changes in survival rate or cell proliferation. However, it clearly promoted the induction of cell hypertrophy.

Conclusion In summary, these preliminary data suggest that SIK2 might modulate the hypertrophic response of cardiac tissue during pathological insults.

Conflict of Interest No

BS25 TAM RECEPTOR AXL LOSS REGULATES SMOOTH MUSCLE CELL DIFFERENTIATION AND ACCELERATES ATHEROSCLEROSIS IN MICE

[1]Lucy Mcshane, [1]Danila Gurgone, [1]Abdullatif Mohammed Bin-Khunayn, [2]Eva Crespo, [1]Gianluca Grassia, [1]Neil MacRitchie, [3]Mauro Giacca, [4]Lorena Zentilin, [5]Glykeria Karadimou, [5]Gabrielle Paulsson-Berne, [5]Peder S Olofsson, [1]Thomas D Otto, [2]Tomasz J Guzik, [1]Mariola Kurowska-Stolarska, [1]Pasquale Maffia. [1]*Centre for Immunobiology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK;* [2]*Institute of Cardiovascular and Medical Sciences, University of Glasgow;* [3]*School of Cardiovascular Medicine & Sciences, King's College London British Heart Foundation Centre;* [4]*Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB);* [5]*Center for Molecular Medicine, Karolinska Institutet*

Introduction The TAM receptors (Tyro3, Axl, and MerTK) are a distinct family of three receptor tyrosine kinases, namely Tyro3, Axl and MerTK, which play critical roles in cancer, inflammatory disorders and cardiovascular diseases. Axl, in particular, has been shown to influence multiple aspects of cardiovascular pathology via diverse effects on cells of both the vasculature and immune system through regulation of vascular remodelling, efferocytosis and inflammation. Clinical studies have shown that Axl is detectable in atherosclerotic plaques; however, the causal relationship between Axl and atherosclerosis is still uncertain, and results from mouse models fell short of defining the specific role(s) of Axl in the disease process.

Methods In order to quantify Axl expression in carotid endarterectomy atherosclerotic plaques we examined data from the Biobank of Karolinska Endarterectomy (BiKE). Using single-cell RNA sequencing (scRNA-seq) data from published atherosclerosis datasets we determined which cell types express Axl during pathology. Finally, we utilised an inducible atherosclerosis model in order to assess atherosclerosis formation in global Axl-deficient mice (Axl-/-). Female C57BL/6NQ (WT; n=15) and Axl-/- (n=21) mice were injected with 3×10^{11} vector genomes of AAV8-proprotein convertase subtilisin/kexin type 9 (PCSK9) and placed on a Western Diet (WD) for 12 weeks. Plaque size and percentage of necrotic core were determined in the aortic sinus using Oil Red O (ORO). Collagen content was determined using picrosirius red and polarised light microscopy. ScRNA-seq was performed to explore differences in Axl-/- vs. WT aortas at the cellular and molecular level.

Results We found expression of Axl in human carotid plaque to be significantly reduced in comparison to healthy control tissue (P=1.96e-06) in the BiKE cohort. Similarly, we detected less Axl RNA expression in the aortas of WD-fed apolipoprotein-E-/- mice compared to WT (P<0.05). Analysis of published scRNA-seq databases found that Axl is expressed predominantly in the vascular smooth muscle cell (VSMC) compartment of the aortas in both healthy and atherosclerotic mice, with expression also observed in fibroblasts and macrophages. Global Axl-deficiency increased lesion size in the aortic sinus (P<0.001). While

collagen content and necrotic core were not affected. ScRNA-seq on the aortas showed a switch versus a less contractile smooth muscle cell phenotype in Axl-/- mice compared to WT.

Conclusions In conclusion, our results indicate a protective role for Axl in atherosclerosis. The TAM receptor is reduced in diseased vessel compared to healthy controls in both human and mouse. Furthermore, global knock-out resulted in significantly increased plaque burden in mice. The necrotic core was not found to be influenced by Axl, suggesting that TAM receptor-mediated efferocytosis is not a key contributor to the role of Axl in atherosclerosis. Axl was found to be predominantly expressed in the VSMC compartment in the aortas of both healthy and diseased mice. Furthermore, Axl deficiency promoted VSMC phenotypic switching. These data support the hypothesis of a beneficial role of Axl in atherosclerosis via modulation of smooth muscle cell phenotype.

Conflict of Interest none

BS26 THE PARTNERSHIPS IN CONGENITAL HEART DISEASE IN AFRICA STUDY (PROTEA): CLINICAL CHARACTERISTICS AND GENETIC FINDINGS FROM A SOUTH AFRICAN CONGENITAL HEART DISEASE COHORT

[1]Timothy Spracklen, [1]Thomas Aldersley, [1]Nicole Saacks, [1]Bianca de Koning, [1]John Lawrenson, [1]Paul Human, [2]James Eales, [1]Blanche Cupido, [1]George Comitis, [1]Rik De Decker, [3]Barend Fourie, [1]Lenise Swanson, [1]Alexia Joachim, [1]Phaphama Magadla, [1]Malebogo Ngoepe, [1]Liam Swanson, [2]Alistair Revell, [1]Raj Ramesar, [1]Gasnat Shaboodien, [1]Andre Brooks, [1]Karen Sliwa, [1]John Anthony, [1]Ayesha Osman, [2]Bernard Keavney, [1]Liesl Zuhlke. [1]*University of Cape Town, Cape Town, South Africa;* [2]*University of Manchester;* [3]*University of Stellenbosch*

Introduction Congenital heart disease (CHD) is the most common birth defect and a significant cause of paediatric morbidity and mortality worldwide. Epidemiological data from Africa are lacking, although this information is of importance in determining the burden of CHD and guiding policy. As a multifactorial disease, the role of genetic factors in CHD is increasingly recognised. However, the genetic contribution to CHD remains relatively unexplored in Africa. The Partnerships in CHD in Africa (PROTEA) project was established to better understand the epidemiology and genetics of CHD in sub-Saharan Africa. The aim of this investigation is to describe the clinical and genetic characteristics of a cohort of CHD patients from the Western Cape, South Africa.

Methods PROTEA is a multicentre, prospective registry of CHD patients, recruited from seven hospitals in the Western Cape, South Africa. Patients with any structural CHD were eligible for inclusion, this excluded patients with isolated patent foramen ovale, peripheral pulmonary stenosis or patent ductus arteriosus in premature infants. Some of these patients were consented into the genetics study, for which a DNA biorepository was established. These patients were investigated using exome sequencing and/or chromosomal microarray (CMA) to identify disease-causing mutations or copy number variants in established CHD genes.

Results A total of 1,473 participants were recruited into the PROTEA registry between April 2017 and March 2019 (median age 1.9 years, 51% male). Compared to international cohorts, ventricular (PR: 1.8, 95%CI: 1.63-1.97) and atrial (PR: 1.4, 95%CI: 1.20-1.57) septal defects were significantly

less prevalent in PROTEA, while atrioventricular septal defects (PR: 2.2, 95%CI: 1.90-2.61), pulmonary stenosis, aortic stenosis, tetralogy of Fallot and double outlet right ventricle were significantly more prevalent. CMA analysis of 89 patients identified likely disease-causing copy number variants in five patients (5.4%), while a further 4.5% had variants of uncertain clinical significance. Using exome sequencing on 95 patients, pathogenic or likely pathogenic mutations were identified in 15 patients (15.8%); 65.3% of the sequenced cohort had variants of uncertain significance in established CHD genes.

Conclusions Preliminary analysis of the PROTEA cohort indicates that the prevalence of CHD subtypes is largely in accordance with international data, although mild lesions had a lower prevalence, and certain severe phenotypes were more prevalent. Genetic analysis of these patients demonstrates that disease-causing variants can be identified using CMA and exome sequencing, and will yield results in the expected range from international studies. Together these findings illustrate the feasibility of conducting epidemiological and genomic research amongst CHD patients in sub-Saharan Africa.

Conflict of Interest None

BS27 **HARNESSING THE POWER OF PALMITOYLATION TO TUNE NCX1 PHYSIOLOGY**

Caglar Gok, Alan D. Robertson, William Fuller. *Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK*

Introduction The cardiac Na+/Ca2+ Exchanger (NCX1) is a primary regulator of Ca2+ handling in cardiac tissue by mediating electrogenic exchange of Na+ for Ca2+. In cardiac muscle NCX1 functions primarily in the forward mode that facilitates Ca2+ extrusion from cytosol. NCX1 is palmitoylated at a single cysteine at position 739 within its large regulatory intracellular loop. Palmitoylation is critical for proper inactivation of NCX1, which is mediated by the endogenous XIP region of the same regulatory loop (Reilly et al., 2015). XIP interacts with a region near the palmitoylation site between 709-728 (Gök et al., 2020). Palmitoylation modifies XIP binding to this particular region by inducing local structural changes within the intracellular loop. Yet, relatively little is known regarding the physiological relevance of NCX1 palmitoylation.

Methods We engineered cell lines stably expressing tetracycline-inducible NCX1: palmitoylatable (WT-NCX1), unpalmitoylatable (C739A), non-functional XIP domain (K229Q and Δ229-232), missing XIP binding site (Δ709-728), and missing XIP domain (ΔXIP). NCX1 current was recorded using whole-cell patch clamp in response to voltage steps from -120mV to +100mV with 20mV steps from a holding potential of -80mV. We prepared FRET sensors for all mutated NCX1 lines listed above to study intermolecular structural changes between NCX1 dimers. We combined our patch clamp and FRET experiments with pharmacological approaches where we either manipulate NCX1 activity using a custom-made XIP peptide or deplete PIP2 (to release the endogenous XIP domain) using a cocktail of Wortmannin/m-3M3FBS. We measured intracellular Ca2+ and Na+ dependent Ca2+ intake using Fluo-4 dye.

Results and Conclusion Disrupting NCX1 inactivation by either mutating the XIP domain (K229Q) or abolishing palmitoylation (C739A) increased NCX1 current amplitude compared to WT-NCX1. Basal intracellular Ca2+ was higher and Na+-dependent cellular Ca2+ uptake was larger in cells expressing K229Q than cells expressing WT-NCX1. However, treating K229Q cells with a custom-made XIP peptide fully compensated the Ca2+ overload caused by impaired inactivation. Strikingly, XIP peptide did not affect NCX1 current in patch clamp recordings made from cells expressing unpalmitoylatable NCX1. This suggests that XIP inactivates K229Q but not C739A because palmitoylation tunes the interaction between XIP and its binding site. Targeting palmitoylation therefore offers great potential to sensitize NCX1 to XIP, and hence devise novel pharmacological strategies to manage cardiac pathologies.

REFERENCES
1. Gök *et al. Cell Reports* 2020 Jun 9;**31**(10):107697.
2. Reilly *et al. FASEB J* 2015 Nov;**29**(11):4532–43

Conflict of Interest None

BS28 **OPTIMISING CULTURE OF IPSC-DERIVED CARDIOMYOCYTES ON A PRO-SURVIVAL SUBSTRATE**

Annalisa Bettini, Daniel Stuckey, Richard Day. *University College London, London, UK*

Introduction Cell therapy is a potential novel treatment for cardiac regeneration. Numerous studies have attempted to transplant cells to regenerate myocardium lost during myocardial infarction. To date, only minimal improvements to cardiac function have been reported in clinical trials. This is likely to arise from low cell retention following delivery and high cell death after transplantation.

The current study aims to improve intramyocardial delivery of viable cells by using an injectable biodegradable substrate that supports cell attachment and growth of cardiomyocytes derived from induced pluripotent stem cells (iPSC).

Methods Highly porous microcarriers <250 μm were fabricated from 2% (w/v) 75:25 poly(DL-lactide-co-glycolide) using Thermally Induced Phase Separation (TIPS). Ultrastructural features of the microspheres were characterised using scanning electron microscopy and image analysis. A range of parameters were investigated for optimising conditions for iPSC attachment to TIPS microspheres. This included pre-conditioning with a protein solution containing recombinant human vitronectin (VTN-N) ranging from 0 to 20 μg/ml and 'wetting' with solutions of HBSS or F10 medium for up to 7 days. 5×10^5 episomal iPSC were incubated with the pre-conditioned microspheres and cell attachment quantified after 24 hours. The distribution of iPSC attached to the surface of the microspheres was evaluated using fluorescence microscopy. Pluripotency of the attached cells was assessed by confocal microscopy and flow cytometry, as well the ability to subsequently differentiate into cardiomyocytes.

Results TIPS microspheres exhibited a highly porous topography, with pore features ranging in shape and size (figure 1A). Pre-incubating the microcarriers with VTN-N; 0.5 μg/ml, resulted in an increase in iPSC attachment (93.4% vs 2.9% control; attached cells as % of seeded cells, n=5, P ≤