26th Northern Cardiovascular Research Group Meeting

Newcastle-upon-Tyne

Tuesday 24th April 2018

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http://photoeverywhere.co.uk/britain/northeast/38-highlevel2.jpg

Welcome to the 26th annual Northern Cardiovascular Research Group Meeting, which is taking place in the Discovery Museum here in Newcastle. We hope you enjoy the day.

Sponsors

We are very grateful to Newcastle University and the British Heart Foundation for providing funds to allow us to organise this meeting. We also appreciate the generous support of Cairn Research in sponsoring the keynote lecture by Professor David Sedmera.

Many thanks to the generous support provided by our sponsors: Abcam, Cambridge Bioscience, Ion Optix, PromoCell, Starlab, ThermoFisher Scientific and World Precision Instruments.

We encourage you to take a moment during the day to visit our exhibitors.

Simon Bamforth Helen Phillips Julie Taggart Rachael Watson

(NCRG 2018 organisers)

Cairn Research Keynote Lecture: Professor David Sedmera

This year's keynote lecture is sponsored by Cairn Research and will be delivered by Professor David Sedmera from the Institute of Anatomy, Charles University, Prague.

Integrative view on evolution and ontogeny of the cardiac conduction system

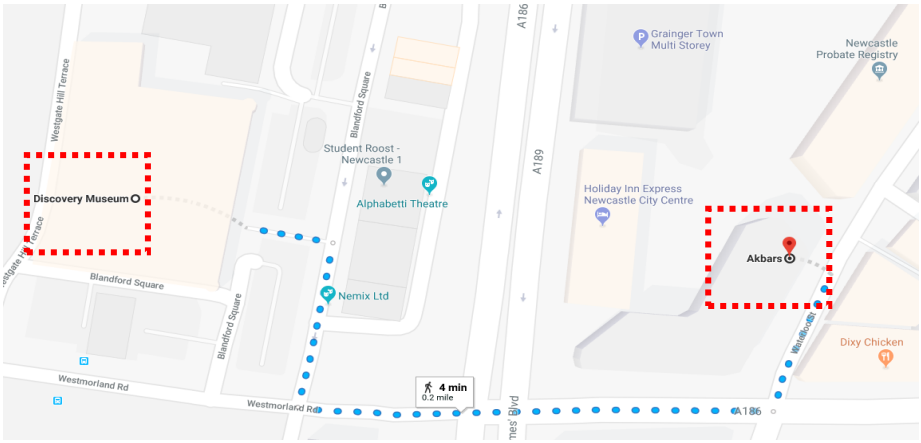
Cardiac conduction system (CCS) is present in its classical description known over a century in adult birds and mammals. Recently, considerable advances were made in the area of molecular mechanisms of its specification and functional deployment in avian and mammalian developmental models. Considerably less information is available, however, along the evolutionary lines about the presence and nature of CCS in the hearts of lower vertebrates. In this lecture, I will collate the currently available information from fish, amphibian and reptilian models, and try to integrate it with the developmental studies in reptilian, but also avian and mammalian hearts. I will focus on the remodelling of the atrioventricular canal myocardium, as the nature of the atrioventricular connections plays an important role in determination of ventricular activation pattern, in addition to presence of a specialized ventricular conduction system. I will also highlight varied physiological conditions as constraints imposed on cardiac structure and function in different species at specific stages of their life cycle.

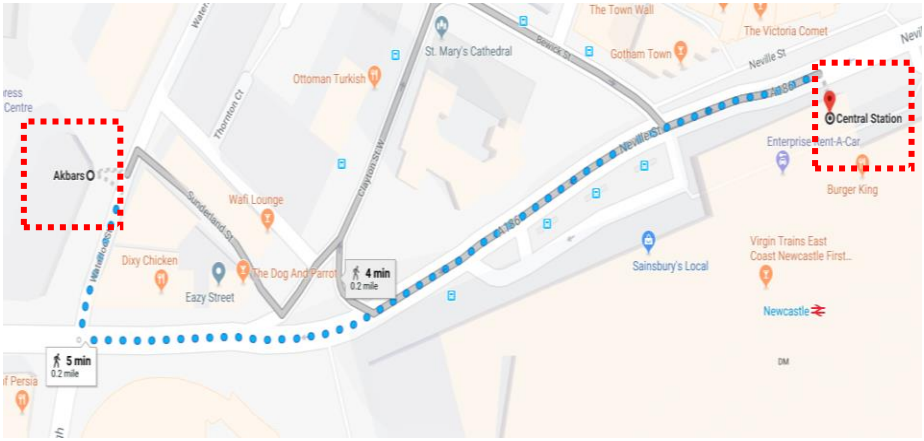
Venue

The day's events will all take place at the Discovery Museum, Blandford Square, Newcastle upon Tyne NE1 4JA.

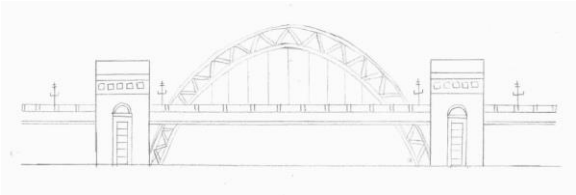
The conference dinner will take place at Akbar's Indian restaurant, City Quadrant, Westmorland Road, NE1 4DP, a short walk from the Discovery Museum and Newcastle Central Station.

Maps





Meeting Programme



Meeting Programme

09.30 – 10.20 **Registration/exhibits/refreshments**

10.20 – 10.30 **Welcome**

Session 1

Chairs: Sarah Calaghan (University of Leeds) and David Greensmith (University of Salford)

10.30 – 10.45 **Simon Tual-Chalot (Newcastle University)**

Endothelial endoglin is required to protect against high output heart failure

10.45 – 11.00 **Peter Barabas (Queen's University Belfast)**

TRPV2 heterozygous knockout rats display diabetic retinopathy-like phenotype

11.00 – 11.15 **Caglar Gok (University of Glasgow)**

The influence of palmitoylation on NCX function

11.15 – 12.00 **Refreshments/poster session 1/exhibits**

Session 2

Chairs: William Fuller (University of Glasgow) and Helen Arthur (Newcastle University)

12.00 – 12.15 **Anna Wilsdon (University of Nottingham)**

Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects identified by exome sequencing

12.15 – 12.30 **Natasha Hadgraft (University of Salford)**

The effects of tumour necrosis factor alpha and interleukin 1 beta on cardiac intracellular calcium handling

12.30 – 12.45 **Ya Hua Chim (University of Liverpool)**

Elastin degradation in ascending aortic aneurysms

12.45 – 13.00 **Gennadiy Tenin (University of Manchester)**

From GWAS to gene function: analysis of the Glypican 6 and its involvement in cardiac septation and Tetralogy of fallot

13.00 – 14.00 **Lunch with time for posters and exhibits**

14.00 – 15.00 **Cairn Research Keynote Lecture**

(Introduction by Martyn Reynolds, Cairn Research)

David Sedmera (Charles University, Prague)

Integrative view on evolution and ontogeny of the cardiac conduction system

Session 3

Chairs: Tim Curtis (Queens University Belfast) and Deborah Henderson (Newcastle University)

15.00 – 15.15 **Florah Moshapa (University of Bradford)**

Therapeutic targeting of JAK-STAT signalling pathways responsible for vascular re-stenosis in type 2 diabetes mellitus

15.15 – 15.30 **Cole Sims (University of Manchester)**

MKK7 knockout by improved CRISPR/Cas9 does not affect cardiac differentiation of human embryonic stem cells

15.30 – 15.45 **Ivona Kelley (Abcam)**

Detection of miRNA and Protein biomarkers using the FirePlex® Technology Platform

15.45 – 16.30 **Refreshments/poster session 2/exhibits**

Session 4

Chairs: David Brook (University of Nottingham) and Kirsten Riches-Suman (University of Bradford)

16.30 – 16.45 **George Madders (University of Manchester)**

Susceptibility to atrial alternans; a role for transverse (t)-tubule loss in heart failure?

16.45 – 17.00 **Adrián Santos-Ledo (Newcastle University)**

Alternative splicing of jnk1 directs cardiac morphogenesis

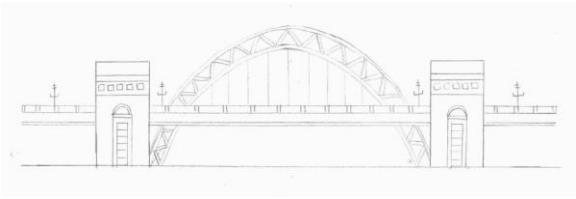
17.00 – 17.15 **Pieter de Tombe (Imperial College, London)**

The ANREP effect: role of titin strain

17.15 – 17.30 **Closing remarks and prizes**

18.00 – 20.30 **Conference dinner at Akbar's Indian restaurant**

Talk Abstracts



Endothelial endoglin is required to protect against high output heart failure.

Simon Tual-Chalot[1], Benjamin J Davison[1], Rachael E Redgrave[1], Esha Singh[1], Allan Lawrie[2] and Helen M Arthur[1]

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Objectives: Endoglin is a co-receptor for TGFbeta/BMP9/10 signalling and *ENG* mutations lead to the vascular disorder hereditary haemorrhagic telangiectasia type I (HHT). Endoglin is also required for normal vascular development and angiogenesis, but little is known about endoglin's role in quiescent adult vascular endothelium.
Methods: To investigate this role, tamoxifen was administered to adult Cdh5(PAC)-CreERT2;Eng$^{fl/fl}$ mice to generate endothelial-specific depletion of endoglin (Eng-iKO^{e}). Cardiac magnetic resonance imaging, myography, vascular casting, microsphere injection, immunohistology, qPCR and aortic telemetry were used to evaluate cardiovascular changes after endoglin knockdown.
Results: Endothelial-specific loss of endoglin leads to an enlarged heart and cardiomyocyte hypertrophy within 5 weeks, progressing to high output heart failure (HOHF). In vivo aortic telemetry revealed significant loss of aortic pressure within a few days of endoglin depletion. Increased cardiac size and reduced cardiac afterload were confirmed by ventricular pressure loop analysis. As HOHF could result from arteriovenous malformations (AVMs), and these are found primarily in mucocutaneous and pulmonary tissues in HHT, we systematically screened for AVMs using microspheres and vascular casting. Although AVMs were absent in the majority of tissues, they were observed in the pelvic region and may account for the rapid increase in cardiac output. Having also observed an increase of VEGF-A protein in tissues from Eng-iKOe mice, we found that inhibition of VEGFR2 was protective against enlargement of the heart and dilatation of the ventricles.

Conclusion: Our results showed the essential role of endoglin in the maintenance of adult cardiovasculature through crosstalk with the VEGF signalling pathway.

TRPV2 heterozygous knockout rats display diabetic retinopathy-like phenotype

Michael O'Hare, **Peter Barabas**, Gema Esquiva, Mary K. McGahon, Jennifer Henry, Richard Knell, David Grieve, Graham McGeown & Tim M. Curtis

Welcome-Wolfson Centre for Experimental Medicine, Queen's University Belfast, Belfast, UK

Disruption of retinal blood flow autoregulation is an early hallmark of diabetic retinopathy. Our prior investigations into the mechanisms underlying these autoregulatory changes suggested a critical role for stretch-activated TRPV2 channels, whose level and activity is decreased in rodent models of diabetes. Here we show that TRPV2 heterozygous (+/-) rats develop retinal pathology, reminiscent of diabetic retinopathy. Methods: TRPV2+/- rats were assessed for retinal pathology at P21, P90 and P360. Immunohistochemical analysis of flat-mounted retinas and retinal cryosections was performed to study the vascular, neuronal and glial components. Vasopermeability was assessed using the Evan's blue dye method and systemic blood pressure measured using tail cuff plethysmography. Retinal neurophysiology was examined by electroretinography (ERG). Results: Blood glucose, haemoglobin A1c and systemic blood pressure were within normal parameters in TRPV2+/- rats. At P90 and P360, TRPV2+/- animals exhibited a significant increase in vasopermeability ($P<0.05$) and acellular capillary formation (collagen IV positive/Isolectin B4 negative vessels; $P<0.05$). A decrease in the mean number of retinal ganglion cells ($p<0.001$) and an increase in the proportion of activated Müller glia (GFAP-positive; $p<0.0001$) was also observed. In addition, microglial cell numbers were increased in P90 and P360 TRPV2+/- retinas ($P<0.05$ & $P<0.01$ respectively). TRPV2+/- animals also had a reduction in the amplitude of both the ERG A & B-waves at P360 ($P<0.01$). Conclusion: TRPV2 heterozygous rats exhibit microvascular pathology, suggesting that even partial loss of TRPV2 channel activity in the retina is detrimental and may be a contributing factor to the pathogenesis of diabetic retinopathy.

The influence of palmitoylation on NCX Function

Caglar Gök, William Fuller

Institute of Cardiovascular & Medical Sciences, University of Glasgow

The sodium-calcium (Na/Ca) exchanger (NCX) is an antiporter membrane protein that regulates cytoplasmic Ca by facilitating its electrogenic exchange for 3 Na. Cardiac NCX (NCX1) is implicated in the pathogenesis of heart failure and a number of cardiac arrhythmias. A single cysteine in position 739 of the NCX1 large intracellular loop is palmitoylated, which regulates NCX1 inactivation. We investigated the impact of palmitoylation on NCX1 function using resin-assisted capture, and Fluorescent Lifetime Imaging combined with Fluorescence Resonance Energy Transfer (FLIM-FRET) in HEK cells transfected with wild type (WT) or unpalmitoylatable (C739A) NCX1. NCX1 functions as a dimer: interactions between NCX1 dimers were visualised as FRET changes between exchanger proteins with CFP or YFP inserted at position 266 within the large cytoplasmic loop. NCX1-NCX1 FRET was greater in cells expressing WT than C739A NCX1, and the broad-spectrum palmitoylation inhibitor 2-Bromopalmitate reduced NCX1-NCX1 FRET only in cells expressing WT NCX1. Affinity purification followed by mass spectrometry identified the thioesterase APT1 as an NCX1-interacting protein: APT1 inhibition increased NCX1 palmitoylation and NCX1-NCX1 FRET. The palmitoylation of NCX1 and NCX1-NCX1 FRET were altered by the ion composition of the extracellular buffer: Ca-free extracellular solutions favoured FRET and elevated NCX1 palmitoylation, whereas Na-free extracellular solutions did the opposite. C739A-NCX1 FRET was unaffected by Ca-free conditions. We conclude that palmitoylation influences the structure of the NCX1 intracellular loop to modify FRET between NCX1 dimers. The impact of extracellular solution composition on NCX1 palmitoylation implies the existence of mechanisms to dynamically couple NCX1 palmitoylation to its function.

Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects identified by exome sequencing

A. Sifrim[1], M. Hitz[1,2], **A. Wilsdon[3]**, J. Breckpot[4], S. H. Al Turki[5], B. Thienpont[6,7], J. McRae[1], T. W. Fitzgerald[1], T. Singh[1], G. J. Swaminathan[1], D. Rajan[1], E. Prigmore[1], The INTERVAL study, The UK10K Consortium, Competence Network for Congenital Heart Defects, German Register for Congenital Heart Defects, S. Mital[8], P. Daubeney[9], B. Keavney[10], J. Goodship[11], R. M. Abu-Sulaiman[12,13], S. Klaassen[14,15], C. F. Wright[1], H. V. Firth[16], J. C. Barrett[1], K. Devriendt[4], D. R. Fitzpatrick[17], J. D. Brook[3], The Deciphering Developmental Disorders Study, M. E. Hurles[1]

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Congenital heart disease (CHD) is the most common type of birth defect. We carried out whole exome sequencing on 1891 probands with CHD, which included 1365 trios. The majority of individuals had non-syndromic CHD (n=1219).

In syndromic-CHD (S-CHD) we identified a significant excess of de novo protein truncating variants (PTVs) in known CHD-associated genes. In non-syndromic CHD (NS-CHD) we identified a significant excess of PTVs in CHD-associated genes that were inherited from healthy parents. We identified three new autosomal dominant S-CHD disorders due to mutations in *PRKD1*, *CHD4* and *CDK13*. Network and gene function analysis has identified a number of over-represented pathways, and gene functions including chromatin modification. We also identified a number of genes that were known to cause developmental disability that we can now show also cause CHD.

This is the first study to explain the low sibling recurrence risk that we see in both S-CHD *and* NS-CHD, and the first time an excess of inherited variants in CHD-associated genes displaying reduced penetrance has been identified within the NS-CHD population. Given NS-CHD accounts for around 90% of CHD, this is an important finding. Our results indicate that there are further undiscovered CHD genes, but that much larger cohorts will be required to discover these. In the future, we hope that understanding the networks of genes involved in CHD will lead to the development of treatments for both CHD and the failing heart.

The effects of tumour necrosis factor alpha and interleukin 1 beta on cardiac intracellular calcium handling

Natasha E. Hadgraft[1] and David J. Greensmith[1]

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Cytokines including tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) are known to mediate systolic and diastolic myocardial dysfunction in inflammatory disease such as sepsis. To provide a cellular basis, we separately studied the effects of 50 ng/ml TNF-α and IL-1β on intracellular Ca handling and contractility in sheep ventricular myocytes.

All procedures used accord with the Animals (Scientific Procedures) Act, UK, 1986 and Directive 2010/63/EU of the European Parliament (Home Office, 1986). Ventricular myocytes isolated from young sheep were loaded with Fura-2 and field stimulated at 0.5 Hz, to measure intracellular Ca and contractility dynamics by epi-fluorescent photometry and video sarcomere detection respectively. Relative changes in SR Ca content were estimated from the amplitude of caffeine evoked Ca transients.

TNF-α and IL-1β reduced SR Ca content by 27% and 41% respectively, accounting for a rapid (<10 s) 17% and 24% reduction each in systolic Ca. With TNF-α, this was associated with a 20% reduction in contractility whereas IL-1β, increased contractility by 22%, suggesting increased myofilament sensitivity. The mechanism by which SR Ca content is reduced remains unclear. The rate constant of systolic Ca decay was unaffected by both cytokines, suggesting SERCA impairment does not play a role. An initial and short-lived increase of systolic Ca was present in 71% of TNF-α treated cells and 52% IL-1β treated cells (by 58 and 52% respectively), which may suggest a role for ryanodine receptor potentiation. Both cytokines can account for aspects of myocardial depression in sepsis on a cellular level.

Elastin degradation in ascending aortic aneurysms

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[2] Institute of Integrative Biology, University of Liverpool, Liverpool, UK
[3] Institute for Risk and Uncertainty, University of Liverpool, Liverpool, UK
[4] Department of Cardiac Surgery, Liverpool Heart and Chest Hospital, Liverpool UK

Introduction: Degradation of medial elastin is thought to be important in aortic aneurysms. However, it is unclear whether elastin degradation differs in different aneurysm aetiologies. Here, we measured the micromechanical properties and characterised elastic fibres in the aortic tissue of two specific groups; bicuspid aortic valve with associated aneurysm (BAV) and idiopathic degenerative aneurysm (DA).
Methods: Aortic tissue was retrieved from 28 age-matched patients undergoing either BAV or DA aneurysmal repair. Dynamic nanoindentation was used to characterise the medial layer with a 100 μm flat punch tip; the storage (G´) and loss modulus (G˝) were obtained. The tissue samples were subsequently stained for elastin with Verhoeff-Van Gieson. Images were captured across the tissue (n=4); the number of elastic fibres and their length were measured.
Results: G´ and G˝ of BAV tissue was found to be significantly higher than DA tissue ($p<0.001$). Similarly, this significant trend was also noted for the elastic fibre lengths ($p=0.02$). No significant difference in the number of fibres was noted between both tissue groups. When the elastin properties were compared with G´ and G˝, a positive correction was found for DA tissue. Meanwhile, a negative correlation was found when micromechanical properties were compared with the number of elastic fibres in BAV tissue.
Discussion: BAV tissue was significantly different to DA tissue, having longer fibres and higher tissue stiffness. Interestingly, depending on the type of aneurysm the number of elastic fibres correlates differently with its mechanical properties. These initial observations provide new insight to ascending aortic aneurysms.

From GWAS to gene function: analysis of the Glypican 6 and its involvement in cardiac septation and Tetralogy of Fallot.

Gennadiy Tenin, Prof Bernard Keavney

University of Manchester

Congenital heart diseases (CHD) are defects in the structure of the heart and great vessels that are present at birth and an important cause of childhood mortality and lifelong morbidity. They are amongst the most common birth defects, present in 9 out of 1000 live births. Tetralogy of Fallot (TOF) is one of the commonest cyanotic CHD and considered to be a multigenic condition as no single causative gene has been found so far. Studies on animal models have linked the development of the TOF to defects in cardiac septation. A recent GWAS study [Cordell et al, 2013] of TOF patients found 2 significantly associated intronic SNPs on chr 13q31; however, genes associated with these variants are unknown. Published data analysis suggested that nearby located gene GPC6 might be associated with CHD. We found GPC6 being expressed in the endocardial cushions, transitory structures critical for cardiac septation. Mice carrying a hypomorphic allele of Gpc6 display abnormalities in cushion development, leading to lower cell density in cardiac valves, thinner great vessels and, in some cases, a ventricular septal defect (VSD). The complete knock-out of Gpc6 leads to double outlet right ventricle with transposition of great arteries, resulting in after birth lethality. Thus, we discovered previously uncharacterized gene GPC6 being a novel candidate for congenital cardiac defects.

Therapeutic targeting of JAK-STAT signalling pathways responsible for vascular re-stenosis in type 2 diabetes mellitus.

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Vascular inflammation emerge as a key event in development of macrovascular complications of type 2 diabetes mellitus (T2DM). Suppressor of cytokine signalling 3 (SOCS3) is a potent inhibitor of inflammatory pathways (JAK/STAT) involved in the propagation of vascular endothelial cell (EC) inflammation, smooth muscle cell (SMC) migration and proliferation all of which are key events in development of coronary artery bypass graft re-stenosis. However, SOCS3 is limited by its short biological half-life. Therefore a mutated SOCS3 transgene that is resistant to ubiquitination and subsequent proteasome-dependent degradation ("Lys-less" SOCS3) has been developed.

Hypothesis being tested is that JAK-STAT signalling pathway is enhanced in HSV-SMCs as a consequence of T2DM. In addition, "Lys-less" SOCS3 may have greater therapeutic potential versus wild type (WT) SOCS3 in limiting JAK-STAT mediated processes responsible for neo-intimal hyperplasia and vein graft failure in T2DM. Immuno-fluorescence and confocal imaging have shown that transduction of WT and Lys-less SOCS3 in HSV-SMCs and ECs by lenti-virus can be highly efficient after 48 hours, importantly, there was prolonged and stable SOCS3 protein expression for up to 2 weeks as assessed by immunoblotting. Lys-less SOCS3 was found to be resistant to ubiquitination and has a biological half-life of at least 4 hours versus 45-60 mins for WT SOCS3 as determined from emetine chase experiments in transduced cells. Overexpressed Lys-less SOCS3 and WT SOCS3 inhibited sIL-6Rα/IL-6 mediated STAT3 activation in HSV-SMCs by 83% ±7 and 79% ±7 respectively ($P<0.05$ versus control response, set at 100%). Furthermore, immunoprecipitation experiments have shown that overexpressed Lys-less SOCS3 precipitated along with Elongin B/C as compared to negative control in SV ECs.

In summary, current in-vitro results suggest a mechanism by which overexpressed SOCS3 in SMCs and ECs may supress vascular inflammation causing graft restenosis in T2DM.

MKK7 knockout by improved CRISPR/Cas9 does not affect cardiac differentiation of human embryonic stem cells

CH Cole Sims, Matias Autio, Albert Dashi, Roger SY Foo, Christine Cheung, Xin Wang

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Human embryonic stem cells (ESCs) present a useful tool to study development however the roadmap of differentiation is not fully realised. We can use CRISPR/Cas9 to edit the genome of ESCs and shed light on previous unknowns. In mouse ESCs, a deletion of mitogen activated protein kinase kinase 7 (MKK7) does not hinder differentiation into various cell types, including cardiomyocytes (CMs). Creating a knockout (KO) ESC line lacking *MAP2K7* would allow us to examine its role in humans. After differentiation, any resultant CM phenotype and the ability of those KO CMs to deal with stress would help our understanding of MKK7 in both development and disease.

We used an improved plasmid based CRISPR/Cas9 system to remove MKK7 from hESCs. Notable improvements in our methods were the use of enhanced specificity Cas9 described recently and the inclusion of a p53 dominant negative particle which has been shown to increase cell survival and viability after double stranded DNA break caused by Cas9. These KO cells were then differentiated into CMs using an adapted protocol, based on a previously published method.

Using these methods, we have shown that MKK7 knockout does not affect maintenance of pluripotency marker expression nor differentiation into CMs. An on-going in vivo study is showing that a cardiac specific knockout of MKK7 in mice leads to a larger left ventricular dilation after chronic myocardial infarction. We hypothesise that this is due to increased cell death over wildtype mice and will use our hESC-KO CMs to investigate the molecular mechanism.

Detection of miRNA and Protein biomarkers using the FirePlex® Technology Platform

Ivona Kelley

Global Director of Sales, Multiplex Assays

The detection of circulating molecular biomarkers is useful for monitoring pathogenic processes and response to therapeutic intervention[1,2]. Research has shown that using a combined signature of multiple biomarkers can better account for patient and epidemiological heterogeneity, and provide a more accurate indication of patient health[2]. In addition, the desire to leverage minimally-invasive samples has necessitated the development of technologies that can profile biomarkers directly from biofluids such as plasma, serum, and urine. To address this need, we developed the FirePlex® Technology platform, which allows for the sensitive and accurate multiplexed detection of protein analytes or miRNAs directly from a wide array of biological samples. The platform uses patented, multi-functional hydrogel particles that allow for in-well multiplexing and panel design flexibility.

For the detection of protein analytes, FirePlex immunoassays use high-performance matched antibody pairs that provide minimal cross-reactivity between individual analytes, up to 5 logs dynamic range, and single-digit pg/ml sensitivity, while requiring only 12.5 µl biofluid input. Similarly, the FirePlex miRNA assay can reliably detect miRNAs directly from as little as 10 µl of biofluid without the need for RNA purification. This assay utilizes single step RT-PCR signal amplification with universal primers, thus leveraging PCR sensitivity while eliminating an independent reverse transcription step and mitigating amplification biases introduced by target-specific qPCR.

FirePlex assays are validated across a wide range of biological samples including plasma, serum, urine and cell culture supernatant. The 96-well plate assay format enables high-throughput sample screening with readout on standard flow cytometers, thereby omitting the need for complex and expensive dedicated instrumentation. Finally, the integrated FirePlex Analysis Workbench software enables rapid data analysis, visualization, and export, and includes key features such as standard curves as well as publication-quality heatmaps and graphs.

Here we present data from studies using the FirePlex Platform to profile miRNAs in cancer. Together, the combination of multiplexed, high-sensitivity assays with powerful bioinformatics tools enables rapid discovery and validation of biomarker signatures from liquid and solid specimens.

References

1. Biomarkers: Potential Uses and Limitations. ***NeuroRx*** (2004).
2. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. **Nat. Biotech.** (2006).

Susceptibility to atrial alternans; a role for transverse (t)-tubule loss in heart failure (HF)?

GWP Madders, L Woods, DC Hutchings, CA Waddell, JL Caldwell, AW Trafford, DA Eisner, KM Dibb

University of Manchester

Atrial fibrillation (AF) is prevalent in HF. Alternans, a beat-to-beat oscillation in the atrial action potential and/or Ca^{2+} transient, has been implicated in AF initiation. However, alternans aetiology is not fully understood.

Atrial contraction is driven by intracellular Ca^{2+} cycling, changes to which are thought to play an important role in alternans. Ca^{2+} handling machinery is concentrated around a network of membrane invaginations, t-tubules. T-tubules are lost in AF and HF, thus t-tubule loss may predispose alternans. The aim of this work was to determine if alternans could be induced more easily in HF and if this was due to t-tubule loss.

Left atrial myocytes were isolated from control and HF sheep, induced by tachypacing of the right ventricle. Fluo-5F loaded myocytes were incrementally paced under current clamp control. The lowest frequency at which alternans was detectable was deemed the threshold. The Ca^{2+} and action potential alternans thresholds were decreased in HF *vs.* control (1.25±0.18 *vs.* 2.40±0.16; 2.00±0.20 *vs.* 2.45±0.11 Hz, $p<0.05$;)s

T-tubule loss observed in HF was mimicked in control cells by formamide induced osmotic shock; lowering alternans thresholds in detubulated cells *vs.* control ((Ca^{2+}) 1.75±0.34 *vs.* 2.40±0.16 Hz; (AP) 1.69±0.25 *vs.* 2.45±0.11; $p<0.05$) with no difference between HF and detubulated cells.

Our data suggests alternans occurs more readily in HF atrial cells, potentially providing a mechanism for increased prevalence of AF in HF. Further work shall determine whether other key components of intracellular Ca^{2+} cycling, further to t-tubule loss, are involved in the earlier onset of alternans in HF.

Alternative splicing of *jnk1* directs cardiac morphogenesis

Adrián Santos-Ledo, Tamilvendhan Dhanaseelan, Sam Washer, Deborah Henderson and Bill Chaudhry

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Congenital heart defects (CHD) occur in one per cent of live born infants. Although both environmental and genetic causes are proposed human candidate genes remain elusive.

Our laboratory has been studying the non-canonical Wnt/Planar Cell Polarity (PCP) signalling and has shown it is essential for heart development. Disturbances of several genes within the pathway lead to outflow tract heart malformation in animal models. The *c-Jun amino-terminal kinase* (*Jnk*) gene is a highly conserved downstream component of the PCP pathway, which has not been shown to play a role in heart development although it participates in several other pathways implicated in CHD.

We set out to investigate the role of Jnk in development using zebrafish. These small tropical bony fish are a valuable laboratory model for the study of cardiac development. There is conservation of developmental processes and genes; the genome has been sequenced and a variety of genetic and imaging techniques are well established.

We first analysed the temporal and spatial expression of the zebrafish *jnk* genes using *in-situ* hybridisation and established presence of unique *jnk1* transcripts within the zebrafish heart. Using both CrispR/Cas9 genome editing and morpholino knockdown we went on to demonstrate the role of jnk1 in cardiac development. Importantly the genome duplication in zebrafish allowed us to dissect the role of alternative spliced jnk1 transcripts and demonstrate requirement in both specification of ventricular cardiomyocytes and left right patterning.

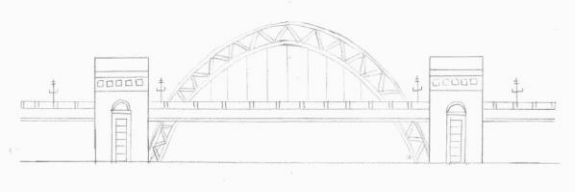
The ANREP effect: role of titin strain

Younss Ait-Mou, Mengjie Zhang, Jody L. Martin, Marion L. Greaser, and **Pieter P. de Tombe**.

Magdi Yacoub Institute, Harefield and Imperial College, London.

Stretch of myocardium, such as occurs upon increased filling of the cardiac chamber, induces two distinct responses: an immediate increase in twitch force followed by a slower increase in twitch force that develops over the course of several minutes. The immediate response is due, in part, to modulation of myofilament Ca^{2+} sensitivity by sarcomere length (SL). The slowly developing force response, termed the Slow Force Response (SFR), is caused by a slowly developing increase in intracellular [Ca^{2+}] upon sustained stretch. A blunted immediate force response was recently reported for myocardium isolated from homozygous giant titin mutant rats (HM) compared to muscle from wild-type littermates (WT). Here, we examined the impact of titin isoform on the SFR. Right ventricular trabeculae were isolated and mounted in an experimental chamber. SL was measured by laser diffraction. The SFR was recorded in response to a 0.2 μm SL stretch in the presence of [Ca^{2+}]o=0.4 mM, a bathing concentration reflecting ~50% of maximum twitch force development at 25 ^{o}C. Presence of the giant titin isoform (HM) was associated with a significant reduction in diastolic passive force upon stretch, and ~50% reduction of the magnitude of the SFR; the rate of SFR development was unaffected. The sustained SL stretch was identical in both muscle groups. Therefore, our data suggest that cytoskeletal strain may underlie directly the cellular mechanisms that lead to the increased intracellular [Ca^{2+}]$_i$ that causes the SFR, possibly by involving cardiac myocyte integrin signalling pathways.

Poster Abstracts



1. Glucagon-like peptide-1 analogues exert differential in vitro actions on macrophages and cardiac fibroblasts in experimental diabetes

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Background: Patients with heart failure and diabetes mellitus show specific cardiac abnormalities, which contribute to poor prognosis. In addition to glycaemic control, glucagon-like peptide (GLP-1) demonstrates cardioprotective effects in experimental diabetes, with selective benefits on inflammation and extracellular remodelling. The aim of this study is to investigate precisely how GLP-1 exerts anti-inflammatory actions in experimental diabetes.

Methods: RAW 264.7 macrophages were exposed to normal (5.5mM) or high glucose (25mM) for 48-72h in the presence of exenatide or liraglutide (1nM) to assess their anti-inflammatory effects on cell and secreted cytokine expression (real-time RT-PCR, proteome array). Effects on paracrine signalling were interrogated by incubating conditioned media from exenatide/liraglutide and/or glucose-treated macrophages with primary mouse cardiac fibroblasts prior to assessment of myofibroblast markers (real-time RT-PCR).

Results: Mouse macrophages, but not cardiac fibroblasts, were confirmed to express the GLP-1 receptor. Interestingly, exenatide and liraglutide exerted differential effects on macrophage cytokine expression under normoglycaemic (e.g. TGFβ: exenatide 0.81±0.13, liraglutide 2.04±0.40; n=3, $P<0.05$) but not hyperglycaemic conditions. In contrast, cytokine/chemokine secretion was unaltered in normal glucose but decreased to a greater extent by liraglutide versus exenatide in high glucose (e.g. CXCL10, TNF-α, CCL12; n=3 pooled samples), whilst liraglutide suppressed high glucose-induced cardiac fibroblast differentiation more than exenatide (α-SMA, procollagen Iα1), which was unaltered by normal glucose.

Conclusions: The results indicate that GLP-1 analogues, exenatide and liraglutide, exert differential actions on in vitro macrophage cytokine/chemokine expression and paracrine signalling, which may at least partly explain the reported differential cardiovascular benefits of these glycaemic agents in clinical diabetes.

2. Mathematical models of neonatal rabbit sino-atrial node (SAN) action potentials (APs)

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Heart disease diagnoses and treatment are based largely on our understanding of the adult myocardium. The marked differences in waveform of action potential (AP) between neonatal and adult cardiac myocytes, however, suggesting a different set of molecular interactions in neonatal myocytes requires different treatment for newborns. Computational modeling is useful for simulate the different experimental data to determine the functional interactions between different ionic channel remodeling current lead to systems-level behaviour, The use of modelling technique has not been used extensively to study neonatal heart cell function. This study presented a new mathematical model of the neonatal rabbit SAN cells by modifying the adult rabbit SAN models developed by Zhang model 2000, based on available experimental data obtained from newborn rabbit SAN cells, the densities or formulation of INa , ICa,L, if, IKr, IKs, and INaCa in an adult cell model. The new model reproduces similar APs to the neonatal APs recorded experimentally, the most differences of the APs characteristic was: a higher upstroke velocity (dV/dt_{max}) and peak amplitude (PA), shorter cycle length (CL) and duration (APD) than the adult, therefore, faster pacemaking rhythm. The simulation results were matching with experimental data. The new model provide a good understanding of the age related changes mechanism on the ionic currents transport , and a good tool for testing the drugs effect on neonatal san cells tool to hypotheses and obtaining a better quantitative understanding of differences between neonatal and adult physiology.

3. Extraction and sequencing of DNA from human tissue, fixed and stored in formalin

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Introduction: Formalin is widely used to preserve human tissues for pathological investigation. However, the DNA isolated is usually fragmented and corrupted, limiting its use for diagnosis or research. We wanted to extract high-quality DNA from formalin fixed tissue suitable for use in Sanger and next generation exome sequencing.

Methods: Using human tissue stored in formalin for 12 months, we created a novel protocol to extract high quality DNA, combining proteinase-K and heating with Chelex resin. We also evaluated the effect of uracil-DNA-glycosylase (UDG), an enzyme suggested to remove formalin artefacts. The yield, purity, fragment size distribution, efficacy as template for PCR and sequences of PCR products were compared.

Using this protocol, next generation whole exome sequencing was performed on DNA extracted from explanted heart that had been stored in formalin for over two years. The results were compared with those from freshly obtained DNA (blood) and the utility of Sanger sequencing as a confirmatory strategy was also evaluated.

Results and conclusions: High quality DNA was obtained from formalin-fixed tissue using our protocol. This increased the PCR product length obtained from 200 to 400bp. However, whilst UDG removed some artefacts it introduced others. Results of NGS exome sequencing on formalin-fixed DNA compared favourably to fresh DNA with 94% specificity, but 73% sensitivity. Thus, it is possible to obtain high quality DNA template from formalin-fixed samples, but limited sensitivity to detect all variants limits its use to confirmation of known, rather than discovery of novel, variants.

4. Acetylation of TBX5 by KAT2A and KAT2B regulates heart and limb development

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TBX5 plays a critical role in heart and forelimb development. Mutations in TBX5 cause Holt-Oram syndrome, an autosomal dominant condition that affects the formation of the heart and upper-limb. Several studies have provided significant insight into the role of TBX5 in cardiogenesis; however, how TBX5 activity is regulated by other factors is still unknown. Here we report that histone acetyltransferases KAT2A and KAT2B associate with TBX5 and acetylate it at Lys339. Acetylation potentiates its transcriptional activity and is required for TBX5 nuclear retention. Morpholino-mediated knockdown of *kat2a* and *kat2b* transcripts in zebrafish severely perturb heart and limb development, mirroring the *tbx5a* knockdown phenotype, which includes pericardial oedema, incomplete heart looping, lack of blood flow into the heart and absence of functional fins. The phenotypes found in MO-injected embryos were also observed when we introduced mutations in the *kat2a* or *kat2b* genes using the CRISPR-Cas system. These studies highlight the importance of KAT2A and KAT2B modulation of TBX5 and their impact on heart and limb development.

5. The Early Role of Rho kinase (ROCK) in the development of the ventricular wall and cardiomyopathy.

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Cardiovascular Research Centre, Institute of Genetic Medicine, Newcastle University[1], The Francis Crick Institute[2] and Charles University[3]

Congenital heart defects are extremely common, affecting over 1% of live births, while adult heart disease is the main cause of death in the UK. Defects acquired during fetal development can have a lasting detrimental effect on adult heart function. Therefore, understanding the underlying mechanisms involved in cardiac development and disease progression are of particular importance. Rho Kinase (ROCK) is expressed in the heart during development and has many cellular functions including cell polarity, proliferation, apoptosis, and migration as well as being a key regulator in actin-myosin contraction. ROCK is required for heart development to occur normally, however, the exact function of ROCK within the developing cardiomyocytes remains unknown.

Transgenic mouse models using *Cre-LoxP* technology have been utilised to downregulate ROCK specifically in the ventricle of the heart. Downregulating ROCK specifically in the cardiomyocytes results in the development of a number of heart defects including an abnormally thin myocardium. Analysis at the cellular level indicates abnormalities in sarcomere assembly in mutant hearts, which are present from early in development. Interestingly these mice survive into adulthood where they develop characteristics associated with cardiomyopathy including hypertrophy, fibrosis and a reduction in heart function. This model highlights the importance of understanding developmental defects and how they contribute to adult disease. This model will help in identifying cellular mechanisms underpinning the development of adult cardiovascular disease.

6. Settling in for the long haul: The transcriptomic changes underlying atrial t-tubule development and maturation

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University of Manchester

In adult atria, the Ca^{2+}-handling machinery concentrates around deep sarcolemmal invaginations called transverse (t)-tubules and produces a synchronous rise of $[Ca^{2+}]_i$. However, Ca^{2+}-dependent contractile mechanisms undergo significant remodelling during neonatal development. Here, in newborn cells rapid Ca^{2+} release is observed mainly at the cell periphery and migrates into the cell interior during the first 3 months of life as t-tubules develop.

This project aims to better understand the pathways responsible for postnatal development of Ca^{2+} handling and t-tubules.

Left atrial samples from female Welsh mountain sheep up to 1 week (1W), 1 month (1M), 3 months (3M) and ~18 months of age (adult) were snap-frozen in liquid nitrogen. Total RNA was extracted (TRIzol) and 75bp paired-end reads sequenced. RNAseq data was analysed using bioinformatics techniques including DESeq2 and FGSEA.

Reads mapped to 20204 unique genes. Most changing genes were simply remained on (13700) or off (5203) between 1W and adult. However, 591 genes switched off and 354 genes switched on, generally changing only once. Expression levels of 5334 genes were found to change in development ($qval<0.05$). The number and magnitude of expression changes was greater at 1W to 1M and 1M to 3M rather than 3M to adult, coinciding with development of t-tubules and central Ca^{2+} release. Expression of 114 genes involved in Ca^{2+} ion transport altered during development including increased CACNAC1C ($qval<0.05$). Conversely BIN1, a t-tubule gene, decreased ($qval<0.05$).

Future work involves identifying common pathways involved in gene expression changes.

7. Insulin Sensitivity in Induced Pluripotent Stem Cell Derived Cardiomyocytes

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Glasgow University

Diabetic cardiomyopathy (DCM) contributes towards the significant cardiovascular mortality rate associated with diabetes. This project assessed the potential for human derived, induced pluripotent stem cell cardiomyocytes (iPSC-CM) to act as a novel cellular model for DCM. The central feature of any diabetic model is an impairment of insulin stimulated glucose transport, therefore the primary requirement of this cell type is that they exhibit a robust baseline response. After adaptation of a [^{3}H]-2-deoxyglucose uptake assay to a 96-well plate format and extensive experimental optimisation, no robust or reliable insulin stimulated glucose uptake response was recorded from the iPSC-CM. Inhibition of contraction revealed its crucial role in regulating metabolic demand, but was not masking any effect of insulin. Activation of intracellular insulin signalling intermediates was detected in response to insulin stimulation, however the primary limitation was found to be very low levels of GLUT4 protein expression – the predominant insulin sensitive glucose transporter. This suggests that iPSC-CMs may be metabolically immature, rendering them unsuitable for modelling DCM. Initial interventions, including maturation medium conditioning and incubation with thyroid hormone (T3) had limited success in increasing GLUT4 expression in these cells. However, iPSC-CMs appear amenable to robust overexpression of exogenous GLUT4 via lipofectamine mediated transfection. Shortly, the functional consequences of this intervention shall be assessed, with the primary aim of inducing an insulin stimulated glucose uptake response.

8. Trophoblast Invasion and Remodelling of Human Spiral Arteries Examined with 3-D Electron Microscopy

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Cardiovascular Research Centre, Institute of Genetic Medicine, Newcastle University

Dramatic remodeling of decidual spiral arteries - to become flaccid, widened conduit vessels - by placental-derived extravillous trophoblast (eVT) is an essential feature of human pregnancy. This enables maternal blood delivery to the placental surface in support of appropriate fetal growth. Knowledge of the cell-to-cell interactions facilitating spiral artery remodelling is incomplete. By using parallel approaches of cell-specific immunostaining, and serial block face-scanning electron microscopy (SBF-SEM) with digital reconstruction, we ask: what heterocellular arrangements are evident during eVT-mediated spiral artery remodeling in early pregnancy?

Human placental bed biopsies (LREC 10/H0906/71) were obtained from women undergoing surgical termination of pregnancy between gestational weeks 9-14. Samples were fixed and processed for (i) immunostaining with cell-specific antibody markers and examination by light microscopy and (ii) for structural examination by SBF-SEM whereby 50-150 serial EM scans (100nm sections) were digitally aligned and reconstructed in Microscopy Image Browser with additional 3D viewing in Amira 6.0.

Intramural rVT closely approached the lumen border and had complex morphologies: most are large, rounded cells with prominent roughened ER; others elongated and dendritic in appearance, run parallel to the long axis of the lumen. Intraluminal eVT bound to the vessel wall lining in partial replacement of endothelial cells. Retained endothelial cells showed evidence of shedding contents to the lumen. 3D reconstruction of serial EM images revealed transluminal eVT migration. Extension of these approaches should inform us of the nature of spiral artery remodelling deficiencies in complicated pregnancies imparting lifelong cardiovascular risk.

Supported by BBSRC (BB/MO12093/1).

9. Trigger versus Substrate: Multi-scale Considerations for Arrhythmia Modulation through Pharmacological Action

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Cardiac arrhythmias such as ventricular fibrillation are intrinsically tissue-scale phenomena, wherein single-cell triggers (e.g. Ca^{2+}-induced spontaneous depolarisation) must interact with tissue-level substrate (e.g. heterogeneous repolarisation) to result in complex and irregular organ excitation (e.g. self-perpetuating re-entrant excitation). Prolongation of the QT interval of the electrocardiogram (pQT), associated with conditions such as heart failure and long-QT syndromes, is linked to increased vulnerability to arrhythmia. Pharmacological management of arrhythmia associated with pQT has been demonstrated to have limited effectiveness; Understanding the impact of pharmacological modulation on the complex interaction between trigger and substrate remains a significant yet important challenge in order to improve intervention efficacy.

We examined the efficacy of a hERG activator (MC-II-157c) to reduce the manifestation of cell- and tissue-scale triggers and its concomitant effects on the tissue substrate using a multi-scale modelling approach: a model of the human ventricular action potential was integrated with a model of stochastic 3D spatiotemporal Ca^{2+} dynamics, which was then coarse-grained for suitability for 1D-3D tissue simulations. Parameters were modified to mimic pQT and MC-II-157c conditions.

pQT conditions promoted the development of spontaneous release events underlying afterdepolarisations during rapid pacing. MC-II-157c applied to pQT conditions shortened the action potential duration, inhibited the development of afterdepolarisations and reduced the probability of afterdepolarisations manifesting as triggered activity in single cells and ectopic activity in tissue. However, it could also increase transmural dispersion of repolarisation, which manifested as an increased vulnerable window for unidirectional conduction block.

The combination of stochastic release event modulation and transmural dispersion of repolarisation modulation by MC-II-157c resulted in an integrative behaviour wherein the arrhythmia trigger is reduced but the substrate is increased. Such variable overall vulnerability to arrhythmia cannot be predicted from single-cell studies alone.

10. Cardiomyocyte senescence accumulates following myocardial infarction and ischaemia-reperfusion injury

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Myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide. While reperfusion via primary percutaneous coronary intervention is the gold-standard therapy, it can also lead to ischemia-reperfusion injury (IRI) characterised by progressive remodelling and heart failure. While the mechanisms contributing to IRI are not fully understood, increased oxidative stress plays a role. Our previous studies demonstrate that during ageing increased oxidative stress drives telomere associated DNA damage foci (TAF) induced cardiomyocyte senescence which is directly associated with a hypertrophic phenotype. Furthermore senescent cardiomyocytes express a pro-fibrotic profile; in particular an up-regulation of TGF-β expression. We now hypothesise that following MI and IRI, cardiomyocyte senescence contributes to remodelling through similar mechanisms and as such represents a potential therapeutic target. Young three month old mice underwent 60 minute surgical ligation of the left anterior descending coronary artery to mimic MI followed by reperfusion. Histological analysis at numerous time points post-MI demonstrated that mice displayed classical pathophysiological aspects related to MI. In support of our hypothesis, in the hearts of these mice we have observed that within the surviving myocardium, proximal to the infarct region, cardiomyocytes accumulate TAF and a senescent-like phenotype. This is demonstrated by an increase in senescence markers including SA-β-Gal, p21 and p19. We are now using *in vitro* studies and transgenic mouse models to better understand the biology underlying cardiomyocyte senescence and to establish the mechanisms that senescence contributes to remodelling. Furthermore we aim to ascertain if modulation or clearance of cardiomyocyte senescence improves outcome following IRI.

11. Prostanoid-mediated inhibition of IL-6 trans-signalling in pulmonary arterial hypertension: a role for "suppressor of cytokine 3" (SOCS3)?

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Inflammation has been highlighted as a key factor in pulmonary arterial hypertension (PAH) development[1], in particular interleukin-6 (IL-6)[2]. IL-6 trans-signalling activates JAK/STAT signalling to induce transcription of pro-inflammatory and pro-angiogenic genes, enabling PAH progression, as well as the transcription of suppressor of cytokine signalling 3 (SOCS3) which limits IL-6 signalling[3]. Current PAH therapies include prostanoid drugs which induce vasodilation via stimulating intracellular cyclic adenosine monophosphate (cAMP) levels. cAMP is also an inhibitor of endothelial dysfunction via induction of SOCS3[4].

Thus, my studies are testing the hypothesis that an important mechanism by which cAMP-mobilising prostanoid drugs limit PAH is by inhibiting IL-6-mediated pulmonary inflammation and remodelling via Epac1-mediated SOCS3 inhibition of IL-6 induced JAK/STAT signalling.

We have demonstrated that prostanoid drugs beraprost and treprostinil both induce SOCS3 mRNA and protein in pulmonary arterial ECs to inhibit IL-6 mediated Tyr705 phosphorylation of STAT3 by 30% ±8 ($P<0.01$) and 25% ±9 ($P<0.05$) respectively for *n=4* experiments. We will also present data assessing the functional significance of prostanoid mediated inhibition of IL-6 signalling in pulmonary arterial ECs, and determine the EPAC1 and SOCS3 dependence of their inhibitory effects on IL-6 signalling.

From these and future studies, it is anticipated that more effective strategies will emerge with which to target the IL-6/JAK/STAT signalling pathway in PAH.

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12. Embryonic origins of the great arteries.

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Background: Developmental abnormalities of the proximal thoracic aorta are a common accompaniment to congenital heart malformation. In adulthood, specific areas are affected by cardiovascular disease processes which have been related to physical forces. However, it has also been suggested that the distribution of different cell lineages in the vessel wall may focus pathologies. Avian and murine studies show that the tunica media in the root of the great arteries is derived from the second heart field (SHF) whereas neural crest (NC) cells give rise to the tunica media in the aortic arch.

Aim: We sought to establish the origins of the tunica intima and tunica adventitia and to determine whether lineage boundaries of the great arteries persist into adulthood. In addition, we asked if cytoskeletal phenotype was linked to these origins.

Methods: R26R eYFP$^{F/F}$ transgenic reporter mice were crossed with mice carrying transgenes for Mef2c-AHF-Cre and Wnt1-Cre to track cells derived from the SHF and NC respectively. The eYFP epitope was detected by immunohistochemistry on paraformaldehyde fixed paraffin embedded tissue sections.

Results and Conclusions: The majority of smooth muscle cells (SMC) in the root of the aorta and pulmonary artery trunk are derived from the SHF. The outer media and adventitia of the ascending aorta and pulmonary artery trunk are also formed from the SHF whereas the inner media of the ascending aorta and pulmonary artery trunk, as well as the media and adventitia of the aortic arch, are derived from the NC. In contrast, the intima of the pulmonary artery trunk, ascending aorta and aortic arch is derived solely from the SHF. These distinct boundaries are maintained as the vessels mature.

The SMC cytoskeletal proteins αSMA and SM22α are expressed at high levels throughout the media of the great arteries; except for regions in the proximal right subclavian artery and in the aortic arch, between the left common carotid and left subclavian arteries. These actin deficient stripes correspond to the regions of the mature aortic tree that are derived from the embryonic fourth pharyngeal arch arteries and share their distal boundary with the NC lineage boundary in the media. Desmin was localised most strongly to the outer media of the ascending aorta and pulmonary trunk, generally in cells from the SHF. Desmin was also strongly expressed in the NC derived ductal ligament.

These studies indicate that cell lineage boundaries differ in the intima, media and adventitia, revealing variation in their embryonic origin throughout the aorta and pulmonary artery. SMC proteins show differential expression patterns which do not map exclusively to lineage boundaries but instead appear to reflect the developmental structure they are derived from. Future studies will elucidate whether the differences in embryonic origin throughout the great arteries influence the development and progression of vessel pathology.

13. Class II histone deacetylases HDAC4 and HDAC5 repress TBX5 activity

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Abstract: TBX5 is a T-box family transcription factor that regulates both heart and forelimb development in vertebrates. Functional deficiencies in TBX5 cause Holt-Oram syndrome that affects both heart and hands structure in human. Recently, we have shown that acetylation of TBX5 potentiates its activity and is important for heart and limb development. Here we report that class II histone deacetylases HDAC4 and HDAC5 associate and repress TBX5-mediated cardiac gene transcription. Both HDAC4 and HDAC5 can deacetylate TBX5 and relocate it to the cytoplasm. We also revealed that HDAC4 antagonizes the physical association and functional cooperation between TBX5 and MEF2C. Protein kinase D1 (PRKD1) relieves HDAC4/5-mediated repression on TBX5. The study unravels a novel regulation of TBX5 transcriptional activity by HDAC4/5 and PRKD1

14. NOX4 NADPH oxidase is a key regulator of endothelial cell function in experimental diabetes

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Introduction: Diabetes-induced hyperglycaemia drives reactive oxygen species (ROS) production in endothelial cells (ECs), leading to microvascular dysfunction and cardiovascular complications. NADPH oxidases are enzymes whose primary function is ROS generation, and which contribute to development/progression of cardiovascular disease. We investigated the role of EC-specific NOX4 in autocrine/paracrine signalling as key determinants of diabetic cardiovascular remodelling.

Methods: Human aortic ECs (HAoECs) treated with normal (NG, 5.5mM) or high (HG, 25mM) glucose for 2-5 days with/without NOX4 siRNA knockdown (KD) were assessed for mRNA/protein expression (real-time RT-PCR, Western blot) and superoxide generation (DHE fluorescence). NIH3T3 fibroblasts were treated with conditioned media from NOX4-modified HAoECs for 24h to interrogate effects on paracrine signalling.

Results: HG treatment of HAoECs for 5 days increased NOX4 mRNA (NG 1.01±0.06, HG 1.27±0.06; n=9, $p<0.05$) and protein expression, associated with increased antioxidant and proinflammatory genes (NRF2: 0.91±0.04 vs. 1.22±0.04; IL6: 1.03±0.13 vs. 2.59±0.58; n=6, $0<0.05$), and increased superoxide production (262±12 vs. 338±11 arbitrary units; n=4, $p<0.05$) at 2 but not 5 days. NOX4KD under HG conditions increased mRNA expression of antioxidant enzymes after 2 days (NRF2: 1.90±0.06 vs. 2.21±0.06; n=3, $p<0.05$) whilst normalising increased superoxide production. Increased TGFβ-induced fibroblast differentiation observed with conditioned media from HG-treated HAoECs was ablated by NOX4KD (α-SMA: scrambled control 1.31±0.04, NOX4KD 0.94±0.07; n=3, $p<0.05$).

Conclusions: HG-induced NOX4 signalling regulates ROS production and endogenous antioxidant expression in ECs, driving paracrine stimulation of fibroblast differentiation. It therefore seems likely that EC NOX4 NADPH oxidase signalling contributes significantly to diabetic cardiovascular remodelling.

15. Energetic regulation of SERCA in LV from a post-MI rat model

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The Ca^{2+} uptake properties were measured in cardiac sarcoplasmic reticulum actively loaded with calcium. Local regulation of ATP/ADP ratio, creatine kinase (CK) and mitochondrial activity upon sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) were retrospectively investigated in frozen left ventricle (LV) biopsies samples from control, sedentary and trained Wistar rats. Chronic high-intensity aerobic treadmill running or untrained control was administered 4 weeks after induction of coronary artery ligation resulting in myocardial infarction (MI). Heart failure (HF) was confirmed by echocardiography and reduced exercise capacity (15-20%), presence of ~35% left ventricular scar tissue, pathologic hypertrophy of cardiomyocyte (20-30%), and reduced cardiomyocyte contraction and Ca2+ transient amplitude (both 30-40%). SR properties were examined (in units of nmol/mg wet weight) using the fluorescent indicator, Fura-2 free acid. Ca^{2+} loading was investigated on multifactorial conditions: samples were supplied with external source of either ATP or ADP with or without phosphocreatine (PCr) towards assessing mitochondrial ATP synthesis and/or CK-system stimulation. Azide (2mM) was employed to eliminate mitochondria-mediated energy production. Present data support that Ca2+ uptake (represented by amplitude of Ca^{2+} transients) occurs through SERCA activity, which was faster in control and trained groups when compared to sedentary, in presence of ATP and PCr. Exogenous ADP-supplied source did not significantly diminish SERCA uptake in control group when compared to sedentary animals, suggesting the pump, as well as the CK-system, can be impaired in HF. Elongation of the decay phase is consistent with a reduction in the efficiency of the SR Ca2+ pump due to azide-induced blockade in all groups.

16. Update on the H2020-RISE-645648- Muscle Stress Relief: An integrated research program linking together basic research on secondary myopathies in stress states to innovative translation in applied myology.

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A variety of medical or lifestyle conditions lead to a progressive loss of muscle force by functionally impairing myofibril contractility and causing ultimately muscle loss. Together, these conditions are predicted to lead to an endemic incidence of muscle weakness in the developed countries. Understanding the mechanisms involved requires a multidisciplinary research approach covering aspects of ageing, metabolism, and the cross-talk of muscle with other key organs including heart, liver, kidney, and lung. To achieve this, six European academic groups with complementary expertise in inter-organ-cross-talk during stress-induced secondary myopathies have teamed-up with third country teams (TC): three leading teams in the U.S. with expertise in the translation of muscle research into therapeutic interventions, and one team from South Africa with cutting-edge expertise in the regulation of regenerative capacities in muscle. Importantly, beneficiary EU small & medium enterprises (SMEs) of this RISE network provide expertise in early muscle disease detection, monitoring, and the development of new technologies. Their knowledge on muscle disease detection at early stages will promote translational innovation. To implement innovation and our joint research program, both early stage and advanced researchers are being seconded from the EU academic teams to the SMEs and TC teams and vice versa. Excellent progress in individual work-packages promises that this RISE scheme will establish a long-term collaborative University-SME driven translational innovative research in this interdisciplinary field of growing socioeconomic importance.

17. MAP1S ablation impairs survival after MI and the hypertrophic response to pressure overload through mediating cardiac autophagy

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Autophagy is an important process to maintain cellular homeostasis in many cell types including cardiomyocytes. Defective autophagy in response to pathological stimuli may lead to the development of adverse remodelling and eventually heart failure. The microtubule-associated protein 1S (MAP1S) has previously been identified as an interacting partner of the major autophagy regulator LC3; however, its role in the heart is not completely understood. Here we investigated the role of MAP1S in regulating autophagy in cardiac pathological conditions.

We used mice with genetic knockout of Map1s (MAP1S-/-) and neonatal rat cardiomyocytes (NRCM) with siRNA-mediated gene silencing to study the role of MAP1S. In NRCM lacking MAP1S, it has showed higher autophagic flux following rapamycin (5uM) and chloroquine (3uM) treatment as detected by analysing GFP-LC3 puncta formation and increased levels of LC3II protein expression.

We subjected MAP1S-/- mice to myocardial infarction (MI) or transverse aortic constriction (TAC). Following MI, we found a significantly higher mortality in MAP1S-/- mice vs WT control although the extent of MI was comparable between MAP1S-/- and WT as indicated by cTnI level and the fibrotic infarct area. However, the surviving MAP1S-/- mice displayed less hypertrophy 4 weeks after MI and was consistent in the pressure overload model as indicated by heart weight/body weight (HW/BW) ratio. This phenotype might be attributable to higher autophagy in the knockout animals.

Our findings suggest that MAP1S modulates autophagy in cardiomyocytes. In vivo ablation of MAP1S might impair survival after MI and lead to an attenuated hypertrophic response following pressure overload.

18. Regulatory Mechanism of SK Channel in Atrial Electrophysiology

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Small conductance calcium-activated potassium (SK) channels are unique as they have the ability to sense changes in intracellular Ca^{2+} concentration and convert it to K^+ conductance. A meta-analysis of genome-wide association (GWAS) studies conducted in 2010 with lone Atrial Fibrillation (AF) cases showed association with KCNN3, the gene encoding SK3 protein and in recent GWAS studies in 2017, genetic loci at KCNN2 has also been found to be associated with AF. In pharmacological studies, blockade of SK channels has demonstrated efficacious in converting AF to normal sinus rhythm, likely by prolonging the atrial effective refractory period (AERP). SK channels tend to be more prominent in the atria than in the ventricles, which elevates its importance as atrial-selective target for AF by reducing the risk of adverse toxicities of the current anti-arrhythmic drugs; however, the molecular mechanism involved is not clear yet. Protein Kinase A (PKA) has been shown to regulate surface expression of SK2 channel in hippocampal neurons. We have tested the role of PKA on SK channel regulation in HEK293, a heterologous system expressing human SK3 by patch clamp technique and immunostaining. We showed that activation of PKA by forskolin (FSK), an adenylyl cyclase activator inhibits SK3 current density, also applying KT 5720, a PKA inhibitor on FSK treated cells reverts SK3 current. Inhibition of PKA by KT 5720 in HEK293-hSK3 resulted in increase in SK3 current considerably, which proves the regulation of SK3 current by PKA. Ongoing projects include testing of the PKA phosphorylation sites on SK2 and SK3 channel by mutating the putative PKA phosphorylation sites.

19. The Role of Plasma Membrane Calcium ATPase 1 in Angiogenesis

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Recently, plasma membrane calcium ATPase 4 (PMCA) has been established as a novel mediator of angiogenesis through its role in endothelial cell migration and tubule formation. In addition to PMCA4, both PMCA1 and PMCA2 are also expressed in human endothelial cells but their contribution to angiogenesis remains unknown. Therefore, we hypothesise that PMCA1 also modulates formation of new blood vessels by altering endothelial cell behaviours.

Transient knockdown of PMCA1 was achieved in human umbilical vein endothelial cells (HUVECs) using siRNA (si-PMCA1) and confirmed with qPCR and western blot. HUVEC viability, proliferation and rate of apoptosis was assessed using Alamar Blue, Ki-67 immunofluorescence and Caspase-Glo 3/7 assay respectively. Live cell imaging was performed to evaluate migration of cells in a wound, cytotoxicity and cell death. Furthermore, tubule formation was assessed using the Matrigel assay and FACs was used to determine cell-cycle distribution.

Transient knockdown of PMCA1 in HUVECs resulted in an 85% reduction of PMCA1 at both mRNA and protein level. Phenotypically, 3 and 6 days post-transfection, loss of PMCA1 significantly reduced HUVEC viability without a concomitant increase in apoptosis or reduction in proliferation. FACs-mediated cell cycle analysis revealed si-PMCA1 HUVECs had a higher percentage of cells in S-phase with fewer in G2/M-phase compared to controls. Additionally, loss of PMCA1 significantly reduced HUVEC migration and tubule formation despite significantly higher protein levels of the pro-angiogenic gene RCAN1.4. Overall, transient knockdown of PMCA1 has adverse effects on HUVEC viability, migration and tubule formation, suggesting loss of PMCA1 is detrimental for *in-vitro* angiogenesis.

20. Caveolar proteins in cardiac myocytes

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Caveolae are small (50–100 nm) invaginations of the cell membrane present in most cells. Caveolar proteins (caveolins and cavins) aid in the formation of caveolae and assist with the multiple functions caveolae perform. Current caveolar research focuses on non-muscle cells which lack the muscle-specific caveolin 3 (cav 3) and cavin 4 proteins. Cardiac myocytes express both muscle specific-caveolar proteins, as well as the ubiquitously expressed caveolin 1 (cav 1) and cavin 1. To date we have little understanding of how caveolar proteins are arranged and interact within the cardiac myocyte. Here, we have quantified protein expression and described protein distribution within the cardiac cell using quantitative western blotting (WB), Airyscan super-resolution and dSTORM microscopy.

Ventricular myocytes isolated from male Wistar rats (250-280 g) were either homogenised in Laemmli sample buffer for WB or attached to laminin-coated coverslips for imaging. Quantitative WB was modelled on the DOSCAT system [1]. For imaging, isolated cells were electrically stimulated at 1 Hz before being fixed in paraformaldehyde. Antibodies against cav 3 and the sodium-calcium exchanger (NCX) were used to map the cell membrane. Cavin 1, cavin 2 and cavin 4 were visualised by immunofluorescence staining in relation to the membrane for Airyscan, or individual cavin staining examined in dSTORM

This is the first quantitative measurement of caveolar protein expression and shows similar concentrations of Cav 3, Cav 1 and cavin 1 in the cardiac cell. Super-resolution imaging highlights differences in the subcellular distribution of the cavin proteins, consistent with spatially distinct caveolar sub-populations.

1. Bennett, R.J., et al., *DOSCATs: Double standards for protein quantification.* Scientific Reports, 2017. **7**: p. 45570.

21. MicroRNA 411 delivery promotes cardiomyocyte proliferation via Hippo pathway modulation

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Modulation of the Hippo-YAP signalling pathway has recently been shown to induce cardiac regeneration post myocardial infarction (MI). Previous studies have found that microRNAs can modulate Hippo pathway activity. In this study, we identified a novel microRNA that effectively regulates the Hippo pathway and induces cardiomyocyte proliferation *in vitro* and *in vivo*.
Following bioinformatics and YAP-luciferase reporter assay screening, two candidate microRNAs, miR-411 and miR-181a, have been identified as potential novel Hippo pathway regulators. We tested the effects of miR-411 and miR-181a expression in vitro using neonatal rat cardiomyocytes (NRCMs). We found that miR-411 could significantly increase YAP activity and cardiomyocyte proliferation whilst miR-181a did not cause any changes. We then injected miR-411 mimics in adult mouse hearts using polyethyleneimine (PEI). Five days post injection, the numbers of Ki-67 and EdU positive nuclei were increased significantly, suggesting that miR-411 can induce proliferation in mature cardiomyocytes. Next, we analysed expressions of 30 genes that are predicted to target miR-411. We found that the expression of Foxo1, a known inhibitor of YAP, was significantly decreased in NRCMs. These data suggest that miR-411 may increase YAP activity by targeting Foxo1.
In conclusion, miR-411 expression induces cardiomyocyte regeneration through a Foxo1-mediated increase of YAP activity.

22. A *de novo* mass spectrometry library of the guinea pig proteome as a tool for cardiovascular research.

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Recent advances in liquid chromatography-mass spectrometry (LC-MS) approaches have enabled the incorporation of proteomic studies in to workflows that increase our understanding of the molecular regulators of cardiovascular physiology and pathophysiology. The outcomes of proteomic experiments, particularly directed approaches e.g. SRM or SWATH, benefits greatly from the availability of an extensive background data resource. The guinea pig is an excellent experimental model for many aspects of human cardiovascular physiology yet the experimental information regarding its proteome is very limited. In an effort to overcome this obstacle, we sought to generate, via numerous LC-MS/MS measurements, a spectral library of the guinea pig proteome.

Homogenates and tryptic digests were prepared from 15 tissues (heart, skeletal muscle, brain, uterus, colon, placenta, ovaries, liver, pancreas, lung, kidney, intestines, duodenum, adipose) isolated from sacrificed guinea pigs (fetal-adult) and subjected to >200 LC-MS/MS runs to (i) extract peptide-specific information including retention time, m/z value, fragmentation pattern and amino acid sequence; and (ii) thereby, with reference to the guinea pig genome, identify protein constituents of the proteome.

Analysis of >250,000 peptide-spectrum matches resulted in the construction of a library of 63,617 peptides that corresponded to 6,695 proteins. This experimentally validated library increases coverage of the guinea pig proteome >50-fold beyond that publicly available (Uniprot, Dec 2017). It thereby will furnish the research community with a comprehensive resource to enable exploration of future molecular-phenotypic studies using the guinea pig as an experimental model.

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23. Regional variations in the micromechanical and biochemical properties of the ovine aorta.

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Investigation of the mechanical properties of the aorta is important for better understanding of aortic diseases such as aortic aneurysms and aortic dissection. While there have been previous studies examining regional differences in the structure and biomechanical properties of the aorta, little is known about how these properties vary across its entire length. The purpose of this study was to map the biomechanical properties and biochemical changes of the ovine aortic wall along its entire length.

Fresh ovine aortas (n-3) were split into nine sections; they were separated by 2 cm intervals from the aortic root to the celiac artery region. For each section, three biopsies were cut out using a 5 mm biopsy punch (a total of 81 biopsies). The adventitia layer was measured using a dynamic nanoindentation method. 4×4 arrays of oscillatory indentations were applied to the surface of the tissue, and the shear storage modulus (G´) was determined. Subsequently, the same samples were used to determine elastin, collagen and glycosaminoglycan (GAG) levels using established biochemical assays. Overall, the shear storage modulus increased by 175.1% from the ascending to abdominal region. The collagen level was lowest at the first point of ascending and steadily increased with distance whereas elastin level and GAG decreased. We conclude that there is a significant correlation between an increase in G′ and collagen (P=0.01) with distance from the aortic root whilst elastin (P=0.05) and GAG (P=0.05) levels were significantly decreased.

24. Disruption of sodium-dependent vitamin transport: a potential novel cause of cardiomyopathy

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Cardiomyopathy is a heterogeneous disorder affecting adults and children and is a leading cause of death. Paediatric cardiomyopathies affect ~1 per 100,000 children and around one third of these children will undergo a heart transplant or die within two years. Using whole exome sequencing we have identified a homozygous missense mutation in the human Sodium Multivitamin Transporter (SMVT) gene, *SLC5A6*, in two sisters with paediatric cardiomyopathy. SMVT is a plasma membrane protein that transports biotin, pantothenic acid and lipoic acid throughout several tissues including the brain, intestine, placenta and heart. These three substrates play an essential role in energy metabolism and homeostasis, suggesting that a reduction in the functionality of SMVT within the heart could result in cardiomyopathy. In order to assess the effect of dysfunctional SMVT within the heart, we have developed a *Slc5a6* cardiac-specific conditional knockout (cKO) mouse model. *Slc5a6*-cKO mice appeared phenotypically normal at weaning but some died suddenly at 20 weeks of age. We are currently carrying out a phenotypic analysis of the mutant mice using body weight and electrocardiography (ECG) readings. At 20 weeks (the study end point due to the severity of the phenotype) the mice will undergo magnetic resonance imaging to observe cardiac function before the hearts are collected for histological analysis. Here we present a summary of the current data detailing the cardiac phenotype of the *Slc5a6*-cKO mice.

25. Heart transplantation in the failing Fontan: Will it work for my patient?

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Introduction: High-risk heart transplantation (HT) currently offers the only sustainable approach to modify the natural history of the failing Fontan circulation. However, it remains unclear at assessment which patients will gain most benefit. The aim of this study was to assess the validity of factors (lower age, shorter Fontan-transplantation interval, lower ejection fraction, pre-transplantation cardiac/renal mechanical support, moderate/severe systemic atrioventricular valve regurgitation, higher MELD-XI score) identified to predict poor post-transplantation outcome [Berg CJ et al AmJCardiol 2017;119(10):1675-1679].
Methods: We reviewed pre-transplantation data for all paediatric and adult patients who underwent HT for failing Fontan between January 2009 and October 2017 at our Institute. The association of all-cause mortality with individual demographic, echocardiographic and clinical variables was assessed using the Cox proportional hazards model.
Results: Thirty-seven patients (median age 21.3 years, range 3.4-43.5) underwent HT for failing Fontan circulation. Median follow-up was 2.8 years (range 0-8.1). Survival was 73% at 1 year and 60.1% at 5 years. Most deaths (10/13) were early and preceded hospital discharge. Factors predictive of higher post-transplantation mortality in our cohort were MELD-XI score, bridging to transplantation with renal replacement therapy and Fontan failure with preserved systolic ventricular function (Table). Transplantation age below 18 years (n=14, 5 deaths) was not a statistically significant predictor of mortality compared to adult age (n=23, 8 deaths) and neither were gender, age at Fontan, Fontan-to-transplantation interval, degree of pre-transplantation systemic AVVR and use of ECMO as a bridge to transplantation.
Conclusion: In this mixed population of children and adults with failing Fontan circulations, higher MELD-XI score, renal replacement therapy and preserved ventricular function predicted post-transplantation mortality. This contrasts with others' findings and underlines the need for multi-centre cooperation to identify robust risk factors that can be utilized in this rare and challenging patient group.

26. Pharyngeal endoderm signalling controls aortic arch artery development

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Congenital cardiovascular defects affecting the outflow tract and aortic arch arteries are a key phenotype observed in DiGeorge Syndrome patients, which is caused by a microdeletion on chromosome 22q11. *TBX1*, a transcription factor contained in the deleted region, has been identified as a gene crucial for correct morphogenesis of the aortic arch arteries. Another transcription factor, *Pax9*, which is specifically expressed within the pharyngeal endoderm at mid-embryogenesis, has been shown to be downregulated in *Tbx1*-null mouse embryos. We tested whether there was a genetic interaction between *Tbx1* and *Pax9* by crossing mice heterozygous for each gene and found that the double heterozygous mice (i.e. $Tbx1^{+/-};Pax9^{+/-}$) presented with a significantly increased incidence of interrupted aortic arch when compared to $Tbx1^{+/-}$ mice. We also found that *Pax9* itself is required for cardiovascular development as *Pax9*-null mice die perinatally with complex cardiovascular defects affecting the outflow tract and aortic arch arteries, including double-outlet right ventricle, interrupted aortic arch and absent common carotid arteries. Analysis at mid-embryogenesis revealed that the 4th pharyngeal arch artery fails to form and the 3rd arch artery forms but collapses by E11.5, giving the later stage phenotypes of interrupted aortic arch and absent common carotid arteries respectively. A concomitant reduction in neural crest cell migration into the pharyngeal arches and the failure of smooth muscle cell recruitment to the 3rd arch artery is likely to be the cause of its collapse. This study demonstrates that a signalling mechanism emanating from the pharyngeal endoderm is vital for the morphogenesis of the cardiovascular system.

27. An imbalance of mitochondrial fission and fusion is a feature of maturity onset of diabetes in the young (mody)

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Maturity Onset Diabetes in the Young (MODY) afflicts several millions globally but yet is poorly characterised. Cardiovascular complications concomitant with mitochondrial dysfunction are common in type 1 and type 2 diabetes[1]. Using the GENA348 mouse model, characterised by a mutation in the glucose kinase gene (recapitulating a form of MODY) we determined by echocardiography that at 6 months of age left ventricular dysfunction develops. RT-qPCR of proteins regulating mitochondrial fusion (mitofusin 1, Mfn1; mitofusin 2, Mfn2; optic atrophy protein1, Opa1) and fission (dynamin-1-like protein, Drp1)[2] identified that Mfn1, Mfn2 and Opa1 are upregulated at the transcriptional level whereas Drp1 is down-regulated. Quantitative mass spectrometry also revealed a down-regulation of protein subunits forming Complex I and Complex IV and the β-fatty acid oxidation pathway indicating impairment of oxidative phosphorylation and metabolic pathways. In summary, we report for the first time that GENA348 mice develop cardiovascular complications, exhibit mitochondrial dysfunction with an imbalance in mitochondrial dynamics. Interestingly, bariatric surgery and exercise leads to increased Mfn2 expression, with a corresponding increase in glucose oxidation and insulin sensitivity[3], suggesting up-regulation of Mfn2 may be beneficial. Work is now examining how changes to fission and fusion properties impact upon mitochondrial morphology using 3-D electron microscopy[4].

28. Arterial stiffness and cardiac power output in patients with heart failure

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Background / Aim: Heart failure (HF) is a debilitating disease presented with reduced ability of the heart to pump blood. It is associated with poor prognosis, poor quality of life for patients and high healthcare costs.[1] In addition to diminished heart function, people with heart failure also demonstrate reduced vascular function as demonstrated with increased arterial stiffness which is an independent cardiovascular risk factor. To date, there is very little evidence on how arterial stiffness effects cardiac function and performance in heart failure. Therefore, the aim of the project is to determine the relationship between arterial stiffness and cardiac function and performance in heart failure.
Methods: Twenty-four patients with stable chronic HF have been recruited into the study. All patients attended Clinical Research Facility for clinical investigations including blood analysis, body composition, vascular function using pulse wave analysis, and maximal graded cardiopulmonary exercise stress test for determination of exercise tolerance using online gas-exchange metabolic analyzer.[3] Haemodynamic measures of cardiac function and performance were monitored during rest and exercise stress testing conditions using non-invasive method based on an electrical signal processing technology called bioreactance.[4] All data will be screened for univariate and multivariate outliers and for normal distribution. Pearson's or Spearman's coefficient of correlation, as appropriate, will be calculated to determine the strength of the relationship between physical activity and cardiac function and performance.
Importance: Better understanding of the interaction between vascular and cardiac function in heart failure provides new insights on pathophysiology of heart failure which may lead to improvement in diagnostic and monitoring pathway for heart failure.

29. Small molecules activating Nrf2 as a therapeutic approach to prevent myocardial ischaemia/reperfusion injury.

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Ischaemic heart disease, often manifesting as acute myocardial infarction was accountable for 66,076 deaths (11% of all deaths) within the UK in 2016. While restoration of blood flow through a blocked coronary artery is necessary to prevent cell death, it paradoxically damages the myocardium; a phenomenon known as 'myocardial ischaemia/reperfusion (I/R) injury'. Such injury occurs via several mechanisms including the rapid production of reactive oxygen species (ROS) upon reperfusion. At the present time, no anti-oxidant therapies are currently approved for clinical use.

Transcription factor NF-E2 p45-related factor 2 (Nrf2) is controlled by the Kelch-like ECH-associated protein 1 (Keap1) and regulates cytoprotective processes associated with ROS. Under homeostatic conditions, Nrf2 is targeted for ubiquitination and proteosomal degradation by Keap1, keeping cytoplasmic levels low. However, in response to electrophiles which chemically modify Keap1, Nrf2 ubiquitination is inhibited, causing it to accumulate and increase transcription of its protective target genes. Interestingly, selected Nrf2 target genes are cytoprotective in the setting of I/R injury, as is the Nrf2 pathway itself, demonstrated by $Nrf2^{-/-}$ mice having an increased susceptibility to myocardial I/R injury and attenuated beneficial responses to ischaemic preconditioning. While dimethylfumarate and sulforaphane induce Nrf2 target genes, their specificity and therapeutic index is poor thus their clinical use is likely limited, and they have not been assessed in post-MI remodelling. Extensive structure-activity studies have identified exceptionally potent triterpenoid 'next generation' inducers (RTA dh404 and 408) of Nrf2 target genes that act via specific chemical modification of Keap1 and induce target gene expression in the heart. The ability of these orally active Nrf2 activators to protect against myocardial I/R injury is currently under evaluation.

30. Effect of flecainide on human atrial cell action potential upstroke velocity at different resting potentials and stimulations frequencies

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Background: Flecainide is a class Ic anti-arrhythmic drug used to treat patients with atrial fibrillation (AF). The mechanism involves use- (frequency-) dependent inhibition of Na^+ current and consequent reduction in action potential (AP) maximum upstroke velocity (V_{max}). However, the effect of altered resting membrane potential (RMP) on V_{max}-reduction by flecainide is unclear.

Aim: To measure effects of flecainide at therapeutic concentration on AP upstroke V_{max} in human atrial cells, at a range of pre-defined RMPs and two stimulation rates.

Methods: Right atrial tissues were obtained from 7 consenting patients undergoing cardiac surgery, all in sinus rhythm. Myocytes were isolated by enzymatic dissociation. APs were recorded (35-37°C) by whole-cell-patch clamp.

Results: At 1 Hz-stimulation, with RMP current-clamped at -85 mV, AP V_{max} was 236±31 V/s (*n*=10 cells). Progressively clamping to more +ve RMPs (-80 mV to -55 mV, in 5 mV steps) markedly and significantly decreased V_{max} (e.g. to 24±2 V/s at -55 mV; *P*<0.05). A similar V_{max}-RMP relationship was observed with 3 Hz-stimulation (*n*=8). Flecainide (1 µM) significantly decreased V_{max} at RMP -85 mV (1 Hz and 3 Hz), and also at -80 mV (3 Hz), but had no effect at RMPs positive to -75 mV. The degree of V_{max}-decrease by flecainide, at RMP -85 mV, was significantly greater (*P*<0.05) at 3 Hz (50±4%-decrease) than at 1 Hz (31±7%-decrease).

Conclusion: The use-dependent effect of flecainide on human atrial cell action potential maximum upstroke velocity requires resting potentials more negative than ~-75 mV, which may have implications for patients with AF.

31. Investigating a potential *Tbx1-Pax9-Gbx2* genetic network in the control of cardiovascular development

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Congenital heart defects are a leading cause of morbidity and occur in 22q11 deletion syndrome (22q11DS) patients where approximately 30 genes, including *TBX1*, are deleted hemizygously. Patients, and mouse models mutated for *Tbx1*, present with cardiovascular defects that include aberrant development of the aortic arch arteries. Formation of these vessels requires the remodelling of the pharyngeal arch arteries (PAA) which depends on regulated gene expression and the interaction of multiple tissues that comprise the pharyngeal arches. The wide spectrum of defects present in 22q11DS patients suggests that modifier genes may contribute to this phenotypic variation. *TBX1*, *PAX9* and *GBX2*, all independently required for cardiovascular development, are co-expressed in the pharyngeal endoderm, a tissue that provides signalling cues during PAA morphogenesis.

In the mouse, *Tbx1* is known to interact with *Gbx2* in cardiovascular development. We have shown that *Tbx1* and *Pax9* interact for 4th PAA formation. Here we have investigated whether *Pax9* and *Gbx2* interact in cardiovascular development using transgenic mouse models. Mouse embryos null for *Gbx2* and heterozygous for *Pax9* (*Gbx2*$^{-/-}$;*Pax9*$^{+/-}$) showed a greater penetrance of cardiovascular defects than *Gbx2*-null embryos. *Gbx2*$^{-/-}$;*Pax9*$^{+/-}$ embryos also had abnormal thymus development, a defect typical of *Pax9*-null mice but not *Gbx2*-null mice. Conditionally deleting *Gbx2* from the endoderm concomitantly with the heterozygous deletion of *Pax9* resulted in abnormal development but to a reduced frequency when compared to *Gbx2*$^{-/-}$;*Pax9*$^{+/-}$ embryos. This data indicates that *Pax9* heterozygosity modifies the *Gbx2*-null phenotype highlighting an *in vivo* interaction between these genes. However, more work is needed to unpick the tissue-specific requirements in PAA morphogenesis.

32. Do human right atria derived cardiac stem cells differ between developmental and adult heart?

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Knowledge of the role of native cardiac derived stem cells during human cardiogenesis remains limited. We have isolated native cardiac stem cells called cardiac mesenchymal stem cell like cells (CMSCLC) from the right atria (RA) of fetal and adult heart that have some of the characteristics of mesenchymal stem cells (MSCs) as determined by the ISCT position paper, 2006. Both adult and fetal CMSCLC are plastic adherent, have a typical MSC morphology and immunophenotype. In addition MSCs should be capable of tri-lineage differentiation to chondrocytes, adipocytes and osteocytes. Fetal RA CMSCLC are able to undergo osteogenic and adipogenic differentiation but this ability is more variable in adult cells. The adult CMSCLC contain some cells that undergo adipogenesis but they had low/no ability to undergo osteogenesis. This may be due to adult CMSCLC having less plasticity compared with developmental cells possibly as a result of adult CMSCLC having undergone changes due to duration/nature of the patient's cardiomyopathy. However, in all cases both fetal and adult cells under cardiomyocyte differentiation culture conditions express tropomyosin. In addition we examined both adult and fetal cell populations based on the expression of 22 genes using principal component analysis (PCA). We showed that the fetal and adult cells are two distinct cell populations but there is an overlap between the two groups. Further analysis of cells from 3 different adult donors showed that these cells expressed genes associated with pluripotency, proliferation and paracrine potential.

33. Post-translational Modification of Chemokines: Implications For Their Biological Function

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Following transplantation, chemokines contribute to acute and chronic rejection. Ischaemia-reperfusion Injury (IRI), which occurs during transplantation, involves the production of reactive nitrogen species (RNS), such as peroxynitrite (ONOO-), which has been shown to nitrate chemokines. Our group has shown that this post-translational nitration can affect chemokine function and detectability [1]. These modified chemokines (if non-functional) could pose a natural mechanism for the regulation of inflammation.

We have shown that nitration of CXCL8 by ONOO- reduces its ability to induce primary human neutrophil migration *in vitro*, and murine neutrophil migration *in vivo*. This was found to be through inhibition of both G-protein coupled receptor (GPCR) signalling and glycosaminoglycan (GAG) binding. We used N-loop mutant and nitrated mutant versions of CXCL8 (Y13F) and CXCL1 (L15Y), to assess which residues are essential for chemotactic function and targets for nitration. In both cases nitration appears to render the chemokines non-functional, reducing migration ($p<0.001$).

Characterising the expression and function of wild-type/nitrated chemokines could lead to use of these molecules as biomarkers of rejection, or anti-inflammatory therapies.

34. Improved analysis procedures for the quantification of cardiac spatiotemporal electrical excitation.

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Optical mapping techniques are commonly used to measure cardiac electrical excitation patterns with high spatial and temporal resolutions. A normal cardiac ventricular action potential (AP) consists of a very fast upstroke and a much slower repolarisation. These differences pose a challenge to adequately separate noise from the optical signals for each entire AP (and at each ventricular pixel). In support of our studies of electrical excitation in isolated guinea pig hearts, we developed algorithms and novel analysis procedures that improve the quantification of several dynamic parameters of cardiac APs.

We have routinely recorded spatiotemporal propagation (1kHz, 128x128 pixels of ~230um) of left ventricular APs from isolated guinea pig hearts for up to 60 seconds. Dynamic features of APs at each pixel are measured using a piece-wise digital filtering algorithm to: (i) securely separate the upstroke of the AP (ranging 8-15 msec depending on biological setting) from the remainder of excitation; this enables accurate quantification of upstroke duration and speed. (ii) apply a suitable noise reduction filter on the plateau and repolarisation phase. This improves signal:noise, consistency of APs recorded between pixels, and facilitates accurate determination of AP durations and repolarisation rates. Another notable improvement is made on the computation of the conduction velocity (CV) across the ventricle by enacting an algorithm that ensures the validity of the spread of data measurements surrounding each and every point of interest. These procedures facilitate the accurate quantification of entire AP spatiotemporal parameters including upstroke duration, AP duration and CV.

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35. Pharmacological modulation of the hippo pathway in cardiomyocytes

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Cardiac remodelling is a key process that can lead to the development of heart failure. Hypertrophic growth, cell death and apoptosis are important processes that are involved during remodelling. The Hippo pathway is known as an important regulator of cardiomyocyte hypertrophy, apoptosis and survival. Genetic inhibition of Mst1 and Mst2, the main core components of the pathway, has been shown to improve cardiac remodelling by regulation of hypertrophy and apoptosis. Here we used a novel inhibitor of Mst1/2 (XMU-MP-1) to examine the effects of pharmacological inhibition of the Hippo pathway in isolated cardiomyocytes.

We found that treatment with XMU-MP-1 induced both transcriptional activity and nuclear translocation of the transcriptional co-activator YAP, the main downstream target of the Hippo pathway, in neonatal rat cardiomyocytes (NRCM). This was accompanied by a significant increase in cardiomyocyte proliferation as indicated by Ki-67 staining and the BrdU incorporation assay. XMU-MP-1 also reduced H_2O_2-induced cell death as detected by MTT and TUNEL assays. Consistent with previous findings on Mst1/2 knock-out, XMU-MP-1 treatment significantly reduced phenylephrine-induced cardiomyocyte hypertrophy as indicated by cell size measurement and assessment of BNP levels. Western Blot analysis has revealed that this compound inhibited the activation of the pro-hypertrophic ERK1/2 pathway in response to phenylephrine.

In summary, XMU-MP-1-mediated inhibition of the Hippo pathway induces YAP activity and proliferation in neonatal rat cardiomyocytes as well as protecting cells from excessive hypertrophy and apoptosis. Thus, targeting the Hippo pathway may become a potential approach to control adverse cardiac remodelling.

36. Senescence as a therapeutic target for myocardial ageing

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Ageing is the biggest risk factor for impaired cardiovascular health, cardiovascular disease being the leading cause of death in 40% of individuals over 65 years old. Ageing is associated not only with an increased prevalence of cardiovascular disease but also with a poorer prognosis, including increased mortality or incidence of heart failure after myocardial infarction (MI). We have demonstrated that aged (23 month old) mice have an accumulation of cardiomyocyte senescence, reduced regenerative potential and display increased mortality as well as impaired recovery following MI. Cellular senescence is defined not only by the irreversible loss of division potential but also by the production of a senescence-associated secretory phenotype (SASP). This cocktail of pro-inflammatory cytokines, chemokines, matrix proteases and growth factors can impact on tissue function, inducing fibrosis, extracellular matrix degeneration and driving inflammation. We have therefore begun to test if clearance of senescent cardiomyocytes, using the senolytic compound Navitoclax, has the potential to improve cardiac health and post MI outcomes in aged animals. Following treatment with Navitoclax, but prior to MI, aged mice demonstrated a reduction in senescent cardiomyocytes, which was associated increased cardiomyocyte generation, a decline in myocardial hypertrophy and a decrease in fibrosis. Following MI, Navitoclax treated mice displayed a tendency towards improved survival and had a significant improvement in cardiac function when compared to vehicle controls. We conclude that clearance of senescent cells is a potential therapeutic strategy for the treatment of age related cardiac dysfunction.

37. Mechanisms of cell death in cardiac stem cells induced by receptor tyrosine kinases.

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The development of the novel anti-cancer drugs receptor tyrosine kinase inhibitors (RTKIs) has improved patient prognosis for certain cancers, however they have also been linked to causing cardiotoxic side-effects (Albini *et al*. (2010) J. Natl. Cancer Inst. 102:14–25). The impact of RTKIs on the endogenous cardiac stem cell (eCSC) population could have long term impact on myocardial ability to repair after diffuse damage (Ellison *et al.* (2013) Cell 154(4):827-42). We therefore investigated the toxicity of three RTKIs (imatinib mesylate, sunitinib maleate and sorafenib tosylate) on c-kit-positive, CD45-negative eCSCs. All three drugs reduced cell viability of eCSCs after 24 hour exposure (10μM imatinib: 70%±11%; 2μM sunitinib: 74%±6%; 10μM sorafenib: 67%±8%; relative to untreated control values of 100±6.5%, n=7, $p<0.05$). Examination of caspase-3/7 activation showed sunitinib caused a 1.2%±0.2 (2μM) and 23%±2.5 (20μM) increase in caspase-3/7-positive cells, whereas there was no increase following sorafenib or imatinib treatment (n=4, $p<0.05$). Real-time qPCR analysis identified increased expression of apoptosis-linked genes after sunitinib (2μM) exposure: 3±0.7 fold-change in calpain, 2.5±1.4 in FASR and 2±0.2 in BAX, with a 3.5±0.5 fold-change reduction in gene expression of anti-apoptotic Bcl-2 also seen (n=3, $p<0.05$). Expression of apoptosis-associated proteins in eCSCs (Bcl-2; BAX; Calpain 1; Calpain 2 and caspase 8) was examined after sunitinib exposures (2μM) of 24 hours and 72 hours. In summary, these data demonstrate that all three RTKIs are toxic to eCSCs and that cells exposed to sunitinib underwent apoptosis, whereas sorafenib and imatinib appear to be linked to necrotic cell death.

38. Single cell RNA-seq reveals the stochastic nature of endothelial gene expression

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Endothelial dysfunction contributes to the pathogenesis of numerous diseases, including diabetic microvascular complications, and understanding this dysfunction requires an in depth knowledge of endothelial cell gene expression and regulation. Single cell RNA-seq is a new technology that captures an individual cell's transcriptome enabling elucidation of cellular heterogeneity, stochasticity of gene expression and gene regulatory networks. This information is lost when using traditional bulk RNA-seq which takes average expression across a cell population.
In this study we optimised a workflow for single cell RNA-seq analysis in retinal microvascular endothelial cells. Primary Bovine Retinal Endothelial cells (BRECs) were captured on the Fluidigm C1 system, cDNA libraries were prepared using a miniaturized Nextera XT prep and sequenced on the Illumina NextSeq 500. Data analysis was performed in R using Scater for QC and pre-processing and generation of violin plots of gene expression and using Single Cell Consensus Clustering (SC3) for unsupervised clustering. The Automated Single Cell Analysis Pipeline (ASAP) was used to create dimensionality reduction plots (PCA and t-SNE) as well as heatmaps of microvascular barrier and angiogenesis gene expression.
Our results show that retinal endothelial cells in culture have relatively homogenous gene expression when analysed using clustering methods, however proliferating cells can be identified by their distinctive gene expression profiles. Heatmaps of genes involved in microvascular barrier function and angiogenesis showed considerable cell-to-cell variation in the level of expression of individual genes.
Our studies provide a quantitative basis for studying the stochastic regulation of endothelial gene expression in health and disease.

39. Characterisation of Caveolar Subpopulations in Ventricular Myocytes.

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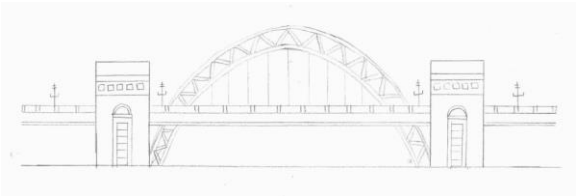
The lipid raft concept proposes that membrane environments enriched in cholesterol and sphingolipids cluster certain proteins and form platforms to integrate cell signalling. Evidence from non-cardiac cells has shown that there are multiple caveolar subpopulations, defined by different content of cholesterol, caveolin isoforms, signal effectors and targets.
The aim of this investigation was to identify caveolar proteins in cardiac muscle, investigate dynamic regulation of caveolar content, and define co-localised subpopulations of caveolar proteins.
To define subpopulations of cardiomyocyte caveolae we enriched caveolar membranes using a sucrose gradient. Membranes were fractionated by size exclusion chromatography, and fractions blotted for Caveolin-1, Caveolin-3, Na pump a1 subunit and Cavin-1. Preliminary results show that Caveolin-1 and Caveolin-3 are not enriched in the same fractions, suggesting a subset of cardiomyocyte caveolae is Caveolin-1-free. We find cavin-1 is associated preferentially with Caveolin-3 over Caveolin-1/3-enriched membranes. To further extend caveolae characterisation we developed a new profile of sucrose gradient which allows separation of caveolar membranes with higher resolution. We characterised these populations by peptide mass spectrometry. All cavins and caveolins are predominantly localised in more buoyant caveolar membranes (60-80%) except Cavin-4 (46%). Moreover, co-localisation fluorescence microscopy (proximity ligation assay) showed that Caveolin-3 share the same location with Cavin-1 in sarcolemma and transverse tubules while with Cavin-4 in sarcolemmal membranes only. This confirms that myocyte caveolae exist in different subpopulations. Hence physical and functional co-localisation of subpopulations of caveolar residents may contribute to the complexity of signalling through these microdomains in cardiac muscle.

Delegates



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