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Scientific Business Abstracts (1) Rare variants in Steroid Receptor Coactivator-1 (SRC-1) associated with obesity, adipose tissue dysfunction and liver fibrosis T.M. Cacciottolo, A. Perikari, A. van der Klaauw, E. Henning, L.K.J. Stadler, J. Keogh and I.S. Farooqi From the University of Cambridge Metabolic Research Laboratories Biography: Tessa M. Cacciottolo graduated from the University of Malta Medical School, then moved to the UK to further her clinical training in general medicine, gastroenterology and hepatology. During this time she became interested in the interface between liver disease and metabolism. She joined Professor Farooqi's group at the Institute of Metabolic Science in Cambridge, to study the mechanisms which are disrupted in people with severe obesity, and how these could be associated with liver and metabolic disease. Aim: The prevalence of obesity and associated cardiometabolic complications has risen sharply in recent years. Within the Genetics of Obesity Study (n = 7000) we identified 15 rare loss of function variants in SRC-1 associated with obesity and liver cirrhosis at a young age. We hypothesized that SRC-1 plays a crucial role in human peripheral lipid metabolism. Methods: To test the effect of SRC-1 on lipid metabolism, we knocked down SRC-1 in hepG2 cells, quantified expression of key enzymes using qPCR and measured exogenous fatty acid oxidation using the Seahorse Bioscience flux analyser. In human variant carriers, we performed open adipose tissue biopsies and assessed histology for fibrosis using Sirius red stain. Hepatic fibrosis was quantified using magnetic resonance elastography. Results: SRC-1 knock-down caused 75% reduction in expression of CYP7A1 and 80% reduction in expression of CPT1a, the rate limiting enzymes for cholesterol catabolism and fatty acid oxidation, respectively; and significant reduction in exogenous palmitate oxidation. In humans, we found significant insulin resistance (mean HOMA-IR = 3.2), severe fibrosis in 40% adipose tissue biopsies and advanced liver fibrosis or cirrhosis in 27% of cases. Conclusions: SRC-1 variants are associated with a high risk of adipose tissue fibrosis. The inability of hepatocytes to handle excess lipid may lead to lipotoxicity and accelerated liver fibrosis.

(2) Glypican-6 (GPC6) is a gene responsible for sporadic, non-syndromic Tetralogy of Fallot in humans G. Tenin and B. Keavney From the University of Manchester Biography: Dr Gennadiy Tenin started his scientific career as a researcher in the University of Dundee (Scotland, UK) studying the termination of body axis elongation in chick embryo, followed by the short period in King's College London investigating molecular control of head mesoderm patterning in chick. He then moved to the University of Manchester to work with Dr Kathryn Hentges, working on several projects, studying different aspects of heart development using mouse as a model organism. As the next step in his career, he started working with Prof Bernard Keavney to identify new genes involved in cardiovascular development and congenital heart disease, using GWAS data in Tetralogy of Fallot patients as the starting point. He currently continues his research in the area, studying the function of the one of the newly identified genes during cardiogenesis. Congenital heart diseases (CHD) are the commonest birth defects and present in 9 out of 1000 live births. Tetralogy of Fallot (TOF) is the commonest cyanotic CHD; some 80% of TOF cases are sporadic and not due to a recognized genetic syndrome. Sporadic, non-syndromic TOF nonetheless exhibits significant heritability, and is considered to be a multigenic condition. Previously, a region of association on chr 13q31 (P = 3.03 10⁻¹¹) was discovered through genome-wide association studies (GWAS) in TOF patients but no causative gene identified. Integration of population genetics data, regional chromosomal interactions and embryonic expression data suggested that the nearby located gene Glypican-6

(GPC6) was the strongest candidate. GPC6 was found to be expressed exclusively in the endocardial cushions, transitory structures critical for cardiac septation. We used mouse gene knock-out to investigate the functional significance of Gpc6 in heart. Mice carrying a hypomorphic allele of Gpc6 displayed abnormalities in cushion development, leading to lower cell density in cardiac valves, thinner great vessels and, in some cases, a ventricular septal defect. The complete knock-out of Gpc6 resulted in double outlet right ventricle with mal-positioned aorta, a phenotype closely related to human TOF. The previously uncharacterized gene GPC6 was confirmed to be a novel causative gene for CHD, and is the first such gene to be identified from a GWAS approach. The glypicans, of which GPC6 is one, are heparan sulfate proteoglycans involved in a number of cell signalling pathways; this work newly identifies the role of the glypican gene family in heart development.

(3) Does altered metabolism drive macrophage dysfunction in COPD? E. Ryan¹, R. Budd², M. Bewley², P. Coelho¹, W. Rumsey³, Y. Sanchez³, J. McCafferty¹, D. Dockrell¹, S. Walmsley¹ and M. Whyte¹ From the ¹ Department of Respiratory Medicine, Centre for Inflammation Research, University of Edinburgh, ² Department of Infection Immunity and Cardiovascular Disease, The Florey Institute for Host-Pathogen Interactions, University of Sheffield and ³ Stress and Repair Discovery Performance Unit, Respiratory Therapy Area Biography: Dr Eilise Ryan currently works as a Clinical Research Fellow with Professors Moira Whyte and Sarah Walmsley at the Centre for Inflammation Research Edinburgh. She was awarded a Wellcome Research Grant for her PhD, exploring mechanisms for impaired macrophage function in chronic obstructive pulmonary disease (COPD), with a particular focus on cellular energetics. COPD patients have defective innate immunity, characterized in part by macrophage dysfunction with impaired macrophage phagocytosis of bacteria and apoptotic cells (efferocytosis). We hypothesized that defective macrophage function in COPD may share a common mechanism related to altered metabolism, and or, as previously suggested in the literature, failure to upregulate the Nrf2-mediated antioxidant response. AM and MDM were isolated from patients with established COPD (GOLD stages 1–3). Macrophage efferocytosis rates were correlated with bacterial internalization of *Streptococcus pneumoniae*. Seahorse technology was utilized to metabolically profile cells in real time. Efferocytosis assays were performed *in vitro* with Sulforaphane (a non-specific Nrf2 agonist) and Compound 7, a highly specific Nrf2 agonist (GSK). Both COPD MDM and AM have significantly impaired bacterial phagocytosis and efferocytosis compared to Healthy Controls ($P < 0.05$). Moreover, there was a correlation between COPD macrophage phagocytosis and efferocytosis ($r = 0.71$). Both Glycolytic Reserve and Spare Respiratory Capacity were significantly reduced in AM and MDM from COPD donors ($P < 0.05$). *In vitro* studies using Sulforaphane and Compound 7 enhanced efferocytosis of apoptotic cells in both COPD AM and MDM ($P < 0.01$). In summary, we observe a correlation between macrophage phagocytosis and efferocytosis in COPD, suggesting a common mechanism. We demonstrate an altered metabolic profile in COPD macrophages, with potential consequences for high energy requiring processes such as efferocytosis. The partial rescue of defective COPD MDM and AM efferocytosis via specific activation of the Nrf2 pathway, suggests that activation of the Nrf2 module may enable metabolic reprogramming in COPD macrophages, leading to enhanced efferocytosis.

(4) STX18-AS1 is an lncRNA governing *in vitro* cardiomyocyte differentiation and predisposing to atrial septal defect via downregulation of NKX2-5 Y. Liu, M.-k. Choy, G. Tenin, S. Abraham, G. Black and B. Keavney From the University of Manchester Biography: Yingjuan Liu was recently awarded her PhD degree at November 2018 from The University of Manchester. She is currently appointed as a research associate working with Professor Bernard Keavney in Division of Cardiovascular Sciences, The University of Manchester. She is focusing on the genetics of Congenital Heart Diseases with particular efforts in investigating the functional roles of long noncoding RNAs during cardiovascular development. Objectives: To identify the gene and mechanism responsible for the Genome-wide

association studies (GWAS) signal (OR \approx 1.46; P \approx 2.61 10⁻¹⁰) which we previously identified at chromosome 4p16 for atrial septal defect (ASD). To date, while a number of risk variants have been identified from GWAS of congenital heart disease (CHD), none has been functionally confirmed. Methods and Results: The linkage disequilibrium in the region indicated association was restricted to the long noncoding RNA STX18-AS1. Since STX18-AS1 is not conserved beyond primates, all experiments were conducted in human tissues and cell lines. In 108 RNA samples from right atrial appendages and corresponding DNA, we first confirmed the risk SNPs of ASD were eQTLs for STX18-AS1 in cardiac tissues. Using qPCR, the transcription of STX18-AS1 in embryonic hearts was detected to be the highest at CS14-CS18, the critical time for atrial septation. Furthermore, using in situ hybridization on whole embryonic hearts at CS16-CS19, we detected substantial expression of STX18-AS1 in the atrial septum. We next identified STX18-AS1 as a regulator of the key cardiac transcriptional factor NKX2-5 using CRISPR/Cas9 knockdown in HepG2 cells. Reduced STX18-AS1 transcription inhibited the expression of NKX2-5, mutations in which cause septal defects in humans. Using in vitro cardiomyocyte differentiation from human embryonic stem cells (hESCs), we demonstrated that the knockdown of STX18-AS1 depleted the potential of hESCs in differentiating into cardiomyocytes without changes in cell viability and pluripotency. Conclusions: STX18-AS1 is the first long noncoding RNA influencing CHD risk identified from GWAS. The mechanism involves downregulation of the NKX2-5 gene.

(5) CorMicA: a randomized controlled trial of coronary function testing in angina and no obstructive coronary disease T. Ford^{1,2}, B. Stanley³, R. Good², P. Rocchiccioli^{1,2}, M. McEntegart^{1,2}, S. Watkins², H. Eteiba², A. Shaikat², M. Lindsay², K. Robertson², S. Hood², R. McGeoch⁴, R. McDade², N. Sidik^{1,2}, P. McCartney^{1,2}, D. Corcoran^{1,2}, D. Collison^{1,2}, C. Rush^{1,2}, A. McConnachie³, R. Touyz¹, K. Oldroyd^{1,2} and Colin Berry^{1,2} From the 1 BHF Centre of Excellence in Vascular Science and Medicine, University of Glasgow, 2 Golden Jubilee National Hospital, 3 Robertson Centre for Biostatistics and 4 University Hospital Hairmyres Biography: Tom Ford is a BHF Clinical Research Fellow (Interventional Cardiology) and PhD Candidate at the University of Glasgow. Passionate about cardiovascular medicine and holds special interests in coronary physiology and interventional cardiology. Graduated with first class honours in Medicine from the University of Dundee in 2007 before gaining his Membership of the Royal College of Physicians (MRCP) in Edinburgh (2010). Undertook specialist training in cardiovascular medicine in Sydney gaining Fellowship of the Royal Australian College of Physicians (FRACP—Cardiology) in 2015. Worked as a cardiologist in Port Macquarie, Australia before embarking on a Fellowship and Clinical PhD with Prof Berry in Glasgow. Background: Patients with angina but no obstructive coronary artery disease (CAD) pose a diagnostic and therapeutic challenge. Objectives: Test whether an interventional diagnostic procedure (IDP) linked to stratified medicine improves angina in patients without obstructive CAD. Methods: We conducted a randomized, controlled, blinded clinical trial of stratified medicine vs. standard care in patients with angina. Patients with angina undergoing invasive coronary angiography (standard care) were recruited. Patients without obstructive CAD were immediately randomized 1:1 to the intervention group (stratified medical therapy) or the control group (standard care). The IDP consisted of invasive vasoreactivity testing using IV adenosine and intracoronary acetylcholine. The primary endpoint was the mean difference in angina severity at 6 months (assessed by the Seattle Angina Questionnaire—SAQ). Results: Three hundred and ninety-one patients were enrolled with obstructive CAD found in 206 (53.7%). One hundred and fifty-one (39%) patients without angiographically obstructive CAD were randomized (n = 76 intervention group; n = 75 blinded control group). The intervention resulted in a mean improvement of 11.7 units in the SAQ score at 6 months [95% confidence interval (CI): 5.0–18.4; P \approx 0.001]. The intervention also led to improved quality of life score (EQ5D index 0.10 units; 95% CI: 0.01–0.18; P \approx 0.024). There were no differences

in major adverse cardiac events at the 6-month follow-up (2.6% controls vs. 2.6% intervention; $P = 1.00$). Conclusion: Coronary function testing changed physician diagnosis for one in two patients, commonly identifying microvascular and vasospastic angina. These distinct disorders treated using stratified medicine to improve angina and quality of life.

(6) Exomic sequencing uncovers novel genetic associations in children with hypospadias and neurodevelopmental abnormalities G. Gazdagh¹, L. Diver², J. Marshall³, R. McGowan², F. Ahmed⁴, DDD Study⁵ and E. Tobias⁶ From the 1 School of Medicine, Dentistry & Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, 2 West of Scotland Regional Genetics Service, Laboratory Medicine Building, Queen Elizabeth University Hospital, 3 Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, 4 Developmental Endocrinology Research Group, Royal Hospital for Children, University of Glasgow, 5 Wellcome Sanger Institute, Wellcome Genome Campus and 6 Academic Unit of Medical Genetics and Clinical Pathology, Laboratory Medicine Building, Queen Elizabeth University Hospital, University of Glasgow
Biography: Dr Gabriella Gazdagh is a Specialist Trainee in Clinical Genetics with a special interest in disorders of sex development. She is currently based at the West of Scotland Regional Genetics Service in Glasgow. Gabriella graduated from the University of Szeged in Hungary and after pursuing training in Paediatrics in the Kent, Surrey and Sussex Deanery, joined the Clinical Genetics training in 2014. Gabriella was awarded the Clinical Research Fellowship by the Glasgow Children's Hospital Charity Research Fund in 2016. Background: Currently, a molecular diagnosis is not reached in the majority of cases of hypospadias. Cohorts such as the UK-wide Deciphering Developmental Disorders (DDD) Study represent a useful resource of large molecular and phenotypic datasets obtained from individuals with undiagnosed conditions including hypospadias. Aims/Objectives: To identify likely pathogenic variants in previously undiagnosed DDD participants with hypospadias and neurodevelopmental disorders. Method: Retrospective review of anonymized phenotype data and bioinformatic re-analysis of variant call format files of 33 DDD participants (22 family trios and 11 singleton cases) manifesting hypospadias and neurodevelopmental abnormalities. A bioinformatic analysis pipeline (GoldenHelix, Varsq 1.4.4) was created and searches were performed in genetic databases (Online Mendelian Inheritance in Man, PubMed and the Jackson Laboratory). Results: Two previously unidentified, de novo variants in two genes, including CTNND1 and TRIO are described in the family trios. The CTNND1 gene encodes a protein, which has a role in cell-cell adhesion and signal transduction, and the TRIO protein is involved in actin remodelling, necessary for cell migration and growth. Variants in CTNND1 and TRIO are linked to blepharochelodontic syndrome 2 and intellectual disability, respectively. Variant analysis of singleton cases revealed an additional variant in HIST1H1E gene, encoding a histone H1 protein. Mutations in HIST1H1E are linked to overgrowth and intellectual disability. Conclusion: Not only has exome sequencing proven a powerful tool for the investigation of conditions associated with hypospadias but it has also extended our understanding of the level of genetic heterogeneity that may be associated with disorders of sex development.

(7) Bone turnover in pregnancy is influenced by vitamin D supplementation and is associated with maternal bone health: the MAVIDOS trial E. Curtis¹, C. Parsons¹, K. Maslin¹, S. D'Angelo¹, R. Moon¹, S. Crozier¹, F. Gossiel², N. Bishop³, S. Kennedy⁴, A. Papageorghiou⁴, R. Fraser⁵, S. Gandhi⁵, A. Prentice⁶, H. Inskip¹, K. Godfrey¹, I. Schoenmakers⁷, M.K. Javid⁸, R. Eastell², C. Cooper¹ and N. Harvey¹ From the 1 MRC Lifecourse Epidemiology Unit, University of Southampton, 2 Academic Unit of Bone Metabolism, University of Sheffield, 3 Academic Unit of Child Health, University of Sheffield, 4 Nuffield Department of Women's & Reproductive Health, John Radcliffe Hospital, University of Oxford, 5 Department of Obstetrics and Gynaecology, Sheffield Hospitals NHS Trust, University of Sheffield, 6 MRC Elsie Widdowson Laboratory, 7 Department of Medicine,

Faculty of Medicine and Health Sciences, University of East Anglia and 8 NIHR Oxford Biomedical Research Centre, University of Oxford Biography: Dr Beth Curtis undertook her undergraduate medical training at the University of Oxford, graduating in 2010. Whilst there, she undertook a BA in Medical Sciences, graduating with a First Class honours degree and developing interests in both rheumatology and human development. Following completion of the Academic Foundation Programme and Core Medical Training at Southampton General Hospital she joined the Wessex rheumatology rotation as an Academic Clinical Fellow in 2014. Following a research placement at the MRC Lifecourse Epidemiology Unit under the supervision of Professors Cyrus Cooper, Nicholas Harvey and Elaine Dennison, working on the Southampton Women's Survey mother-offspring cohort, she developed an interest in early-life determinants of osteoporosis. In 2016, she was awarded a Wellcome Trust Clinical Research Training Fellowship and is now undertaking her doctoral studies supervised by Professors Cooper and Harvey and epigenetics specialists Professor Karen Lillycrop and Dr Christopher Bell. Her research is based on the mechanisms in early life which determine future bone health, using the Southampton Women's Survey children, now 12 years of age, and the offspring of the MAVIDOS randomized controlled trial of vitamin D supplementation in pregnancy, aged between 4 and 7 years. Dr Curtis has received Young Investigator awards from the British Society for Rheumatology (BSR), National Osteoporosis Society, the Bone Research Society and the International Osteoporosis Foundation for her work in this area. Other parallel projects include leading the authorship of the most comprehensive assessment of UK fracture epidemiology to date, using data from the Clinical Practice Research Datalink, co-authorship of the BSR safety guidelines on use of biologic agents in rheumatoid arthritis, and working within a European collaboration towards a systematic review on the safety of COX2 inhibitors and opioids in osteoarthritis. Objectives: Markers of bone turnover in pregnancy have been poorly characterized. We investigated changes in a marker of maternal bone resorption, C-terminal telopeptide of type-I collagen (CTX), in pregnancy, the influence of gestational vitamin D supplementation, and associations between CTX and maternal postnatal bone indices. Methods: MAVIDOS is a randomized, double-blind, placebo-controlled trial of 1000 IU/day cholecalciferol vs. placebo from 14/40 to birth. Maternal second-void urinary a and b CTX (ELISA) were measured at in early (14/40) and late pregnancy (34/40); Dual X-ray absorptiometry was performed within 2/52 postpartum. Wilcoxon signed-rank, Spearman's correlation and linear regression were used to test changes in median CTX from 14/40 to 34/40, compare CTX values at these timepoints and explore associations with maternal bone outcomes. Results: Six hundred and thirty-six women were studied (314 placebo, 322 cholecalciferol). Median (interquartile range) CTX (lg/mmol creatinine) increased from 14/40 to 34/40 in both groups [224 (155–309) to 445 (319–657) and 218 (155–305) to 420 (252–609), both $P < 0.0001$, in placebo and cholecalciferol groups, respectively]; the higher 34/40 CTX in the placebo group was of borderline statistical significance ($P \approx 0.06$). CTX values at 14/40 and 34/40 were correlated in both placebo ($r \approx 0.32$) and treatment ($r \approx 0.45$) groups (both $P < 0.0001$). Greater 34/40 CTX was associated, similarly in both groups, with lower maternal total hip and lumbar spine (LS) bone area and bone mineral content (BMC) (all $P < 0.02$); e.g. LS BMC[b ≈ 1.63 g/SD increase in CTX; (2.80, 0.46), $P \approx 0.006$]. Conclusion: Maternal CTX, a bone resorption marker, rises in pregnancy, appears to be reduced by cholecalciferol supplementation, and is inversely associated with maternal post-partum bone mass. These findings inform our understanding of bone resorption in pregnancy and potential relationship with vitamin D metabolism.

(8) Hypoxia drives a hyperinflammatory neutrophil phenotype in the lung E.R. Watt¹, A. Howden², A. Mirchandani¹, P. Coelho¹, J.L. Hukelmann², P. Sadiku¹, T.M. Plant¹, D.A. Cantrell², M.K.B. Whyte¹ and S.R. Walmsley¹ From the ¹ University of Edinburgh and ² School of Life Sciences, University of Dundee Biography: Emily is a Wellcome Trust Clinical Training Fellow and Specialty

Trainee in Respiratory Medicine in Edinburgh. Aims: Neutrophils are critical mediators of innate immunity but may contribute to inflammatory lung diseases such as acute respiratory distress syndrome which are also associated with hypoxia. Therapies specifically targeting neutrophilic inflammation are lacking. This project aimed to define the proteome of inflammatory lung neutrophils and determine how this is regulated by hypoxia. Methods: We utilized a murine model of acute lung injury (ALI) to investigate hypoxic regulation of neutrophilic inflammation. Mice were treated with LPS to induce neutrophilic lung inflammation and housed in either room air or in hypoxia (10% oxygen). We analysed sickness and lung damage in normoxic and hypoxic mice. Lung neutrophils were isolated from bronchoalveolar lavage and analysed by high resolution mass spectrometry. Raw data were analysed using MaxQuant software. Results: Mice subjected to hypoxia in the context of ALI were found to have more severe lung injury with significant hypothermia and increased lung damage compared to normoxic controls. Proteomic analysis of normoxic and hypoxic neutrophils demonstrated distinct proteomic signatures. Hypoxia resulted in a hyperinflammatory neutrophil phenotype with increased inflammatory receptor expression. This was associated with evidence of enhanced *in vivo* neutrophil degranulation, independent of neutrophil numbers. Discussion/conclusions: Hypoxia is a common feature of inflammatory environments. In the context of ALI, hypoxia is a damaging stimulus resulting in more severe and persistent neutrophilic inflammation. Hypoxia drives a distinct neutrophil proteomic signature which is hyperinflammatory. Through dissecting the pathways which regulate this hyperinflammatory phenotype we aim to identify novel therapeutic targets to combat neutrophilic lung inflammation.

(9) Repurposing of metformin as a treatment for adverse left ventricular remodelling I. Mordi, C. Forteach, A. Wong, M. Mohan, C. Palmer, A. Doney, G. Rena and C. Lang From the University of Dundee Biography: Ify Mordi is a Clinical Lecturer and Specialty Registrar in Cardiology, University of Dundee. Aims: Left ventricular hypertrophy (LVH) is independently associated with adverse cardiovascular outcome. The pathophysiology of LVH development remains unclear although observational studies have reported its association with blood pressure, insulin resistance (IR) and the transcription factor KLF15, a master regulator of anabolic branched chain amino acids (BCAAs) in cells. The current study aims to determine the potential of the diabetes drug metformin to ameliorate these aspects of LVH. Methods: We used a multidisciplinary approach encompassing population genomics and a clinical randomized trial. Results: We constructed genetic risk scores (GRS) for IR and systolic blood pressure (SBP) and, using the GoDARTS study which consists of over 10 000 patients with diabetes and over 8000 controls, found that IR and SBP GRS were significantly associated with LVH after adjustment for relevant clinical variables (IR: $P = 0.012$; SBP: $P = 0.025$). Patients with the AA genotype of the rs9838915 KLF15 variant were more likely to have LVH (OR 1.19; 95% CI 1.05–1.35, $P = 0.006$). This association was attenuated in used metformin users (OR 1.01; 95% CI 0.96–1.08, $P = 0.64$, interaction P -value 0.027), suggesting that metformin might also reduce BCAA uptake into tissues. Our analysis of BCAAs in plasma samples from a clinical trial of metformin vs. placebo confirmed this. Finally, in a randomized-controlled clinical trial, metformin reduced indexed LV mass compared to placebo (3.1 \pm 1.9 g/m^{1.7} vs. 1.2 \pm 2.7 g/m^{1.7}; $P = 0.005$) and SBP ($P < 0.001$). Conclusions: We have used genomic studies to help underpin a randomized trial showing that metformin significantly reduced LV mass compared to placebo.

(10) Faecal cytokine profiling provides novel insights into intestinal barrier disruption and bacterial translocation in acute decompensation of cirrhosis E. H. Gray^{1,2}, S. Azarian¹, A. Riva^{1,2}, H. Edwards^{1,2}, M.J.W. McPhail^{2,3}, R. Williams^{1,2}, S. Chokshi^{1,2}, V.C. Patel^{1,2,3} L.A. Edwards^{1,2} From the 1 Institute of Hepatology, Foundation for Liver Research, 2 School of Immunology and Microbial Sciences, King's College London and 3 Institute of Liver Studies & Transplantation, King's College Hospital Biography: Vishal C. Patel is a Principal Investigator and group lead for Advanced Chronic

Liver Disease with a focus on the role of the gut microbiome, at the Institute of Hepatology (Foundation for Liver Research), working with Prof Roger Williams CBE. His clinical role is as an appointed Honorary Consultant Hepatologist & Endoscopist with triple accreditation in Hepatology, Gastroenterology and General Internal Medicine. He is an Honorary Clinical Lecturer within the School of Immunology & Microbial Sciences at King's College London. VCP has been involved in a several observational and interventional clinical translational studies in chronic and acute liver failure syndromes. He is the recipient of the NIHR CRN Greenshoots Award and more recently an NIHR HTA £2.3 million grant for the BOPPP trial (beta-blockers in small varices to prevent variceal haemorrhage in cirrhosis). He was previously awarded a Young Investigator of the Year grant from the Intensive Care Foundation to facilitate his work examining the gut microbiome, bacterial translocation and immune dysfunction in acute-on-chronic liver failure syndromes as part of his doctoral thesis. VCP is now focusing on metagenomic and metabonomic profiling with primary interests based around characterizing and understanding the pathophysiological role of the gut microbiome in driving chronic liver failure syndromes, and therapeutic interventions that can target gut dysbiosis. Linked to this are how metabolic profiling and other high throughput techniques can better elucidate the mechanisms behind these changes. He is a Gut Microbiota for Health Expert Panel member (British Society for Gastroenterology Research Section), and joint BSG-BASL Liver Research Development Group member where national strategy for hepatology research is formulated. Aims: Intestinal dysbiosis with gut barrier impairment (GBI) and excessive bacterial translocation (BT) to the liver are recognized as central features of acutely decompensated cirrhosis (AD). To date, interrogation of mucosal inflammation has been technically challenging with difficulty obtaining representative tissue and paucity of non-invasive techniques. We have developed a novel, reliable and validated assay to quantify faecal cytokines (FC) as surrogate makers of intestinal inflammation and compared to GBI markers and systemic immunological profiles. Methods: Protein was isolated from faeces of patients with stable cirrhosis (SC; n = 16), AD (n = 48) and healthy participants (HC; n = 31). Faecal and plasma cytokine expression, intestinal epithelium-associated fatty acid binding protein 2 (FABP2) and intestinal-microbiota metabolite D-lactate were quantified by electrochemiluminescence and ELISA. Results: Faecal IL-1b, IFN-c, TNF-a, IL-21, IL-17A/F and IL-22, which mediate intestinal epithelial barrier damage or repair, were significantly elevated in AD vs. SC (P = 0.0085; 0.01; 0.0194; 0.0014; 0.0026; 0.01 and 0.025, respectively) and HCs. Along with IL-1b, faecal IL-23 was significantly elevated in AD vs. HC (P = 0.0007), important in promoting deleterious Th17 effector function. Faecal FABP2 (P < 0.0001) and plasma D-lactate (P = 0.0004) were significantly elevated in AD vs. SC and HC, consistent with GBI. Discussion: FC profiling represents a novel targeted approach to localized measurement of the intestinal cytokine milieu in cirrhosis. In combination with markers of GBI, elevated FC profiles provide evidence for the inability to defend and repair the intestinal barrier in AD. These data provide the first elusive link between intestinal mucosal injury, GBI and BT which predispose to AD.

(11) Whole exome sequencing reveals the major genetic contributors to non-syndromic Tetralogy of Fallot D. Page1,2, M. Miossec2,3, S. Williams1, R. Monaghan1, E. Fotiou1, CHANGE Study Collaborators, CheartED Study Collaborators, M. Santibanez-Koref2 and B. Keavney1 From the 1 University of Manchester, 2 Manchester Metropolitan University and 3 University of Newcastle Biography: I worked as a Research Associate for Professor Bernard Keavney at the University of Manchester and continue to work closely with the Keavney group. We are primarily interested in understanding the functional association between genetics and complex cardiovascular diseases. Prior to this, my PhD was in Developmental Biology in Dr Shane Herbert's group, also at the University of Manchester. I used the zebrafish embryo model to study the molecular control of angiogenesis. I'm currently a Lecturer at Manchester Metropolitan University where I teach in the

area of Haematology and Transfusion Science, although my research interests remain focused on cardiovascular development and the genetics of complex cardiovascular diseases. Objectives: Familial recurrence studies provide strong evidence for a genetic component to the predisposition to sporadic, nonsyndromic Tetralogy of Fallot (TOF), the most common cyanotic congenital heart disease (CHD) phenotype. Rare genetic variants are important contributors to CHD risk, but relatively small numbers of TOF cases have been studied to date. Methods/Results: We used whole exome sequencing to assess the prevalence of unique, deleterious variants in 829 patients. The clustering of variants in two genes, NOTCH1 and FLT4, surpassed thresholds for genome-wide significance (assigned as $P < 5 \times 10^{-8}$) after correction for multiple comparisons. NOTCH1 was most frequently found to harbour unique, deleterious variants. Thirty-one changes were observed in 37 probands (4.5%; 95% CI: 3.2–6.1%) and included 7 loss-of-function variants, 22 missense variants and 2 in-frame indels. Sanger-sequencing of the unaffected parents of seven cases identified five de novo variants. Three NOTCH1 variants were subjected to functional evaluation and two showed a reduction in Jagged1-induced NOTCH signalling. FLT4 variants were found in 2.4% (95% CI: 1.6–3.8%) of TOF patients, with 21 patients harbouring 22 variants. The variants identified were distinct to those that cause the congenital lymphoedema syndrome Milroy Disease. In addition to NOTCH1, FLT4 and the well-established TOF gene, TBX1, we identified potential association (exomewide corrected $P < 0.05$) with variants in several other candidates including RYR1, ZFPM1, CAMTA2, DLX6 and PCM1. Conclusion: The NOTCH1 locus is the most frequent site of genetic variants predisposing to non-syndromic TOF, followed by FLT4. Together, variants in these genes are found in almost 7% of TOF patients.

(12) Genome editing of haemopoietic stem cells for treatment of thalassaemia M. Badat¹, S. Mettananda², P. Hua¹, R. Schwessinger¹, J. Hughes¹, D. Higgs¹ and J. Davies¹ From the 1 MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital and 2 Department of Paediatrics, Faculty of Medicine, University of Kelaniya

Biography: James Davies is a clinician scientist with a specialist interest in haemopoietic stem cell transplantation, genome editing, genomics and bioinformatics. He studied medicine at Oxford University before going on to complete specialist training in haematology and intensive care medicine in London. He is an honorary consultant haematologist with the allogeneic transplant service in Oxford at present. He was awarded Wellcome Trust Clinical Research Training Fellowship to do a DPhil with Prof Doug Higgs and Prof Jim Hughes at the Weatherall Institute of Molecular Medicine. This focussed on developing next generation sequencing-based methods for interrogating the physical structure that genes form with their distal regulatory elements. This work led to important insights into how genes are controlled and he was awarded the RDM Graduate Prize for this project. He has subsequently developed an interest in the clinical application of genome editing technology. In 2017, he was awarded an MRC Clinician Scientist fellowship to leverage his expertise in gene regulation, bioinformatics and development of high throughput sequencing assays to develop novel safe approaches for treating inherited disorders of haemopoiesis, such as thalassaemia. Aim: Thalassaemia is commonly due to mutations at the beta globin (HBB) locus, and this causes transfusion dependent anaemia in severe cases. A key pathophysiological factor is the imbalance of alpha and beta globin production. This results in accumulation of excess alpha globin chains, which are toxic and cause cell death. Patients who co-inherit partial deletions of the alpha globin genes with beta thalassaemia usually have a mild phenotype and are transfusion independent. We aim to develop genome editing strategies of haemopoietic stem cells to exploit this for use as part of an autologous transplant to treat thalassaemia. Methods: CRISPR-Cas9 was used to edit the most important enhancer of the alpha globin gene to elicit a controlled reduction in alpha globin expression. In silico methods were used to define the key sequences to delete to

abrogate transcription factor binding. This allowed us to develop a strategy to disrupt single transcription factor binding sites using Cas9 ribonucleoprotein. Results: Our in silico approaches allowed us to define three key transcription factor binding sites within the enhancer. We were able to achieve indel efficiencies in excess of 75% as measured by next generation sequencing. This resulted in a much more controlled reduction in alpha globin expression than was achieved by deletion of the whole enhancer. Discussion: In silico prediction allows the identification of the sites within enhancers that allow genome editing to be used to reduce gene expression in a highly controlled manner.