and impact of alanine-scanning mutations were determined by performing chemotaxis assays using synthetic peptides. NNK saturation mutagenesis coupled with phage display and NGS was used to study the impact of mutations on chemokine binding, estimated by calculating delta.log2E i.e. the difference in enrichment (E) compared to the parental peptide. Modelling of peptide-chemokine docking was performed using AlphaFold2-Multimer.
Results: Mapping of peptides identified by phage display to the originating protein identified regions coincided with those previously documented to interact by structural methods. Four peptides identified from the phage-display screen based on their enrichment were synthesised and were shown to inhibit the chemokine CCL8. One of these peptides, HD2, derived from EVA4, was investigated further and found to weakly inhibit CCL2 and CCL3, but strongly inhibit CCL5, CCL7 and CCL8-induced THP1 migration, with median IC50s of 7.9E-07M, 8.8E-06M and 1.9E-08M respectively. HD2 also unexpectedly inhibited CXL10 and CXL11-induced activated T-cell migration. HD2 bound to CCL8 and CXL10 in biolayer interferometry experiments. Alanine-scanning mutagenesis identified residues that were important in inhibiting chemokine function which correlated well with delta.log2E ($R = -0.86$, $p=1.5E-11$). Modelling of HD2 binding to chemokine indicates that it targets parts of the chemokine "active-sites" i.e. the N terminus of CCL8 and the first beta-sheet of CXL10, suggesting it functions at least in part by steric hindrance.
Conclusions: These experiments provide proof-of-concept that the phage display-NGS method can identify hexadecapeptides from CKBPs that bind and inhibit multiple chemokines and identify key residues responsible for these functions. The limitations of these peptides are that they still do not bind enough chemokines with adequate potency to be useful therapeutic agents. To address this, I will be using *in vitro* molecular evolution of the active peptides obtained in this study to improve their range of binding and potency.

References:

1. Darlot, B., Eaton, J. R. O., Geis-Asteggiante, L., Yakala, G. K., Karuppanan, K., Davies, G., Robinson, C. V., Kawamura, A., and Bhattacharya, S. (2020) Engineered anti-inflammatory peptides inspired by mapping an evasin-chemokine interaction. *The Journal of biological chemistry* **295**, 10926

POSTER NO: 9

Title: Hexadecapeptides targeting T-cell recruiting chemokines
Authors: Megan Payne* (DPhil Student, non-clinical), Serena Vales, Graham Davies, Shoumo Bhattacharya
Departmental affiliations: RDM Cardiovascular Medicine

Research Rationale: Chemokines, small chemotactic proteins, drive leukocyte migration toward sites of inflammation. Selective targeting of chemokines that mediate inflammatory T-cell (e.g. $CD8^+$) recruitment in chronic cardiovascular diseases such as atherosclerosis and myocarditis may be of benefit in these disease conditions. Redundancy within the chemokine network has prevented the clinical development of anti-inflammatory therapeutics. Chemokine binding proteins (CKBPs) identified from ticks and viruses inhibit multiple T-cell recruiting chemokines overcoming redundancy but are unsuitable for clinical use. Our lab has shown that short peptides identified from tick CKBPs inhibit redundant chemokine activity[(1)] and has utilised phage-display to systematically identify CKBP-derived hexadecapeptides that bind multiple chemokines.

Hypothesis: The hypothesis underlying this project is that hexadecapeptides derived from tick and viral CKBPs will inhibit T-cell recruiting chemokines.

Methodology: $CD8^+$ T-cells were isolated from human leukocyte cones, and then activated and expanded. Activated T-cells were used in 96-well transwell migration assays, where cells migrating in response to chemokines CXCL9, 10, and 11 were quantified by flow cytometry. Hexadecapeptides identified by phage-display were screened for their ability to inhibit T-cell migration in response to CXCL10 and CXCL11. Screen hits were then confirmed in single-dose inhibition assays. The antagonistic activity of confirmed hits was then assessed with dose-response curves to measure IC50.

Results: Phage-display results were examined to select 23 hexadecapeptides derived from tick and viral CKBPs that showed binding to CXCL10 and 11. These hexadecapeptides were screened for the ability to inhibit CXCL10/11-induced T-cell migration. Several peptides that had inhibitory activity were identified. A single peptide, EB429, derived from the tick CKBP E1096, was shown to significantly and reproducibly inhibit activated T-cell migration in response to CXCL9, 10, and 11. The potency of EB429 as measured by IC50 against CXCL10 is 3.8E-6M.

Conclusions: This work confirms the hypothesis that hexadecapeptides derived from tick and viral CKBPs will inhibit T-cell recruiting chemokines. A limitation of the current work is the relatively low potency of EB429. In future work, I will attempt to overcome this by using saturation mutagenesis and phage-display selection to identify mutations and mutation combinations that enhance the potency of EB429. As CXCL9, 10, and 11 are present in multiple cardiovascular disease states, such as atherosclerosis and myocarditis, EB429 or mutated versions may have therapeutic potential in these diseases.

1. Darlot B, Eaton JRO, Geis-Asteggiante L, Yakala GK, Karuppanan K, Davies G, Robinson CV, Kawamura A, Bhattacharya S. Engineered anti-inflammatory peptides inspired by mapping an evasin-chemokine interaction. J Biol Chem. 2020;295(32):10926-39.

POSTER NO: 10

Title: Small peptide engineering for the development of chemokine network inhibitors.
Authors: Jhanna Kryukova* (DPhil Student, non-clinical), Serena Vales, Megan Payne, Graham Davies, Shoumo Bhattacharya.
Departmental affiliations: Radcliffe Department of Medicine Cardiovascular Medicine.

Research rationale: Aberrant inflammation is implicated in cardiovascular diseases such as atherosclerosis and myocarditis. There is a need for precision therapeutics to tackle inflammation without suppressing the systemic immune response. Inflammation is driven by chemokines, which activate migration of leukocytes to sites of tissue injury and disease. Chemokines have differential expression across diseased tissues, making them a promising target for the development of precision therapeutics. However, the chemokine-receptor network is highly redundant, and drugs targeting individual chemokines fail as anti-inflammatory agents. Promiscuous chemokine-binding proteins such as tick salivary evasins and short peptides derived from them have been shown to overcome this redundancy in models of immune cell migration[1]. HD2, a lead candidate short peptide developed in our lab, has shown strong inhibition of 4 chemokines. This level of promiscuity is yet insufficient to overcome the broad range of chemokines expressed in diseased tissues.

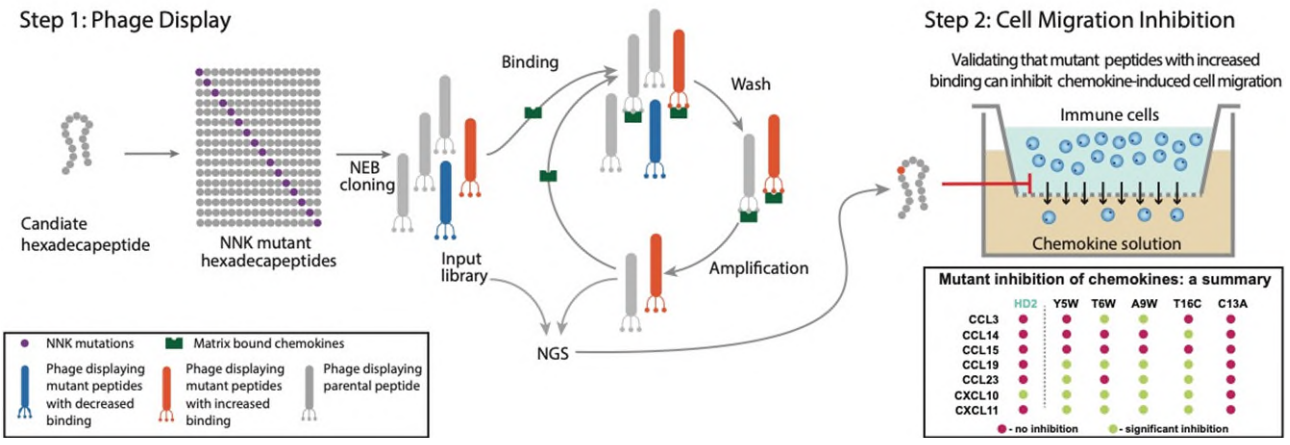
Hypothesis: *In vitro* molecular evolution will identify promiscuous HD2 variants that inhibit a wider profile of chemokines.

Methodology: NNK scanning saturation mutagenesis coupled with phage display and next generation sequencing was used for directed evolution of HD2. A phage library of 304 single-residue HD2 mutants was generated and screened for increased binding to 23 chemokines. Enriched peptides from the screen were analysed in chemotaxis assays with either THP1 monocytes or activated T cells in 96-well Boyden chambers.

Results: We identified 12 mutations that had marked enhancement of chemokine binding. To validate that they were also capable of inhibiting chemokine function, four exemplar mutants were selected and analysed by chemotaxis assays. All four peptides showed improved inhibition with different chemokines compared to the parental HD2. The best result was achieved with HD2-A9W and HD2-T16C, which inhibited 4 more chemokines than HD2. There is a clear correlation between phage display data and cell migration validation showing that peptides predicted to bind better in phage display translated well into improved inhibition. Importantly, peptides predicted to bind worse exhibited poor potency, which provides scope for fine-tuning the therapeutic peptide for a specific chemokine expression pattern.

Conclusions: This work provides clear proof-of-concept that *in vitro* molecular evolution can be used to enhance the promiscuous binding of a peptide to multiple targets, and that chemokine selectivity can be engineered by changing a single peptide residue. Additive or cooperative effects between different mutations will be explored by screening a combinatorial mutant library, followed by studies of binding (e.g., using biolayer interferometry). This approach can be applied to future peptide candidates and brings us closer to engineering precise peptide therapeutics for specific chemokines and indications.

References: *1. Darlot, B. et al (2020). Engineered anti-inflammatory peptides inspired by mapping an evasin-chemokine interaction. JBC, 295(32)*



POSTER NO: 11

Title: Peripartum Interventions to Prevent Cardiovascular Disease after Hypertensive Disorders of Pregnancy

Authors: Hannah R Cutler* (DPhil Student) (Non-clinical), Annabelle Frost, Jamie Kitt, Logan Barr, Adam Lewandowski, Winok Lapidaire, Annabelle McCourt, Jill Mollison, Katherine Louise Tucker, Katie Suriano, Yvonne Kenworthy, William Woodward, Cheryl Tan, Rebecca Mills, Maryam Kham, Elizabeth Tunnicliffe, Betty Raman, Mauro Santos, Cristian Roman, Henner Hannsen, Lucy Mackillop, Alexandra Cairns, Basky Thilaganathan, Lucy Chappell, Christina Aye, Richard Mcmanus, Keith Channon, Cheryl Tan, Surawee Chuaiphichai, Eric Ohuma, Manu Vatish, Paul Leeson

Departmental affiliations: Cardiovascular Clinical Research Facility, Radcliffe Department of Medicine, University of Oxford, Oxford, Oxfordshire.

Research rationale

High blood pressure during pregnancy affects more than 80,000 women annually in the UK and identifies women and children at significant risk of early onset cardiovascular diseases such as myocardial infarction, stroke, and hypertension. We have shown that women who had a hypertensive pregnancy have evidence of adverse cardiovascular remodelling in the years after pregnancy, which identifies those at risk of future cardiovascular disease. We are undertaking a programme of work to identify whether interventions around the time of pregnancy can impact peri-partum cardiac remodelling and reduce long-term risk.

Methodology

(1) *Systematic review* – I have undertaken a literature review exploring the short and long-term complications of hypertensive disorders of pregnancy and whether these are related to a distinct hypertensive phenotype. (2) *Post-partum blood pressure self-management:* the Physician Optimised Post-Partum Hypertension Treatment (POP-HT) is a randomised controlled trial involving 200 women with pre-eclampsia and gestational hypertension using blood pressure self-management via an app that provides algorithm-driven individualised care in the postpartum period. (3) *Vascular protection during pregnancy:* the Clinical Randomised Antenatal study to Characterise Key Roles of Tetrahydrofolate in Hypertensive Pregnancies (CAREFOL-HT) is a randomised controlled trial involving 96 women with early onset pre-eclampsia randomised to either a placebo, high dose, or low dose of tetrahydrofolate supplementation. Both trials include follow up to 6 months post-partum with assessment of blood pressure profile and cardiovascular remodelling with echocardiography and magnetic resonance.

Results

(1) The systematic review has shown that women with histories of hypertensive pregnancies have renal, ocular, cardiac and neural alterations which remain years after their pregnancy. These complications vary across the spectrum of hypertensive disorders of pregnancy and suggest a unique hypertensive phenotype is involved. (3) The POP-HT trial has demonstrated that a period of self-management for six weeks post-partum results in significantly reduced systolic and diastolic clinical and ambulatory BP 6 to 9 months postpartum. Additionally, echocardiography and cardiovascular magnetic resonance has found blood pressure reductions were associated with improved left ventricular systolic and diastolic function, aswell as beneficial atrial and ventricular remodelling and increased aortic compliance. (2) Our CAREFOL-HT trial is currently ongoing, but our results seek to demonstrate whether tetrahydrofolate supplementation improves cardiovascular outcomes for women with pre-eclampsia.

Conclusions

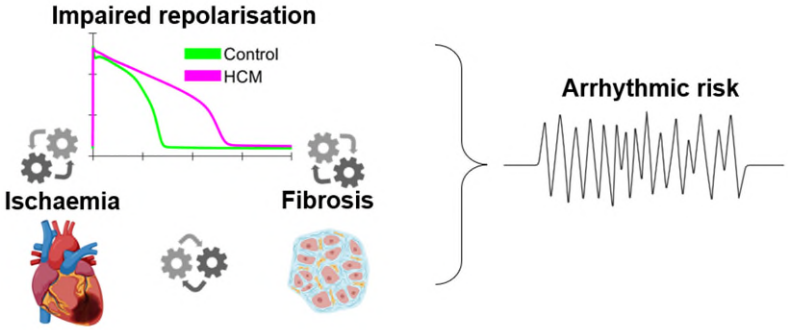
Findings so far provide evidence that peri-partum interventions can have long-term benefits for mothers including improved blood pressure and beneficial cardiovascular remodelling. Work is in progress to understand the mechanisms driving these long-term cardiovascular changes in the post-partum period to refine management strategies and improve the long-term cardiovascular health of women after hypertensive pregnancies.

POSTER NO: 12

Pro-arrhythmic interactions between myocardial ischaemia, fibrosis and ionic remodelling in human hypertrophic cardiomyopathy ventricles

James A Coleman[1*], Rubén Doste[1], Zakariye Ashkir[2], Rafael Sachetto[3], Betty Raman[2], Alfonso Bueno-Orovio[1]
[1]Department of Computer Science, University of Oxford, United Kingdom
[2]Department of Cardiovascular Medicine, University of Oxford, United Kingdom
[3]Department of Computer Science, Federal University of São João del-Rei, Minas Gerais, Brazil
*Presenting author – non-clinical DPhil student.

Research rationale – Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease and a leading cause of sudden cardiac death (SCD) in the young, with most SCDs being unpredicted. Myocardial ischaemia is acknowledged as a key contributor to SCD events in HCM, yet the mechanisms underlying how ischaemia interacts with other HCM disease factors (fibrosis, ionic remodelling) are incompletely understood, and this is further complicated by the impossibility of invasive studies in these high-risk patients. The present study aims to investigate the electrophysiological mechanisms by which arrhythmic risk during ischaemia is modulated by fibrosis and ionic remodelling in HCM. We hypothesise that: (1) hypertrophy-associated ionic remodelling increases arrhythmic risk from ischaemia by enhancing gradients of refractoriness, and that (2) fibrosis increases arrhythmic risk from ischaemia by exacerbating conduction slowing.



Methodology – Computational models of human cardiac electrophysiology were used to simulate the effects of phase 1A acute myocardial ischaemia in tissue and in HCM ventricles, where ventricular simulations were informed by common distributions of subendocardial/transmural ischaemia as analysed in perfusion CMR scans (N=28 patients). Using S1-S2 pacing protocols, arrhythmic risk was quantified for scenarios in which regions of septal obstructive hypertrophy are affected by (i) ischaemia only, (ii) ischaemia and ionic remodelling, and (iii) ischaemia, ionic remodelling, and diffuse fibrosis.

Results – Ionic remodelling in HCM enabled arrhythmia induction earlier in the onset of ischaemia than otherwise possible in control conditions, by enhancing gradients of refractoriness. In ventricular simulations, arrhythmias were more sustained in transmural ischaemia than subendocardial ischaemia. Competing effects were identified related to diffuse fibrosis. Because re-entries required propagation through ischaemic tissue that had scarcely recovered excitability, diffuse fibrosis could impair conduction and interrupt arrhythmia. However, where re-entries were possible, the fibrotic microstructure could force re-entrant wavefronts to emerge adjacent to refractory tissue, promoting wavefront-wavetail interaction and sustained ventricular tachycardia.

Conclusions – Our findings suggest that deleterious interactions between ischaemia, ionic remodelling and fibrosis underlie arrhythmic risk in HCM, contributing to an improved characterisation of disease-specific SCD mechanisms.

POSTER NO: 13

Title: Regulation of cAMP in response to α-adrenergic stimulation in atrial cells is dependent upon NAADP

Authors: **E. C. Akerman**[1*] (DPhil, non-clinical), S. J. Bose[1], M. Read[1], R. A. Capel[1], A. Koschinsky[2], D. A. Terrar[1], M. Zaccolo[2], R. A. Burton[2]

Departmental affiliations: University of Oxford, [1] Department of Pharmacology, [2]Department of Physiology, Anatomy and Genetics, Oxford, United Kingdom

Research rationale: Calcium (Ca2+) signalling is highly coordinated in atrial cells and abnormal intracellular Ca2+ homeostasis occurs in chronic atrial fibrillation (AF) [1]. Inositol trisphosphate (IP3), a Ca2+-mobilizing second messenger, modulates adenylyl cyclase (AC) activity in atrial myocytes [2]. Lysosomes participate in Ca2+ homeostasis regulation, acting as Ca2+ stores by mobilising Ca2+ in response to NAADP (Nicotinic Acid Adenine Dinucleotide Phosphate). Lysosomes have been shown to associate with IP3R clusters and sequester Ca2+ released by IP3R [3], suggesting the involvement of IP3Rs in regulating Ca2+ exchange between the sarcoplasmic reticulum and lysosomes. We postulate that both downstream activation of Ca2+ sensitive AC (AC1/8) and lysosomal Ca2+ signalling in response to IP3R activation via phenylephrine (PE) contribute to atrial myocytes function.

Methodology: All experiments complied with the United Kingdom Home Office Guide on the Operation of Animal (Scientific Procedures) Act of 1986. Guinea pig atrial cells were isolated enzymatically, fixed within 1h of isolation and stained using standard protocols. Intact murine right atrial preparations were maintained in oxygenated physiological solution at 37oC and allowed to beat spontaneously. IP3-mediated Ca2+ release was stimulated by cumulative additions of phenylephrine (PE, 0.1 to 30 μM) and dose-response curves fitted. Neonatal rat atrial myocytes (NRAMs) were isolated from 3-day old Sprague Dawley rats by enzymatic digestion. FRET imaging experiments were performed on day 3 of culture and 24 h after infection of the NRAMs with adenovirus carrying EPAC-SH187 cytosolic biosensor [4]. Data are presented as mean ± 95 % confidence interval (CI, dose response curves) or mean ± SD (FRET and colocalization analysis) relative to maximum response by addition of Forskolin (10 μM) and 3- Isobutyl-1-methylxanthine (IBMX, 100 μM).

Results: Maximum rate change is reduced from 24.5 ± 6 % in control conditions to 10.7 ± 3.2 % (n = 5) in the presence of 500 μM BZ-194 (inhibitor of NAADP binding), 6.3 ± 4 % (n = 6) in the presence 10 μM SAN4825 (inhibitor of cADPR and NAADP synthesis) and 11.9 ± 3.2 % (n=3) in the presence of bafilomycin A1 (vacuolar-type H+-ATPase inhibitor), in response to PE ($P<0.001$, 2-way ANOVA). We show for the first time, stimulation of the IP3 pathway in NRAMs using PE resulted in an increase in cyclic adenosine monophosphate (cAMP) level. Control conditions resulted in FRET change with average initial peak corresponding to 48.1 % (n= 17). In the presence of 10 μM SAN4825 or 100 nM Bafilomycin A1, the peak cAMP response to PE was reduced to 18.8 % (n= 62) and 31 % (n= 43) respectively. In the presence of the NAADP inhibitors Ned-19 (1 μM) and BZ-194 (500 μM), the cAMP peak response was reduced to 11.9 % (n= 73) and 8.1 % (n= 35) respectively ($P<0.0001$).

Conclusions: We have shown a crosstalk between IP3 and NAADP pathways, highlighting that lysosomal calcium is an important component of α-adrenergic stimulation in the cardiac atria and warrants further investigation.

References:

[1] Landstrom et al. 2017.
[2] Capel et al. 2021.
[3] Atakpa et al. 2018.
[4] Surdo et al.2017.

POSTER NO: 14

Title: **Modelling and Simulation Reveals Density-Dependent Re-Entry Risk in the Infarcted Ventricles after Stem Cell-Derived Cardiomyocyte Delivery**

Authors: [1]Leto L Riebel*[(DPhil student)], [1]Zhinuo J Wang, [1]Hector Martines-Navarro, [1]Cristian Trovato, [2]Jacopo Biasetti, [3]Rafael Sachetto Oliveira, [3]Rodrigo Weber dos Santos, [1]Blanca Rodriguez

Affiliations: [1]Department of Computer Science, University of Oxford, UK; [2]AstraZeneca AB R&D, Sweden; [3]Universidade Federal de Sao Joao del-Rei, Brazil; [4]Universidade Federal de Juiz de Fora, Brazil

Abstract

Research rationale: Recent research has explored delivery of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) to repair and prolong cardiac function after cardiac injury. However, hPSC-CMs express immature electrophysiological and structural properties presenting a potentially pro-arrhythmic substrate when introduced into the adult human heart.

Methodology: Here we simulate the delivery of hPSC-CMs into a computational human-based multiscale electrophysiological model of infarction. Membrane kinetics of adult and hPSC -CMs are based on state-of-the-art single cell models. A transmural infarct zone with a surrounding border zone is introduced, exhibiting conduction slowing and ionic remodelling based on experimental data. We introduce hPSC-CMs by sampling from a normal distribution taking the middle of the infarct as delivery centre and considering experimentally reported hPSC-CM patch sizes. Three densities are modelled by varying the sampling size and cover 4, 22, and 39% of the infarct and border zone (Figure 1). Arrhythmogenic re-entrant wavefronts are induced by applying an ectopic stimulus in the remote zone proximal to the border zone at varying coupling intervals from 360 to 480ms after the last sinus beat.

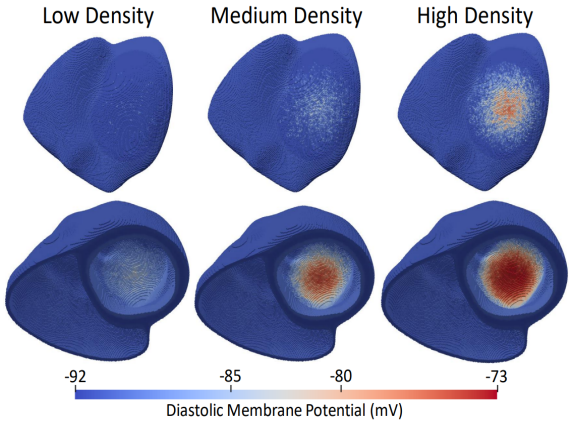


Figure 1. Simulated hPSC-CM densities in the injured human-

Results: Arrhythmic risk increases once hPSC-CMs are introduced and is highest in the medium and high-density scenarios. Increased refractory period of the hPSC-CMs compared to the infarcted adult tissue enables re-entrant wavefronts. Repolarisation times in the hPSC-CM region are increased from 410 in the low to 490ms in the high-density scenario, which causes an increase in local repolarisation dispersion around the delivery site, thus facilitating re-entry (Figure 2). We furthermore model the effect of different therapeutic agents on the arrhythmic risk and show its sensitivity to blocking the rapid delayed outward recitifier potassium current and the L-type calcium current.

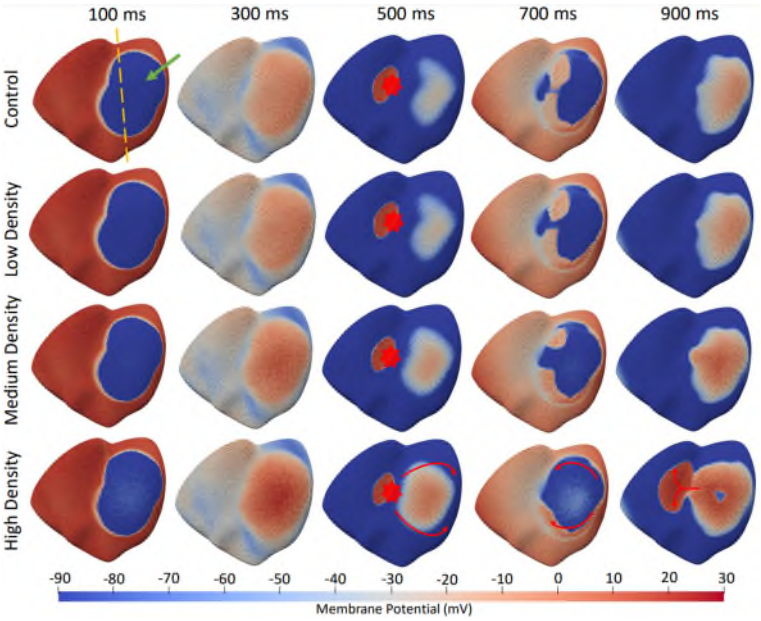


Figure 2. Electrical propagation across different hPSC-CM densities. Green arrow: infarct zone; yellow dashed line: axis between left and right ventricle; red star: application of ectopic stimulus; red arrows (bottom row): re-entry pattern.

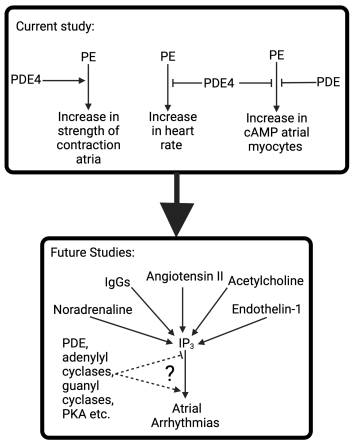
Conclusion: Our results highlight the density-dependent effect of hPSC-CMs delivery on arrhythmic risk in the infarcted ventricles and show the effect of drugs on the increased arrhythmia susceptibility.

POSTER NO: 15

Title: Targeting α-adrenergic signalling in cardiac atria through cyclic nucleotide phosphodiesterase (PDE) 4

Authors: Matthew J. Read[1*], Emily Akerman[1], Samuel J. Bose[1], Rebecca A. Capel[1], Andreas Koschinski[2], Manuela Zaccolo[2], Rebecca A.B. Burton[1]

Departmental affiliations: [1]*Department of Pharmacology*; [2]*Department of Physiology, Anatomy and Genetics*
*MSc(Res) Student (non-clinical). **I would NOT like to be considered to give a short talk at the symposium.**



Research Rationale: There is a need for novel atrial specific anti-arrhythmic drug targets due to current treatments adverse ventricular effects. Inositol 1,4,5 trisphosphate (IP_3) is pro-arrhythmic and preferentially affects the atria vs. ventricles[1]. IP_3 signalling is reported increased in atrial fibrillation contributing to its pathogenesis[2,3]. Thus, atrial IP_3 signalling is a potential atrial specific anti-arrhythmic drug target. At present, there is no clear strategy to specifically modulate atrial IP_3 signalling. Nevertheless, the positive chronotropic and inotropic effects of atrial IP_3 signalling, such as stimulated by the α-1 adrenergic agonist phenylephrine (PE), are dependent on IP_3 receptors, adenylyl cyclases and protein kinase A activity[4,5], suggesting cyclic nucleotide hydrolysing PDEs might also be involved. We hypothesised that inhibition of PDEs would alter the atrial response to IP_3 signalling.

Methodology: Non-specific PDEs were inhibited using IBMX. PDE4 was specifically inhibited with rolipram. To stimulate atrial IP_3 signalling PE was used after the addition of 2.5μM metoprolol to inhibit β-adrenergic signalling. The positive inotropic or chronotropic response was assessed by measuring the strength of contraction of electrically stimulated left atrial preparations or rate of spontaneous beating in right atrial preparations containing the intact sinoatrial node, respectively, from male CD1 mice, in 37°C physiological saline gassed with 95%O_2 5%CO_2. 3-parameter best-fit [PE]-response curves were fitted using Prism and curve parameters compared using Extra-sum of squares F-tests with a Bonferroni correction. To assess atrial cAMP signals Sprague-Dawley neonatal rat atrial myocytes (NRAM) expressing the cytosolic FRET (fluorescence resonance energy transfer)-based cAMP sensor EPAC2-S^{H187} were used.

Results: Pre-treatment with 10μM rolipram (n=6) increased the EC_{50} of the positive inotropic dose-response curve (**14.7μM** 5.03-41.5μM **vs. 0.849μM** 0.384-1.86μM; best-fit value, 95% CI; **P<0.0001**) vs. 0.002% DMSO control (n=7). 10μM or 100μM rolipram (n=9; n=10) increased the maximum of the positive chronotropic dose-response curve vs. 0.002% DMSO control (n=9) (**15.8%** 14.4-17.2% or **15.2**% 13.4-17.1% **vs. 11.8%** 10.2-13.4%; best-fit value 95% CI; **P=0.0002; P=0.0048**). In NRAMs prior treatment with 3μM IBMX or 25nM rolipram increased the average FRET % change of the response to PE (100μM) vs. DMSO control (**6.52%** 4.76-32.9 or **9.16%** 6.92-15.3% **vs. 3.72%** 1.72-5.02% respectively; n=8; **P=0.043** or **P=0.0144;** Kruskal-Wallis test**;** median 25th percentile- 75th percentile).

Conclusions: PDE4 inhibition alters the chronotropic, inotropic, and cAMP response to PE in rodent atria suggesting PDE4 is a regulator of the atrial response to PE and thus possibly IP_3 signalling. This data highlights the potential of targeting atrial IP_3 signalling through cyclic nucleotide signalling. Exploration of the effects of PDE modulation on IP_3 signalling induced ectopic activity in atria and the identification of the cyclic nucleotide nanodomains regulating and or stimulated by arrhythmic atrial IP_3 signalling is warranted.

Key References: [1]Lipp et al. 2000 (Current Biology); [2]Yamada J et al. 2001 (Journal American College Cardiology.); [3]Xiao J et al. 2010 (Experimental Biology and Medicine); [4]Wang YG et al. 2005 (Journal of Physiology); [5]Capel RA et al. 2021 (American Journal Physiology)

POSTER NO: 16

Title: **Immunophenotyping of the diabetic heart**

Authors: *Stephanie Anderson[1], Robert Hedley[2], Dunja Aksentijevic[3], Andrew Lewis[4], Damian Tyler[1,4]
Departmental affiliations: [1]Department of Physiology, Anatomy and Genetics, [2]Dunn School of Pathology, William Harvey Research Institute, Queen Mary University London, [4]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford. *non-clinical DPhil student

Research Rationale: The association between diabetes, increased risk of cardiovascular disease and inflammation is widely recognised but incompletely understood. Whilst increased systemic markers of inflammation have been observed in patients with diabetes and cardiovascular disease, it is still unclear exactly what the inflammatory phenotype of the diabetic heart is. Cardiac macrophages have gained increasing recognition for their canonical and non-canonical functions in supporting electrical and energetic homeostasis in the heart and sub-populating cardiac macrophages is an important part of understanding their functional roles. This work aims to elucidate how cardiac macrophages are affected by diabetes.

Methodology: Flow cytometry was used to investigate cardiac macrophage phenotypes in the diabetic *db/db* mouse model through a uniquely developed antigen profiling panel that supported identification and quantification of cardiac resident, anti-inflammatory, activated, antigen-presenting macrophages and infiltrating monocyte derived macrophages. This panel enabled deep phenotyping of cardiac macrophage subpopulations, optimally analysed using dimensional reduction analysis followed by unsupervised hierarchical clustering to identify clusters of macrophage subpopulations expressing novel combinations of antigen markers.

Results: Diabetic mouse hearts (n=15) had significantly fewer leukocytes, including a significant reduction in the number of macrophages compared to healthy controls (n=15, 58.3 v 138.5±31.8 SEM cells/mg, p=0.02, 20.3 v 36.9±4.7, p=0.002). Cardiac macrophages in diabetic hearts displayed significantly lower expression of markers associated with residency in the heart (CX3CR1, median fluorescence intensity (MFI) 6796 v 8788± 642.2, p=0.004), anti-inflammatory function (CD163, 1458 v 3773±860.5 MFI, p=0.01, CD206, 1547 v 4382±817 MFI, p=0.009), reparation and tissue healing (Ly6C, 5883 v 691±36.6 MFI, p=0.01) and canonical macrophage activation and antigen presentation capacity (CD86, 3187 v 3855±298.8 MFI, p=0.03, MHCII, 171 v 811±305.6 MFI, p=0.04). Changes in the relative frequencies of macrophage subpopulations also revealed a consistent reduction in cardiac resident populations (43.0 v 54.9±4.6 relative frequency (RF) of macrophages, p=0.01), anti-inflammatory populations (31.3 v 43.7±3.4 RF of macrophages, p=0.001) and activated, antigen presenting populations (64.7 v 76.6±4.3 RF of macrophages, p=0.01). High dimensional analysis of cardiac myeloid populations revealed an increase in the abundance of monocytes infiltrating diabetic hearts (38 v 24.2±3.9 RF of myeloid, p=0.03) along with more detailed phenotypic changes in the cardiac macrophage populations, including a loss of antigen presenting function in the cardiac resident populations (42.33 v 54.27±5.1 RF of myeloid, p=0.02) and a decrease in the population of infiltrating monocytes able to differentiate towards macrophage phenotypes (5.9 v 18.0±4.5 RF of myeloid, p=0.01).

Conclusions: Diabetic hearts undergo substantial remodelling of their immunophenotype, with significant changes in macrophage populations. Diabetic cardiac macrophages are polarised away from resident, anti-inflammatory populations supporting canonical macrophage functions and towards an increase in monocyte derived populations that are unable to acquire macrophage specific functional markers. Loss of resident cardiac macrophages could contribute to energetic and functional deficit in diabetic cardiomyopathy.

POSTER NO: 17

Title: The role of *ALPK3* in autosomal dominant Hypertrophic Cardiomyopathy – a chronic adrenergic challenge study

Authors: Lisa Leinhos[1]*, Giulia Poloni[1], Julia Beglov[1], Adam Lokman[1, 2], Gillian Douglas[1], Manuel Schmid[1], Evie Singer[3], Sophie Broadway-Stringer[3], Kate Thomson[1], Liz Ormondroyd[1], Ben Davies[4], Charles Redwood[1], Hugh Watkins[1], Katja Gehmlich [1,3]

Departmental affiliations:
[1]Division of Cardiovascular Medicine, Radcliffe Department of Medicine and British Heart Foundation Centre of Research Excellence Oxford, University of Oxford, UK
[2]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK
[3]Institute of Cardiovascular Sciences, University of Birmingham, UK
[4]Transgenic Core, Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK

*Presenting author, PostDoc

Funding: BHF project grant PG/19/45/34419, BHF CRE pump priming award.

Abstract:

Homozygous truncation variants in the *ALPK3* gene, which codes for alpha-protein kinase 3 (ALPK3), have been shown to cause recessive cardiomyopathy in infancy or early childhood. Our new genetic data indicate that *ALPK3* variants can also cause autosomal dominant adult onset Hypertrophic Cardiomyopathy (HCM), as an excess of heterozygous truncation variants in *ALPK3* was found in sarcomere-gene negative HCM patients compared to controls.

To investigate the underlying disease mechanisms, a mouse model carrying one of the *ALPK3* truncating variants found in HCM patients has been generated (*Alpk3* K201X). To unmask pathogenic effects of the *ALPK3* variant and to document the progression of cardiomyopathy, comprehensive *in vivo* phenotyping, including echocardiography and chronic adrenergic challenge have been performed.

Homozygous animals show significant reduced cardiac function, as well as cardiac hypertrophy compared to wildtype and heterozygous animals. In contrast, heterozygous animals had normal cardiac function and structure at baseline, as it has been the case for other cardiomyopathy genes. Therefore, heterozygous mice were challenged by adrenergic stimulation, delivered via osmotic minipumps, to induce hypertrophic pathways and to explore consequences on cardiac performance via echocardiography. Upon adrenergic stimulation, no differences in cardiac function parameters were identified across wildtype and heterozygous mice. However, heterozygous *Alpk3* K201X mice had increased left ventricular mass and left ventricular wall thickness upon adrenergic stimulation, indicating an aggravated hypertrophic response in these mice.

This novel mouse model will help to establish *ALPK3*'s role as a disease gene for autosomal dominant HCM and understanding its protein functions, interacting partners and the substrates is of high clinical relevance for future studies.

POSTER NO: 18

Title: Key role of endothelial cell Jcad in exercise induced vascular remodelling

Authors: *SAV Draycott[1,2], KE Shimell[1,2], E Drydale[2], KM Channon[1,2] and G Douglas[1,2]
* = presenting author (non-clinical postdoc)
Departmental affiliations:
[1]BHF Centre of Research Excellence, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, John Radcliffe Hospital, University of Oxford, UK
[2]Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, UK

Research Rationale

Acting as a powerful angiogenic stimuli, exercise results in beneficial vascular remodelling (VR) and is implicated as a key driver in the modulation of coronary artery disease (CAD) risk. The cellular mechanisms mediating these changes are however, poorly understood. We hypothesise that novel CAD implicated genes, which mediate their effects via changes in the vasculature, may offer novel insights into the mechanisms of exercise induced VR. In this study, we aim to elucidate whether the novel CAD gene JCAD, which we have shown has a key role in pathological VR, is also a critical regulator of exercise capacity.

Methodology & Results

Jcad null mice ($Jcad^{-/-}$) were given free access to a voluntary running wheel for 4 weeks. Global loss of Jcad was associated with a significant reduction in running capacity, both during the training and plateau phase, with $Jcad^{-/-}$ mice running ~6km less per night than their wildtype (WT) counterparts which was accompanied by a reduced gastrocnemius capillary to muscle fibre ratio. However, no difference in the number of running bouts was observed between groups suggesting Jcad does not influence initial exercise effort. To further investigate the specific role of endothelial cell Jcad, we developed an inducible endothelial cell specific Jcad knockdown mouse model ($Jcad^{EC\ -/-}$). Interestingly, during the first 8 days of training there was no difference in exercise capacity between $Jcad^{EC-/-}$ mice and WT counterparts, however after this point $Jcad^{EC-/-}$ mice ran on average ~1.7 km less per night than their WT counterparts.

Conclusions

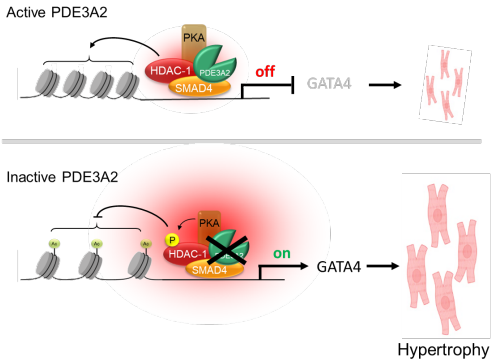
These findings suggest that the CAD implicated gene Jcad plays a key role in exercise capacity potentially acting through altered angiogenic responses of the vasculature to exercise.

POSTER NO: 19

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

Authors: Gunasekaran Subramaniam*, Katharina Schleicher, Duangnapa Kovanich, Anna Zerio, Andreas Koschinki, 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 subfamilies, PDE3A and PDE3B, and at least three PDE3A splice variants, PDE3A1, PDE3A2, and PDE3A3. PDE3 inhibitors are used in the clinic as a last resort positive inotropes to control acute heart failure that is unresponsive to other treatments. However, they are associated with increased mortality in the long term, characterised by worsened cardiac remodeling and fatal arrhythmic events. The mechanisms responsible for the cardiac long-term detrimental effects of PDE3 inhibition remain 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 in vitro hypertrophy assays. **Results:** GO term analyses of the PDE3 phospho-proteome and PDE3A1 and PDE3A2 isoform-specific interactomes identified several nuclear proteins. Among these, SMAD4, a mediator of Transforming growth f*actor-beta* (TGF-β) signaling, was significantly enriched in the PDE3A1 and PDE3A2 interactomes. Co-immunoprecipitation experiments confirmed the interaction of endogenous PDE3A with SMAD4. 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-PDE3A complex includes the epigenetic regulator Histone deacetylase 1 (HDAC1), a protein that is significantly enriched in the PDE3-specific phosphoproteome. Further analysis confirmed that inhibition of PDE3 increases the PKA-dependent phosphorylation on HDAC1 and that this phosphorylation results in inhibition of HDAC1 deacetylase activity. Further, PDE3A regulated PKA-dependent phosphorylation on HDAC1 leads to increased GATA4 expression and cardiac myocyte hypertrophic growth. **Conclusions:** Our findings are compatible with a model whereby PDE3A-SMAD4-HDAC1 constitutes a nuclear nanodomain where local regulation of cAMP levels by PDE3A modulates 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: 20

Title: Association of Cardiovascular Disease Mortality with Externally Validated Wrist-Based Step Count in the UK Biobank

Authors: *Scott Small[1, 2] (Non-clinical Postdoc), Shing Chan[1], Rosemary Walmsley[1], Benjamin Feakins[1], Lennart von Fritsch[2], Andrew Creagh[3], Andrew Price[2], Sara Khalid[2], Derrick Bennett[1], Aiden Doherty[1]
Departmental affiliations: [1]NDPH, [2]NDORMS, [3]Dept. of Engineering Science

Research Rationale
Step count is an intuitive measure of physical activity, regularly used in a range of clinical research areas. Wrist-based step counting is popular; however, accurate quantification of wrist-based step count can be difficult in the free-living environment, with step counting error frequently above 20% in both commercial and research-grade devices. This study aims to describe the development and validation of step count derived from a wrist-based accelerometer and to assess its association with cardiovascular disease mortality and all-cause mortality.

Methodology
We collected a ground-truth annotated, free-living step count dataset (n=39, aged 19-81) for development and externally validating a machine learning step detection model. This model was applied to raw wrist-based accelerometer data from UK Biobank participants to ascertain daily step counts. Participants with prevalent cardiovascular disease and cancer diagnoses were excluded from analysis. Cox Regression was used to obtain hazard ratios (HR) and 95% confidence intervals (CI) for the association of daily step count with fatal cardiovascular disease (CVD) and all-cause death after adjustment for adjusting for potential confounders.

Results
In external validation, the hybrid step algorithm predicted step count with an overall -1.1% mean overestimation bias and a 7.3% error collectively across a combination regular and interrupted gait. UK Biobank participants took a median 10,844 [8,314-11,268] steps per day. Participants in the most active quintile (≥14,527 daily steps) had HR (95% CI) all-cause and CVD mortality of 0.56 (0.50-0.64) and 0.43 (0.33-0.56) respectively, compared to the least active participants. Generally, there was an inverse dose response relationship between step count and CVD and all-cause mortality.

Conclusions
An externally validated step detection pipeline was developed from free-living data. In a large-scale cohort of UK adults, aged 40-79, a step count was inversely associated with CVD and all-cause mortality. Reduced mortality relative to the least active participants was apparent even at daily step counts below the popular 10,000 steps-per-day threshold.

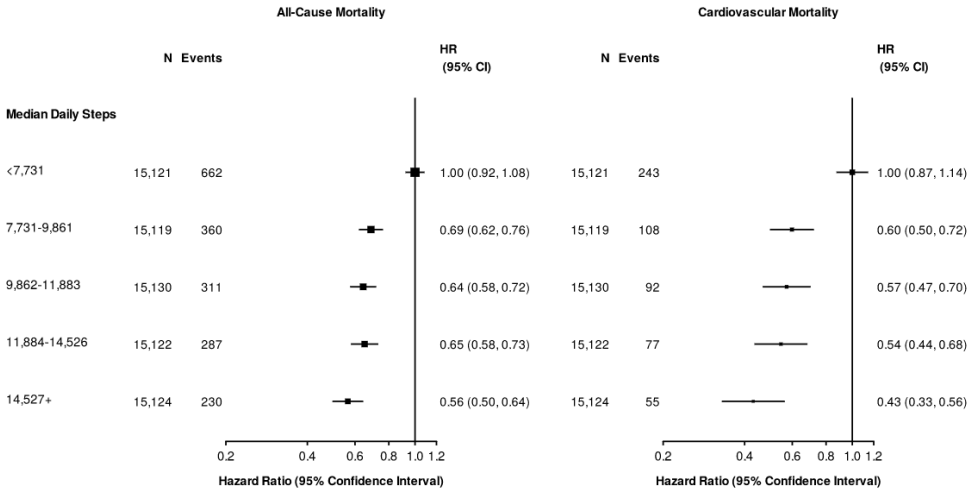


Figure 1: Forest plots for cardiovascular disease mortality and all-cause mortality associations with quintiles of daily step count. Hazard ratios (HR) and 95% confidence intervals calculated using age as a timescale, adjusted for demographic and dietary factors, and Charlson Comorbidity Index.

POSTER NO: 21

Title: Lysosomal calcium signalling contributes to acute physiological control of heart rate at the sino-atrial node pacemaker

Authors: Rebecca A Capel[1]*, Emily Akerman[1], Razik Bin Abdul Mu-u-min[1], Ifan Jenkin[1], Veerle Brans[1], Samuel J Bose[1], Margarida Ruas[1], Barry VL Potter[1], Antony Galione[1], Manuela Zaccolo[2], Derek A Terrar[1], Rebecca AB Burton[1]

Departmental affiliations: 1. Department of Pharmacology, 2. Department of Physiology, Anatomy and Genetics

* Presenting author, postdoctoral researcher, non-clinical

Research Rationale

Pacemaking at the sino-atrial node (SAN) is highly Ca^{2+}-sensitive and driven by a coupled system of Ca^{2+} and membrane 'clocks'. Acidic Ca^{2+} stores make a significant contribution to basal Ca^{2+} transient amplitude and β-adrenergic responses in contractile cardiac myocytes[1,2], requiring TPC2 Ca^{2+} release channels[3] at lysosome-SR membrane contact sites[4]. We have investigated whether endolysosomal Ca^{2+} signalling plays a role in SAN pacemaker function.

Methodology

Spontaneously-beating right atrial preparations with intact SAN were dissected from NCRL, *Tpcn2*$^{-/-}$, C57BL/6 or CD38$^{-/-}$ mice[3,5]. For dose-response curves (0.1 – 1000 nM isoprenaline [ISO]), spontaneous beating rate was measured by force transducer and log[agonist] vs. response curves fit by least-squares regression (GraphPad Prism v9). Guinea pig SAN myocytes were isolated by enzymatic digestion for Ca^{2+} transient imaging (Fluo-5F-AM). Murine and guinea pig experiments were carried out at 36°C in modified Tyrode's solution equilibrated with 95% O_2/5% CO_2. Data are presented as mean±SEM or mean (95% CI). Cellular cAMP synthesis was measured at room temperature using neonatal rat atrial myocytes expressing the cytosolic FRET (fluorescence resonance energy transfer)-based cAMP sensor EPAC2-S^{H187}.

Results

Abrogation of NAADP-mediated endolysosomal Ca^{2+} signalling by TPC2 knock-out significantly reduced SAN beating rate increase to cumulative ISO doses in spontaneously-beating right atrial preparations. NCRL wild-type mice exhibited a maximum response of 76% (69 to 83%, n=9), which was significantly blunted to 52% (42 to 62%, n=9) in *Tpcn2*$^{-/-}$ (P<0.05, F-test).

Voltage dye mapping (Di-4-ANBDQPQ) of intact murine SAN was used to investigate the requirement for NAADP synthesis in SAN responses. In preparations from wild type C57BL/6 the beating rate increase to 10 nM ISO was 46.3±3.7% (n=6). This was significantly reduced in the presence of Bafilomycin-A1 (Baf, 5 µM), to abolish acidic store Ca^{2+} loading (to 25.7±2.3%, n=6, P<0.05, ANOVA with Tukey's). Intact SAN preparations lacking the enzyme responsible for NAADP synthesis (CD38[5]), however, had a blunted rate increase to ISO (26.5±2.1%, n=6), which was not affected by Baf (27±2% increase, n=6, P=0.66, ANOVA with Tukey's).

Observations were maintained at the single cell level. In controls, 3 nM ISO caused a 77±7% rate increase (n=5) in spontaneously-beating isolated guinea pig SAN myocytes. In the presence of Baf (100 nM), this was reduced to 43±5% (n=6, P<0.05, T-test). In cultured neonatal cells, the presence of Baf (100 nM) or NAADP inhibitor BZ194 (500 µM) significantly reduced cAMP generation as measured by FRET (P<0.05, ANOVA).

Conclusions

This is the first study to investigate the role of acidic store Ca^{2+} in cardiac pacemaker physiology. Our data support the hypothesis that NAADP-mediated Ca^{2+} signalling plays a significant role in pacemaker modulation in the cardiac SAN, and that this may occur through modulation of cellular cAMP generation.

1. Collins *et al.* (2011) *Cell Calcium* 50(5): 449-58, 2. Macgregor *et al.* (2007) *J Biol Chem.* 282(20): 15302-11
3. Capel *et al.* (2015) *J Biol Chem.* 290(50): 30087-98, 4. Aston *et al.* (2017) *Sci Rep.* 7: 40620, 5. Lin *et al.* (2017) *J Biol Chem.* 292(32): 13243-57

POSTER NO: 22

Title: Machine Learning for the Computational Modelling of Hypertension Progression in the UK Biobank

Authors:
Zhaohan Xiong (Postdoc), Winok Lapidaire, Sam Krasner, Andrew Fletcher, Mohanad Alkhodari, Natalie Savage, Tobias Baumeister, Eric Ohuma, Ana Namburete, Pablo Lamata, Yasser Iturria-Medina, Adam Lewandowski, Paul Leeson

Abstract:
Background – Hypertension is associated with an increased risk of developing myocardial infarction, stroke, and heart failure. Imaging studies have shown that subclinical end organ changes in structure and function are evident in patients with hypertension and predict likelihood of later disease. Blood pressure measures may not always accurately reflect these changes as blood pressure can be transiently influenced by environment or medication. Identification of phenotypic characteristics associated with different stages of hypertension disease progression from multi-organ multi-modality data could improve the early detection and aid the development of preventative measures. In this study we aim to test the feasibility and performance of a computational modelling approach to describe phenotypic changes associated with hypertensive disease progression.

Methods – To date, 1086 imaging and clinical features from 27,338 UK Biobank participants have been used for model development. Participants were excluded if they had pre-existing cardiovascular diseases and then partitioned into healthy and diseased groups based on diastolic and systolic blood pressure thresholds (<120/80 and >160/100 respectively). Data cleaning was performed to remove patients who have experienced a heart attack or stroke. The healthy group were restricted to patients not on blood pressure medication, those without prior hypertension diagnosis, and those with normotensive blood pressure readings throughout all 3 visits. Features were equalized with z-score normalization, and outliers outside of 5 standard deviations from the mean were removed. The dataset was adjusted for age and sex, and harmonized between the different imaging centres. We used a contrasted trajectory inference (cTI) method to model variation in imaging (heart, brain, carotid arteries, and abdomen) as well as clinical features (measurements from electrocardiograms, spirometry, blood samples, and body composition) attributable to blood pressure in participants in the Imaging Enhancement of UK Biobank. The cTI methodology uses unsupervised dimensionality reduction and clustering of features which are enriched in the diseased group compared to the healthy population, allowing for the identification of patterns of end organ changes as hypertensive disease progresses. The reduced dimensional feature space was then used to construct a minimum spanning tree from which the normalized disease progression score (*HyperSCORE*) was computed. The stability of the cTI model for disease scoring was evaluated with ten-fold cross-validation and root mean squared error (RMSE).

Results – The cTI model was able to effectively identify a subset of 183 features which explained 84.3% of the total data variance. Brain imaging and body composition features contributed to 81.9% and 13.3% respectively in the cTI model, while cardiac imaging and blood sample features contributed to 2% each. cTI also effectively separated patients in the healthy and diseased populations with significant differentiation between groups (sensitivity = 96.0%, specificity = 96.8%, AUROC = 0.99, $p < 0.001$). Stability validation showed an RMSE of 6.6% ± 0.5% for score generation.

Conclusions – This preliminary data demonstrates feasibility of developing a pseudo-temporal disease progression model of end organ changes related to hypertension in the UK Biobank dataset. The work has revealed various feature modalities and trajectories of disease related to hypertension for further analysis. Current work is focused on using larger scale datasets, improved feature selection and validation of findings. In the future, HyperSCORE could be used to better asses patients' hypertensive disease progression and provide personalised treatment recommendations.

POSTER NO: 23

Title: The effects of chronic neuropeptide-Y exposure on human induced pluripotent stem cell-derived cardiomyocytes

Authors: Kun Liu* (non-clinical postdoc), Carla Handford, Ni Li, Thamali Ayagama, Dan Li, Neil Herring
Departmental affiliations:
Burdon Sanderson Cardiac Science Centre and BHF Centre of Research Excellence,
Department of Physiology, Anatomy and Genetics, University of Oxford

Background Acute myocardial infarction and chronic systolic heart failure result in high levels of cardiac sympathetic activation, and the release of the co-transmitter neuropeptide-Y (NPY). Moreover, our data show that circulating NPY levels correlate with morbidity and mortality in both conditions. Whilst the acute physiological actions of NPY have been well studied, the effects of chronic NPY exposure are poorly understood. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a powerful and evolving technology that has been applied to investigate the role of NPY.

Methods and Results We demonstrated the expression of the Y1, Y2 and Y5 receptors in hiPSC-CMs with immunostaining and western blotting and validated this using qPCR on human left ventricular muscle samples from patients undergoing valve surgery (n=5). Incubation with 1nM, 10nM and 100nM of NPY over 2 days significantly ($p<0.05$) increased cell area (following staining for troponin-T and α-actinin). The increase in cell size in response to NPY (10nM) was associated with increased expression of ANP assessed via qPCR and a decrease in ATP based cell viability (CellTiter-Glo 2.0). The effects of NPY on both cell size and viability could be blocked with the Y1 receptor antagonist (BIBO 3304, 1mM) and Y5 receptor antagonist (CGP-71683A, 1mM). We expressed forster resonance energy transfer–based sensor Epac-SH187 to monitor the cyclic adenosine 3',5'-monophosphate (cAMP) response to NPY in hiPSC-CMs. A reduction in cAMP levels in response to low dose NPY (1 and 10nM) was observed after adenylate cyclase activation with forskolin (Fsk 1 µmol/L) with higher doses (100nM) causing an increase in cAMP levels. Saturation of the sensor was achieved by using 25 µmol/L Fsk and the broad-spectrum PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX, 100 µmol/L) at the end of each experiment.

Conclusion These data support the hypothesis that chronic NPY exposure promotes cellular hypertrophy and reduces viability at least in part by modifying cellular cAMP dynamics. The NPY Y1 and Y5 receptors could be potential drug targets to treat chronic heart failure.

POSTER NO: 24

Title: **Circulating neuropeptide-Y dynamics during exercise in heart failure and young healthy controls**

Authors: Thamali Ayagama[1*], Peregrine Green[1], Cheryl M. J. Tan[2], Adam J. Lewandowski[2], Paul Leeson[2], Neil Herring[1]

Departmental affiliations: *Presenting author, Post-doctoral research scientist (Non-clinical). [1]Department of Physiology, Anatomy and Genetics, and [2]Oxford Cardiovascular Clinical Research Facility, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford.

Research Rationale:

Chronic heart failure (CHF) causes high levels of cardiac sympathetic drive and the release of the sympathetic co-transmitter neuropeptide-Y (NPY)[1]. Resting venous NPY levels have been associated with morbidity and mortality[2], but how levels change during exercise in relation to functional capacity is unknown. Cardiopulmonary exercise testing (CPET) is the established method of quantitative assessment of exercise performance via measurement of ventilation, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production. It is well validated in the heart failure population where quality of life and exercise capacity are inextricably linked. We sought to establish the dynamics of circulating NPY levels both in heart failure and a young healthy population and correlate this with indices of performance and cardiac function linked to long term prognosis.

Methodology:

Patients 6 months post cardiac resynchronisation therapy (CRT) device implantation underwent CPET with blood sampling at rest, peak exercise, and recovery as part of the SyncAV study (n=15). These were compared to normotensive, healthy controls who underwent CPET testing as part of the Young Adult Cardiovascular Health (YACHT) study (n=51). Data is expressed as mean±standard deviation or median [interquartile range].

Results:

15 heart failure patients (9 male, age 70.3±10 years, ejection fraction 29±7 % pre-CRT and 44±7 % post-CRT, p<0.00001) and 51 healthy controls (32 male, age 23.5±3.7 years, ejection fraction 63.3±5%) were recruited. Resting NPY levels were significantly higher in the heart failure group compared to healthy control group (39.2 [35.5-44.5] vs 17.1 [13.0-21.8] pg/ml, *p <0.0001*), however NPY levels at peak exertion were significantly lower (80.6 [62.2-129.6] vs 168.7 [101.6-250.6] pg/ml, *p=0.0015).* Peak NPY levels were positively correlated performance indices in healthy controls including peak oxygen consumption (r=0.35, *p= 0.006*), peak work (watts) (r=0.43, *p=0.001*) and heart rate at peak (r=0.33, *p=0.009*), but this was not observed in the heart failure group. In heart failure, peak NPY levels (r=0.58, *p=0.02*), and the ability to increase NPY from baseline (r=0.53, p0.04) significantly correlated with heart rate recovery at 1 minute.

Conclusion:

Patients with heart failure have impaired and abnormal NPY dynamics during exercise. Instead of correlating with performance indices, the ability to increase NPY levels on exertion in heart failure correlates with heart rate recovery, a known prognostic indicator for mortality.

References:

1. AJIJOLA, O. A. *et al*. 2020.

2. HERRING, N. *et al*. 2019.

POSTER NO: 25

Title: **PDE4D9 selectively regulates troponin I phosphorylation and cardiomyocyte relaxation**

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

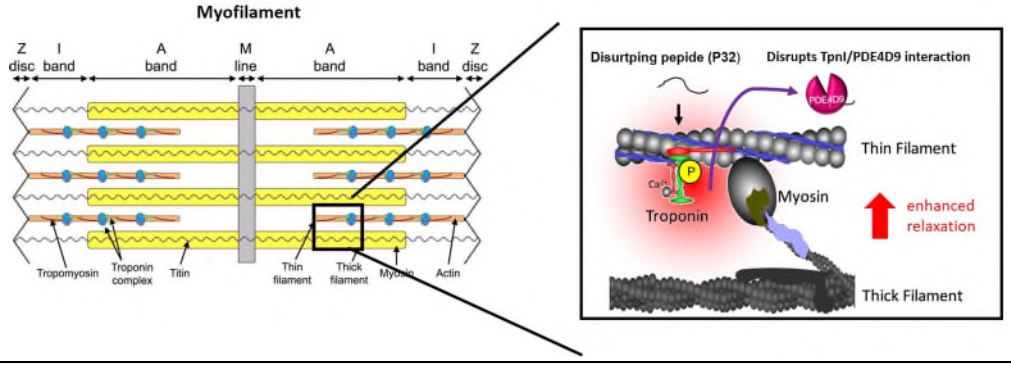
Rationale: Compartmentalisation of cAMP/PKA signalling underpins sympathetic regulation of the heart. Signalling compartmentalisation is achieved via spatial segregation of the molecular components of this pathway into distinct multiprotein complexes, or signalosomes, localised to different subcellular 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 a P32 disrupting peptides to interfere with this interaction. Furthermore, we explored the functional relevance of this regulation both in adult rat ventricular myocytes (ARVM) and human iPS-dervied cardiomyocytes (hiPSC-CM) as well as in a rat myocardial infarction disease model.

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 P32 peptide attenuate the PDE4D9-TPNI interaction. Detection of TPNI phosphorylation level and contractility experiments demonstrated that interfering with the interaction between PDE4D9 and TPNI affects β-ARs/cAMP/PKA signalling at TPNI and impacts on the myocyte contractile properties in $[Ca^{2+}]_i$ independent manner. These results were confirmed both in ARVM and hiPSC-CM . In addition, we found that the regulation of TPNI phosphorylation by PDE4D9 is impaired in infarcted hearts and normal regulation can be rescued by myocyte treatment with P32.

Conclusion: Our results demonstrate that TPNI phosphorylation is selectively regulated by a local cAMP nanodomain under the control of PDE4D9 and that MyBPC phosphorylation is regulated by a distinct cAMP domain. 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+} hypersensitivity.

Graphic Abstract:

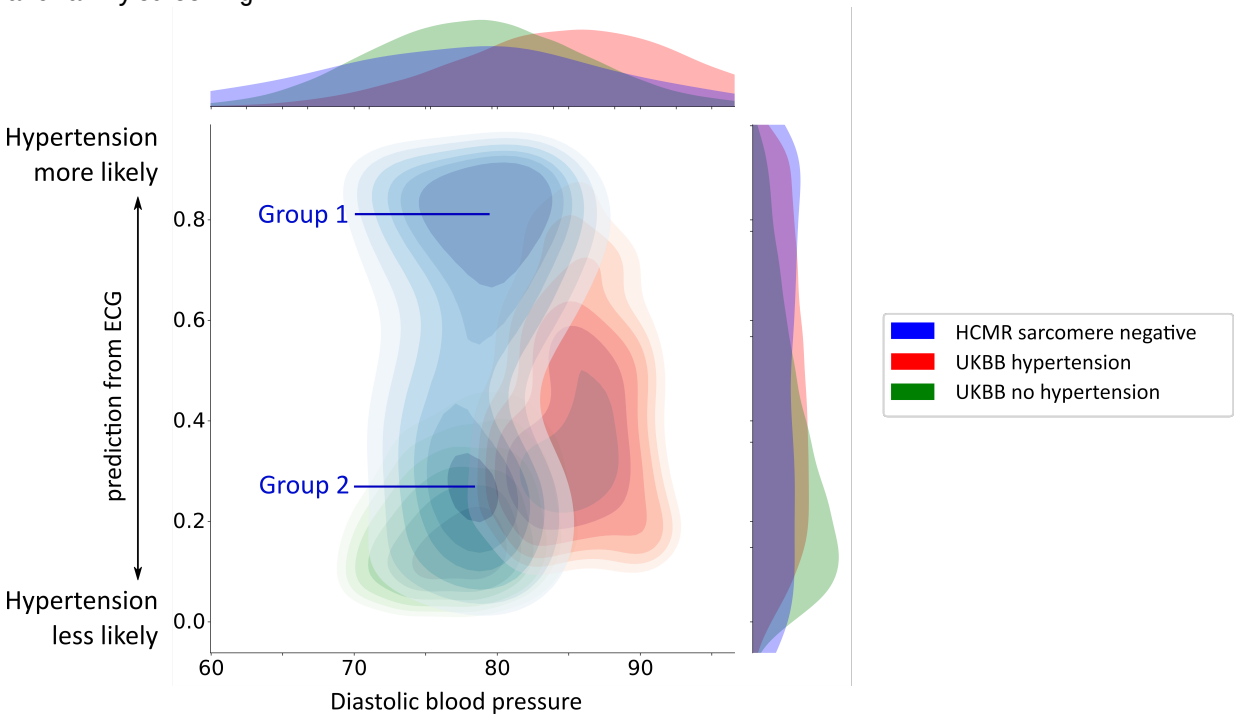


POSTER NO: 26

Title: Dissecting the aetiology of sarcomere-negative hypertrophic cardiomyopathy using convolutional neural network-ECG analysis

Authors: Sunil Manohar*[1,2], Vicente Grau[3], Stefan Neubauer[1], Hugh Watkins[1], Sanjay Manohar[4], Rina Ariga[1]
Departmental affiliations: [1]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, [2]Acute General Medicine, Oxford University Hospitals NHS Foundation Trust, , [3]Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, [4]Nuffield Department of Clinical Neurosciences, University of Oxford *Consultant Physician (clinical, unfunded)

Rationale: The majority of patients with hypertrophic cardiomyopathy (HCM) have a negative gene-panel test (sarcomere-negative; SN-HCM). The aetiology is likely heterogeneous, with known contributions from polygenic risk and modifiable risk factors, notably diastolic hypertension[1]. Abnormal electrocardiography (ECG) is common in hypertension and HCM. We hypothesised that ECG analysis could identify influences of hypertension in SN-HCM patients.
Methodology: A convolutional neural network (CNN), trained on hypertension diagnosis and 12-lead ECGs from 35,398 UK Biobank (UKBB) participants and 1,928 ECGs from patients in the HCM Registry[2], quantified ECG features of hypertension using 10-fold cross-validation. This trained model then scored features of hypertension in 1,225 ECGs from SN-HCM patients in the HCM Registry[2].
Results: Modelling reveals a bimodal distribution of ECG-hypertension scores within SN-HCM, suggesting two distinct phenotypic groups. Group 1 produces extreme ECG-hypertension scores well above those of the UKBB hypertensives, whilst Group 2 produces scores slightly above the UKBB with no hypertension, despite both groups demonstrating only marginally raised blood pressure readings (see figure).
Conclusions: CNN-ECG analysis suggests that SN-HCM patients can be partitioned into two groups: some have an exaggerated response to mild hypertension (matching previously reported Mendelian Randomisation findings[1]), while others do not (presumably indicating other drivers). We propose that CNN-ECG provides insight into the underlying aetiology of SN-HCM and could help guide patient management and family screening.



References

1. Harper, A. R., Goel, A., Grace, C. et al. Common genetic variants and modifiable risk factors underpin hypertrophic cardiomyopathy susceptibility and expressivity. *Nature Genetics*. **53,** 135–142 (2021).
2. Neubauer, S., Kolm, P., Ho, C.Y. et al. Distinct subgroups in hypertrophic cardiomyopathy in the NHLBI HCM registry. *Journal of the American College of Cardiology*, 74(19), pp.2333-2345 (2019).

POSTER NO: 27

Title: Empagliflozin prevents Doxorubicin cardiotoxicity and alters branched chain amino acid metabolism as measured by hyperpolarised [1-^{13}C]-Ketoisocaproate

Authors: Brett Kennedy*(Clinical; postdoc);Damian Tyler;Kerstin Timm
Departmental affiliations: CV Med; DPAG; Pharmacology

Research Rationale: Doxorubicin (DOX), an important cancer chemotherapy, can cause heart failure (HF). The cause is unknown but may be due to abnormal 'rewiring' of cardiac metabolism. We show that empagliflozin (EMPA), a diabetes medicine, prevents DOX cardiotoxicity and normalises cardiac metabolism. This may translate clinically into a way to prevent cancer patients developing DOX-HF. It also gives insight into how EMPA works in the heart. This study was funded by a BHF CRE pump-priming award.

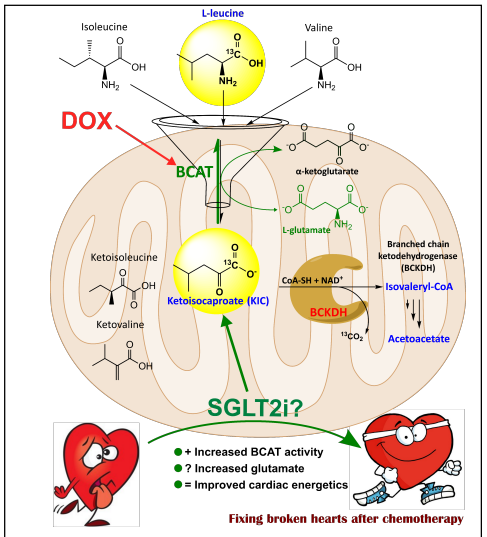


Figure 1: Graphical abstract. Metabolism of [1-^{13}C]-ketoisocaproate is altered following treatment with doxorubicin and is restored with concomitant empagliflozin treatment. This may improve cardiac metabolism and provide protection against the negative effects of chemotherapy

Methodology

Male Wistar rats were divided into 3 groups. Controls received drinking water/chow *ad libitum* and 5 x weekly IV injections (saline; 4ml/kg). DOX animals received the same as controls but received IV 0.75mg/ml doxorubicin. DOXEMPA animals received the same as the DOX group but had EMPA in their drinking water (final dose ~10mg/kg/rat). Cardiac function (ejection fraction; EF) was assessed using CINE MR imaging. Hyperpolarised ^{13}C data were acquired using [1-^{13}C]-ketoisocaproate (KIC). Following metabolic studies, hearts were rapidly excised, washed in ice-cold buffer and freeze-clamped. These hearts were used to make homogenates for biochemical analyses.

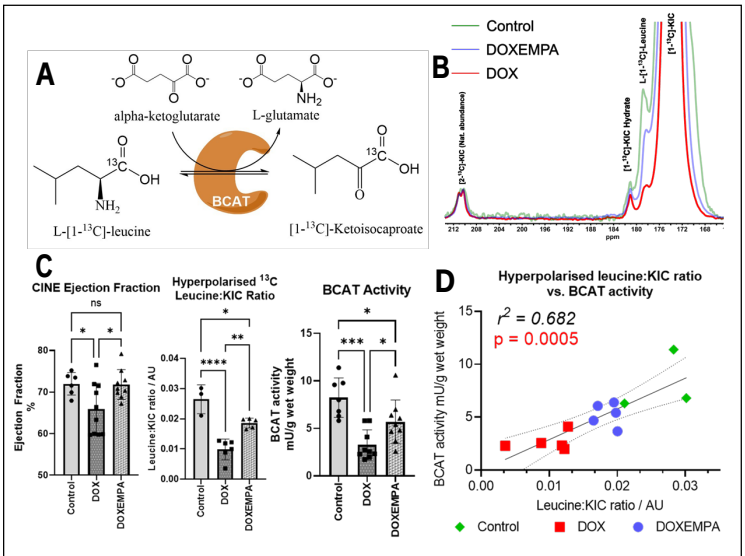


Figure 2: A) Diagram showing the enzymatic metabolism observed using hyperpolarised [1-^{13}C]-KIC. **B)** Representative spectra obtained after injection of hyperpolarised [1-^{13}C]-KIC into control, DOX and DOXEMPA rats. **C)** Panel showing a decrease in EF with DOX which is restored with EMPA treatment. This is mirrored by changes in observed ^{13}C metabolism and biochemical enzymatic activity in heart homogenates. **D)** The observed ^{13}C metabolism *in vivo* is directly correlated to enzymatic activity of heart homogenates *ex vivo*.

Results

DOX reduced EF compared to control animals. EMPA restored EF to that of controls. In metabolic studies, BCAA flux of hyperpolarised [1-^{13}C]-KIC to L-[1-^{13}C]-leucine was reduced following DOX. However, EMPA increased BCAA metabolic flux towards control values. Hyperpolarised flux measurements of BCAA *in vivo* were mirrored by changes in BCAT enzymatic activity of heart homogenates *ex vivo*.

Conclusions

EMPA prevents DOX induced reductions in EF. BCAA flux, catalysed by BCAT, is also restored following treatment with EMPA as measured by hyperpolarised [1-^{13}C]-KIC. The rewiring of BCAA metabolism by EMPA may have important cardiac implications beyond DOX cardiotoxicity.

POSTER NO: 28

Title: Mechanical Forces pull the strings on EndMT and Atherosclerosis via an Alk5-Shc Pathway

Authors: Vedanta Mehta[1,2]*,[#](post-doc), Kar Lai Pang[1,2#], Christopher S. Givens[3], Zhongming Chen[3], Jianhua Huang[3], Daniel T. Sweet[3], Hanjoong Jo[4], John S. Reader[1,2], Ellie Tzima[1,2]

Departmental affiliations:

1. Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.
2. Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK.
3. McAllister Heart Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
4. Coulter Department of Biomedical Engineering, Emory University and Georgia Institute of Technology, Atlanta, GA, USA.

[#]*Equal Contribution*

Rationale – Endothelial cells (ECs) lining blood vessels are constantly exposed to haemodynamic forces due to blood flow. The frictional force of fluid shear stress is a critical determinant of vessel health. In straight regions of arteries, shear stress is laminar and ECs are protected from disease. In contrast, regions where blood vessels bend/bifurcate are characterized by disturbed/oscillatory shear stress and ECs lining these regions show an upregulation of inflammatory markers, making these areas more susceptible to endothelial-to-mesenchymal transition (EndMT) and atherosclerosis. Our previous work has shown that the adaptor protein Shc mediates endothelial responses to shear stress *in vitro* and *in vivo*. We also hypothesize that TGFβR1 (Alk5), one of the principal receptors for EndMT, may play a role in pathological shear stress-dependent mechanotransduction pathways. The objective of the current study is to examine the requirement for endothelial Shc and Alk5 in flow-dependent EndMT and atherosclerosis.

Methods – Conditional endothelial specific Shc knockout mice (Shc^{ECKO} mice) were bred on to an ApoE-/- background and fed a high fat diet (HFD) for 8 weeks. In complementary experiments, partial carotid ligation (a model of accelerated atherosclerosis) was performed on control and Shc^{ECKO} mice prior to feeding a HFD for 3 weeks. Plaque deposition was assessed by Oil-Red-O staining, while EndMT was assessed by immunostaining of different markers. *In vitro* experiments involving application of atheroprone shear stress or tensional force on Alk5 were performed to elucidate the mechanistic role of Alk5 and Shc in EndMT.

Results – Reduced EndMT and plaque deposition were observed in Shc^{ECKO} mice compared to controls. Exposure of ECs to disturbed flow *in vitro* also resulted in reduced upregulation of EndMT gene expression in Alk5-depleted ECs and Shc-knockout ECs compared to control ECs. Tensional force application experiments demonstrated that Alk5 is a unique mechanosensor for EndMT responses that does not require other mechanosensors (like PlexinD1 or PECAM-1) but is dependent on co-operation with Shc.

Conclusion – Alk5 is mechanosensor that is *sui generis* for EndMT, and requires Shc to initiate vascular pathology.

POSTER NO: 29

Title: Prognostic value of exercise-induced ventricular ectopy in asymptomatic individuals

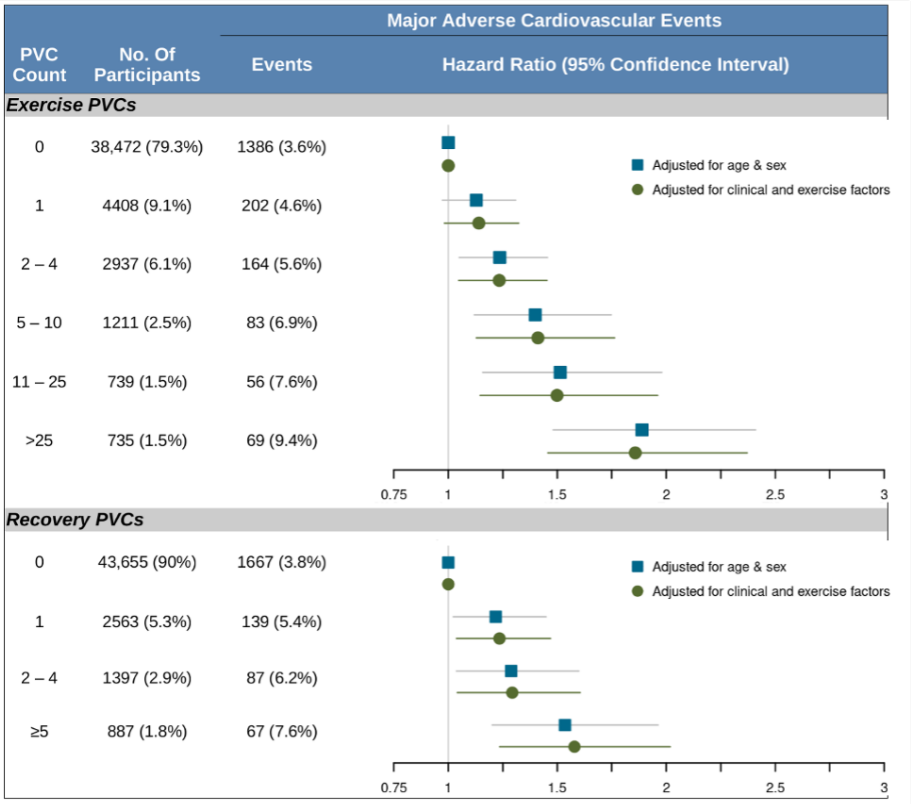
Authors: *Dr Stefan van Duijvenboden (non-clinical), Dr Julia Ramirez, Dr Michele Orini, Prof Andrew Tinker, Prof Patricia B. Munroe, Prof Pier D. Lambiase, Prof Aiden Doherty
Departmental affiliations: Nuffield Department of Population Health

Background: The consequences of exercise-induced premature ventricular contractions (PVCs) in asymptomatic individuals remain unclear. We aimed to study the association between PVC burdens during stress testing and major adverse cardiovascular events (MACE; myocardial infarction, life-threatening ventricular arrhythmia (LTVA), and heart failure (HF)).

Methods: A neural network was developed to count PVCs from ECGs recorded during exercise (6 min) and recovery (1 min). Associations with PVC counts were studied using multivariable Cox proportional hazard models in 48,502 asymptomatic participants from UK Biobank.

Results: Mean age was 56.8 (+/-8.2 years); 51.1% were female, and median follow-up was 11.5 years. After adjusting for clinical and exercise factors, >1 PVCs during exercise were associated with MACE, and risk increased with PVC count: hazard ratio [HR]: 1.2 (2-4 PVCs, p = 0.012) to 1.9 (>25 PVCs, p < 0.001). A similar trend was found for PVCs during recovery (HR: 1.2, (1 PVC, p < 0.026) to 1.5 (>4 PVCs, p < 0.001). Only high PVC burden was associated with ACM: HRs: 1.4 (11-25 exercise PVCs, p = 0.007) and 1.5 (>4 recovery PVCs, p < 0.001). PVCs were also strongly associated with incident HF and LTVA. The presence of complex PVCs further increased risk.

Conclusions: PVC count during exercise and recovery are both associated with MACE, ACM, HF, and LTVAs, independent of clinical and exercise stress test factors, indicating a “dose response” between PVC count and risk. Complex PVCs rhythms are associated with higher risk compared to PVC count alone.



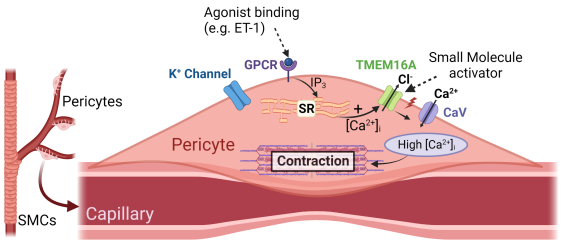
POSTER NO: 30

Title: Pharmacological stimulation of the TMEM16A anion channel results in prominent arterial and capillary constriction.

Authors: Rumaitha Al-Hosni*[1] (non-clinical post-doc), Emilio Agostinelli[1], Zeki Ilkan[1], Lara Scofano[1], Kathryn Acheson[1], Andrew MacDonald[2], Dean Rivers[2], Martin Gunthorpe[2], Frances Platt[1] and Paolo Tammaro[1]

Departmental affiliations: [1]Department of Pharmacology, University of Oxford, Mansfield Road, OX1 3QT, Oxford, United Kingdom. [2]Autifony Therapeutics Ltd., Stevenage Bioscience Catalyst, Gunnels Wood Road Stevenage, SG1 2FX, United Kingdom

Research rationale:



The TMEM16A Ca^{2+}-activated chloride channel constitutes a key depolarising force in arterial smooth muscle cells (SMCs) and contractile pericytes. The channel is a proposed target for diseases associated with impaired vascular tone including hypertension, stroke, and vascular dementia (Al-Hosni et al., 2022). However, the pharmacodynamics of synthetic inhibitors and activators of the TMEM16A channel is incompletely understood. Here, we provide insights into the mode of action of a recently disclosed positive allosteric modulator (PAM) of the TMEM16A channel and explore its effect on the tone of isolated arteries and capillaries.

Methodology:

Patch-clamp electrophysiology, wire-myography, and differential interference contrast (DIC) imaging were employed to define the mechanisms of action of small molecule modulators of the TMEM16A channel and their effects on vessel tone. Pericyte cell death in response to oxygen and glucose deprivation (OGD) conditions was assessed in freshly isolated cortical brain slices with propidium iodide assay, as previously described (Korte et al., 2022). For heterologous expression of wild-type and mutant channels (Dinsdale et al., 2021), TMEM16A was subcloned into the pCDNA3.1 vector and transfected into HEK293T cells. Data are given as mean±SEM of N independent observations.

Results:

In the presence of sub-maximal (300 nM) intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), PAM activated the heterologously expressed TMEM16A channels at positive and negative potentials (EC_{50}≈4 nM), while being almost ineffective on the closely related TMEM16B channel. PAM did not activate the TMEM16A currents in either the absence of intracellular Ca^{2+} or in the presence of saturating $[Ca^{2+}]_i$ (12 μM). Mutant TMEM16A channels with the intracellular gate constitutively open were much less sensitive to PAM, suggesting that PAM may act as a modifier of TMEM16A channel gating. Consistent with the effects observed in heterologously expressed TMEM16A channels, PAM activated endogenous TMEM16A currents in isolated rat aortic SMCs, promoted contraction of isolated aortic rings and enhanced cerebral capillary (pericyte) constriction evoked by endothelin-1 or OGD to stimulate cerebral ischemia. Conversely, inhibiting TMEM16A pharmacologically facilitated arterial and pericyte relaxation, and protected against OGD-mediated pericyte cell death.

Conclusions:

Our observations (i) enhance our understanding of fundamental mechanisms of pharmacological modulation of the TMEM16A channel and (ii) highlights TMEM16A as a key determinant of SMCs and pericytes tone. Collectively, these data suggest the TMEM16A channel may constitute a potential therapeutic target for the treatment of disorders of altered vascular tone.

References:

AL-HOSNI, R., ILKAN, Z., AGOSTINELLI, E. & TAMMARO, P. 2022. The pharmacology of the TMEM16A channel: therapeutic opportunities. *Trends Pharmacol Sci,* 43**,** 712-725.

DINSDALE, R. L., PIPATPOLKAI, T., AGOSTINELLI, E., RUSSELL, A. J., STANSFELD, P. J. & TAMMARO, P. 2021. An outer-pore gate modulates the pharmacology of the TMEM16A channel. *Proc Natl Acad Sci U S A,* 118(34).

KORTE, N., ILKAN, Z., PEARSON, C. L., PFEIFFER, T., SINGHAL, P., ROCK, J. R., SETHI, H., GILL, D., ATTWELL, D. & TAMMARO, P. 2022. The Ca^{2+}-gated channel TMEM16A amplifies capillary pericyte contraction and reduces cerebral blood flow after ischemia. *J Clin Invest,* 132(9).

POSTER NO: 31

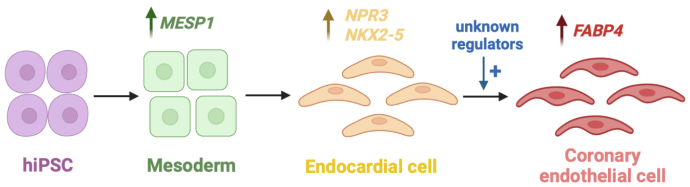
Title: Development of a human iPSC-based model of coronary endothelial cell development.
Authors: Dr Ian McCracken[1]* (post-doc, non-clinical) and Prof Nicola Smart[1]
Departmental affiliations: [1]IDRM/Department of Physiology, Anatomy and Genetics

Research Rationale: The embryonic heart develops as a two layered tubular structure consisting of an outer layer of cardiomyocytes and inner layer of endocardium. The endocardium acts as a vital source of multiple cell lineages in the developing heart, including a substantial proportion of the endothelium which is essential for the development of the coronary blood vessels[1]. Despite the evidence of endocardial plasticity during development, there is virtually no insight into how the endocardium becomes specified to form coronary endothelium. New human relevant models of coronary formation are required to identify novel factors which promote endocardial to coronary endothelial cell transition and may have a therapeutic benefit following reactivation in adult disease. Endocardial cells derived from human induced pluripotent stem cells (hiPSC) have the potential to be used as an innovative model of human coronary endothelial cell formation. However, improved methods are required to efficiently differentiate hiPSC-derived endocardial cells (hiPSC-ECC).

Methodology: hiPSC are first seeded onto laminin-221 coated plates before promoting commitment towards a mesodermal fate by day 2 of differentiation. Mesodermal cells are then cultured in media containing VEGF-A, FGF2, BMP-10 and XAV-939 (a Wnt pathway inhibitor) for four days to induce endocardial cell specification. This highly reproducible differentiation protocol uses only defined animal-free components, reducing batch variability and enabling application for potential cell therapy purposes.

Results: Induction of mesoderm fate revealed an increase in mesodermal marker *MESP1* by day 2 of differentiation. Following mesoderm specification, the addition of BMP10 and XAV-939 alongside VEGF-A and FGF2 was found to strongly induce expression of endocardial genes *NPR3* and *NKX2-5* in day 6 CD31+ cells. Flow cytometric analysis revealed ~60% of day 6 cells to be CD31+/NPR3+ with cells having a characteristic cobblestone morphology. Culture of CD31+/NPR3+ hPSC-ECC in media containing VEGF-B, a known regulator of endocardial angiogenesis in mouse[2], resulted in increased expression of coronary endothelial cell marker, *FABP4*.

Conclusions: I have developed a robust, xeno-free hiPSC differentiation protocol to generate hiPSC-ECC. The addition of BMP10 and XAV-939 following the specification of an intermediary mesodermal population strongly induced an endocardial identity by day 6 of differentiation. Upregulation *FABP4* in response to culturing hiPSC-ECC with VEGF-B suggests that hPSC-ECC can undergo a transition to form coronary endothelial cells, thus recapitulating the developmental mechanism and validating the application of this model to identify novel regulators of endocardial angiogenesis.



Differentiation of hiPSC to endocardial cells to provide a novel human relevant model of coronary endothelial development.

1. Wu B, Zhang Z, Lui W, Chen X, Wang Y, Chamberlain AA, Moreno-Rodriguez RA, Markwald RR, O'Rourke BP, Sharp DJ, et al. Endocardial cells form the coronary arteries by angiogenesis through myocardial-endocardial VEGF signaling. *Cell*. 2012;151:1083-1096. doi: 10.1016/j.cell.2012.10.023
2. Rasanen M, Sultan I, Paech J, Hemanthakumar KA, Yu W, He L, Tang J, Sun Y, Hlushchuk R, Huan X, et al. VEGF-B Promotes Endocardium-Derived Coronary Vessel Development and Cardiac Regeneration. *Circulation*. 2021;143:65-77. doi: 10.1161/CIRCULATIONAHA.120.050635

POSTER NO: 32

Altered expression of SCD1 is a key feature of hypercholesterolaemia induced trained immunity

Gareth S.D. Purvis[1*], Thomas Hiron[2], Jiahao Jiang[2], David R Greaves[3], Chris O'Callaghan[2] and Keith M. Channon[1]

[1]Wellcome Trust Centre for Human Genetic, Division of Cardiovascular Medicine, [2]Wellcome Trust Centre for Human Genetic, Nuffield Department of Clinical Medicine and [3]Sir Willian Dunn School of Pathology.
*Post-Doctoral Researcher

Introduction: Many patients who present with an acute cardiovascular event have undiagnosed hyperlipidaemia. Hyperlipidaemia is an independent risk factor for developing CVD, and lipid-lowering confers major clinical benefits. However, even patients in whom lipid levels are optimised many continue to experience coronary artery disease progression and/or recurrent acute cardiovascular events. It is possible that a component of this 'residual risk' may be mediated by the effects of prolonged exposure to hyperlipidaemia on monocyte and macrophage function, that are not reversed by lipid lowering in the short term. Therefore, we hypothesised that exposure to high cholesterol levels would cause a programmed trained immunity effect in bone derived marrow monocytes and macrophages.

Methods: To induce hypercholesterolaemia male C57BL/6 mice were injected with either gain-of-function mutation of mouse proprotein convertase subtilisin/kexin type 9 (AAV8- mPCSK9) AAV8-mPCSK9 and fed a high-fat diet, or control AAV8 and fed a chow diet for 12 weeks. Mice were then challenged with zymosan, and recruited cells collected for single cell RNA-Seq. Bone marrow monocytes were isolated from normo- or hyperlipidaemic mice for ATAC-sequencing analysis. Bone marrow derived macrophages (BMDM) were grown for metabolomic and functional analysis.

Results: Hyperlipidaemic mice have increased peritoneal recruitment of monocytes following zymosan challenge, while neutrophil recruitment remained the same, suggesting monocytes are primed by exposure to hyperlipidaemic for increased recruitment. BMDM from hyperlipidaemic mice had an enhanced pro-inflammatory profile, while M(IL-4) macrophages, failed to fully polarise. Metabolomic analysis revealed that M0 and M(IL-4) macrophages from hyperlipidaemic mice, had breaks in the TCA cycle, and decreased capacity for oxidative phosphorylation. ATAC-Seq analysis of bone marrow monocytes from hyperlipidaemic mice revealed a pattern of open chromatin located in the regions around stearoyl-CoA desaturase 1 (SCD1), while transcriptional analysis revealed that SCD1 was decreased in bone marrow monocytes, MO and M(IL-4) BMDM from hyperlipidaemic mice. The enzymatic produce of SCD1 activity mono-unsaturated fatty acid (MUFA) were also decreased in M0 and M(IL-4) BMDM from hyperlipidaemic mice.

Discussion: Hypercholesterolaemia causes an induced and sustained trained innate immunity effect in monocytes and macrophages. We propose that dysregulated expression of SCD1 resulting in decreased MUFA production maybe a potential mechanism by which monocytes and macrophages have an altered metabolic phenotype limiting their inflammation resolution capacity.

POSTER NO: 33

Title: Measurements of General and Central Adiposity in the Prediction of Cardiovascular Disease Risk and Hypertension Among South Asian Populations: A Systematic Review and Meta-Analysis

Authors: Federica Re MSc*, Ayodipupo S. Oguntade MSc, Fiona Bragg PhD, Jennifer L. Carter PhD
Presenting author (*) is a Graduate-Entry Medicine student at Oxford University and is clinical

Departmental affiliations: Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU), Nuffield Department of Population Medicine, University of Oxford.

Background and rationale: The increasing prevalence of obesity is a global public health concern. Body mass index (BMI)—a measure of general adiposity—has been used to determine obesity and cardiovascular disease (CVD) risk. Recently, measures of central adiposity, like waist circumference (WC), which better discriminate visceral fat, have been suggested to be more closely associated with morbidity. This is particularly relevant to South Asians who, compared to Caucasians, manifest larger WCs for equivalent BMIs. However, these populations remain understudied, with much of the evidence consisting of small cross-sectional studies prone to biases precluding true associations. Concurrently, while strong, positive associations have been reported between higher BMI and WC and CVD risk among Caucasians, there are mixed results regarding the shape and strength of the association in South Asians. This systematic review and meta-analysis provide an overview of the literature on the association of different anthropometric measures with CVD risk and hypertension among South Asians.

Methodology: MEDLINE and Embase were searched from 1990 to present for studies conducted on adult South Asian populations investigating two or more measures of adiposity against at least one CVD outcome. After duplicate removal, titles and abstracts were screened for 7,327 studies, yielding 151 full-text reviews. The final sample for the systematic review (n=34) included four prospective, three case-control, and 27 cross-sectional studies. Meta-analyses were conducted on the association of BMI (n=23) and WC (n=13) with hypertension. A fixed effects model was used, with heterogeneity determined using I^2 statistic.

Results: Overall, studies (n=28) reported significant, positive associations of adiposity measures with CVD risk, with no clear superiority of general or central adiposity measures for discriminating risk among South Asians. Ten cross-sectional studies reported that measures of central adiposity were more strongly associated with hypertension than those of general adiposity. Two prospective studies concluded similar findings. Nine studies concluded that the risk of hypertension was higher with a high BMI compared to WC. Meta-analysis showed that, compared with normal-weight individuals, overweight individuals ($BMI \geq 25 kg/m^2$) were at greater risk of hypertension, with a pooled odds ratio (OR) of 1.12 (95%CI: 1.11-1.14). Similarly, women with a WC>88cm and men with a WC>102cm were also at higher risk of hypertension (OR: 1.53, 95%CI: 1.46-1.59).

Conclusions: It remains unclear whether measures of central or general adiposity are superior in predicting CVD and hypertension risk among South Asians, and whether the association between these measures and CVD is indeed as strong and positive among South Asian populations as it is among Caucasians. Most evidence comprises small, cross-sectional studies, which are prone to biases, particularly reverse causality. Large cohort studies, comparing different measures of adiposity with risk of CVD and hypertension are needed to further clarify the relationship. Given CVD prevalence, this may have implications on primary prevention from a clinical and public health perspective.

POSTER NO: 34

Title: Selective palmitoylation of CD36 drives metabolic dysfunction in the type 2 diabetic heart

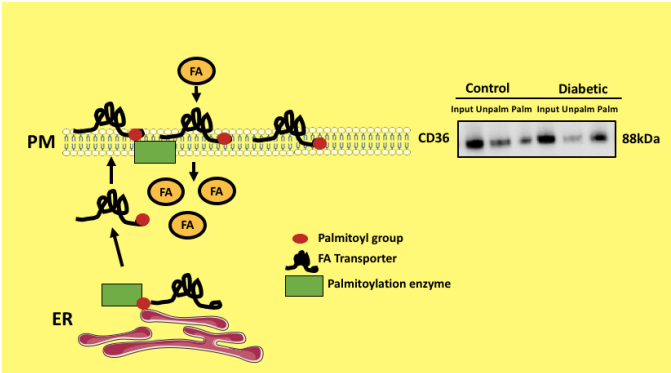
Authors: Kaitlyn Dennis *[1], U. Purnama[1], CN. Montes Aparicio[1], K. Timm[1], M. Castro-Guarda[1], D. Aksentijevic[2], W. Fuller[3], DJ. Tyler[1], LC. Heather[1].

Departmental affiliations: * DPhil student. [1] Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford. [2] William Harvey Research Institute, Queen Mary University of London, London [3] Institute of Cardiovascular and Medical Sciences, University of Glasgow

Research Rationale - Cardiac metabolism is altered in type 2 diabetes (T2D) and is associated with impaired cardiac function. The sarcolemma fatty acid transporter FAT/CD36 is the primary regulated step in fat metabolism and has been implicated in the development of diabetic cardiomyopathy. We questioned how CD36 changes in T2D, and whether palmitoylation, a reversible post-translational modification associated with protein trafficking, was driving metabolic remodelling in the diabetic heart.

Results - T2D was induced in the rat using a combination of high fat diet and a low dose of streptozotocin, increasing blood glucose from 5 to 12 mmol/l thereby generating a mild model of T2D. T2D hearts had abnormal substrate metabolism, with increased fatty acid oxidation, increased triacylglycerol storage and decreased glycolysis, as measured using radioisotopes in the perfused contracting heart. Total and surface-membrane CD36 protein content was increased in T2D, demonstrating increased translocation of this fatty acid uptake protein to the sarcolemma. Palmitoylation of CD36 was significantly increased in T2D hearts, but was absent for other substrate transporters including the glucose transporters GLUT1 and 4, and other members of the fatty acid transporter family. These findings were confirmed in human insulin resistant iPSC-cardiomyocytes and db/db mouse hearts. The enzymes regulating depalmitoylation did not change in diabetes, indicating they are not driving the hyper-palmitoylation observed. Interestingly, the regulatory enzymes involved in palmitoylating CD36, DHHC4 and DHHC5, trended towards an increase.

Conclusion- Increased cardiac fat metabolism is associated with redistribution of CD36 to the surface membrane and enhanced CD36 palmitoylation. Moreover, the palmitoylating enzymes DHHC4 and DHHC5 appear to be working in concert to enhance CD36 palmitoylation in diabetes. We suggest that enhanced palmitoylation of CD36 may contribute to the metabolic derangements in T2D, and future experiments will aim to reverse CD36 palmitoylation as a therapeutic strategy to improve cardiac function in the T2D heart.



POSTER NO: 35

Title: Human iPSC derived cardiac myocytes and sympathetic neurons in disease modelling

Authors: Ni Li * [1,2], Dan Li [1], Michael Edel[3], Kun Liu[1], Chris Denning[4], David J. Paterson[1]
Departmental affiliations:
1. Burdon Sanderson Cardiac Science Centre and BHF Centre of Research Excellence, Department of Physiology, Anatomy and Genetics, University of Oxford
2. Chinese Academy of Medical Sciences Oxford Institute, Nuffield Department of Medicine
3. Autonomous University of Barcelona Faculty of Medicine Dept Anatomy and Embryology Barcelona Spain; and Centre for Cell Therapy and Regenerative Medicine, Harry Perkins Research Institute, University of Western Australia, Perth 6009, Australia.
4. University of Nottingham Biodiscovery Institute, Faculty of Medicine & Health Sciences
* presenting author, DPhil student, non-clinical

Background
Human induced pluripotent stem cells (hiPSCs) offer an unprecedented opportunity to generate a potentially unlimited source of cells to develop model systems that facilitate a mechanistic understanding of human disease. However, the predictive ability of hiPSC derived neurocardiac co-culture systems to recapitulate the human phenotype in diseased modelling is limited. Here, we optimized current methods for efficient and replicable induction of cardiac myocytes (hiPSC-CMs) and sympathetic neurons (hiPSC-SNs). The utility of healthy hiPSC-CMs was tested with pressor agents to develop a model of cardiac hypertrophy. Mono-cultures and co-cultures were also made from a patient with a catecholaminergic polymorphic ventricular tachycardia (CPVT) genotype (with isogenic pairing) to generate a model of triggered arrhythmia.

Method
hiPSC-CMs and hiPSC-SNs were characterized by immunofluorescence, flow cytometry and calcium (Ca2+) imaging. Healthy hiPSC-CMs were incubated with angiotensin II (AngII) or endothelin-1 (ET-1) for 48 hours. Immunostaining, qPCR and ELISA were performed to measure cell size, pro-BNP expression and secretion in hypertrophic and control groups. cAMP was measured by Förster resonance energy transfer (FRET). Neurocardiac co-cultures were established by re-seeding spontaneously beating hiPSC-CMs (day 20) with mature hiPSC-SNs (day 40). Intracellular Ca2+ transients were measured in myocytes in response to isoprenaline, and in neurons in response to nicotinic stimulation.

Results
Healthy hiPSC-SNs possessed neurite outgrowth, stained positive for PHOX2B, tyrosine hydroxylase and peripherin. Derived myocytes showed spontaneous beating, stained positive for cardiac troponin T and a-actinin. Cell surface area was significantly increased in older iPSC-CMs (day 40) (606.3±290.4μm² vs 3299.8±967.9μm², p<0.0001) compared to younger cells (day 20). Sarcomere length extended from 1.59μm to 1.88μm (p<0.0001). Healthy hiPSC-CMs exposed to AngII 0.1 μM (n=23), or ET-1 100nM (n=13) resulted in cell and nuclear enlargement, as well as enhanced proBNP gene expression and proBNP secretion. This overexpression was reversed by losartan in AngII treated cells. Cytoplasmic cAMP levels were increased by isoprenaline, which was higher in hypertrophic cells. CMs from the CPVT hiPSC line expressed a higher Ca2+ responsive to isoprenaline, caffeine and KCl stimulation when compared with healthy hiPSC-CMs. They also displayed spontaneous Ca2+ oscillations after isoprenaline. CPVT hiPSC-SNs had greater Ca2+ transients to nicotinic stimulation, indicating a diseased phenotype also resides in the neuron as well as the myocyte.

Conclusion
We have recapitulated many features of the anatomy and (patho)physiology of SN and CM, where co-culture preparations behave in a manner that mimics key physiological responses seen in other mammalian systems. Whether our cell types have the full transcriptomic atlas of actual human cells remains to be established.

POSTER NO: 36

Title: Human engineered heart tissue cultured in a “diabetic” media recapitulates the type 2 diabetic heart: A multi-omics study.

Authors: Ryan Carter *#[1], Ujang Purnama #[1], Anandhakumar Chandran [2], Richard Mbasu [2], Charlotte Daly [2], Maxwell Ruby [2], Lisa Heather [1] and Carolyn Carr [1]
Departmental affiliations: [1] Department of Physiology, Anatomy and Genetics, University of Oxford [2] Novo Nordisk Research Centre Oxford. * Presenter (non-clinical DPhil). # Contributed equally.

Diabetes affects nearly half a billion individuals worldwide and increases the risk of developing cardiovascular disease (CVD. Therefore, biological models representing the human diabetic heart are needed to understand the disease pathology and develop new treatments. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) can be used to produce engineered heart tissue (EHT), and have facilitated the study of the adult human heart in vitro. We questioned whether culturing human EHT in a “diabetic-like media” would recapitulate changes seen in the type 2 diabetic (T2D) heart. Utilising multi-omics approaches we investigated known metabolic pathways disrupted in diabetes in our EHT model, and identified novel and emerging mechanisms that could be targeted therapeutically.

Parallel differential abundance and expression analysis of proteomics and transcriptomics identified expected changes in proteins/genes within the KEGG-defined pathways ‘insulin signalling’, ‘PPAR-alpha signalling’, and ‘oxidative phosphorylation’ within EHTs exposed to hyperlipidemic, hyperglycaemic and hyperinsulinemic media. Hypothesis-agnostic approaches identified significant negative enrichment for the HALLMARK-defined ‘PI3K-Akt-mTOR signalling’ pathway, validating our EHT model as capturing the human in vivo diabetic phenotype. We also identified a reduced abundance of the V-type proton ATPase subunit with increased CD36 expression in the diabetic-EHT, an emerging mechanism of lipid-induced cardiomyopathy Furthermore, the abundance of proteasomal subunits decreased alongside significant negative enrichment of the KEGG-defined ‘ubiquitin-mediated proteolysis’ pathway, establishing the proteasome as an exciting area for future work in T2D.

EHT cultured in a diabetic media recapitulate many key pathway changes present in the T2D heart, but also identifies novel pathways not previously associated with the disease. This validates the use of EHTs as a model for studying the T2D heart and for future drug development.

POSTER NO: 37

Title: **Beyond the Krebs cycle: Fumarate reprogrammes metabolic preferences within human cardiomyocytes.**

Authors: **Marcos Castro-Guarda[1*#]**, Claudia N. Montes Aparicio[1], Ujang Purnama[1], Kaitlyn M.J.H Dennis[1], Thomas Nicol[2], Mark J. Crabtree [2], Lisa C. Heather[1].

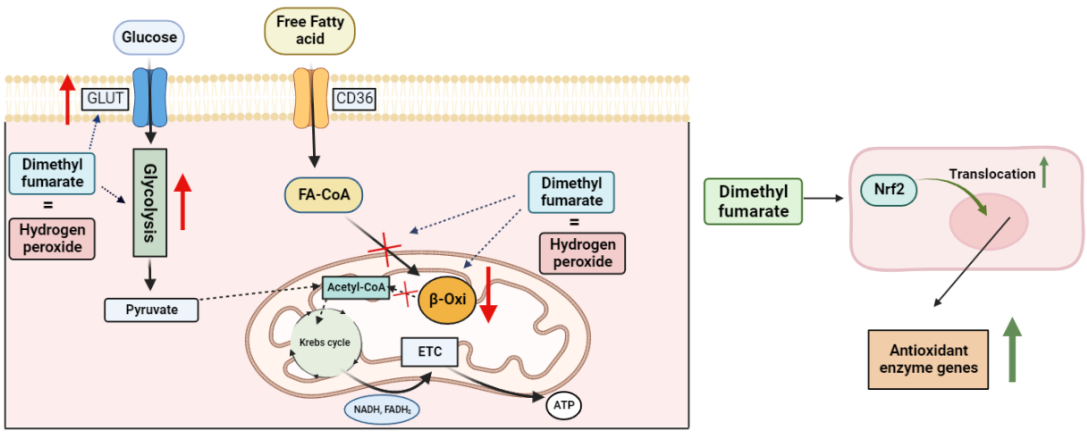
Departmental affiliations: * DPhil student. # BHF CRE funded research. [1] Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford. [2] Division of Cardiovascular Medicine, Radcliffe Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford.

Research rationale: Intracellular fumarate concentrations increase dramatically in the heart during ischemia. Fumarate can activate Nrf2 (Nuclear factor erythroid 2 [NF-E2]-related factor 2), a well-known transcription factor, which regulates antioxidant enzymes expression. However, there is evidence in the liver that Nrf2 can also regulate metabolism. Therefore, the purpose of this study is to evaluate if the use of exogenous fumarate derivatives can influence cardiomyocyte metabolism, through the derivative that has already been approved for use in humans for the treatment of autoimmune diseases.

Methodology: Matured human iPSC-derived cardiomyocytes (hiPSC-CM) were treated with dimethyl fumarate or hydrogen peroxide. The effect on gene and protein expression was evaluated using qPCR and western blotting, respectively. For the measurement of palmitate oxidation and glycolytic rates, media was supplemented with [9,10-^{3}H] palmitate or [^{3}H] glucose, respectively. Mitochondrial oxygen consumption was measured using a Seahorse analyser.

Results: In human cardiomyocytes, dimethyl fumarate metabolically reprogrammed cardiac substrate utilisation by decreasing genes involved in fatty acid metabolism, followed by upregulating glucose metabolism genes. This resulted in decreased palmitate oxidation rates and increased glycolytic rates and lactate production following treatment with dimethyl fumarate. No changes in the mitochondrial Krebs cycle or oxidative phosphorylation genes, protein levels or mitochondria oxygen consumption were detected, indicating a shift in fuel preference within the cardiomyocyte. To understand the mechanism driving this metabolic reprogramming we showed that dimethyl fumarate promoted Nrf2's nuclear translocation and upregulation of antioxidant genes, known to be the traditional targets of Nrf2. When we exposed cells to hydrogen peroxide to induce oxidative stress, we found Nrf2 translocation, antioxidant gene induction but also the same metabolic shift from fat to glucose metabolic genes.

Conclusions: Dimethyl fumarate emerges as a key molecule to modulate cardiomyocyte metabolism, decreasing fatty acid oxidation and increasing glycolysis. Our data indicates this is via the same Nrf2 mechanism activated by oxidative stress.



POSTER NO: 38

Title: Does machine learning of the electrocardiogram improve 10-year risk prediction of cardiovascular disease?

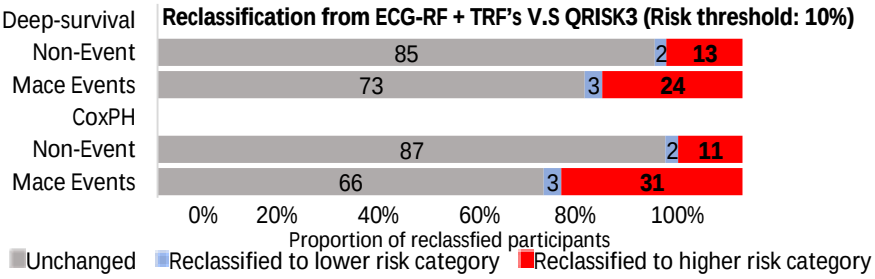
Authors: *Adam Sturge (DPhil Student, non-clinical), Dr Stefan van Duijvenboden, Prof Barbara Casadei, Prof Aiden Doherty,
Departmental affiliations: Nuffield Department of Population Health

***Research rationale*:** Recent state-of-the-art performance in diagnosing cardiovascular disease risk factors, comparable to that of a human expert, has been demonstrated using deep-learning techniques. However, the utility of these methods in predicting the future risk of Major Adverse Cardiovascular/Cerebrovascular Events (MACCE) is unknown. Therefore, we aimed to investigate the added value of deep learning of electrocardiogram (ECG) signals to traditional MACCE risk prediction and stratification methods.

***Methodology*:** We analysed short-duration (15s), single-lead ECG data (Lead I) from the UK Biobank submaximal cardiorespiratory fitness assessment sub-study recorded between 2006-2013. All selected participants had 10-years of follow-up, no known cardiovascular disease, and a complete history of traditional risk factors specified in the QRISK3 algorithm. Participants with missing or poor-quality ECG data were excluded from the analysis. The study endpoint was MACCE, defined as death or hospitalisation for (1) myocardial infarction, (2) arrhythmia, (3) heart failure, or (4) Stroke/transient ischaemic attack. Cardiovascular disease history and incident MACCE status were obtained from hospital inpatient data. In a training and validation subset (60%/20%, respectively), we derived an ECG risk factor (ECG-RF), independent of traditional risk factors, via a convolutional and deep-survival neural network. We compared three 10-year risk scores for MACCE: (1): traditional-risk-factors only, (2): ECG-RF, and (3): ECG-RF + traditional-risk-factors. The performance of each score was evaluated using conventional Cox proportional hazard (CoxPH) models and deep survival networks in a separate testing set (20%). The performance of each score was evaluated against QRISK3 by its ability to successfully reclassify participants, reported as Net-Reclassification Index (NRI) and model discrimination.

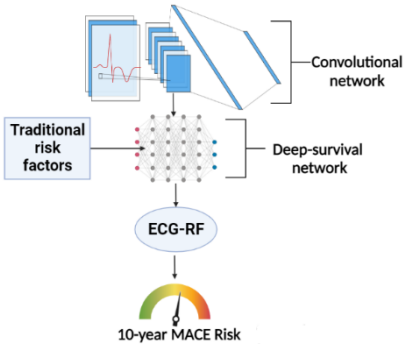
Results: 59,962 participants were followed up for a median of 10.9 years (IQR:0.2), with 1,923 (3.21%) incident cases of MACCE reported. ECG-RF was not significantly associated with incident MACCE (Hazard ratio (95% CI): 1.04(0.99-1.10), p = 0.14). Despite this, ECG-RF+QRISK3 showed improved reclassification vs QRISK3 alone in both CoxPH and deep-survival risk scores; NRI (95% CI): CoxPH 0.08(0.05-0.11) p < 0.001, deep-survival 0.09 (0.06-0.12) p <0.001. However, there were no observable changes in model discrimination in either approach: C-index (95% CI) QRISK3: 0.71(0.70-0.73) vs. CoxPH: 0.72(0.70-0.73), p = 0.34; deep-survival: 0.72(0.70-0.73), p = 0.99.

Conclusions : For individuals without known cardiovascular disease, the addition of ECG-RF, when adjusting for population-specific traditional risk factors, successfully reclassified high-risk individuals with incident MACCE. Further analysis is needed to identify specific sub-populations that most benefit from an ECG. Future work will investigate the utility of deep learning in extended wearable ECG measurements for predicting acute coronary syndromes and MACCE relative to contemporary approaches (QRISK3).



Proportion of reclassified individuals by combined ECF-RR + traditional risk factors ,risk scores, in Cox proportional hazards and deep-survival models, vs QRISK3.

**Abbriavtions: TRF = Traditional risk factor*



Derivation of ECG-RF and 10-year incident risk of MACCE

POSTER NO: 39

Title: Dietary Short-Term Upregulation of *De Novo* Lipogenesis in Healthy Adult Humans Does Not Increase Atherogenic Lipoprotein Production

Authors: David Dearlove, Nikola Srnic* (DPhil Student, Non-Clinical), Elspeth Johnson, Paige O'Rourke, Sion Parry, Leanne Hodson

Departmental affiliations: DPAG, OCDEM-RDM

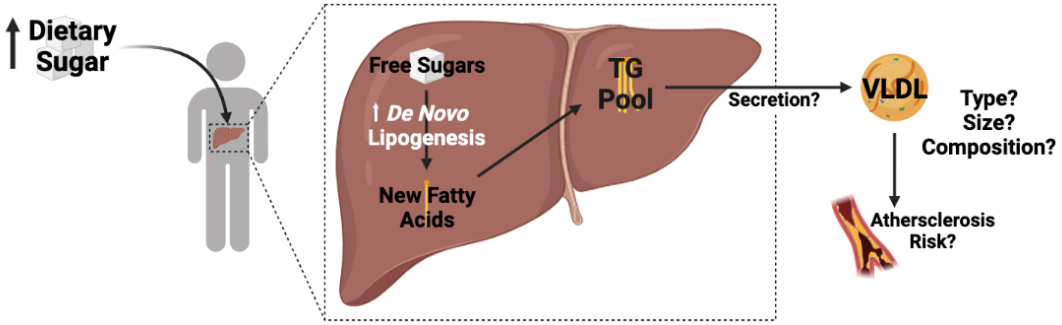
Research Rationale: The mechanism through which high consumption of dietary free sugars increases cardiovascular disease risk remains unclear. Within the liver, excess free sugars are shuttled into *de novo* lipogenesis (DNL) to synthesize novel fatty acids (FA) which are preferentially secreted into the plasma as very-low-density lipoprotein (VLDL)-bound triglycerides (TG). While a high-sugar diet would increase DNL and lipoprotein production, it is unknown if an increase in DNL increases atherogenic lipoprotein species and risk of atherosclerosis.

Methods: To upregulate DNL, sixteen human volunteers (56% men, 48.8 ± 3.8y, BMI 27.6 ± 3.8kg/m^2) were recruited from the Oxford BioBank and underwent a metabolic study visit before and after consuming up to a 21-day isocaloric high-sugar (35% TE) diet. Following an overnight fast and drinking [^{2}H]H_2O, fasting plasma samples were collected for plasma metabolite analysis and NMR-profiling of their lipoprotein concentration, size, and composition, while DNL was quantified by measuring the percent incorporation of [^{2}H]palmitate into plasma VLDL-TG by isotope-ratio mass-spectroscopy.

Results: Following a short-term high-sugar diet, DNL significantly increased ($p < 0.001$) as 17.85 ± 9.44% of newly synthesized palmitate was incorporated into VLDL-TG post-diet compared to 7.72 ± 6.55% pre-diet. Similarly, our NMR plasma and lipoprotein profiling showed a significant increase in DNL products such as saturated FA ($p = 0.0058$), monounsaturated FA ($p = 0.012$), and total-TG content ($p = 0.0051$). As DNL increased, there was a significant increase in TG bound to VLDL ($p = 0.006$), high-density lipoprotein (HDL) ($p = 0.0043$), and intermediate-density lipoproteins ($p = 0.0071$) and an increase in total VLDL lipid content ($p = 0.0067$) and VLDL size ($p = 0.0058$). This was matched by a decrease in the polyunsaturated FA (PUFA) to monounsaturated FA ratio ($p = 0.0109$) and the percent of total FA made up of omega-6 FA ($p = 0.0067$) and PUFA ($p = 0.0091$). Notably, an increase in DNL did not change atherogenic lipoprotein species like small dense low-density lipoproteins (sdLDL), nor total lipoprotein number, total cholesterol, HDL-cholesterol, or non-HDL-cholesterol which are all cardiovascular disease risk factors.

Conclusion: We demonstrate here that while a short-term isocaloric high sugar diet upregulates DNL and leads to an increased TG content, VLDL size, and VLDL lipid content, it does not increase atherogenic lipoprotein species in healthy humans. This suggests that increased hepatic DNL may not mediate the increased risk of cardiovascular diet in individuals consuming a high-sugar diet.

This work was funded by the British Heart Foundation.



POSTER NO: 40

Genome-wide association study of blood pressure traits in 140 000 Mexican adults

Michael Turner (DPhil student, clinical)*[1], Jason Torres[1,2], Richard Haynes[1,2], William Herrington[1], Natalie Staplin[1,2], Michael Hill,[1,2] Rachel Wade,[1,2] Rory Collins[1], Jonathan Emberson[1,2], Raúl Ramírez-Reyes[3], Jesus Alegre-Diaz[3,4], Jaime Berumen[4], Pablo Kuri-Morales[3], Roberto Tapia-Conyer[3]

1. Clinical Trial Service Unit & Epidemiological Studies Unit, Nuffield Department of Population Health (NDPH), University of Oxford, Oxford, UK
2. MRC Population Health Research Unit, NDPH, University of Oxford, Oxford, UK
3. Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico
4. Experimental Research Unit from the Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico

Research Rationale: Genome-wide association studies (GWAS) have identified hundreds of variants associated with blood pressure but such studies have been done mostly in people of European ancestry. Using data from 150,000 Mexican adults recruited into the Mexico City Prospective Study (MCPS) we analysed the associations between 42 million genetic variants and systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (P) and mean arterial pressure (MAP).

Methodology: Between 1998 and 2004, 159,755 participants from Mexico City were recruited into a prospective study, answered health and lifestyle questions, had blood pressure and other measurements taken, and gave a blood sample. Genotyping of ~650 000 variants was done with the Illumina Global Screening Array v2 at the Regeneron Genetics Center. Imputation was done using the ancestrally-diverse Trans-Omics for Precision Medicine (TOPMed) reference panel. Quality control (QC) removed those with imputation $r^2<0.4$ and $N_{eff}<30$. Genetic principal components (PCs) were calculated using the *bigsnpr* package. GWAS was performed using SUGEN, with adjustment for age, age^2, sex, body mass index (BMI) and the first seven genetic principal components. The threshold used for a variant to be considered GWAS-significant was $p<5x10^{-8}$. Covariance-adjusted linkage disequilibrium (LD) scores, computed from a subset of 9674 unrelated participants who were whole-genome sequenced, were used to estimate genomic inflation. Conditional analyses were performed using GCTA-COJO. We considered a locus to span 1Mb and required variants to have $\geq$2 supportive loci to be considered potentially-novel. Results were compared to previous published reports.

Results: We analysed ~42 million variants in 140 559 MCPS participants passing genetic and SBP QC. SUGEN analyses had LD score regression intercepts of 1.03, 1.02, 1.03 and 1.01 for SBP, DBP, MAP and PP respectively, implying good control of genomic inflation. For SBP, conditional analyses refined 3593 variants with $p<5x10^{-8}$ to 71 independent signals at 66 separate loci. Of these, 15 were at novel loci and a further 10 were at known loci but conditionally-independent of all previously reported variants. For DBP, 1953 GWAS-significant variants were refined to 52 signals at 52 loci. Of these, 12 were at novel loci and 11 were at known loci but independent of all previously reported variants. For MAP, 3200 GWAS-significant variants were refined to 73 signals at 71 loci. Of these 27 were at novel loci and 7 were at known loci but independent of all previously reported variants. For PP, 928 significant variants were refined to 24 independent signals. Of these, one was a novel locus and two were at known loci but independent of all reported variants. The vast majority of the novel variants identified are non-coding, with only 2, 1, 1 and 0 lead variants in coding regions for SBP, DBP, MAP and PP respectively.

Conclusions: These results constitute the largest GWAS of blood pressure traits in a Latin-American population. Potentially-novel associations were found with all traits, but replication is now required to ascertain which are robust. As is typical in GWAS, the majority of associations identified are non-coding. Functional analyses are planned to ascertain the biological impact of the variants in question.

POSTER NO: 41

Title: **CHEMICAL TOOLS TO UNDERSTAND FATTY ACID UPTAKE, TRAFFICKING, METABOLISM AND SIGNALLING IN TYPE 2 DIABETES**

Authors: Ríona Devereux*,[a] Dr. Josie Gaynord,[b] Prof. Angela J. Russell,[a,c] Prof. Lisa Heather[d]

Departmental affiliations: *[a]**non-clinical DPhil student** at the Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK; [b]Merck Sharp & Dohme (UK) Limited. (MSD), 1 Midland Rd, London NW1 1AT; [c]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, UK; [d]Department of Physiology, Anatomy and Genetics, Sherrington Building, Sherrington Rd, Oxford OX1 3PT,*
*riona.devereux@chem.ox.ac.uk

Research rationale - Fatty acids are the predominant fuel in the body, with many important functions. Too much fatty acid metabolism can contribute to many diseases including the cardiac complications that occur in type 2 diabetes. Despite the prevalence of clinical data demonstrating this role of fatty acid metabolism in type 2 diabetes, we do not currently understand with which proteins the fatty acids, or their metabolites interact within cells and its impact on the disease. Therefore, this project aims to develop chemical probes to begin to address the following research questions: which long chain fatty acids or metabolites are most prevalent in type 2 diabetes, with which biomolecules do these long chain fatty acids/metabolites interact with and what are the outcomes of these lipid-biomolecule interactions with regards to type 2 diabetes?

Methodology - The synthesised chemical probes contain both photoaffinity fatty acid probes (containing both a diazirine photo-crosslinking group and a pulldown "Click" alkyne handle) and photocatalytic fatty acid probes (μMap). Using both probe types in parallel will allow for elucidation of both the covalent interactions and non-covalent associations between specific fatty acids and proteins of interest. To screen for lipid-protein interactions in vivo, the cells are dosed with the chemical probes. The probe becomes incorporated into the cellular fatty acid pool and any proteins in direct contact with the probe are then crosslinked through irradiation of the photo-crosslinking group, using UV light. This results in a covalent linkage between the lipid and the protein of interest. Click chemistry is then used to attach a reporter molecule (e.g., fluorophore or affinity tag) onto the terminal alkyne of the probe. This reporter molecule can then be used for affinity purification, followed by proteomics or visualisation of the crosslinked protein. These probes will be used as tools for elucidating lipid function – through exploring molecular interactions and live cell imaging.

Results and Conclusions - The palmitic acid photoaffinity probe **1** and photocatalytic probe **2** have been successfully synthesised and fully characterised. The photoaffinity probe **1** has been used in preliminary experiments in MDA-MB-231 cells. A concentration series of probe has been carried out and demonstrated a concentrations-dependent increase in proteins interacting with the probe following an 18-hour incubation, as visualised using SDS-PAGE and in-gel fluorescence. The proteins captured without the UV radiation step are likely due to due to metabolic incorporation of probe **1** into the protein structure (e.g., post-translational palmitoylation), UV exposure increased the number of proteins identified as interacting with the fatty acid probe, demonstrating that the inclusion of the photo-crosslinking diazirine group improved our ability to detect lipid interacting partners.

Van De Weijer *et al.*, *Cardiovasc. Res.* **2011**, *92* (1), 10–18.
Haberkant *et al. Angew. Chemie Int. Ed.* **2013**, *52* (14), 4033–4038.
Geri *et al.*, **2020**, *Science* 367, 1091–1097.
Trowbridge *et al. Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (34), 1–8.