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VisitDerry

Scottish Cardiovascular Forum

Ulster University

7th May, 2022

25th Annual Meeting of the Scottish Cardiovascular Forum

Welcome / Fair Fa' ye

The committee of the Scottish Cardiovascular Forum, would like to offer you a warm welcome to Derry for the 25th annual meeting of the Scottish Cardiovascular Forum. Our programme features presentations on a very broad range of topics that span clinical practice, laboratory studies and informatics, and it highlights the quality and diversity of research taking place across our countries. The Scottish Cardiovascular Forum has always striven to provide a relaxed and informal environment in which openness and questioning is encouraged. We hope that you will find the meeting enjoyable, engaging and thought-provoking.

We also hope that you will take the opportunity to meet your peers, make new contacts and develop the network of professional relationships that can support your research career. In recent years, that opportunity has been sorely missed. As for so many meetings, the pandemic forced the 24th meeting of the Scottish Cardiovascular Forum to move online. It partly worked to our advantage, increasing the number of people who could access our meeting, but disadvantageously it robbed our attendees of the ability to spend time together, sharing thoughts, discussing ideas and, ultimately, learning about each other. Science is a social activity and physical meetings have an important role to play. We are delighted to be able to move away from online meetings of avatars in virtual rooms and return to physical meetings of peers in actual rooms.

Scotland and Northern Ireland have a great deal of shared history and nowhere is that more evident than in our language. The committee wishes you welcome and, in both the Scots and Ulster-Scots dialects, *Fair Fa' ye.*

Wifi access

Eduroam is available throughout the building and can be accessed using the credentials of your home institute.

Alternatively, you can connect to the Guest wifi and, on the landing page, click on the link to create an account. Create a username and password for yourself and, where it asks for the email address of a member of staff to validate/sponsor your account, enter s.watterson@ulster.ac.uk You should be able to access the Guest wifi with those credentials soon after.

What is in a name?

A Celtic monastery was founded where Derry stands in the 6th Century, named *Doire* ("Oak-wood"). This was subsequently anglicised to *Derry*. During the plantation of Ulster, the trade associations and guilds of London invested heavily in the city and in recognition its name was formally changed to Londonderry. However, the two names Derry and Londonderry have since become somewhat politicised and to try to appease all, the city is often officially referenced as Derry~Londonderry or even Derry~Londonderry~Doire.

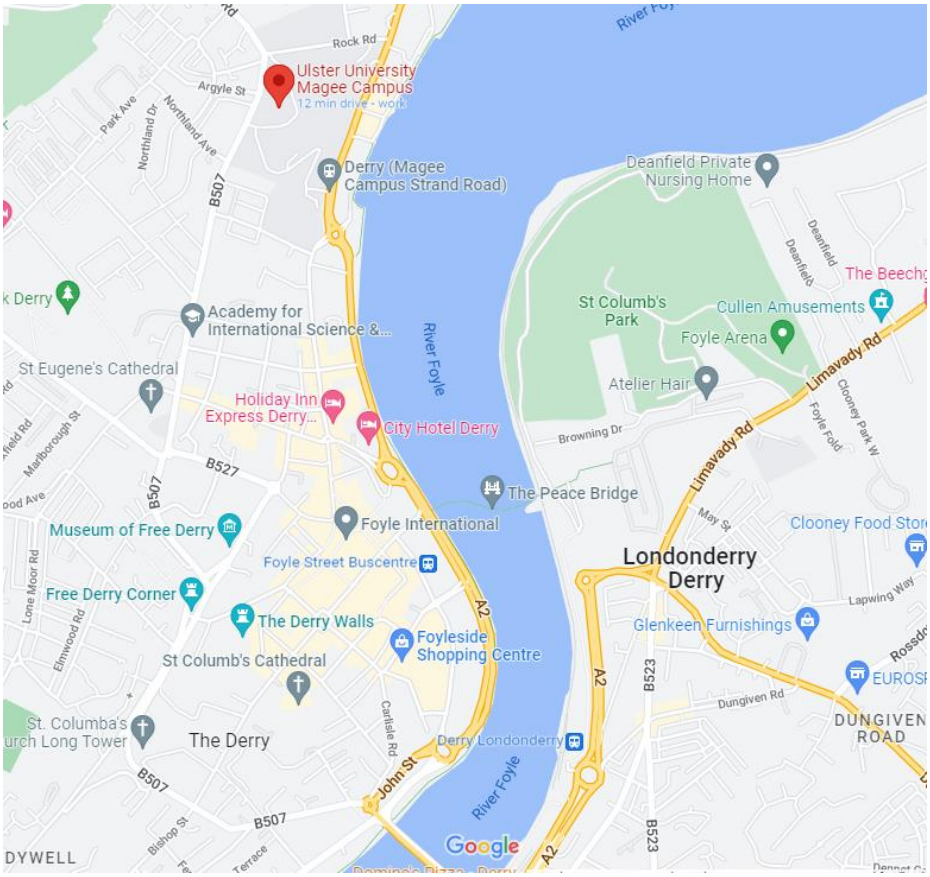
However, everyone who lives here just calls the place *Derry*; it's less of a mouthful.

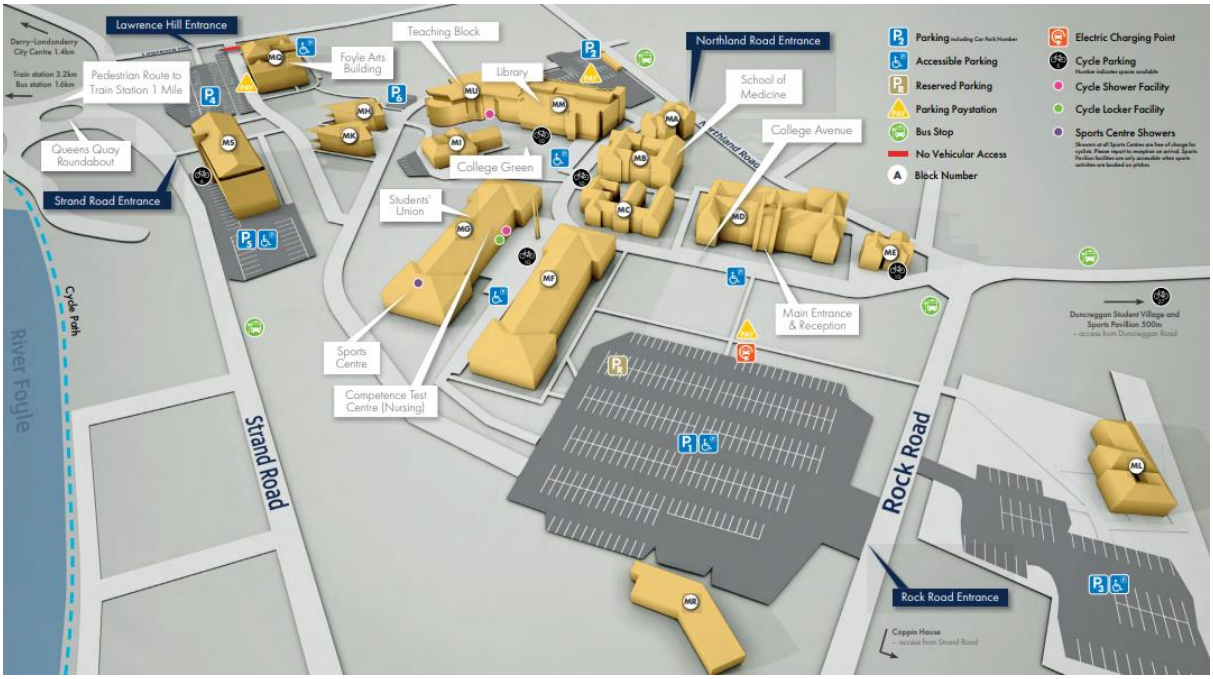
An interesting approach to this problem was taken in the USA. New Hampshire has two separate towns, called Derry and Londonderry, 5 miles apart.

Local amenities

As you walk north from the city centre along the quayside, towards Magee campus, you will pass a large Tesco superstore. The main entrance is on the Strand Road. If you continue a little further on the quayside (and slightly past Magee campus), you will find an even larger Sainsbury's superstore.

How to find us





The meeting will take place in the MU building, top-left labelled Teaching Block, in the ground-floor rooms MU125 (posters, tea/coffee) and MU114 (lecture theatre).

Things to do in Derry

The city walls. Derry is the only remaining completely walled city in Ireland. The Walls were built during the period 1613-1618 as defences for early seventeenth century settlers. The Walls are approximately 1.5km in circumference and form an easy-to-navigate walkway around Derry's centre that is freely open to the public all day. The walls are lined with 22 cannons from the 16th, 17th and 18th centuries and the west side of the walls provides a spectacular view of the Bogside region of the city with its wonderful murals. https://thederrywalls.com/

Walking tours. Fully guided tours lasting approximately 1 hour leave from 11 Carlisle Road, all year round at 10:00, 12:00, 14:00 and 16:00 (no need to book in advance). An opportunity to learn more about the varied and poignant stories of the city - The Bloody Sunday Story, the Apprentice Boys history, the traditions of the marching season, the famous murals and of course the walls themselves. https://www.visitderry.com/things-to-do/tours/walking-tours

The Guildhall. Derry's most distinctive landmark. Completed in 1890, the Guildhall is home to the district council. The clock tower was modelled on the Elizabeth Tower in London and the main hall houses a 3,132-pipe organ. Free to enter, the ground floor houses a small museum of the history of Derry and a coffee shop with views of the Foyle river. https://guildhallderry.com/

The Tower Museum. Immediately inside the city walls and close to the Guildhall, the Tower Museum has two permanent exhibitions, "The Story of Derry" and "La Trinidad Valencera - An Armada Shipwreck", describing one of the largest ships in the Spanish Armada which sank off the Donegal Coast in 1588 and was rediscovered by local divers in 1971. The top of the Tower Museum provides open air viewing with panoramic views. https://towermuseumcollections.com/

The Craft Village. Sandwiched between Shipquay St and Magazine St, the Craft Village is a reconstruction of an 18th century street and 19th century square and provides an eclectic mix of artisan craft shops, coffee shops and a licensed restaurant. https://www.derrycraftvillage.com/

The Void Gallery. A short walk from the Guildhall, on Waterloo Place, the Void is currently hosting a solo exhibition, Black Med Secco by Invernomuto (Simone Bertuzzi and Simone Trabucchi). https://www.derryvoid.com/

Our Place in Space. An epic scale model of the solar system designed by artist/children's author Oliver Jeffers. The 10km sculpture trail of the solar system will animate the riverside, beginning at the 'Sun' in Bay Road Park moving along the park by the River Foyle, running through the city centre, before heading across the river to the Waterside greenway to 'Pluto'. https://www.visitderry.com/whats-on/

Take a lap of the Foyle. Starting from the Guildhall, a walk along the quayside to the Craigavon bridge, across the Foyle, past the train station and along the greenway through St Columb's Park to the Foyle Bridge, before crossing the Foyle river again and walking along the quay back to the Guildhall is 10.4 km.

Programme

9.00 - 9.25am. Registration, Tea/Coffee, Room MU125

9.25 – 9.30am. Welcome remarks

Session 1: 9.30 – 11.15am, Room MU114

9.30 – 10.15am. Keynote 1: Dr Glenda Fleming, Medicines Optimisation Innovation Centre, Northern Health and Social Care Trust. *Medicines optimisation – what's it all about?*

10.15 – 11.15am. Oral Communications 1

10.15: Karla O'Neill, Queen's University Belfast. *Reduced pro-angiogenic function of endothelial colony-forming cells in hyperglycaemia is mediated by NOX4*

10.30: Caitlin Dollin, Ulster University. *MTHFR 677TT genotype is associated with hypertension in the Generation Scotland: Scottish Family Health Study*

10.45: Cameron Malcolm, University of Aberdeen. *GPR75, a G protein-coupled receptor target for metabolic syndrome*

11.00: Kanchan Phadwal, University of Edinburgh. *p53 regulates mitochondrial dynamics in vascular smooth muscle cell calcification*

11.15 – 11.35am. Tea/Coffee, MU125

Session 2: 11.35 – 12.35pm, MU114

11.35 – 12.35pm. Oral Communication 2

11.35: Matthew Ennis, Ulster University. *The contribution of cardiovascular disease to the development of multimorbidity for males and females*

11.50: Claire Tonry, Queen's University Belfast. *Proteomics identification and evaluation of a collagen sub-type with potential to support improvements in diagnosis and management of heart failure*

12.05: Matthew Manktelow, Ulster University. *Identifying opportunities for technological innovation in the pPCI pathway through ECG timing and location*

12.20: Narainrit Karuna, Queens University Belfast. *The study of diabetic cardiomyopathy progression in a mouse model using high-fat diet and high-dose of streptozotocin*

Lunch and Poster Session: 12.35 – 1.45pm, MU125

1.45 - 2.00pm Sonia Patton. *Public Enhancement Involving Research (PIER-NI)*

Session 3: 2.00 – 4.10pm, MU114

2.00 – 3.40pm. Oral Communication 3: The Roger M Wadsworth Prize Session

2.00: Guangran Guo, Ulster University. *Combination of machine learning and senescence signatures predict the risk of myocardial infarction*

2.20: Holly Woodward, University of Edinburgh*. Models and mechanisms of sexual dimorphism in cardiovascular calcification*

2.40: Giovanni Levate, University of Aberdeen. *The intracellular mediator phosphoprotein enriched in astrocytes (PEA)-15 is a novel regulator of cell size and lipid storage in brown adipocytes.*

3.00: Shun Hay Pun, Queen's University Belfast. *Activation of NOX4 NADPH oxidase signalling restores angiogenic function of cord blood endothelial colony-forming cells in hypoxia*

3.20: Qiyu Tang, University of Edinburgh. *Pharmacological inhibition of PI3K signaling in canine myxomatous mitral valve disease (MMVD)*

3.40 - 4.10pm. Oral Communication 4

3.40: Claire Tonry, Queen's University Belfast. *Identification and evaluation of novel protein biomarkers for atrial fibrillation*

3.55: Sphamandla Ntshangase, University of Edinburgh. *Illuminating spatial lipidomic profiles of atherosclerotic plaques by mass spectrometry imaging*

4.10 - 4.30pm. Tea/Coffee, MU125

Session 4: 4.30 – 5.35pm, MU114

4.30 – 5.15pm. Keynote 2: Dr Victoria McGilligan, Ulster University. *The NLRP3 inflammasome as a target for therapy development in cardiovascular disease*

5.15 – 5.35pm. Prize giving and closing remarks.

Abstracts

Roger M Wadsworth Prize Session

Roger M Wadsworth, Professor of Cardiovascular Pharmacology at Strathclyde University, dedicated his working life to cardiovascular research and was one of the founding members of the Scottish Cardiovascular Forum. During his academic career, he made huge contributions to teaching, both of undergraduate and postgraduate students, and in nurturing the scientists of the future. A number of the attendees at today's meeting will have benefitted from collaboration with and/or supervision by Roger. In recognition of Roger's work, the Scottish Cardiovascular Forum awards a prize in his name at each meeting.

The Roger M Wadsworth Prize was introduced in 2014 with a fund established by Roger's family. It is open to PhD students in their final year of study and the prize is awarded to the student judged to have given the most outstanding oral presentation at the SCF meeting.

Combination of machine learning and senescence signatures predict the risk of myocardial infarction

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Acute coronary syndromes (ACS) are a group of cardiac conditions that reduced blood flow into the heart. One of these conditions is myocardial infarction (MI), which affects 26 million people and is responsible for 4 million deaths in Europe and more than a third of deaths from all causes in developed countries. 33% to 50% acute MI happens in patients >70 years old. Further, 80% acute MI caused death occurred in people over 65 years old implicating aging as biggest risk factor for MI. One cellular process that causes ageing and ageing-related diseases is cell senescence. We hypothesize that accumulation of senescent cardiac cells may contribute to MI causing high mortality rate. Discovering specific senescence biomarkers may be able to predict MI risk or provide therapeutic targets. It is well known that senescent cells secrete senescence-associated secreted phenotypes (SASP) to enhance senescence and affect neighboring tissues causing further tissue damages and deepen diseases. In this study, ACS patients (n=171, mean age=65.61 ± 10.71) and control group (n=73, mean age=43.88 ± 10.48, 10-year cardiac-episode free) were recruited and their blood plasma was analyzed for senescence biomarkers by using Proseek Multiplex Proximity Extension Assay (Olink Bioscience). We found senescence biomarkers when combined with machine learning algorithms can accurately predict risk of Myocardial infarction (MI). To validate these findings in senescent cells we developed several senescence cardiac cell models. We introduced senescence to human primary cardiac fibroblasts (from human donors) and human iPSC-derived cardiomyocytes, followed by SA-β-GAL staining, DNA damage assays, proliferating assay to confirm the senescence state. Multi-omic analysis of these cardiac cells confirmed the in-vivo findings showing a successful deployment of a bedside to bench model of cardiac disease. To conclude, our research not only provides potential senescence biomarkers for diagnostic and therapeutic target for the MI patients but also an in vitro model to study these target proteins.

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Models and mechanisms of sexual dimorphism in cardiovascular calcification.

H Woodward[1], AJW Thomson[2], CJ Alcaide-Corral[2]., ADA Tavares[2], VE MacRae[1], PWF Hadoke[3]
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[2]Edinburgh Pre-Clinical Imaging, The University of Edinburgh.
[3]Centre for Cardiovascular Sciences, The University of Edinburgh.

Calcific aortic valve disease (CAVD) is the most common valve disease in the world, which typically causes significant cardiac dysfunction and eventually the need for a valve replacement. There is no effective pharmaceutical treatment for CAVD due to the lack of mechanistic understanding underpinning the disease; including why it is more common in male patients. I hypothesised that androgens would augment cardiovascular calcification in preclinical models. In vitro studies revealed that calcification of both rat valve interstitial cells ($P<0.01$), and murine vascular smooth muscle cells ($p<0.01$) was enhanced (2-3 fold) with testosterone treatment (concentration and length of exposure) whereas estrogen (concentration and length of exposure) had no effect. I next investigated sex differences in the Apolipoprotein E null (ApoE-/-) western diet murine model of CAVD. Increased microcalcification ($p<0.05$) and larger atherosclerotic plaques ($p<0.01$) in the aortae of female compared to male mice were observed. However circulating cholesterol and LDL were significantly lower in females concentrations ($p<0.05$). Female mice also displayed reduced tibial trabecular bone volume ($P<0.001$) and trabecular number ($P<0.001$). To conclude, sex hormones may drive the sexual dimorphism seen in cardiovascular calcification but further development of translational in vivo models is required to fully elucidate the underpinning mechanisms.

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The intracellular mediator phosphoprotein enriched in astrocytes (PEA)-15 is a novel regulator of cell size and lipid storage in brown adipocytes.

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In light of their critical fat-storing and lipo-metabolic roles, white and brown adipose tissue (WAT and BAT) are implicated in many disease states. In addition to lipid storage in WAT, BAT also actively captures and stores both glucose and triglycerides from the plasma, and its impaired activity contributes to type-2 diabetes and cardiovascular disease. Our recent in vivo project has identified the cytoplasmic phosphoprotein enriched in astrocytes (PEA)-15 as a novel regulator of WAT expansion. The present study aimed to investigate the role of PEA-15 in BAT.

We initially compared PEA-15 global knockout (KO) mice to wild type (WT) animals on chow and high fat diet (HFD). For the first time, we found PEA-15 was expressed in BAT. There was a significant increase in brown adipocyte size between KO and WT chow mice (222±9 μm2 vs 116±6 μm2 average area, $p<0.005$, n=4), as well as KO and WT HFD mice (309±10 μm2 vs 233±19 μm2 average area, $p<0.005$, n=5). In parallel, lipid droplets were found to be slightly larger in KO vs WT chow mice (17±1 μm2 vs 7±0.9 μm2 average area, $p<0.05$, n=4), while more significantly between KO and WT HFD animals (32±3 μm2 vs 14±1 μm2 average area, $p<0.005$, n=4). This clearly indicates PEA-15 plays a key role in cell size and lipid storage in BAT.

Having a well-characterised brown adipocyte phenotype, Simpson-Golabi-Behmel Syndrome (SGBS) pre-adipocytes were differentiated and transfected with either PEA-15 siRNA or control siRNA. SGBS adipocytes were larger after PEA-15 siRNA treatment compared to the control (2250±103 μm2 vs 1707±108 μm2 average area, $p<0.005$, n=4). PEA-15 knockdown did not result in increased lipid droplets size between the two groups. Importantly, these adipocytes were more mature than the control, as evidenced by fatty acid-binding protein-4 marker levels. Consequently, a substantial overexpression of enzymes involved in lipolysis and lipid droplets development, such as hormone-sensitive lipase and perilipin-1 was also observed. Finally, we detected increased expression of total (0.70±0.22 vs 1.10±0.15, $p<0.05$, n=4) and phosphorylated p70s6k (0.81±0.16 vs 0.17±0.1, $p<0.05$, n=4), a ribosomal kinase controlling cell growth, in PEA-15 KO cells versus the control. Together, these findings indicate PEA-15 limits BAT expansion and maturation, possibly through inhibition of p70s6k activity.

In summary, our data show PEA-15 is expressed in BAT and PEA-15 knockdown results in increased cell and lipid droplets size in vivo. We also show PEA-15 knockdown leads to changes in lipid metabolism and adipocyte size and maturation in vitro, as well as increased expression of p70s6k.
In conclusion, we reveal that PEA-15 could regulate cell size and lipid storage in BAT and this mechanism may involve PEA-15 dependent regulation of p70s6 kinase.
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Activation of NOX4 NADPH oxidase signalling restores angiogenic function of cord blood endothelial colony-forming cells in hypoxia

SH Pun[1], KM O'Neill[1], H Naderi-Meshkin[1], S Malla[2], W King[1], B Botezatu[1], PD Dunne[2], DP Brazil[1], CJ Watson[1], DJ Grieve[1]

[1]Wellcome-Wolfson Institute for Experimental Medicine,
[2]The Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, UK

Objective: Myocardial infarction leads to adverse cardiac remodelling, driven largely by tissue ischaemia and hypoxia. Whilst cord blood-derived endothelial colony-forming cells (CB-ECFCs) show clear potential to promote angiogenesis and endothelial function post-infarction, their therapeutic application is limited by reduced efficacy in ischaemic tissue. As we reported NOX4 NADPH oxidase to enhance CB-ECFC-mediated neovascularisation, this study aimed to define specific impact of hypoxia, as a highly relevant stress to the ischaemic microenvironment.

Methods: CB-ECFCs (typically n=9, 3 clones) were exposed to hypoxia (1% O_2) or normoxia (21% O_2) for 48h prior to analysis of angiogenic function (3D Matrigel assay), gene expression (qRT-PCR, western blot, microarray), reactive oxygen species (H_2O_2, DHE assay), and gene modification (plasmid overexpression [OE], siRNA knockdown [KD]).

Results: CB-ECFCs showed impaired tubulogenesis in hypoxia (tube area: normoxia 19.1+4.0, hypoxia 2.5+0.7μm^2; $p<0.001$) which was associated with impaired metabolism (MTT, 59% vs normoxia, $p<0.001$) with maintained viability, and reduced expression of NOX4 and other functional genes (eNOS, HMOX1, VEGFR2), together with decreased H_2O_2 (normoxia 0.46+0.03, hypoxia 0.08+0.02 arbitrary units; $p<0.001$) and increased superoxide (normoxia 0.33+0.02, hypoxia 0.44+0.01 arbitrary units; $p<0.001$). Parallel microarray analysis highlighted NOX4 as central to altered signalling observed in hypoxic CB-ECFCs and identified PLAC8 and AK4 as upstream negative regulators. Whilst NOX4 OE partially restored angiogenic function in hypoxic CB-ECFCs (tube area: empty vector 18.7+2.1, OE 31.1+2.5μm^2; $p<0.05$), PLAC8 or AK4 KD reversed hypoxia-induced dysfunction (PLAC8 tube area: non-targeting control 8.2+2.9, KD 33.1+6.5μm^2; $p<0.05$) in parallel with NOX4 activation.

Conclusions: Together, these findings highlight impaired NOX4 signalling as a key determinant of CB-ECFC angiogenic dysfunction in hypoxia, which is likely to influence *in vivo* function and capacity for vascular repair. PLAC8 and AK4 may therefore represent novel targets to enhance NOX4-dependent vasoregenerative capacity of CB-ECFCs with potential therapeutic application to reduce progression of adverse post-infarction cardiac remodelling.

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Pharmacological inhibition of PI3K signaling in canine myxomatous mitral valve disease (MMVD)

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Myxomatous mitral valve disease (MMVD) is one of the most important chronic degenerative valvulopathies in people and dogs with no effective therapeutic inventions to control the onset and progression of disease. It is a common cause of heart failure leading to significant morbidity and mortality in both species. The transition of activated valvular interstitial cells (aVICs; activated myofibroblasts) from a quiescent VIC (qVIC) phenotype is believed to be the primary driver of the myxomatous degeneration characteristic of this disease, and is under transforming growth factor beta (TGF-β) control. The complex TGF-β signalling pathway includes phosphatidylinositol-3-kinase (PI3K) signalling, which has been shown to be important in cancer and non-valvular cardiovascular diseases, providing options for novel treatments. The aim of this study was to investigate the role of PI3K in the pathogenesis of MMVD in the dog, with particular reference to control of cellular apoptosis, autophagy and senescence, using a combination of protein immunoblotting and immune-staining.

VICs from normal and dogs diagnosed with MMVD were isolated and cultured under low-serum conditions (2% FBS). Pharmacological inhibition of PI3K in aVICs by LY249002, copanlisib and alpelisib significantly reduced α-smooth muscle actin (SMA) expression, the main aVIC marker ($p<0.001$), returning cells to a more quiescent phenotype. PI3K inhibition reduced expression of the phosphorylated forms of the downstream effectors Akt and mTOR in the PI3K pathway ($p<0.001$). PI3K/Akt/mTOR inhibition was also found to affect the cellular activities of apoptosis, autophagy and senescence. PI3K inhibition increased the expression of caspase-3 and cleaved caspase-3 (apoptosis), LC3-II (autophagy) and decreased expression of p16, p21 and p53 (senescence) ($P<0.05$). Cell staining identified enhanced TUNEL (apoptosis), LC3-II (autophagy) and attenuation of (senescence-associated-β-galactosidase) SA-β-gal and γ-H2AX staining after PI3K inhibition ($P<0.05$).

These data indicate that aVICs in canine MMVD are in a senescent state with reduced capacity for apoptosis and autophagy and that pharmacological inhibition of PI3K pathway can transition aVICs to qVICs by promoting apoptosis and autophagy and inhibiting senescence. It is likely cell senescence contributes to MMVD pathogenesis and these data provide insight into potential novel therapeutic targets that may be applicable to both the dog and human.

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Abstracts
Oral Communications

Investigating the mechanical properties of alginate-based hydrogels: towards the development of compliant aortic grafts

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Despite numerous advancements in vascular graft technologies, commercially available state-of-the-art Dacron (polyethylene terephthalate, PET) grafts used to surgically treat aortic disease or trauma, demonstrate a mismatch in mechanical properties when compared to the native aorta. The high rigidity and poor compliance of these grafts results in the disruption of cardiovascular homeostasis; conditions including haemodynamic changes and arterial stiffening, culminating in clinical complications such as systolic hypertension, left ventricular hypertrophy, and reduced coronary perfusion. Therefore, improved graft technologies are required that address this mismatch in mechanical properties.

The use of hydrogels for the development of small-diameter blood vessels has been widely investigated, primarily due to the versatility in gel fabrication methods and controllability of the mechanical properties. However, this has yet to be translated to large-diameter structures such as the aorta. In this study, we investigated the mechanical properties of alginate-based hydrogels. Alginate, a natural polysaccharide derived from seaweed, is biocompatible and can easily form a hydrogel in the presence of divalent ions (Ca2+,Ba2+) in a process known as ionic crosslinking. Alginate hydrogels were mechanically characterised via uniaxial tensile testing at a crosshead speed of 1 mm/min until specimen failure occurred (n=5; mean±SD). Initial investigations demonstrated weak mechanical properties for alginate alone gels (ultimate tensile strength; UTS: 0.02 ±0.01 MPa, elastic modulus; E: 0.04 ±0.01 MPa) when compared to aortic tissue data from the literature (UTS: 0.61 ±0.07, E: 1.82 ±0.10 MPa). Ideally, vascular graft materials should be resilient and strong enough to withstand the surgical implantation procedure and the high pulsatile pressure of the aorta in vivo. Therefore, to improve the mechanical properties of the alginate hydrogels, an interpenetrating polymer network (IPN) was designed comprising two separate crosslinking mechanisms: ionically crosslinked alginate and a photocurable polymer. This novel alginate-polymer IPN resulted in a significant increase in strength and stiffness (UTS: 0.39 ±0.05 MPa, E: 1.61 ±0.19 MPa) over the alginate alone gels, with mechanical properties close to aortic tissue.

In conclusion, these results demonstrate how alginate-based hydrogels can be modified to mimic the mechanical properties of the human aorta. This hydrogel formulation will now be coupled with a suitable tubular structure fabrication method with the aim of developing optimised large diameter grafts.

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MTHFR 677TT genotype is associated with hypertension in the Generation Scotland: Scottish Family Health Study

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Background: The C677T polymorphism in the gene encoding methylenetetrahydrofolate reductase (MTHFR) is associated with an increased risk of hypertension, however, few studies have investigated the contribution of this genetic variant to blood pressure (BP) within population cohorts generally.

Objectives: The aim of this study was to investigate the effect of MTHFR genotype on BP within a representative sample of the Scottish population.

Methods: Data was accessed from Generation Scotland: Scottish Family Health Study (GS:SFH; n=19,994) and participants were age and sex matched by MTHFR genotype. Using one-way ANCOVA, the association between the MTHFR C677T polymorphism and BP was examined across different age and sex categories. Logistic regression was performed to predict determinants of hypertension.

Results: The variant MTHFR 677TT genotype was identified in 11.4% of adults in this cohort. Significantly higher systolic (+9.2 mmHg) and diastolic (+9.6 mmHg) BP was observed in the MTHFR 677TT compared to CC genotype group ($p<0.001$). A significantly greater proportion of TT individuals (30.6%) compared to non-TT were observed to be hypertensive (CC=6.4%, CT=7.9%; $p<0.001$). Furthermore, MTHFR 677TT genotype was found to be a significant determinant of hypertension (OR 7.52, 95% CI, 6.00-9.40) and high BP (OR 1.87, 95% CI, 1.48-2.36; $p<0.001$) following adjustment for covariates. In TT adults <30 years mean systolic BP (121.8 mmHg) was +6.6 mmHg higher than CC's of a similar age and was similar of that observed in non-TT adults aged >50. In addition, there was a significant effect of BP prevalence within family history.

Conclusion: We show, for the first time, in a large representative cohort of Scottish adults, that the MTHFR 677TT genotype is associated with higher BP compared to non-TT genotype groups. In those with the TT genotype <30 years BP was typical of an adult >50 without this genetic variant. Further studies are required to investigate the underpinning mechanisms associated with this increased genetic risk.

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The contribution of cardiovascular disease to the development of multimorbidity for males and females

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Cardiovascular disease (CVD) is a significant contributor to multimorbidity, the presence of 2 or more diseases within a patient. CVDs often frequently co-occur with other CVDs as well as non-CVDs. How CVDs contribute to trajectories of multimorbidity, the order in which diseases occur, and how these contributions differ between sex is not well understood. The UK Biobank is a large cohort of ~500,000 elderly adults and includes a wealth of collected data for each participant including linkage to secondary care diagnoses from hospital admissions. These secondary care diagnoses contain the full trajectory of participant hospital diagnoses and provide a useful source of data for investigation of multimorbidity trajectories. Here we apply a form of Dynamic Time Warping (DTW) and cluster analysis to participant disease trajectories, stratifying our analysis by sex. We identify several disease trajectory clusters in males and females. CVDs were found to be contributors to several of the identified clusters. Hypertensive diseases were frequently identified upstream in disease trajectories including those involving circulatory, respiratory, musculoskeletal, digestive, and genitourinary diseases. Cardiovascular trajectories formed a dominant cluster in males while no such cluster was found in females. These clusters included several male-enriched trajectories involving hypertensive diseases and ischemic heart diseases, hypertensive diseases and hernia, and diabetes and hypertensive diseases. The top female-enriched relative to male trajectories included thyroid disorders and hypertensive diseases and benign neoplasms and hypertensive diseases.

3

The study of diabetic cardiomyopathy progression in a mouse model using high-fat diet and high-dose of streptozotocin

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[1]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, Northern Ireland
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Background: Heart failure with preserved ejection fraction (HFpEF) is a multifactorial disease characterised by a variety of unique phenotypes, including cardiometabolic disorders with type 2 diabetes mellitus and obesity. Although there is a growing amount of knowledge on the pathogenesis of diabetic cardiomyopathy, questions remain on various aspects, particularly diabetic cardiomyopathy progression. Therefore, an animal model that recapitulates the complexities of diabetic cardiomyopathy is necessary. This study aimed to elucidate longitudinal follow-up in cardiac structure and function alterations in the context of the diabetic heart.

Methods: Male C57BL/6J mice were fed a control diet (CD) or high-fat diet (HFD) for 24 weeks. Streptozotocin (STZ) 100 mg/kg or vehicle was intraperitoneally administered to induce diabetes in the mouse at 8 weeks of diet. Echocardiography was monthly performed to study cardiac function and structure changes. In addition, left atrial volume and left atrial area were measured to indicate chronic diastolic dysfunction. Mice were tested for HbA1c and fasting blood glucose levels to confirm that mice developed diabetes. Blood pressure was measured by a tail-cuff method.

Results: In our model, HFD mice were injected with high-dose STZ resulted in establishing an evident phenotype in diabetic cardiomyopathy by significant changes in echocardiography parameters, including cardiac hypertrophy (LVPW), diastolic dysfunctions (MV E/A, IVRT, LA area, and LA volume) from 12 weeks to 24 weeks of study, compared to control. It is notable that only MV E/A began to change at 8 weeks of study. Furthermore, our model illustrated that HFD/STZ mice had higher HbA1c and fasting blood glucose levels than CD mice ($p<0.05$) with no differences in systolic blood pressure and ejection fraction ($p>0.05$).

Conclusion: We developed a mouse model that captures the complexities of diabetic cardiomyopathy both early and chronic progression, providing important information on the stage of disease and enhancing studies of therapeutic approaches. Importantly, this study indicated that left ventricular diastolic dysfunction was the earliest manifestation of diabetic cardiomyopathy progression.

3

GPR75, a G protein-coupled receptor target for metabolic syndrome

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Metabolic syndrome is a group of conditions, which include insulin resistance, hypertension, and obesity, that increase your risk of heart disease, diabetes, and non-alcoholic fatty liver disease. The exponential rise in metabolic syndrome means there is an urgent need for new therapeutics. G-protein coupled receptors (GPCRs), due to their tissue selective distribution and expression on the plasma membrane make great pharmacological targets. We and others have found that variants of the orphan GPCR, GPR75, are associated with BMI. Using CRISPR/Cas9 technology, we generated mice lacking GPR75 (GPR75-/-) to investigate whether the absence of this receptor is protective for the development of high fat diet (HFD)-induced metabolic syndrome. We found GPR75-/- mice gained less weight on HFD (4.5kcal/g, 42.7% carbohydrate, 15.2% protein, 42% fat, 12 weeks) compared to wild type (WT) mice (male GPR75-/- 4.8g vs. WT 15.6g, female GPR75-/- 1.5g vs. WT 11.2g, n=5-6, $P<0.0001$). Echo MRI scanning showed the difference in weight was primarily associated with reduced fat mass. In addition, female GPR75-/- mice exhibited improved glucose homeostasis compared to WT controls (30 minutes post glucose administration, GPR75-/- 21.1±2.3mmol/L vs. WT 41.1±0.6mmol/L, n=5-6, $P<0.05$). QPCR and immunoblotting indicated GPR75-/- mice had reduced liver fibrosis (male GPR75-/-: Collagen II 0.33±0.05 and αSMA 0.36±0.14; female GPR75-/-: Collagen II 0.47±0.05 and αSMA 0.34±0.09 fold change vs. WT liver, n=5-6, $P<0.05$) and lipid storage (male GPR75-/-: PPARγ 0.45±0.08 and Fabp4 0.34±0.05; female GPR75-/-: PPARγ 0.28±0.08 and Fabp4 0.52±0.06 fold change vs. WT liver, n=5-6, $P<0.05$). These data indicate that genetic deletion of GPR75 significantly reduced HFD-induced obesity and improved hepatic health therefore providing support for GPR75 as a drug target for metabolic syndrome.

1,3

Identifying opportunities for technological innovation in the pPCI pathway through ECG timing and location

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Primary Percutaneous Coronary Intervention (pPCI) refers to the emergency implantation of a stent to restore coronary blood flow in a person with a STEMI (heart attack due to coronary occlusion) identified by diagnostic ECG criteria. To consistently deliver treatment within the therapeutic time window, an efficient clinical pathway is essential.

In the pPCI centre considered, ECGs from possible STEMI patients are transmitted directly for evaluation by cardiology nurse activators with on-call consultant support. The vast majority of decisions are taken on the basis of a single ECG; however, marginal or evolving indications for intervention as well as uncertainty as to patient fitness for treatment can lead to multiple ECGs being evaluated.

These repeated ECGs indicate situations where improvements in diagnostic technology could provide a more prompt activation of the pathway and therefore plausibly improve patient outcomes. To identify in what context these ECGs are taken and the extent to which activation of the pathway was delayed, we considered the pathways and ECG timings of the patients accepted locally for pPCI in 2017, particularly the 44 patients accepted only after evaluation of multiple ECGs. We find substantial variation in ECG timings both within and between particular pathways, suggesting that different service providers may find greatest utility from different types of technological innovation.

3

Illuminating spatial lipidomic profiles of atherosclerotic plaques by mass spectrometry imaging

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Introduction: Atherosclerotic cardiovascular disease (ASCVD) is a chronic inflammatory disorder characterised by the gradual build-up of plaques in the arterial wall. Unstable plaques are more dangerous than stable plaques since they are prone to rupture and cause obstruction to blood flow, leading to heart attacks and strokes. Lipids play a key role in plaque progression, yet their exact involvement remains elusive. We hypothesise that plaques will have distinct spatial lipid phenotypes, allowing us to characterise them as stable or unstable more accurately. We sought to characterise the lipid composition of atherosclerotic plaques to address their link with ASCVD.

Methods: Matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry imaging (MSI) was used for spatial lipidomic profiling of rabbit and human plaques at different stages of ASCVD. Rabbit plaques were harvested from male New Zealand White rabbits (aged 6-9 months, n=6) following double-balloon injury to the abdominal aorta and maintenance on a high-cholesterol diet (0.2%) to induce atherosclerosis. With ethical approval, human carotid endarterectomy specimens were collected from NHS Lothian patients (men aged 50-80 years, n=6). Following MSI data acquisition, tissue sections were stained with hematoxylin and eosin. Immunohistochemistry was performed on adjacent sections to highlight macrophages and vascular smooth muscle cells using CD68 and α-smooth muscle actin antibodies, respectively. MS images were co-registered with histopathological images to reveal the metabolic and spatial information associated with ASCVD.

Results: Unique histologically-discriminant lipids were identified in both rabbit and human plaques, including sphingomyelins, phosphatidylcholines, cholesteryl esters, triglycerides and oxidised phospholipids, among others. MSI enabled mapping of lipid/lipid classes that define histologically important regions such as the lipid-necrotic core, fibrous tissue and macrophage-rich regions. In both rabbit and human plaques, relatively high levels of sphingomyelins were observed in macrophage-rich regions, supporting their central role in promoting lesional inflammation, while cholesteryl esters were among lipids enriched in the lipid-necrotic core.

Conclusions: The lipid profile in a rabbit model mimics that observed in human plaques serving as a good model for early-stage ASCVD. We have shown that important pathophysiological plaque features that define plaque stability can be distinguished based on their lipid signatures. This work also emphasises the value of MSI in biomarker applications, especially in elucidating molecular characteristics associated with ASCVD.

Reduced pro-angiogenic function of endothelial colony-forming cells in hyperglycaemia is mediated by NOX4

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Objective: Cord blood-derived endothelial colony-forming cells (CB-ECFCs) are a defined progenitor subtype with established roles in vascular homeostasis and angiogenesis. They possess low immunogenicity and show allogeneic or autologous therapeutic potential for improved management of ischaemic cardiovascular disease, whilst targeting key angiogenic signalling pathways remains a key focus for enhancing CB-ECFC intrinsic function. Emerging evidence indicates that CB-ECFCs are regulated by NADPH oxidase (NOX)-derived reactive oxygen species (ROS), with their angiogenic capacity negatively impacted by hyperglycaemia. The aim of this study was to investigate specific influence of CB-ECFC NOX-dependent ROS signalling in experimental diabetes.

Methods: CB-ECFCs (n≥3, up to 4 clones) were cultured in high glucose (DG, 25mmol/L) or normal glucose control (CTL, 5mmol/L) for 72h, with or without PMA stimulation (500nmol/L), prior to analysis of ROS generation (DHE fluorescence), angiogenic function (Matrigel assay), and gene expression (qRT-PCR), and impact of NOX4 plasmid overexpression (OE).

Results: DG treatment increased CB-ECFC ROS generation (CTL 35.3±1.9, DG 63.1±2.2 arbitrary units; P<0.001) and prevented PMA-induced angiogenesis (tube length: CTL+vehicle 9026±111, CTL+PMA 10968±125, DG+vehicle 7710±190, DG+PMA 6349±336, arbitrary units; CTL+PMA vs DG+PMA, P<0.001). Notably, upregulation of NOX4 mRNA expression was observed in PMA-treated CB-ECFCs under CTL conditions (vehicle 0.96±0.19, PMA 1.53±0; P<0.05), but this response was lost with DG culture (vehicle 0.79±0.13, PMA 1.01±0.09; P=NS), suggesting an important role for NOX4-derived ROS. Indeed, NOX4OE completely rescued pro-angiogenic response of CB-ECFCs after DG treatment both basally (tube length: vehicle EV 8104±136, vehicle NOX4OE 9435±420 arbitrary units; P<0.05) and with PMA stimulation (tube length: PMA EV 7454±141, PMA NOX4OE 12212±718 arbitrary units P<0.01) to levels above those observed in CTL.

Conclusions: These data indicate that CB-ECFC angiogenic dysfunction in hyperglycaemia may be mediated by reduced NOX4 signalling, thus highlighting this major NOX isoform as a potential target to enhance the vasoreparative capacity of CB-ECFCs in diabetes.

3

p53 regulates mitochondrial dynamics in vascular smooth muscle cell calcification.

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Arterial calcification is hallmark of aging cardiovascular system. It has many similarities with skeletal mineralization, however; the cellular mechanisms responsible have yet to be fully explained. Mitochondrial dynamics regulate both bone development and vascular function. In the present study, we thus investigated mitochondrial function in vascular smooth muscle cell (VSMC) calcification.

Phosphate (Pi)-induced VSMC calcification was associated with elongated mitochondria (3.04 fold increase, $p<0.001$), increased mitochondrial ROS production (2 fold increase; $p<0.001$) and reduced mitophagy (14.5 fold decrease; $p<0.01$). An increase in protein expression of Optic Atrophy Protein 1 (OPA1; 2 fold increase, $p<0.05$) and a converse decrease in expression of Dynamin-related protein 1 (Drp1; 1.6 fold decrease $p<0.05$), two crucial proteins required for the mitochondrial fusion and fission process respectively, were noted too. Furthermore, the phosphorylation of Drp1 Ser637 was increased in mitochondria from calcified VSMCs (2.8 fold increase; $P<0.05$), suppressing mitochondrial translocation of Drp1. Calcified VSMCs show enhanced expression of p53 (6.09 fold increase, $p<0.05$) and β-galactosidase activity (1.8 fold increase, $p<0.001$), both associated with cellular senescence.

siRNA-mediated p53 knockdown reduced calcium deposition (4 fold decrease; $P<0.01$), mitochondrial length (3.04 fold decrease, $P<0.001$) and β-galactosidase activity (2.4 fold decrease, $P<0.001$), with concomitant mitophagy induction (3 fold increase, $P<0.05$). Reduced OPA1 (2.9 fold decrease, $p<0.05$) and increased DRP1 protein expression (2.6 fold increase; $p<0.05$) with decreased phosphorylation of Drp1 Ser637 (6.83 fold decrease, $p<0.05$) was also observed upon p53 knockdown in calcifying VSMCs. Furthermore, treatment with senolytic quercetin and epigallocatechin gallate enhanced mitophagy and inhibited arterial calcification. Our study has revealed for the first time that p53 modulates DRP1 to regulate mitochondrial morphology and function in arterial calcification. Our data suggests senolytics as a timely and innovative treatment strategy to promote healthy aging through the delay of vascular calcification.

3

Proteomics identification and evaluation of a collagen sub-type with potential to support improvements in diagnosis and management of heart failure.

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Introduction: Heart failure (HF) is an extremely debilitating condition that currently affects at least 16,500 people in Northern Ireland. Ischemic heart disease and cardiac fibrosis are the primary causes of end-stage HF. Greater understanding of molecular changes associated with this underlying pathophysiology could lead to the identification of novel biomarkers and therapeutic targets for improved diagnosis and management of HF.
Methods: Unbiased, deep proteomic analysis of individual left ventricular tissue samples from patients with HF (n=30) and patients without HF (NF; n=9) was performed using the diaPASEF workflow on a timsTOF Pro mass spectrometer. Validation of notable protein expression changes were performed by ELISA. Protein expression changes and correlations with clinical data were assessed using appropriate non-parametric testing of log-transformed data. Differentially expressed proteins were identified based on an observed fold change of ≥ 1.5 or ≤-1.5 and q-value ≤ 0.005.
Results: HF patients included patients with hypertrophic obstructive cardiomyopathy (HOCM; n=12), dilated cardiomyopathy (DCM; n=9) and ischemic cardiomyopathy (ISCM; n=9). One hundred and eighteen proteins were identified as being significantly associated with HF, irrespective of the underlying aetiology. Among these, a collagen sub-type, with a reported role in myocardial development, (referred to as COL-CT), was identified as being significantly elevated in HF (p=0.012), with greatest increase observed in patients with ISCM. Existing transcriptomic data for these samples corroborated these findings at gene level. Measurement of COL-CT is more predictive of HF than combined measurement of the cardiac-specific collagen subtypes I and III (AUC 0.847 vs AUV 0.778). HF-associated changes in COL-CT protein expression were validated in silico in an independent LV tissue dataset (n=7 NF v n=20 HF, p<0.001), and further confirmed in-house by ELISA-based analysis of serum samples from an independent patient cohort (n=50 NF v N=54 HF, p= 0.0004). COL-CT is enriched in atrial regions of the heart and serum levels were found to be significantly positively correlated with left atrial volume (p<0.0001).
Conclusions COL-CT has yet to be fully investigated in the context of myocardial disease. Here we report a significant association between COL-CT and HF. These observations have been validated in multiple independent clinical cohorts, in various sample types. Circulating levels of COL-CT in serum provide evidence that COL-CT may have clinical utility as a minimally invasive biomarker for HF. Moreover, strong association with ISCM and a likely role in cardiac fibrosis suggest that COL-CT should be further investigated as a therapeutic target for management of HF. 3

Identification and evaluation of novel protein biomarkers for atrial fibrillation

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Introduction: There is a need for improved biomarkers to diagnose atrial fibrillation (AF) earlier and reduce risk of future serious comorbidities. Quantitative protein profiling of atrial appendage tissue from patients with atrial fibrillation (AF n=10) and age/sex matched controls with normal sinus rhythm (control, n=10) was performed using mass spectrometry. Similarly, serum samples, collected longitudinally from patients with and without AF (n=186), were analysed to establish a comprehensive dataset that depicts changes in both the atrial tissue and circulating proteome as result of AF.

Methods: Sections of formalin fixed paraffin embedded (FFPE) tissue were mechanically homoginsed in Preomics™ LYSE buffer. Protein lysates were digested with trypsin and Lys-C using the Preomics ™ iST kit. Serum samples were enriched for low abundant serum proteins using High Select™ Top Abundant Protein Depletion Resin (Thermo). Unbiased, deep proteomic profiling of individual tissue and serum samples was performed using the diaPASEF workflow on a timsTOF Pro mass spectrometer. Nonparametric statistical tests were applied for subsequent data analysis in R and SPSS (version 27). Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) software.

Results: Label-free MS analysis led to the identification of over 6,000 proteins in FFPE tissue and over 500 serum proteins. More than 300 proteins were found to be significantly differentially expressed between AF and control samples at tissue level, with stringent cut off criteria applied (observed fold change of ≥ 1.5 or ≤-1.5 and q-value ≤ 0.005). Pathway analysis revealed that significantly up and down-regulated proteins mapped to Epithelial Adherens Junction Signalling and Atherosclerosis Signalling canonical pathways. The most up-regulated protein in AF correlated with tissue BNP levels ($r=1.0$, $p<0.0001$) and markers of tissue ischaemia ($r=1.0$, $p<0.0001$). The most down-regulated protein was inversely correlated with tissue levels of TGFb, the primary pro-fibrotic cytokine in the heart ($r = -0.9$, $p=0.037$). Thirty-one significantly changed tissue proteins were also identified in serum samples and were found to be associated with (i) new-onset AF, (ii) paroxysmal AF and (iii) risk of future stroke and/or heart failure in patients with AF.

Conclusions: The dataset has highlighted significant proteins associated with AF. We have verified that circulating levels of a number of these proteins are significantly associated with AF and, importantly, may be predictive of future cardiovascular comorbidities in patients with AF. 3

Abstracts
Poster Presentations

1. Endothelial interferon signalling represents a key signalling pathway implicated in the pathogenesis of experimental diabetic cardiomyopathy

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Objective: Diabetic cardiomyopathy (DCM) is a major complication of type 2 diabetes mellitus, which is specifically linked with coronary microvascular endothelial dysfunction, and determines adverse cardiac remodelling and heart failure progression. Whilst many pathological pathways are implicated in DCM pathogenesis, such as oxidative stress and dysfunctional nitric oxide signaling, specific underlying mechanisms remain poorly defined. The aim of this study was to determine differentially regulated signalling in experimental DCM with specific enrichment in endothelial cells which may underlie microvascular dysfunction.

Methods: Experimental DCM was induced in adult male C57BL6/J mice (n=4) by high-fat diet (5450kcal/kg) and single-dose streptozotocin (100mg/kg i.p.) with comparison to chow-fed vehicle-injected controls. Mice were sacrificed at 6 months and left ventricular tissue isolated for RNA sequencing and bioinformatics analysis.

Results: R and Partek were used to generate two lists of genes differentially expressed between DCM and control hearts (adjusted p-value 0.05, log2 fold change -1.5 to +1.5), which were filtered for enrichment in capillary endothelial cells based on a published human cardiac cell atlas. This analysis identified 619 common genes with significant endothelial expression, which were uploaded to Ingenuity Pathway Analysis (IPA) to interrogate conical pathways associated with microvascular dysfunction in the diabetic heart. IPA highlighted 7 endothelium-enriched pathways which were differentially regulated between DCM and control hearts. Of these, the most significantly altered were interferon and interferon regulatory factor signalling, with several component genes significantly upregulated within the dataset. Interestingly, interferon signalling has previously been linked to endothelial dysfunction but not in the context of the diabetic heart.

Conclusions: Endothelial interferon signalling may represent a key driver of coronary microvascular dysfunction in experimental diabetes which determines adverse cardiac remodelling. Validation and manipulation of key targets within related pathways is justified towards development of novel therapeutic approaches.

2

2. Sex and age play a key role in determining the cardiac phenotype of pdgfbret/ret mice

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Platelet-derived growth factor B (PDGFB) released by endothelial cells regulates pericyte recruitment in angiogenesis. In microvessels and capillaries, pericytes control vascular permeability and maintain vessel integrity. Deletion of the PDGFB retention motif in pdgfbret/ret (RET) mice alters the range of action of PDGFB, reducing pericyte coverage of blood vessels. Previous ultrasound investigation in male RET mice was interpreted as showing development of eccentric hypertrophy between 10 and 20 weeks of age (Nystrom et al., 2006). However, the effect of this mutation in longer term aging and in females has not been evaluated.

We hypothesise that loss of pericyte coverage will result in changes to left ventricular structure and altered cardiac function in aging male and in female RET mice.
High resolution ultrasound (Visualsonics Vevo 3100) was performed at 3, 6, 9 and 12 months of age in male and female pdgfb+/+ (WT) and RET mice. Structural parameters measured were left ventricular; end-diastolic area (LVEDA) and end-systolic (LVESA) areas, wall thickness (WaT) and mass (LV Mass). Function parameters measured were ejection fraction (EF), fractional shortening (FS) and fractional area change (FAC).

At 3 months old male and female RET mice had significantly increased LVEDA relative to WT littermates consistent with left ventricular dilation. In male RET mice, dilation resolved over time, with no difference in cardiac structure compared to WT at 9 and 12 months. In female RET mice, increased LVEDA persisted at 6, 9 and 12 months, in line with a more pronounced phenotype. Despite this structural change, there was no impact of the pdgfbret/ret mutation on their cardiac contractile function (EF, FAC and FS) over time in either sex. Ultrasound indicated a significant increase in LV mass in male and female RET mice, but heart weight/body weight ratio and heart weight/tibia length ratio at 12 months were similar between male and female WT and RET mice, suggesting that cardiac hypertrophy was not present.

Here, we demonstrate that female RET mice exhibit a persistent change to left ventricular structure in aging while male RET mice exhibit a transient dilatory phenotype that resolves over time. Neither male nor female mice have reduced contractile function suggesting that changes to left ventricular structure may be compensatory. The exact mechanisms underpinning these changes are unclear. Further examination of cardiomyocyte cross sectional area and blood vessel density is required to understand structural changes in the hearts of RET mice.

3. Sex Specific Biomarkers of Ischaemic Heart Disease in Type 2 Diabetes.

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Diabetes Mellitus is a group of metabolic disorders linked to hyperglycaemia. The most common form of diabetes mellitus is Type 2 Diabetes (T2D), with its global incidence continually increasing as a result of increasing rates of obesity and lack of physical exercise. Ischaemic heart disease (IHD) is the leading cause of death worldwide, with T2D being one of the most significant risk factors. Individuals with T2D have a three-fold increased risk of developing IHD compared to age-matched individuals without T2D. Proteomic biomarkers have the capability to improve the diagnosis and prognosis of T2D and may also be advantageous in predicting the likelihood of IHD amongst those with T2D.

This study aims to assess how comorbid ischaemic heart disease affects glycaemic control and comorbidity profiles and to evaluate proteomic biomarkers and potential pathways associated with comorbid T2D and IHD generally and in men and women specifically. Analysis was conducted on the Diastrat cohort of T2D patients, consisting of 310 men and 184 women with and without IHD, recruited from the Western Health and Social Care Trust (the Diastrat cohort).

Both men and women with IHD have comparable glycaemic control to those without IHD. However, those with comorbid T2D and IHD had a significantly increased level of polypharmacy for the entire cohort ($p<0.0001$), men ($p<0.0001$) and women ($p=0.001$). Total cholesterol ($p=0.001$), High density lipoprotein cholesterol (HDL) ($p<0.0001$) and low density lipoprotein cholesterol (LDL) ($p=0.0001$) were all reduced in those with IHD and T2D compared to those without IHD.

Unique sex-specific proteins were identified for both men (n=57) and women (n=10) with comorbid T2D and IHD. The most significant proteins for males included: LAMP3 ($p<0.0001$), TGF-□ ($p=0.001$), IL-17C ($p=0.0001$) and ADM ($p=0.004$). For women, these significant proteins included PI3 ($p=0.0004$), CCL25 ($p=0.002$), IL22 ($p=0.01$) and TNF ($p=0.01$). Proteins identified in men were associated with cytokine receptor interactions, pneumonia and coronary artery disease, whilst proteins identified in women were linked to brain ischaemia, cerebrovascular disease and the MAPK signalling pathway. Discovery of these sex-specific proteins suggests the development of IHD in men and women may be different and requires further investigation.

2

4. Combination of machine learning and senescence signatures predict the risk of myocardial infarction

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Acute coronary syndromes (ACS) are a group of cardiac conditions that reduced blood flow into the heart. One of these conditions is myocardial infarction (MI), which affects 26 million people and is responsible for 4 million deaths in Europe and more than a third of deaths from all causes in developed countries. 33% to 50% acute MI happens in patients >70 years old. Further, 80% acute MI caused death occurred in people over 65 years old implicating aging as biggest risk factor for MI. One cellular process that causes ageing and ageing-related diseases is cell senescence. We hypothesize that accumulation of senescent cardiac cells may contribute to MI causing high mortality rate. Discovering specific senescence biomarkers may be able to predict MI risk or provide therapeutic targets. It is well known that senescent cells secrete senescence-associated secreted phenotypes (SASP) to enhance senescence and affect neighboring tissues causing further tissue damages and deepen diseases. In this study, ACS patients (n=171, mean age=65.61 ± 10.71) and control group (n=73, mean age=43.88 ± 10.48, 10-year cardiac-episode free) were recruited and their blood plasma was analyzed for senescence biomarkers by using Proseek Multiplex Proximity Extension Assay (Olink Bioscience). We found senescence biomarkers when combined with machine learning algorithms can accurately predict risk of Myocardial infarction (MI). To validate these findings in senescent cells we developed several senescence cardiac cell models. We introduced senescence to human primary cardiac fibroblasts (from human donors) and human iPSC-derived cardiomyocytes, followed by SA-β-GAL staining, DNA damage assays, proliferating assay to confirm the senescence state. Multi-omic analysis of these cardiac cells confirmed the in-vivo findings showing a successful deployment of a bedside to bench model of cardiac disease. To conclude, our research not only provides potential senescence biomarkers for diagnostic and therapeutic target for the MI patients but also an in vitro model to study these target proteins.

2

5. Perivascular adipose tissue (PVAT) lacking AMPKα1 releases less Nitric Oxide compared with Wild type: role of cav-1/eNOS binding

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Introduction: Perivascular adipose tissue (PVAT) surrounds blood vessels and releases a variety of bioactive molecules such as NO which may contribute to its anti-contractile effect. Our previous data has demonstrated a key role of the adenosine monophosphate-activated protein kinase (AMPK) in modulating release of PVAT-derived substances. In addition, we have previously shown that thoracic aortic PVAT produces significantly more NO compared to abdominal PVAT in WT, but not in AMPKα1 KO micE. Here, we sought to investigate the protein–protein interactions that regulate eNOS activity and function.

Methods: Wild type (Sv129) and AMPKα1 global knockout mice were euthanised by a rising concentration CO2. Twenty mg of PVAT tissue from WT, HT and KO mice was collected and incubated for 30 min in 1 mL oxygenated physiological buffer solution to produce a conditioned media (CM). Co-immunoprecipitation was carried out to study CAV-1/eNOS interaction. An ELISA assay was performed to investigate ROS generation by PVAT. To examine the role of CAV-1 in regulating eNOS activity, 3T3-L1 adipocytes were treated for 24h with 25 µM and 100 µM of the NO donor (SNAP), and either 5 µM of Geldanamycin (HSP-90 inhibitor) or 25 mM Methyl-β-cyclodextrin CAV-1 inhibitor.

Results: There were no differences in eNOS expression between thoracic and abdominal PVAT between genotypes. Neither iNOS nor nNOS were expressed in fat tissues. CAV-1 expression was higher in abdominal PVAT compared with thoracic PVAT in both WT and KO mice (figure 1). AMPK inhibition significantly reduce CAV-1/eNOS coupling in abdominal PVAT but not in thoracic PVAT. SNAP activated 3T3-L1 NO production via a reduction in cav-1 expression.

Conclusion: AMPK has an important role in regulation of NO production by PVAT and CAV-1/ eNOS coupling. Higher expression of CAV-1 in abdominal PVAT could account for the lower generation of NO by this PVAT depot.

2

6. The study of diabetic cardiomyopathy progression in a mouse model using high-fat diet and high-dose of streptozotocin

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Background: Heart failure with preserved ejection fraction (HFpEF) is a multifactorial disease characterised by a variety of unique phenotypes, including cardiometabolic disorders with type 2 diabetes mellitus and obesity. Although there is a growing amount of knowledge on the pathogenesis of diabetic cardiomyopathy, questions remain on various aspects, particularly diabetic cardiomyopathy progression. Therefore, an animal model that recapitulates the complexities of diabetic cardiomyopathy is necessary. This study aimed to elucidate longitudinal follow-up in cardiac structure and function alterations in the context of the diabetic heart.

Methods: Male C57BL/6J mice were fed a control diet (CD) or high-fat diet (HFD) for 24 weeks. Streptozotocin (STZ) 100 mg/kg or vehicle was intraperitoneally administered to induce diabetes in the mouse at 8 weeks of diet. Echocardiography was monthly performed to study cardiac function and structure changes. In addition, left atrial volume and left atrial area were measured to indicate chronic diastolic dysfunction. Mice were tested for HbA1c and fasting blood glucose levels to confirm that mice developed diabetes. Blood pressure was measured by a tail-cuff method.

Results: In our model, HFD mice were injected with high-dose STZ resulted in establishing an evident phenotype in diabetic cardiomyopathy by significant changes in echocardiography parameters, including cardiac hypertrophy (LVPW), diastolic dysfunctions (MV E/A, IVRT, LA area, and LA volume) from 12 weeks to 24 weeks of study, compared to control. It is notable that only MV E/A began to change at 8 weeks of study. Furthermore, our model illustrated that HFD/STZ mice had higher HbA1c and fasting blood glucose levels than CD mice ($p<0.05$) with no differences in systolic blood pressure and ejection fraction ($p>0.05$).

Conclusion: We developed a mouse model that captures the complexities of diabetic cardiomyopathy both early and chronic progression, providing important information on the stage of disease and enhancing studies of therapeutic approaches. Importantly, this study indicated that left ventricular diastolic dysfunction was the earliest manifestation of diabetic cardiomyopathy progression.

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7. An important role for hypomethylated Integrin beta-like 1 in ischaemic cardiac fibroblasts

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Cardiomyopathy describes a range of cardiac cell alterations which manifest in structural and functional myocardial abnormalities that ultimately result in heart failure. Ischaemic cardiomyopathy (ICM) is currently the most common cause of heart failure in the developed world causing around 9 million deaths per year making it a leading cause of mortality.

Unremitting myocardial ischaemia causes propagated fibrosis, resulting in diminished left ventricular function. Aberrant epigenetic regulation in cardiac fibroblasts contributes to the development of ICM phenotype. DNA methylation is the most common epigenetic modification and occurs when a methyl group is added to a cytosine within a CpG dinucleotide site, which often prevents transcriptional binding and therefore causes gene repression. Abnormal changes to DNA methylation patterns have been shown to play a role in cardiac remodelling in ICM. The functional impact of dysregulated DNA methylation in specific genes in the ischaemic heart has not been fully elucidated.

In this study, we aimed to identify specific DNA methylation sensitive genes in human ICM left ventricular (LV) tissue through integrated use of targeted bisulphite sequence capture sequencing and RNA sequencing. We identified the gene integrin beta-like 1 (ITGBL1) as a gene of interest though comparative analysis. ITGBL1 was hypomethylated in LV tissue from patients diagnosed with ICM, and its gene expression was upregulated. We validated its aberrant expression in both an ischaemic pig model and an ischaemic mouse model. We examined the effect of siRNA knockdown and plasmid DNA overexpression of ITGBL1 on cardiac fibroblast migration to uncover a role for this gene in disease-relevant cardiac fibroblast activation and migration. Lastly, we assessed whether DNA methylation regulation is a plausible, potential mechanism driving ITGBL1 transcription in ICM by measuring CpG methylation status of specific regions within the ITGBL1 gene sequence in genomic DNA from ICM LV tissue and from stimulated cardiac fibroblasts.

Our findings show that ITGBL1 is a key player in the development of cardiac fibrosis in ICM pathophysiology, and that dysregulated gene expression of ITGBL1 is caused by gene specific hypomethylation.

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8. Investigating the role of microRNA-26b in vascular calcification

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Vascular calcification (VC) is the abnormal deposition of calcium phosphates within the blood vessels. Despite significantly contributing to the development of cardiovascular disease, much remains unknown about the mechanisms driving this process. Recent advances have shown that microRNAs play critical roles in the regulation of biological processes in both health and disease. Based on previous phenotypic screening microRNA-26b (miR-26b) was identified as having a protective role within the vasculature, by promoting endothelial cell growth and survival.

Additionally, micro-PET/CT imaging using 18–Sodium Fluoride (18F-NaF) to detect hydroxyapatite demonstrated increased microcalcification in the aortic arch and descending aorta of 6-month-old miR-26b KO mice compared to wildtype (WT).

To understand how miR-26b may influence gene expression during this process, bulk RNA-seq was performed using aortic tissue of 6-month-old male WT, miR-26b KO and miR-26b KO mice administered with LDN (miR-26b KO_LDN), a known BMP signalling inhibitor (n=3 per group). The resulting transcript counts were compared between groups to identify differentially expressed genes (DEGs). Upregulated DEGS in the WT vs miR-26b KO groups were analysed to identify miR-26b targets, while downregulated DEGs in the miR-26b KO vs miR-26b KO_LDN group allowed identification of miR-26b targets belonging to the BMP signalling pathway.

Using Gene Ontology (GO) enrichment analysis, it was observed that upregulated DEGs between WT and miR-26b KO strains were enriched in GO terms associated with cell cycle, immune system development, and antigen receptor-mediated signalling pathway.

Of these upregulated DEGs, LEF1 (Lymphoid Enhancer Binding Factor 1) was identified as a miR-26b target gene of interest due to functioning as a key mediator of the Wnt/BMP signalling pathways, an important pathway in VC development. Further investigation is necessary to validate the involvement of this gene in the miR-26b vascular phenotype to identify possible therapeutic targets for VC.

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9. Biomarker discovery using machine learning methods for COVID-19 patient outcomes

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COVID-19, a disease caused by the SARS-COV-2 virus, is responsible for the recent global pandemic with remaining uncertainties in regard to why certain patients experience worse symptomologies. Despite COVID-19 becoming the central area of clinical research over the previous two years, there are few predictive markers which can differentiate COVID-19 patients based on health outcomes. Here we applied machine learning models in the form of support vector machines on protein samples obtain from over 500 patients to identify individual and combinatory proteins that can effectively differentiate patients based on severity and ICU admission. We managed to discover four highly accurate individual proteins (LGALS9, AGRN, PRSS8 and TREM2) that could act as biomarkers and two protein combinations that can effectively differentiate patients based on COVID-19 severity; (ITGA11, LTA, OSCAR, CCL28, IL32, IL33) and ICU admission status; (IL2RB, MICA + MICB, SIT1, IFNLR1, PSPN, CCL23).

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10. Illuminating spatial lipidomic profiles of atherosclerotic plaques by mass spectrometry imaging

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Introduction: Atherosclerotic cardiovascular disease (ASCVD) is a chronic inflammatory disorder characterised by the gradual build-up of plaques in the arterial wall. Unstable plaques are more dangerous than stable plaques since they are prone to rupture and cause obstruction to blood flow, leading to heart attacks and strokes. Lipids play a key role in plaque progression, yet their exact involvement remains elusive. We hypothesise that plaques will have distinct spatial lipid phenotypes, allowing us to characterise them as stable or unstable more accurately. We sought to characterise the lipid composition of atherosclerotic plaques to address their link with ASCVD.

Methods: Matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry imaging (MSI) was used for spatial lipidomic profiling of rabbit and human plaques at different stages of ASCVD. Rabbit plaques were harvested from male New Zealand White rabbits (aged 6-9 months, n=6) following double-balloon injury to the abdominal aorta and maintenance on a high-cholesterol diet (0.2%) to induce atherosclerosis. With ethical approval, human carotid endarterectomy specimens were collected from NHS Lothian patients (men aged 50-80 years, n=6). Following MSI data acquisition, tissue sections were stained with hematoxylin and eosin. Immunohistochemistry was performed on adjacent sections to highlight macrophages and vascular smooth muscle cells using CD68 and α-smooth muscle actin antibodies, respectively. MS images were co-registered with histopathological images to reveal the metabolic and spatial information associated with ASCVD.

Results: Unique histologically-discriminant lipids were identified in both rabbit and human plaques, including sphingomyelins, phosphatidylcholines, cholesteryl esters, triglycerides and oxidised phospholipids, among others. MSI enabled mapping of lipid/lipid classes that define histologically important regions such as the lipid-necrotic core, fibrous tissue and macrophage-rich regions. In both rabbit and human plaques, relatively high levels of sphingomyelins were observed in macrophage-rich regions, supporting their central role in promoting lesional inflammation, while cholesteryl esters were among lipids enriched in the lipid-necrotic core.

Conclusions: The lipid profile in a rabbit model mimics that observed in human plaques serving as a good model for early-stage ASCVD. We have shown that important pathophysiological plaque features that define plaque stability can be distinguished based on their lipid signatures. This work also emphasises the value of MSI in biomarker applications, especially in elucidating molecular characteristics associated with ASCVD.

11. Improving the screening and diagnosis of familial hypercholesterolaemia

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Familial hypercholesterolaemia (FH) is an inherited disorder causing chronically elevated LDL-C, affecting 1 in 250–311 of the general population and 1 in 17 of the Atherosclerotic Cardiovascular Disease population. Internationally, systematic identification of FH has not been widely adopted. In 2019, the NHS Long Term Plan (LTP) set a target to identify ≥25% of the UK FH population in 5 years. However, this year an estimated 9.1% of the FH population will be identified, and at current rates, the target will be met in 2045.

We analysed FH screening data from the UK, NI, Netherlands, and Norway, showing that for every index case 2.01, 10.15, 7.54, and 7.15 relatives are tested, identifying 0.68, 3.31, 2.12, and 2.51 relatives with FH, respectively. Under the UK detection rate, with the addition of index cases identified through GP case-ascertainment tools, each strategy would detect 13.67%, 35.11%, 28.6%, and 25.42% of the UK FH population, respectively.

We evaluated the cost of screening in the context of the NHS LTP target, which were calculated for index cases as £629.5 – 824.5, for relatives DNA tested and identified with FH as £325.5 - £435.5 and for relatives DNA tested but not identified with FH as £247.5 - £357.5. Total costs were calculated in the range of £33.41 - £54.03 million. The UK model was the cheapest at £33.41 – 45.20 million, compared with the most expensive at £39.07 - £54.03 million under Norway

The NHS LTP target can be achieved by 2024 at the same detection rate if the number of relatives tested was increased to identify 2.07 relatives per index case from 0.68. The total cost will amount to £34.89 - £47.67 million, a difference of £1.48 - £2.47 million from the cheapest rate.

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12. Clinical profiles and biomarkers associated with comorbid hypertension and Type 2 Diabetes Mellitus (T2DM)

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Comparable HbA1c levels were evident between hypertensive men and women compared to those without hypertension. Creatinine levels were significantly increased in hypertensive patients ($p<0.001$), specifically in men. LDL- ($p<0.001$) and total cholesterol ($p=0.002$), in contrast were significantly reduced in patients with comorbid hypertension. Sex-specific analysis revealed disparities between men and women. HDL, LDL and total cholesterol levels were significantly higher in hypertensive women than men. Increased levels of polypharmacy were observed in men and women with comorbid hypertension compared to those without hypertension. This was corroborated with significantly increased risk of numerous comorbid diseases in both men and women with comorbid T2DM and hypertension compared to patients without hypertension. Eye disease ($p<0.0001$) and endocrine, nutritional and metabolic diseases ($p<0.0001$) were significantly increased in the hypertensive cohort. Upon sex-stratification, hypertensive men had increased risk of neoplasms ($p=0.001$) whilst women had increased risk of diseases of the blood/immune system ($p=0.0026$). Proteomic biomarkers of comorbid T2DM and hypertension unique to men ($n=29$) and women ($n=4$) were identified, revealing potential differences in disease mechanisms in men and women. Further prospective studies into the mechanistic pathways associated with comorbid T2DM and hypertension may aid in identification of novel treatment strategies and prediction of long-term outcomes of hypertension in T2DM.

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13. Pharmacological inhibition of PI3K signaling in canine myxomatous mitral valve disease (MMVD)

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Myxomatous mitral valve disease (MMVD) is one of the most important chronic degenerative valvulopathies in people and dogs with no effective therapeutic inventions to control the onset and progression of disease. It is a common cause of heart failure leading to significant morbidity and mortality in both species. The transition of activated valvular interstitial cells (aVICs; activated myofibroblasts) from a quiescent VIC (qVIC) phenotype is believed to be the primary driver of the myxomatous degeneration characteristic of this disease, and is under transforming growth factor beta (TGF-β) control. The complex TGF-β signalling pathway includes phosphatidylinositol-3-kinase (PI3K) signalling, which has been shown to be important in cancer and non-valvular cardiovascular diseases, providing options for novel treatments. The aim of this study was to investigate the role of PI3K in the pathogenesis of MMVD in the dog, with particular reference to control of cellular apoptosis, autophagy and senescence, using a combination of protein immunoblotting and immune-staining.

VICs from normal and dogs diagnosed with MMVD were isolated and cultured under low-serum conditions (2% FBS). Pharmacological inhibition of PI3K in aVICs by LY249002, copanlisib and alpelisib significantly reduced α-smooth muscle actin (SMA) expression, the main aVIC marker ($p<0.001$), returning cells to a more quiescent phenotype. PI3K inhibition reduced expression of the phosphorylated forms of the downstream effectors Akt and mTOR in the PI3K pathway ($p<0.001$). PI3K/Akt/mTOR inhibition was also found to affect the cellular activities of apoptosis, autophagy and senescence. PI3K inhibition increased the expression of caspase-3 and cleaved caspase-3 (apoptosis), LC3-II (autophagy) and decreased expression of p16, p21 and p53 (senescence) ($P<0.05$). Cell staining identified enhanced TUNEL (apoptosis), LC3-II (autophagy) and attenuation of (senescence-associated-β-galactosidase) SA-β-gal and γ-H2AX staining after PI3K inhibition ($P<0.05$).

These data indicate that aVICs in canine MMVD are in a senescent state with reduced capacity for apoptosis and autophagy and that pharmacological inhibition of PI3K pathway can transition aVICs to qVICs by promoting apoptosis and autophagy and inhibiting senescence. It is likely cell senescence contributes to MMVD pathogenesis and these data provide insight into potential novel therapeutic targets that may be applicable to both the dog and human.

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14. The Role of Connexin 43 in Proliferation and Migration of Mouse Pulmonary arterial fibroblasts

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Introduction: Connexin 43 (Cx43) is involved in cellular communication and regulation of both the systemic and pulmonary vasculature. Pulmonary arterial fibroblasts are involved in pulmonary vascular remodelling which is characteristic of pulmonary arterial hypertension (PAH). Previous work has shown that Cx43 may be important in the pathophysiology of PAH. Here, we assess the role of Cx43 in proliferation and migration of mouse pulmonary arterial fibroblasts (MPAFs), using both mice heterozygous for Cx43 (Cx43+/− mice) and pharmacological inhibition of Cx43.

Methods: Both wildtype (WT) and Cx43+/− littermate mice (C57BL/6 background, 2-4 months old) were used. Mice were euthanised by intraperitoneal injection of pentobarbital sodium (700 mg/kg) containing lidocaine (20 mg/ml). Primary MPAFs were obtained from the main and branch pulmonary arteries and grown in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS) plus 100 µg/ml primocin and 2 mM L-glutamine. Cells were pre-treated with 37,43Gap27 (300 µM; an inhibitor of Cx37 and Cx43) before being placed in normoxic or hypoxic (5% O2) conditions for 24 h. Proliferation of MPAFs was assessed using an automated cell counter, while migration of MPAFs was assessed using a scratch assay.

Results: MPAFs derived from Cx43+/- mice had a reduced proliferative and migratory response to serum under normoxic conditions compared to WT MPAFs. However, proliferation and migration under hypoxic conditions was similar between WT and Cx43+/- MPAFs. Addition of 37,43Gap27 to Cx43+/- MPAFs was necessary to reduce proliferation and migration under hypoxic conditions.

Conclusion: This study has shown that Cx43 plays a role in proliferation and migration of mouse pulmonary arterial fibroblasts. The partial reduction in Cx43 protein found in Cx43+/- mice was sufficient to reduce proliferation and migration under normoxic but not hypoxic conditions. Pharmacological inhibition of Cx43 was required to inhibit proliferation and migration of MPAFs under hypoxic conditions.

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Above all, we would like to extend a huge thank you to all of you for attending the meeting, especially the attendees who have had to travel long distances. For many, this will have been the first meeting post-pandemic and a step into the unknown. We are grateful to you for your support. We hope that you have enjoyed your time here in Derry.

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