and lymphocytes within expansive aneurysms and co-localisation of NE with MMPs in AAA human samples.

This study suggests NE could be a regulator of aortic expansion and dissection, and therefore a potential target for AAA and TAD treatment. Further work is still needed to elucidate the causal mechanism by which NE takes its effect on AAA and TAD.

Conflict of interest no

BS39 TRIB3 DEFICIENCY INCREASES PLAQUE STABILITY BY ALTERING MACROPHAGE FUNCTION

¹Laura Martinez Campesino*, ¹University of Sheffield

10.1136/heartjnl-2019-BCS.201

Laura Martinez Campesino; Jessica M Johnston; Sheila E Francis; Endre Kiss-Toth; Heather L Wilson.

Department of Infection, Immunity & Cardiovascular Disease, Medical School, Beech Hill Road,

University of Sheffield, UK.

Background Metabolic dysfunction and chronic inflammation under obesity are key factors promoting atherosclerosis. Macrophages are the major immune cell type found in atherosclerotic lesions, where plaque macrophages engulf oxidised lipids forming foam cells and also regulate plaque stability. Tribbles (Trib) 3 is a pseudokinase known to be involved in the regulation of adipose homeostasis, lipid and glucose metabolism and macrophage lipid uptake. We propose that Trib3 has a role in regulating metabolic and immune responses to obesity ultimately affecting atherosclerosis development.

Methodology. Trib3KO and Trib3WT mice were injected with rAAV8/mPCSK9 and fed with western diet for 12 weeks to promote atherosclerosis development. Atherosclerotic plaques were quantified using Oil-red-O and aortic root lesions were characterised histologically. Primary macrophages from bone marrow (BMDMs) were isolated from these strains and human monocyte derived macrophages (MDMs) were used to further characterise the effects of Trib3 regulating macrophage function.

Results En face analysis of the aortae showed no statistical differences in atheroma burden, while histological examination of the plaques indicated an increase in plaque stability in the absence of Trib3. Trib3KO BMDMs showed differential expression of genes involved in macrophage metabolism and regulation of the cytoskeleton. The differential expression of these genes was assessed in human MDMs following control or siTRIB3 treatment.

Conclusion These results suggest a role for Trib3 in the regulation of macrophage function promoting plaque stability, and may also have an impact on atherosclerosis development.

Conflict of interest No COI

BS40 HYPOXIA INDUCES GENE-SPECIFIC EPIGENETIC MODIFICATIONS IN HUMAN CARDIAC FIBROBLASTS

¹Adam Russell-Hallinan*, ²Nadezhda Glezeva, ²Bruce Moran, ³Sudipto Das, ²John Baugh, ¹Chris Watson. ¹Queens University; ²University College Dublin; ³Royal College of Surgeons

10.1136/heartjnl-2019-BCS.202

Introduction Ischemia caused by coronary artery disease and myocardial infarction leads to aberrant ventricular remodelling and cardiac fibrosis. This occurs partly through accumulation of gene expression changes in resident fibroblasts, resulting in an overactive fibrotic phenotype. We have recently shown that human myocardial tissue hypoxia is associated with an enhanced pro-fibrotic gene profile in the tissue and, more significantly, that hypoxia-induced pro-fibrotic changes in cardiac fibroblasts are associated with global DNA hypermethylation.

Purpose Based on this epigenetics data, we have conducted a gene-specific methylation study to investigate methylation changes that occur in hypoxic ventricular fibroblasts and to gain novel insights into mechanisms that may contribute to post-ischemic cardiac remodelling.

Methods Human ventricular cardiac fibroblasts were exposed to 1% oxygen for up to 8 days. Global methylation changes were assessed using anti-5-methylcytosine (5MeC) staining, flow cytometry, QPCR, and western blot. Gene-specific methylation changes associated with hypoxia and an increased fibrotic state were determined by 5MeC immunoprecipitation and GeneChip human promoter arrays (Affymetrix), and validated by bisulphite genomic sequencing (BGS).

Results Hypoxia-induced pro-fibrotic changes in cardiac fibroblasts included increased cell proliferation and increased alpha smooth muscle actin, collagen 1, DNMT1 and DNMT3B expression which associated with global DNA hypermethylation. Array analysis revealed 37 gene-specific hypermethylation changes and 133-hypomethylation changes occurred in response to chronic hypoxia.

Conclusion Epigenetic modifications and changes in the epigenetic machinery identified in cardiac fibroblasts during prolonged hypoxia may contribute to the pro-fibrotic nature of the ischemic milieu in the heart during disease. The application of epigenetic-based therapy, such as DNA methylation modifiers, as a treatment option for cardiac pathologies associated with fibrosis and ischemia may provide therapeutic benefit.

Conflict of interest None

BS41 ASYMMETRIC DIMETHYLARGININE INCREASES CALCIUM SENSING RECEPTOR SIGNALLING TO PROMOTE ENDOTHELIAL NO PRODUCTION

¹Laura Dowsett*, ²Lucie Duluc, ¹Erin Higgins, ¹James Leiper. ¹Institute of Cardiovascular and Medical Sciences, The University of Glasgow; ²MRC London Institute of Medical Research, Imperial College London

10.1136/heartjnl-2019-BCS.203

Objective The post-translational methylation of arginine residues by PRMT enzymes can yield asymmetric dimethylarginine (ADMA); which, following proteolysis is released into the cytosol and enters the circulation. Elevated plasma ADMA concentrations are recognised as a risk factor for cardiovascular disease including hypertension, atherosclerosis and stroke. In part, this is due to ADMA acting as a competitive inhibitor of the NOS enzymes reducing NO production and contributing to endothelial dysfunction. However, recently we have identified the Calcium sensing receptor (CaSR) as a target for physiological concentrations of ADMA. CaSR is a GPCR which binds both Ca²⁺ and amino acids, and has been shown to positively modulate NO production in endothelial cells

(ECs). Our objective was to determine whether physiological concentrations of ADMA had a positive effect on NO production via CaSR stimulation.

Methods Inducible CaSR overexpressing HEK293 cells (Hek-CaSR) were generated using the FLP-IN T-REX transfection method. Intracellular Ca²⁺ mobilisation was assessed using the Ca²⁺ sensitive dye CAL520, while cAMP accumulation was measured using an ELISA kit. CaSR was stimulated by Gd³⁺ in the presence of 10µM ADMA. ADMA-CaSR signalling in ECs was investigated using human lung microvascular ECs (HuLMVECs). NO production was assessed using the fluorescent sensor DAF-AM. The CaSR antagonist NPS-2143 was used to show specificity to CaSR signalling.

Results Gd³⁺ releases stored intracellular Ca²⁺ and suppresses cAMP production in a dose dependent manner in HEK-CaSR cells. Incubation with 10µM ADMA for 1 hour prior to Gd³⁺ stimulation was found to sensitise CaSR and shift the dose-response curve for Ca²⁺ mobilisation leftward (CTRL EC₅₀ 0.16mM, ADMA EC₅₀ 0.05mM $p=0.01$). ADMA pre-treatment suppressed forskolin induced cAMP production to 70% of the maximal response. Ca²⁺ stimulation of HuLMVECs releases intracellular Ca²⁺ in a dose dependent manner, in the presence of 10µM ADMA maximal fluorescence is increased with no change to the EC₅₀. Given cytosolic Ca²⁺ release increases NOS activity we determined whether low concentrations of ADMA (10µM) promoted NO production. At maximal CaSR stimulation ADMA increased NO production by 40% ($p=0.04$). Consistent with previous reports higher concentrations of ADMA (100µM) blocked NO production through inhibition of NOS. The action of

ADMA was blocked by the addition of the CaSR inhibitor NPS-2143.

Conclusion Physiological concentrations of ADMA increase CaSR-mediated intracellular Ca²⁺ release and increase CaSR-mediated suppression of cAMP synthesis. In ECs, physiological concentrations of ADMA increase Ca²⁺ stimulated NO synthesis. In contrast pathophysiological concentrations directly inhibit EC NO synthase. These data suggest that physiological concentrations of ADMA positively regulate vascular reactivity while high concentrations of ADMA seen in cardiovascular disease reduce vascular function through NO blockade.

Conflict of interest None

BS42

HOW WARFARIN AND ANTIPLATELETS AFFECT CLOT STRUCTURE IN ATRIAL FIBRILLATION

¹Ahsan Khan*, ²Eduard Shantsila, ²Y.C. Lau, ³Lewis Hardy, ³Helen Philippou, ¹Gregory Lip.
¹Liverpool Heart & Chest Hospital NHS Foundation Trust; ²University of Birmingham;
³University of Leeds

10.1136/heartjnl-2019-BCS.204

Introduction Atrial fibrillation (AF) and coronary artery disease (CAD) are two common cardiovascular conditions associated with increased morbidity and mortality. Both conditions bring a risk of thrombosis and thus antithrombotic therapy is indicated. Despite recognised treatment, stroke and myocardial infarctions still occur. Accordingly, the identification of patients whose haemostasis remains unimpaired by treatment is valuable.

Abstract BS42 Table 1 Demographic, clinical and haemostatic characteristic of patients with atrial fibrillation and coronary artery disease (control group)

		AF Warfarin n=184	AF Antiplatelets n=46	CAD n=40	p Kruskal- Wallis
Demographic and clinical characteristics					
Age, years		76 [67-80]	75 [71-83]	74 [61-78]	0.12
Males, n (%)		82 (45%)	12 (26%)	20 (50%)	0.04
Systolic BP, mmHg		130 [117-145]	130 [120-142]	139 [126-150]	0.08
Diastolic BP, mmHg		76 [68-80]	75 [64-85]	74 [65-80]	0.81
Body mass index, kg/m ²		29 [26-33] ^b	25 [23-30]	28 [24-31]	0.002
Smoking, n (%)		7 (4%)	3 (7%)	6 (15%)	0.02
Creatinine, n (%)		90 [75-121] ^a	90 [76-114]	82 [71-95]	0.04
eGFR, n (%)		66 [48-80] ^a	76 [52-87]	80 [63-88]	0.02
Creatinine clearance, n (%)		69 [45-85]	60 [46-85]	76 [57-96]	0.10
Coronary artery disease, n (%)		75 (41%)	17 (40%)	40 (100%)	<0.001
Diabetes, n (%)		76 (41%)	7 (15%)	17 (43%)	0.003
Hypertension, n (%)		151 (83%)	40 (87%)	37 (93%)	0.26
Chronic kidney disease, n (%)		64 (35%)	15 (33%)	4 (10%)	0.008
Peripheral artery disease, n (%)		17 (9%)	1 (2%)	0	0.04
Parameters of clot turbidity and lysis (non-normalised values)					
Maximum OD	PPP	0.55 [0.46-0.66]	0.52 [0.45-0.72]	0.56 [0.45-0.63]	0.78
	APPT	0.50 [0.43-0.63]	0.47 [0.42-0.63]	0.51 [0.41-0.59]	0.83
	Thrombin	0.58 [0.51-0.69]	0.58 [0.50-0.76]	0.56 [0.48-0.67]	0.62
Time to 50% maximum OD	PPP	454 [400-526] ^{ab}	328 [296-372] ^a	270 [238-318]	<0.001
	APPT	352 [318-398] ^{ab}	322 [288-360] ^a	280 [232-304]	<0.001
	Thrombin	268 [222-336] ^{ab}	232 [200-272] ^a	180 [146-220]	<0.001
Time between 50% clotting and 50% lysis	PPP	546 [456-700]	514 [464-604]	498 [436-598]	0.16
	APPT	626 [520-806] ^{ab}	562 [496-648]	558 [466-598]	<0.001
	Thrombin	560 [488-690]	530 [472-600]	534 [444-610]	0.04
Time to 50% lysis from maximum OD	PPP	360 [284-506]	332 [276-428]	334 [274-430]	0.30
	APPT	496 [398-692] ^a	484 [392-544]	462 [372-510]	0.03
	Thrombin	358 [306-500]	340 [296-400]	352 [288-422]	0.19