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POSTER NO: 1

Gene-specific modelling of mutational hotspots in six hypertrophic cardiomyopathy genes

Waring A.J.[1], Harper A.R.[1,2], Thomson K.L.[2,3], Watkins H.[1,2], Farrall M.[1,2]

[1]*Wellcome Centre for Human Genetics, Roosevelt Drive, Oxford, UK.*
[2]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.*
[3] *Oxford Medical Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust, Churchill Hospital, Oxford, UK.*

In hypertrophic cardiomyopathy (HCM), ~15% of patients that undergo genetic testing carry a rare-missense variant in a known disease-causing gene, but it cannot be confidently interpreted as pathogenic. However, the aggregate burden of rare-variants in these genes suggests many are in fact, pathogenic. Variant clustering is a gene-specific evidence metric (ACMG criteria PM1) currently only applied to *MYH7.* Here we develop data-driven models to extend the application of this criteria to more genes.

Using a flexible statistical modelling framework, generalized-additive models (GAMs), we model mutational hotspots in six well-established pathogenic HCM genes; *MYH7, MYBPC3, TNNI3, TNNT2,* MYL2 and MYL3 (Figure 1). The largest jointly processed HCM dataset in the world (n = 5,338) and the population cohort gnomAD (n = 125,748), were used as training data. The models allow the application of criteria PM1 as either supporting or moderate for many variants in five out of six genes considered increasing the number of clinically actionable variants.

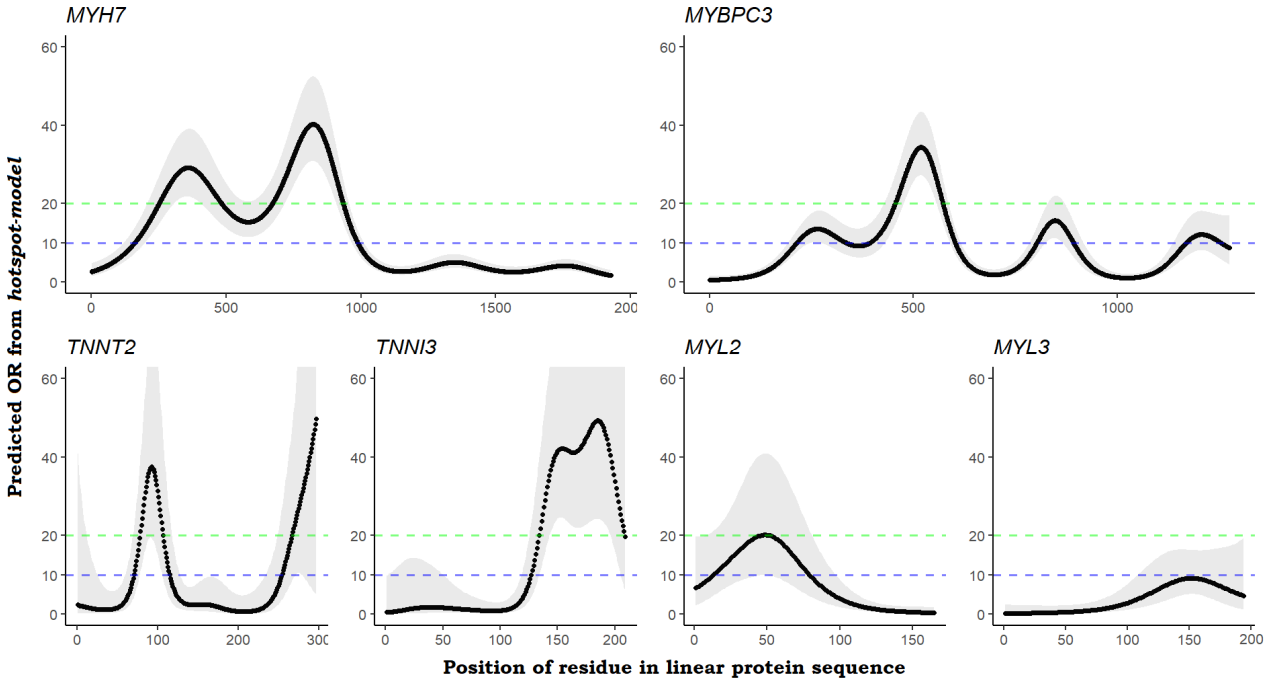


Figure 1: Predicted odds-ratios (ORs) for missense variants affecting each possible residue in the six firmly established HCM genes. Blue (OR=10) and green (OR=20) lines represent thresholds for supporting and moderate evidence of pathogenicity by criteria PM1.

POSTER NO: 2

A Multi-Omics Investigation of a Patient-Derived HNF-1A MODY iPSC Disease Model Reveals Insights into its Role in Pancreatic Islet Development and Function

Claire E. Duff[1], Rikke Rejnholdt Jensen[4], Marta Pérez-Alcántara[2], Mikkel Rasmussen[3], Antje Grotz[1], Max Jansen[2], Bjørn Holst[3], Christian Clausen[3], Nicole A.J. Krentz[2], Mattias Hansson[4], Mark I McCarthy[1,2,4], Anna L. Gloyn[1,2,5,6], Agata Wesolowska-Andersen[1], Christian L. F. Honoré[4]

[1]*Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK.*
[2]*Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK.*
[3]*Bioneer A/S, Hørsholm, Denmark.*
[4]*Novo Nordisk Department of Stem Cell Biology, Novo Nordisk A/S, Måløv, Denmark.*
[5]*Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford, UK.*
[6]*Department of Pediatrics, Stanford School of Medicine, Stanford University, CA, USA.*

Heterozygous loss of function mutations in *HNF1A,* encoding the transcription factor hepatocyte nuclear factor 1 alpha (HNF-1A) are the most common cause of monogenic diabetes. HNF-1A is involved in both beta cell development and mature cell function. However, model organisms have often failed to faithfully recapitulate the human phenotypes. As such, we used a patient-derived *HNF1A* Pro291fsinsC iPSC model and CRISPR-Cas9 genome editing to correct the mutation and generate an isogenic control. iPSC clones (n=6) were differentiated towards the endocrine lineage in triplicate, with RNA-seq (bulk and single-cell) and ATAC-seq performed at definitive endoderm (DE), pancreatic endoderm (PE) and beta-like cells (BLC) stages.

RNA-seq data confirmed *HNF1A* haploinsuffiency in the patient cell lines, with corrected cell lines demonstrating restored *HNF1A* expression at both transcript and protein level. At the BLC stage, there was a significant overlap in differentially expressed genes and *HNF1A* targets identified in an independent *HNF1A* knock-down experiment in the EndoC-βh1 cell line (p=4.4e-132).

Corrected clones demonstrated a higher expression of *INS* (p=7.2e-03), insulin secretion genes and effects on pancreas progenitor cell genes indicating that *HNF1A* may mediate the cellular composition of the pancreatic islet. Bulk RNA-seq data were also supported in the preliminary analysis of the single-cell RNA-seq data. Open chromatin, assessed by ATAC-seq was significantly different between patient and corrected lines, with >90% of the sites being more accessible at both PE and BLC stages in the corrected clones. These sites were enriched for *HNF1A* binding motifs, implicating *HNF1A* in the establishment of open chromatin.

In conclusion, our patient-derived HNF1A-MODY iPSC model recapitulates a number of features of HNF1A-MODY, as well as providing insights into the cellular and molecular phenotypes caused by HNF-1A deficiency.

POSTER NO: 3

Lentiviral Vector-based Gene Therapy for Cystic Fibrosis: Preparations for Clinical Trial Manufacture

Miah KM[1], Conway CC[1], Dean RJ[1], Viegas M[1], Gamlen T[1], Hyde SC[1], Gill DR[1]

[1]*Nuffield Division of Clinical and Laboratory Sciences, Gene Medicine Group, University of Oxford, UK.*

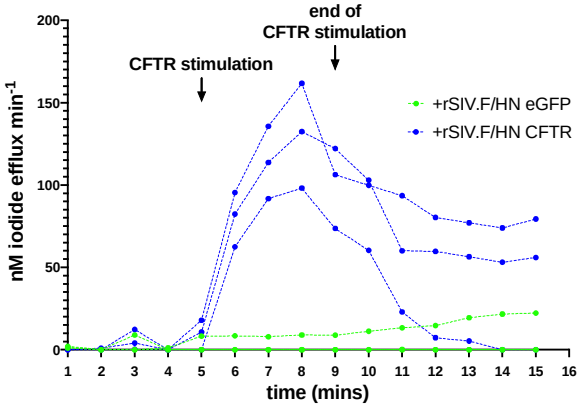
Cystic Fibrosis (CF) is an autosomal recessive disorder caused by mutations in the *CFTR* gene encoding the CFTR chloride-channel. CF results in chronic lung disease caused by ongoing infection, exaggerated inflammatory responses, pulmonary tissue damage, decline in lung function and premature death. Most treatment options are palliative and do not correct the underlying cause. We are developing a gene replacement therapy using recombinant lentiviral vectors (LV) as a curative approach. In particular, we use recombinant Simian Immunodeficiency Virus (SIV) pseudotyped with the Fusion (F) and Haemagglutinin-Neuraminidase (HN) glycoproteins from Sendai virus (rSIV.F/HN) to target lung cells; and this is proving promising for pulmonary gene transfer[1].

We are now working towards an upcoming first-in-human clinical trial to test the safety and efficacy of this vector for CF. This requires vector production to Good Manufacturing Practice (GMP) standards for clinical studies.

To prepare the vector for clinical manufacture, we are investigating deletion of the wildtype SIV R*ev-response element* (RRE) and codon optimisation of SIV *gag-pol* sequence in the LV packaging components, to avoid/minimise *psi-gag* recombination between the LV genome and SIV *gag-pol* during manufacture. This should improve the safety profile, reducing the chance of replication competent LV (RCL) formation due to recombination between homologous vector sequences. We are also generating candidate cell line(s) suitable for use as a surrogate model for RCL formation, which will be used to test rSIV.F/HN clinical product to ensure that RCL has not been generated during manufacture.

Maximising the stability of lentiviral vectors is crucial and so we evaluated several new buffers for final formulation of the vector. We compared vector stability (functional titre) in our standard buffer (TSSM) with several alternative buffers, after multiple freeze-thaw cycles, and found a new buffer that offered improved vector stability ($P < 0.05$; multiple *t*-tests). Whilst LV functional titre can be impacted by aggregation after freeze-thaw, this alternative buffer could offer additional protection during GMP manufacture.

Finally, we needed to develop a test for the functional activity of our vector - a 'potency' assay for different batches. We measured the CFTR-specific efflux of iodide ions (as a surrogate for chloride ions) from cells transduced with our vector, and established an assay that shows statistically significant iodide efflux relative to mock controls ($P < 0.05$; un-corrected Dunn's test). Such evidence of functional CFTR is needed for GMP-compliant manufacture, release testing, and progression to late-stage clinical trials.



Overall, we demonstrate positive progress towards GMP manufacture of rSIV.F/HN vector in preparation for clinical trials in CF patients.

[1] Alton *et al.* 2017, *Thorax* 72:137

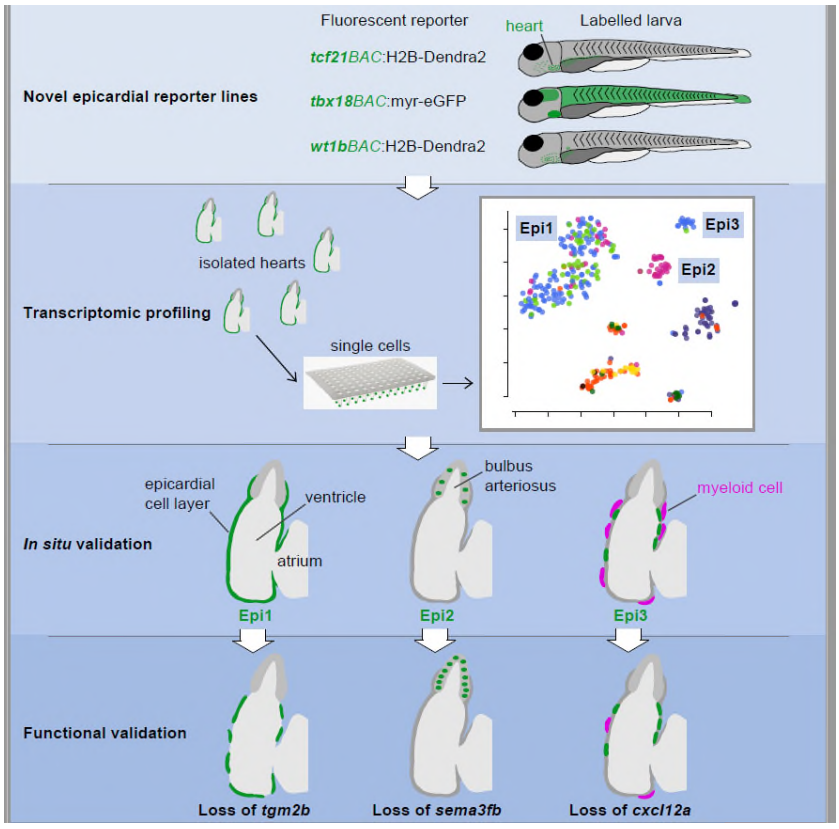
POSTER NO: 4

Functional heterogeneity within the developing zebrafish epicardium

Michael Weinberger[1,2], Filipa C. Simões[1,2], Tatjana Sauka-Spengler[1], Paul R. Riley[2]

[1]*MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Department of Physiology, Anatomy and Genetics, University of Oxford, UK.*

The epicardium is a sheet of cells enveloping the heart muscle and is essential during cardiac development, homeostasis and repair. Yet fundamental insights into epicardium formation, lineage heterogeneity and functional cross-talk with other cell types in the heart are currently lacking. Here, we investigated epicardial heterogeneity and the functional diversity of discrete epicardial subpopulations in the developing zebrafish heart. Smart-seq2 based single-cell RNA-sequencing uncovered three epicardial subpopulations (Epi1-3) with specific genetic programmes and distinctive spatial distribution in the developing heart. Functional perturbation identified *tgm2b*, a transglutaminase gene highly enriched in Epi1, as necessary for the proper development of the epicardial cell sheet. Epi2 was spatially localised in the cardiac outflow tract and expressed the chemokine *sema3fb*. Loss of *sema3fb* increased the number of *tbx18*$^{+}$ cells in the outflow tract, suggesting it controls the spatiotemporal access of epicardial cells to this tissue. Epi3 was enriched for cell guidance cues such as *cxcl12a*, loss of which decreased the number of *ptprc/CD45*$^{+}$ leukocytes on the epicardial surface. Understanding which mechanisms cells employ to establish a functional epicardium and to communicate with other cardiovascular cell types during development will bring us closer to repairing cellular relationships that are disrupted during cardiovascular disease.



POSTER NO: 5

Common genetic modifiers of a rare inherited cardiac condition: hypertrophic cardiomyopathy

Andrew R. Harper[1,2], Anuj Goel[1,2], Kate Thomson[1,2,3], Chris Grace[1,2], Chris Kramer[4], Stefan Neubauer[1], HCMR Investigators, Martin Farrall[1,2], Hugh Watkins[1,2]

[1] Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.
[2]Wellcome Centre for Human Genetics, Roosevelt Drive, Oxford, UK.
[3]Oxford Medical Genetics Laboratories, Churchill Hospital, Oxford, UK.
[4]University of Virginia Health System, Charlottesville, VA, USA.

Research rationale
Rare genetic variants across core sarcomere genes are causal for hypertrophic cardiomyopathy (HCM). However, the majority of HCM patients do not possess a disease-causing variant (sarcomere-negative HCM). The contribution common genetic variants make towards HCM is unknown. We hypothesised that the aggregate burden of common genetic variants explains why some individuals without a disease-causing variant develop HCM.

Methods
A multi-ancestry meta-analysis of two genome-wide association studies, involving 2,780 HCM cases and 47,486 age and sex-matched controls, was performed for variants with a minor allele frequency >1%. Using GCTA-cojo, independent variants associated with HCM, and stratified by sarcomere variant carrier status, were identified. SNP-heritability was estimated using GREML-LDMS. HCM phenotypes were evaluated and compared using bivariate GREML, GWAS-PW and a genetic risk score. Two-sample Mendelian randomisation (2SMR) was performed to evaluate heritable risk factors for HCM.

Results
Common variants contribute towards the genetic architecture of HCM (SNP-heritability of HCM: 35.2% ± 1.81; sarcomere-negative HCM: 34.0% ± 2.4%; sarcomere-positive HCM: 15.8% ± 3.8%). Multi-ancestry meta-analysis and subsequent conditional analysis revealed 29 independent variants (15 variants p-value < 5×10^{-8} and 14 variants < 5% FDR threshold). Sarcomere-positive/negative HCM are highly correlated. Risk associated with the aggregate burden of common variants associated with HCM is higher for sarcomere-negative HCM (OR 2.05 [95% CI: 1.97 – 2.14]) than sarcomere-positive HCM (OR 1.59 [95% CI: 1.50 – 1.69]). 2SMR suggests diastolic blood pressure (DBP) is a substantial heritable risk factor for the development of sarcomere-negative HCM, with a 1 standard deviation predicted increase in DBP (11.3 mmHg) conferring a four-fold increased risk (OR:3.93 [95% CI:3.61 – 4.25]; p-value = 3.80×10^{-17}).

Conclusion
Common genetic variants demonstrate major contributions towards the genetic architecture of HCM, particularly sarcomere negative HCM. These findings have the potential to influence the clinical management of HCM patients.

POSTER NO: 6

AKR1D1 knockout mice show signs of dysbiosis, intestinal damage and increased gut permeability

Arvaniti Anastasia[1,2], Harris Shelley[1], Nikolaou Nikolaos[1], Cox Roger[3], Odermatt Alex[4], Gathercole Laura[2], Tomlinson Jeremy[1]

[1]*Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Department of Biological and Medical Sciences, Oxford Brookes University, UK.*
[3]*MRC Harwell Institute, Harwell Campus, Oxford, UK.*
[4]*Department of Pharmaceutical Sciences, University of Basel, Switzerland.*

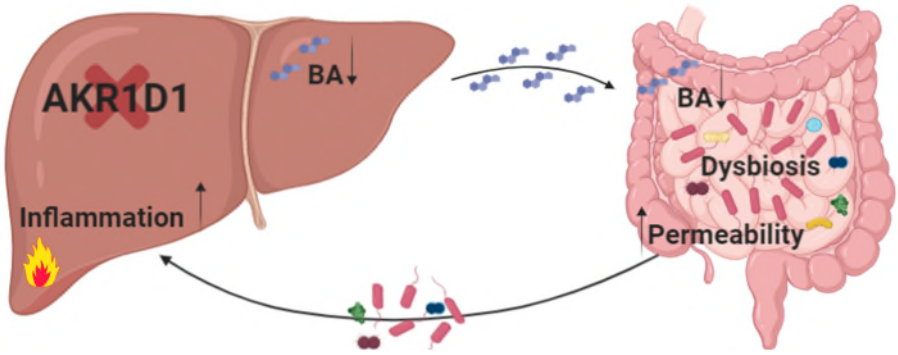


Figure1: Graphical abstract

Gut dysbiosis and metabolic endotoxemia contributes to the progression of non-alcoholic fatty liver disease (NAFLD) from simple steatosis to steatohepatitis. Bile acids (BA) are potent antimicrobials that support gastrointestinal health and dysregulation of BA homeostasis in NAFLD is hypothesised to drive dysbiosis[1]. We have previously shown that expression and activity of a key enzyme in BA synthesis, AKR1D1, is decreased in patients with NAFLD[2] and we have generated a novel AKR1D1-knockout (KO) mouse which at 52-weeks has increased hepatic inflammation. Here we demonstrate the impact of AKR1D1 deletion on gut microbiome and gastrointestinal health.

In female and male mice, AKR1D1 deletion decreased BA levels and altered composition. 16s rRNA analysis of cecal chyme showed family level changes in bacterial composition. Consistent with ileal damage, female AKR1D1-KO mice had decreased villi length and increased crypt depth. Suggestive of increased intestinal permeability and endotoxemia the expression of the tight junction genes *Claudin 1, ZO-1* and *Occludin* were reduced. The presence of bacterial DNA in the liver was increased, as was hepatic mRNA and protein levels of TLR4 along with expression of TLR4 mediated genes, *Nfkb1* and *Tnfa*. Interestingly, male AKR1D1-KO mice had a milder gastrointestinal phenotype with decreased expression of *Claudin 1, ZO-1* and *Occludin* but without signs of damage in ileum or of endotoxemia in liver.

Collectively, our results propose that reduced AKR1D1 activity, as that seen in patients with NAFLD, has a sex specific impact on intestinal health, driving intestinal damage, gut permeability and liver endotoxemia in females.

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1. Mouzaki, M. & Loomba, R. Insights into the evolving role of the gut microbiome in nonalcoholic fatty liver disease: rationale and prospects for therapeutic intervention. *Therap. Adv. Gastroenterol.* **12,** 1756284819858470 (2019).
2. Nikolaou, N. *et al.* AKR1D1 is a novel regulator of metabolic phenotype in human hepatocytes and is dysregulated in non-alcoholic fatty liver disease. *Metabolism* **99,** 67–80 (2019).

POSTER NO: 7

Active Monitoring for AtriaL Fibrillation (AMALFI): protocol and pilot from a mail-based randomized trial of screening for subclinical atrial fibrillation in high-risk individuals

Guilherme Pessoa-Amorim[1,2], Barbara Casadei[2], Nicholas Jones[3], Christine A'Court[3], Richard Bulbulia[1], Georgina Buck[1], Louise Bowman[1]

[1]*Clinical Trial Service Unit, MRC Population Health Research Unit, Nuffield Department of Population Health, University of Oxford, UK.*
[2]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[3]*Nuffield Department of Primary Health Care Sciences, University of Oxford, UK.*

Background: It is unknown whether screening for subclinical atrial fibrillation (SCAF) is effective at preventing strokes of unknown origin.

Methods: AMALFI is a streamlined mail-based trial comparing 14-day continuous electrocardiographic monitoring for SCAF (Ziopatch) versus usual care in people without previous AF or atrial flutter (AFL). Patients ≥65 years with CHA_2DS_2-VASc score ≥3 in men or ≥4 in women are eligible. The primary endpoint is AF prevalence at 2.5 years in primary care records.

Participants are identified via electronic search of primary care records and invited to join by mail. Those randomized to the active arm are sent a patch to wear for 2 weeks. The device is returned for analysis and results provided to the participant's GP.

We estimate an annual incidence of AF of ~3.75% in the Ziopatch group and ~0.7% in the control group. Hence, a trial of 2500 participants gives >90% power at 2p<0.05 to detect the estimated difference.

Results: In a pilot stage, 1208 invitations were sent out and 284 (23.5%) positive replies received. 82% (117/143) of patients allocated to receive a patch both wore and returned it. Median wear-time was 13 days 22 hours, with a median of 97.6% analysable tracing. AF/AFL was detected in 6 reports (5.1%).

Conclusions: This mail-based approach harnessing routine health records seems feasible. Around a quarter of invited patients are recruited, and most seem willing to wear and return their patches. AF detection rates are in line with predictions. Recruitment is ongoing, with main study results expected in 2023.

POSTER NO: 8

Combining single cell-based technologies and cellular barcoding tool for the characterisation of human haematopoietic stem cell heterogeneity

Merve Aksoz[1], Yiran Meng[1], Ryan Beveridge[1], Sally Ann-Clark[1], Paresh Vyas[1], Claus Nerlov[1]

[1]MRC Molecular Hematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK.

Haematopoietic stem cells (HSCs) are defined by their inherent capacity to self-renew and to differentiate into all blood cell types. HSCs form the basis of bone marrow (BM) transplantation and enable treatment of patients with haematological malignancies. Until recently, it was thought that all HSCs had the capacity to produce all blood lineages. By transplanting single murine HSCs, our group has demonstrated that HSCs constitute a functionally heterogeneous population and display a restricted pattern of lineage output; these are known as lineage-biased HSCs [1, 2], which have not been shown for human HSCs yet. Their definition relies largely on work performed with xenografting of umbilical cord blood-derived HSCs; however, adult BM HSCs remain poorly characterized. Here, we use cellular barcoding method [3] to track human BM-derived HSCs and their progenitor and mature cells of the megakaryocyte, erythroid, myeloid and B-cell lineages *in vivo* to identify and quantify HSC clones that contribute to haematopoiesis, including lineage-biased and multi-lineage HSCs. For this purpose, human HSCs purified from adult BM are labelled using lentiviral delivery of unique genetic barcodes and transplanted into non-irradiated NSGW41 mice. We combine single-cell RNA sequencing of the barcoded HSCs and bulk RNA sequencing of their *in vivo* cellular output using Smart-seq+ mRNA targeting and targeted Smart-seq2, respectively. This allows us to correlate HSC lineage output with HSC transcriptome simultaneously and in particular to identify gene signatures associated with specific haematopoietic lineages. In conclusion, this study aims to characterise human HSC heterogeneity, as well as any transcriptomic signatures and potential surface markers that can help to identify HSC subsets. A more complete picture of HSC heterogeneity would allow clinicians to have a better understanding of how transplanted HSCs may behave in patients.

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POSTER NO: 9

Real-World Use and Accuracy of Stress Echocardiography: Preliminary Insights and Development of Clinical Tools from the EVAREST Study

Cameron Dockerill*[1], William Woodward*[1], Annabelle McCourt[1], Arian Beqiri[2], Angela Mumith[2], Andrew Parker[2], Gary Woodward[2], William Hawkes[2], Neelam Hassanali[2], Nikant Sabharwal[3], Henry Boardman[3], Nancy Spagou[2], Elena Benedetto[1], Ross Upton[2], Paul Leeson[1,2,3]

[1]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, Cardiovascular Clinical Research Facility, University of Oxford, UK.*
[2]*Ultromics Ltd, Oxford, UK.*
[3]*Oxford University Hospitals NHS Foundation Trust, Oxford, UK.*
*Contributed equally

Background Stress echocardiography is widely used to identify prognostically significant coronary artery disease. However, interpretation is based on visual assessment of myocardial wall motion and accuracy varies with operator training. The EVAREST study links stress echocardiography clinics in 30 NHS Hospital Trusts and provides the first large-scale data on 'real world' stress echocardiography performance. In addition, imaging data from the study has contributed to development of FDA-cleared software that uses deep learning to automatically process and quantify echocardiograms. This may provide a method to shift the interpretation of stress echocardiography to be more quantitative and reproducible.

Methods Analysis was performed on the first 7415 patients recruited prospectively between 2015 and January 2020. Data is collected on medical history and stress echocardiography performance. Participants are followed up to 12 months. One-year outcome data is currently available for 1892 participants. To analyse feasibility of automated measures in clinical scans 408 sets of images were analysed using the novel AI-based quantitative image analysis software (EchoGo, Ultromics Ltd, Oxford, UK) of which 322 were contrast scans (100 Luminity, 222 SonoVue) and 86 did not have contrast.

Results Mean age of patients undergoing stress echocardiography is 65 $\pm$ 12.3 years and 56% are male. Average BMI is 28.9 $\pm$ 5.6 kg/m^2. 71.4% undergo dobutamine stress echocardiography and 28.4% exercise with <1% having a pacemaker-mediated stress. Contrast was used in 71.4% of studies. Stress echocardiograms were interpreted as positive for inducible ischaemia in 18.2% of patients. Sensitivity and specificity for clinician prediction of a positive cardiac outcome was 88.7% and 94.4%, respectively. Automated values of global longitudinal strain (GLS) and ejection fraction (EF), at both rest and stress stages were acquired from Luminity studies (Rest GLS = -16.4 ± 4.8 %, Rest EF = 63 ± 13 %; Stress GLS = -17.7 ± 5.8 %, Stress EF = 68 ± 11 %), SonoVue studies (Rest GLS = -16.8 ± 5.8 %, Rest EF = 63 ± 10 %; Stress GLS = -19.1 ± 6.7 %, Stress EF = 71 ± 9 %) and non-contrast studies (Rest GLS = -15.7 ± 5.3 %, Rest EF = 57 ± 10 %; Stress GLS = -17.3 ± 6.4 %, Stress EF = 61 ± 14 %).

Conclusion EVAREST demonstrates stress echocardiography has high sensitivity and specificity across multiple NHS centres, consistent with best practice. In addition, the study has demonstrated automated extraction of EF and GLS is feasible from stress echocardiograms at high heart rates and in the presence of different contrast agents, suggesting quantitative analysis of stress echocardiography should be clinically deliverable in the future.

POSTER NO: 10

Breathlessness in a Virtual World

Martin Sergeant[1], Sarah Finnegan[2], Kyle Pattinson[2]*, Stephen Taylor[1]*

[1]*MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Nuffield Division of Clinical Neurosciences, Radcliffe Department of Medicine, University of Oxford, UK.*
*Joint corresponding authors

Chronic breathlessness is a debilitating condition that profoundly affects quality of life for its sufferers. Affecting 1 in 10 adults, and costing the UK £11 billion per year in healthcare and economic costs, breathlessness is often difficult to treat with many people remaining symptomatic despite maximal medical therapy. For these people, the sensation of breathlessness may not match the physical status of the lungs.

Because perceptions, including those of breathlessness are generated in the brain, a disconnect between the lungs and perception can be explained as a mismatch between the senses. Therapies designed to manipulate and realign these pathways may offer a new route to treat breathlessness. To achieve this we intend to capitalise on the power of the brain as a predictive machine to shape our perception of reality and have designed a Virtual Reality (VR) application connected to an exercise bike (Figure 1). Information from a respiratory belt and heart rate monitor are fed into the application and the user's breathing sounds can be amplified and distorted. This enables us to able to manipulate the relationship between the visual world (i.e. cycling along a flat virtual road) from the physical effort (i.e. work required to peddle the static bike/) and the auditory sensory feedback (i.e. the sound of breathing)

Using this apparatus, we propose to examine how the brain weights and combines different sensory inputs to create sensations of breathlessness. In doing so we hope to disentangle the relationship between the brains expectations from physical effort and identify points of potential therapeutic manipulation to realign the brain with the body.

The work is currently in the development stage and we will be demonstrating the VR set up for conference attendees to try.

Figure 1 The application being tested and screen shots of the VR world

POSTER NO: 11

Establishing an ATRX deficient CD34+ cell model to elucidate how mutations in *ATRX* lead to alpha thalassaemia phenotype in ATR-X syndrome

Yuqi Shen[1,2], Chris Fisher[1], Douglas Higgs[1], Richard Gibbons[1]

[1]*MRC Molecular Hematology Unit, MRC Weatherall Institute of Molcular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Chinese Academy of Medical Sciences (CAMS) Oxford Institute, Nuffield Department of Medicine, University of Oxford, UK.*

Background ATRX is a chromatin remodeller involving in a wide range of nuclear processes, including transcription. Mutations in *ATRX* give rise to alpha-thalassaemia/mental retardation X-linked (ATR-X) syndrome and downregulate alpha globin expression as observed by the presence of cells with beta tetramers through a mechanism which is poorly understood[1,2]. The aim of this study is to develop a cellular system to reproduce the *in vivo* observation and to determine the manner by which alpha globin expression is perturbed in this genetic disease.

Methods *ATRX* was knocked out via CRISPR-Cas9 system in CD34+ hematopoietic stem/progenitor cells, and then the cells were differentiated to erythrocytes, during which knockout efficiency, cellular morphology, erythroid proteins and globin expression were detected to evaluate the genotype and phenotype. At the single cell level, early erythroid progenitors (EEPs) were sorted and plated into Methocult media to generate burst-forming unit-erythroid (BFU-E) colonies, after which the genotype and phenotype were analyzed in individual BFU-E colonies.

Results *ATRX* was efficiently knocked out in the CD34+ cells and it had no significant effect on cellular morphology and differentiation, but there was no obvious alpha thalassaemia phenotype observed judging from the globin expression in bulk cells. Determination of genotype and phenotype in single BFU-E colonies reported abundant knockout colonies and some exhibited alpha thalassaemia phenotype.

Conclusions and future directions Despite efficient editing of CD34+ cells, no alpha thalassaemia phenotype was observed on a bulk level, indicating the possible involvement of cellular heterogeneity. Therefore we examined the phenotype in single BFU-E colonies, some of which displayed alpha thalassaemia phenotype. The future work will focus on enriching those knockout cells with alpha thalassaemia phenotype, not only by sorting those ATRX negative and low globin expression cells by FACS, but also considering fusing a fluorescent protein with globin for easy enrichment.

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POSTER NO: 12

Macrophages directly contribute collagen to scar formation during zebrafish heart regeneration

Filipa C. Simões[1,2], Amy Kenyon[1], Daria Gavriouchkina[1], Eva Masmanian[1], Michael Weinberger[1,2], Sarah Mayes[1,2], Le A. Trinh[3], Scott E. Fraser[3], Tatjana Sauka-Spengler[#1], Paul R. Riley[#2]

[1]*MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*

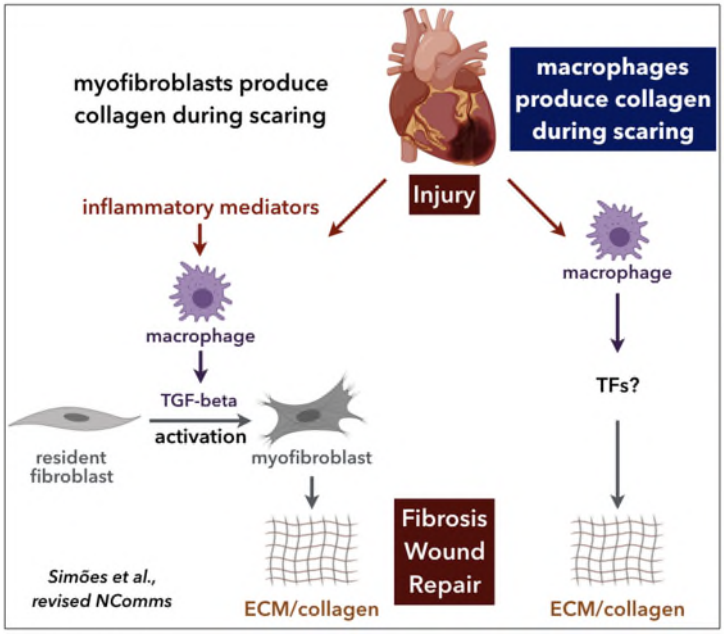
[2]*Department of Physiology, Anatomy and Genetics, University of Oxford, UK.*

[3]*Translational Imaging Centre, University of Southern California, USA.*

[#]Co-senior authors

ABSTRACT

Canonical roles for macrophages in mediating the fibrotic response after a heart attack (myocardial infarction) include turnover of the extracellular matrix and activation of cardiac fibroblasts to initiate collagen deposition. Here we reveal that macrophages can directly contribute collagen to the forming scar through studying the functional kinetics of fibrosis during zebrafish heart regeneration. Unbiased transcriptomics revealed an upregulation of collagen isoforms in zebrafish macrophages following injury. Adoptive transfer of macrophages from collagen-tagged transgenic zebrafish donors enhanced scar formation and induced fibrosis in the heart, via cell autonomous production of collagen. The majority of tagged collagen was deposited proximal to the injury, within the overlying epicardial region, suggesting a possible distinction between macrophage-collagen deposition and that predominantly laid-down by activated myofibroblasts. Macrophage-specific targeting of collagen 4a binding protein and cognate collagen 4a1 followed by adoptive transfer led to significantly reduced pericardiac scarring in cryoinjured hosts. These findings contrast with the current model of scarring whereby collagen deposition is exclusively attributed to myofibroblasts, and implicate macrophages as direct contributors to fibrosis during heart repair.



POSTER NO: 13

_SF3B1_ mutations induce R-loop accumulation and DNA damage in MDS and leukemia cells with therapeutic implications

Shalini Singh[1], Doaa Ahmed[1,2], Hamid Dolatshad[1], Dharamveer Tatwavedi[1], Ulrike Schulze[3], Andrea Sanchi[1], Sarah Ryley[4], Ashish Dhir[5], Lee Carpenter[6], Suzanne M. Watt[6], Amal M. Abdel-Aal[2], Sohair K. Sayed[2], Somaia A. Mohamed[2], Anna Schuh[7], Andriana G. Kotini[8], Eirini P. Papapetrou,[8] Daniel H. Wiseman, Andrea Pellagatti[1], Jacqueline Boultwood[1]

[1]MRC Molecular Haematology Unit, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.
[2]Clinical Pathology Department, Assiut University, Assiut, Egypt.
[3]MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.
[4]Oxford Medical Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust, Churchill Hospital, Oxford, UK.
[5]Centre for Genomic and Experimental Medicine, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK.
[6]Stem Cell Research, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.
[7]Molecular Diagnostic Centre, Department of Oncology, University of Oxford, Oxford, UK.
[8]Department of Medicine, Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY.
[9]Leukaemia Biology Laboratory, Cancer Research UK Manchester Institute, University of Manchester, Manchester, UK.

The myelodysplastic syndromes (MDS) are among the most common myeloid malignancies. Genes involved in pre-mRNA splicing (including SF3B1, SRSF2, U2AF1 and ZRSR2) are commonly mutated in MDS and in other myeloid malignancies. Previous studies showed that mutations in the splicing factors SRSF2 and U2AF1 lead to increased formation of R-loops (RNA-DNA hybrids with a displaced single stranded DNA) in leukemia cell lines, resulting in increased DNA damage, replication stress and activation of the ATR-Chk1 pathway. Given that SF3B1 is the most frequently mutated splicing factor gene in MDS (25-30% of cases), we investigated whether SF3B1 mutations result in elevated R-loop formation in MDS and leukemia cells.

We found that increased R-loops and DNA damage occur in association with the presence of SF3B1 mutations in the myeloid leukemia cell line K562, in MDS-patient derived induced pluripotent stem cells and in MDS patient bone marrow CD34+ cells. We showed activation of the ATR pathway, a major player in the DNA damage response, in SF3B1 mutant hematopoietic cells. SF3B1 mutant K562 cells and primary MDS patient bone marrow cells showed preferential sensitivity to the ATR inhibitor VE-821 and to UCN-01, an inhibitor of Chk1 (a critical substrate of ATR), suggesting that ATR-Chk1 activation is important for the survival of SF3B1 mutant cells and could represent a therapeutic vulnerability. Importantly, we found that the effects of VE-821 or UCN-01 on SF3B1 mutant K562 cells and primary MDS patient bone marrow cells were enhanced by the splicing modulator Sudemycin D6, indicative of synergy between these drugs.

These emerging data indicate that different mutated splicing factors have convergent effects on R-loop elevation leading to DNA damage in hematopoietic cells.

POSTER NO: 14

Lentiviral vector pseudotyped with Sendai virus F and HN proteins uses sialylated glycan receptors to efficiently target human airway cells

Rosie J. Munday[1], Stephen C. Hyde[1], Deborah R. Gill[1]

[1]Gene Medicine Research Group, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, UK.

Lentiviral vectors pseudotyped with the F and HN coat proteins from murine Sendai virus are being developed for lung gene therapy. The F/HN pseudotype facilitates efficient transduction of a range of cells in the murine lung (Alton et al. 2017 Thorax 72:137) and we are investigating its transduction profile in human cells and tissues. Sendai virus is reported to utilise subtypes of sialylated glycans as receptors for cell entry. However, due to differences in the distribution of these receptor subtypes between species, it is unclear whether this efficient targeting will translate to the human lung. Here, we investigate the ability of the F/HN pseudotype to transduce human airway cells, grown in human air-liquid interface (hALI) cultures to assess dependency on sialylated glycan receptors.

The hALI cultures were treated with equivalent doses of recombinant HIV vectors expressing eGFP, pseudotyped with F/HN or the more commonly used Vesicular Stomatitis Virus glycoproteins (VSVg) as a negative control. The F/HN pseudotype resulted in high levels of eGFP (18.5 - 51.6% area eGFP; n=4, day 14) which persisted for at least 4 months. As expected, there was no transduction by VSVg pseudotyped vectors (0-0.8% area eGFP; n=4, day 14), presumably due to lack of VSVg receptors.

The F/HN targeting of basal, goblet, and ciliated cells was confirmed by colocalization of eGFP with cell-type specific markers using hALI cryosection immunohistochemistry. Lectins specific for α2,3 and α2,6 subtypes of glycan sialylation were used to stain for the receptor distribution in hALI cultures, revealing an abundant distribution of α2,6 (and a subtype of α2,3) in hALI cultures (n=4), similar to their distribution in human airways described in the literature. To confirm that F/HN vector cell entry is dependent on sialylated glycans, hALI cultures that were pre-treated with sialidase to cleave sialic acid prior to vector administration, showed reduced transduction levels. Studies are underway to identify the specific glycans targeted by F/HN glycoproteins.

These data confirm the importance of sialylated glycans in F/HN-mediated transduction of human airway cells and suggest that lentiviral vectors pseudotyped in this way should be able to target the human lung during clinical studies.

POSTER NO: 15

A cardiomyopathy-related point mutation in alpha-actinin causes embryonic lethality in mice in the homozygous setting

Austin Jiang[1], Charlotte Hooper[1], Nikita Ved[2], Mathias Gautel[3], Benjamin Davies[4], Duncan Sparrow[2], Hugh Watkins[1], Katja Gehmlich[1, 5]

[1]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.
[2]Department for Physiology, Anatomy and Genetics, University of Oxford, UK.
[3]Cardiovascular Division and The Randall Division of Cell and Molecular Biophysics, King's College London, UK.
[4]Transgenic Core, Wellcome Centre for Human Genetics, University of Oxford, UK.
[5]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, UK.

Background: The Z-disc is important for the structural integrity of the contractile unit, the sarcomere. In the cardiac Z-disc, thin filaments are cross-linked by two proteins, filamin C and alpha-actinin. Filamin C has been implicated in inherited cardiac conditions i.e. cardiomyopathies. However, the role of alpha-actinin in cardiomyopathies is less clear. A pathogenic missense variant in the actin binding domain of alpha-actinin 2 was reported to cause cardiomyopathy in a multi-generation family with autosomal-dominant trait [1].

Methods: We have generated a mouse model to study the consequences of this missense variant M228T in alpha-actinin 2 (*Actn2*). Cardiac function of the adult mice was assessed *in vivo* by ultrasound echocardiography. Cardiac morphology of the embryos was investigated by microMRI.

Results: Heterozygous mice at 3 months of age failed to show an overt cardiac phenotype, and we are currently assessing the cardiac function in aged mice (9-12 months). Attempts to generate homozygous neonates failed (>20 litters), indicative of embryonic lethality. Viable embryos were consistently recovered at E15.5, but not all at later stages. Analysis of embryonic hearts at E15.5 using microMRI is currently underway.

Discussion: While heterozygous mice do not show an overt phenotype at young age, homozygous mutation in mice are embryonic lethal with death occurring between E15.5 and E17.5. This suggests that the missense variant in alpha-actinin 2 has detrimental effects on the cardiac Z-discs. Future work will focus on: a) challenge experiments to provoke a phenotype in the adult heterozygous mice, and b) investigating the underlying cause of Z-disc dysfunction in the homozygous embryos.

Reference: [1] Girolami F et al. Circ Cardiovasc Genet. 2014;7(6):741-50.

POSTER NO: 16

Targeting minimal residual disease in ovarian cancer

Mara Artibani[1,2], Mohammad Karami Nejad Ranjbar[2], Garry Mallet[1,2], Nobina Mukerjee[3], Katrina Gadsby[1,2], Hagan Bayley[3], Ahmed Ahmed[2], Tatjana Sauka-Spengler[1]

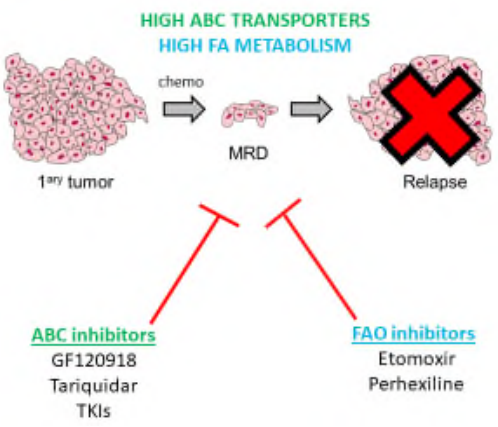
[1]*Gene Regulatory Networks in Development and Disease Laboratory, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.*
[2]*Ovarian Cancer Cell Laboratory, MRC Weatherall Institute of Molecular Medicine, Nuffield Department of Women's & Reproductive Health, University of Oxford, Oxford, UK.*
[3]*Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Oxford, UK.*

Ovarian cancer is the deadliest gynaecological malignancy, claiming nearly 200,000 lives every year. Despite an extremely positive response to chemotherapy in a fraction of patients, the majority suffer from recurrence within a year of completion of primary treatment. Microscopic abdominal minimal residual disease (MRD) at the end of therapy is the most likely source of tumour recurrences. Therefore, eliminating MRD would have a profound effect on improving survival outcomes in ovarian cancer. However, this long-sought goal has been difficult to achieve due to the technical challenges of isolating such small cell populations as well as to the lack of appropriate experimental models that recapitulate MRD biology.

Here, we have performed an in-depth characterisation of ovarian cancer MRD using RNA-sequencing of Laser Capture Microdissected tumour islets from high-grade serous ovarian cancer patients. The MRD tumour cells showed significantly higher expression of ATP-binding cassette (ABC) transporters as well as other known cancer stem cells markers and several genes involved in fatty acid metabolism.

We have established *in vitro* 2D and 3D models that faithfully recapitulate the key characteristics observed in the MRD patients and used them to further characterise MRD, as well as for drug screening. These models have led to the discovery that the resistant cells have a higher mitochondrial oxygen consumption than naïve cells and are more reliant on lipid metabolism than their untreated counterpart. We exploited this vulnerability of MRD cells *in vitro* by applying etomoxir and perhexiline, compounds which inhibit the carnitine palmitoyltransferase (*CPT1*), a mitochondrial membrane enzyme necessary for the β-oxidation of fatty acids. Our successful targeting of fatty acid metabolism in MRD cells paves the way for new treatments in ovarian cancer patients with MRD.

Graphical abstract



POSTER NO: 17

Calcitonin paracrine signaling controls heart fibrogenesis and arrhythmia

Lucia M Moreira[1], Abhijit Takawale[2], Constantinos Psarros[1], David A Menassa[3], Neil Evans[1], Marc-Antoine Gillis[2], Angela Lee[1], Patrice Naud[2], Mary Norris[1], Michele V Clarke[5], Patricia K Russell[5], Barbara Casadei[1], Shoumo Bhattacharya[1], Jeffrey D Zajac[5], Rachel A Davey[5], Martin Sirois[2], Rana Sayeed[4], George Krasopoulos[4], Keith M Channon[1], Jean-Claud Tardif[2], Stanley Nattel[2], Svetlana Reilly[1]

[1]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Research Centre, Montreal Heart Institute, Department of Pharmacology and Therapeutics, McGill University, Canada.*
[3]*Clinical Neurology, Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, UK.*
[4]*Cardiothoracic Surgery, Oxford Heart Centre, John Radcliffe Hospital, Oxford, UK.*
[5]*The Department of Medicine, Austin Health, University of Melbourne, Heidelberg, Victoria, Australia.*

Objectives – Atrial fibrillation [AF] is a very common cardiac arrhythmia and a major contributor to population mortality and morbidity. Structural remodelling in AF is hallmarked by atrial fibrosis, the mechanisms of which are not understood. Studies in bone and skeletal muscle pathophysiology implicate calcitonin [CT] via its binding to the CT receptor [CTR] in collagen turnover; here we explored whether CT-CTR signalling plays a role in atrial fibrosis and arrhythmogenesis in AF.

Methods & Results – Using right atrial tissue specimens and cells (fibroblasts [CFs] and cardiomyocytes [CMs]) isolated from 124 patients (43 with permanent AF *vs* 81 controls in sinus rhythm [SR]), we found that CTR is expressed in CFs (qPCR, immunofluorescence and immunoblotting) and is activated by its ligand CT. *In vitro* assays in SR CFs showed that treatment with CT for 72 hours decreased collagen 1 accumulation by 56% (scar-in-a-jar & hydroxyproline assays), cell migration by 63% (scratch assay), cell proliferation (impedance-based assay) and accumulation of calcium-enriched deposits by CFs (Alizarin red stain); these effects were reversed by the CTR inhibition with sCT8-32 or knock-down (antisense LNA oligonucleotides). Similar CT-mediated effects were observed in TGFβ1-stumulated atrial CFs. By contrast, in AF CFs, CT had no effect on the collagen content, cell migration and proliferation (BrDU assay) despite evident CTR expression (qPCR & immunoblotting). Immunofluorescence revealed that AF CFs possess predominant intracellular CTR localization, while CTR is primarily localised to the CF membrane and cytoplasm in control cells. Studies in mice revealed an increase in atrial fibrosis (Masson Trichrome stain) in CTR-KO (n=27) vs control CTR-flox (n=40) mice and a greater susceptibility to AF (trans-jugular atrial burst electrostimulation). These findings suggest mechanistic link between disrupted CTR signalling and increased induction of AF *in vivo*.

Conclusions – Myocardial CT-CTR signalling cascade is a new regulator of atrial fibrogenesis and arrhythmia in human atrial myocardium. Patients with AF are characterised by a dysfunctional CT-CTR axis due to both reduced myocardial CT secretion and altered CTR cellular localisation. Disruption of the CT-CTR pathway permits atrial fibrosis and promotes AF *in vivo* in mice. Strategies to restore physiological membrane-associated CTR localisation and myocardial CT secretion may offer a new tool to control excessive atrial fibrosis in patients with AF.

POSTER NO: 18

LanceOtron: a deep learning peak caller for ATAC-seq, ChIP-seq, and DNase-seq

Lance Hentges[1], Martin Sergeant[1], Jim Hughes[1], Steve Taylor[1]

[1]MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.

Finding the genomic locations of regulatory events generally require the use of sequencing-based chromatin profiling assays, such as ChIP–seq, DNase-seq, ATAC-seq. The signals produced by these experiments are noisy and complicated, however biological events have characteristic “peak” shapes in genome coverage maps. While experts can readily identify areas of interest by eye, this is unfeasible on the genomic scale. Typical whole genome tools rely on comparing observed versus expected peak heights, but with mixed results. Given the vast number of statistical tests carried out over a genome, many false positives are likely to occur; importantly noise may also be present at significant levels. This can be compensated for by raising the statistical cut-offs, but this then increases the likelihood of false negatives. Further complicating matters, visualising and assessing results should be done for every dataset, but can only be carried out using additional tools. We sought to address these challenges with our peak calling software, LanceOtron. Our tool utilises a powerful computer vision, deep learning algorithm to analyse the shape of the enriched region. When testing 1000 hand-labelled peaks, LanceOtron was able to distinguish true peaks from noise in over 99.9% of cases. Our intent is for our software to be useful to bench biologists and bioinformaticians alike, and as such it is deployed as a webtool with a graphical interface for visualisation, complete with interactive filters for further refining results. We believe LanceOtron’s high accuracy and intuitive graphical implementation give it a competitive advantage over traditional peak callers.

POSTER NO: 19

Improved Diagnosis and Classification of Myeloproliferative Neoplasms Through Deep Phenotyping

Korsuk Sirinukunwattana[1-3], Alan Aberdeen[1], Helen Theissen[1,2], Nikolaos Sousos[4], Bethan Psaila[4], Adam J. Mead[4], Gareth D.H. Turner[5], Gabrielle Rees[5], Jens Rittscher[1-3,6], Daniel Royston[5,7]

[1]*Institute of Biomedical Engineering (IBME), Department of Engineering Science, University of Oxford, UK*
[2]*Big Data Institute, University of Oxford, UK.*
[3]*Oxford NIHR Biomedical Research Centre, Oxford University Hospitals Trust, Oxford, UK.*
[4]*MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK.*
[5]*Department of Cellular Pathology, Oxford University NHS Foundation Trust, Oxford, UK.*
[6]*Ludwig Institute for Cancer Research, Nuffield Department of Medicine, University of Oxford, UK.*
[7]*Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, UK.*

Myeloproliferative neoplasms (MPNs) are clonal disorders characterized by excessive proliferation of myeloid lineages. Accurate classification and appropriate management of MPNs requires integration of clinical, morphological and genetic findings. Despite major advances in understanding the molecular and genetic basis, morphological assessment of the bone marrow trephine (BMT) remains paramount in differentiating between MPN subtypes and reactive conditions (1). However, morphological assessment is heavily constrained by a reliance on subjective, qualitative and poorly reproducible criteria (2). To address this, we have developed a machine-learning strategy for the automated identification and quantitative analysis of megakaryocyte morphology and topography using clinical BMT samples. Using sample cohorts of recently diagnosed or established MPN subtypes (Essential Thrombocythaemia [ET; n = 48], Polycythaemia Vera [PV; n = 22], Primary Myelofibrosis [PMF; n = 26]) and reactive control cases (n = 42) we demonstrated a high predictive accuracy (AUC = 0.95) of automated tissue MPN diagnosis. The identified megakaryocyte morphological phenotypes showed evidence of specific genotype associations, which offers promise of an automated cell phenotyping approach of clinical diagnostic utility as an adjunct to standard genetic and molecular tests. This has great potential to assist in the routine assessment of newly diagnosed or suspected MPN patients and those undergoing treatment / clinical follow-up. The extraction of quantitative morphological data from BMT sections will also have value in the assessment of new therapeutic strategies directed towards the bone marrow microenvironment and can provide clinicians and researchers with objective, quantitative data without significant demands upon current routine specimen workflows.

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POSTER NO: 20

The Embryonic zeta-globin gene is Silenced by Deacetylation mediated in part by BCL11A and LRF1

Siyu Liu[1], Duantida Songdej[1,2], Andrew J King[1], Damien J Downes[1], Maria C Suciu[1], Lars LP Hanssen[1], Danuta Jeziorska[1], James OJ Davies[1], Nigel Roberts[1], Stephanie Hill[1], Jacqueline Sharpe[1], Jacqueline Sloane-Stanley[1], Jelena Telenius[1], Len A Pennacchio[3,4], Jim R Hughes[1], Douglas R. Higgs[1], Christian Babbs[1]

[1]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK.
[2]Division of Hematology/Oncology, Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.
[3]Functional Genomics Department, Lawrence Berkeley National Laboratory, Berkeley, CA.
[4]Comparative Biochemistry Program, University of California, Berkeley, CA.

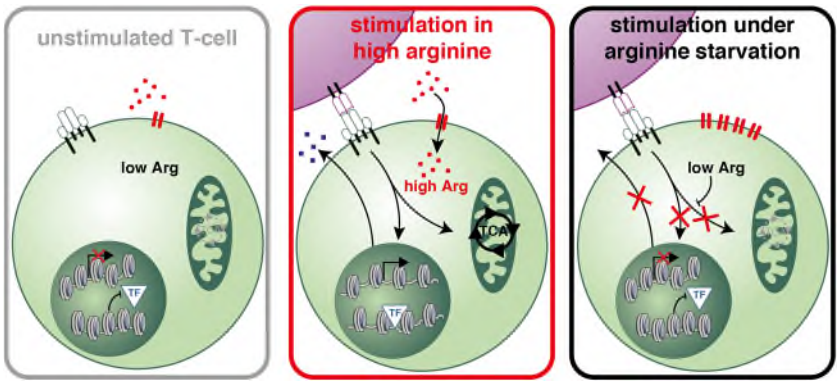
The embryonic α-like globin gene, ζ-globin is silenced in definitive erythroid cells, despite lying adjacent to the α-globin super-enhancer. We have studied the chromatin landscape in mouse when ζ-globin expression is maximal in primitive erythroid cells, and when it is silenced, in definitive erythropoiesis. We show that the ζ-α-globin locus is contained within an identical ~80kb erythroid-specific self-interacting chromosomal domain in both primitive and definitive erythroid cells and is constrained by the same CTCF elements. However, ζ-globin gene interactions with the enhancer elements significantly decrease when definitive erythroid cells are compared to primitive. Dissection of the super-enhancer *in vivo* shows that no single enhancer is necessary for normal ζ-globin expression, in contrast to α-globin expression. ζ-globin silencing is associated with localised histone deacetylation in definitive erythropoiesis and inaccessible chromatin. Changes in chromatin are mediated in part by two transcription factors important in controlling the γ to β-globin switch: Leukaemia related factor (LRF) and B-cell lymphoma/leukemia 11A (BCL11A). Their knockout leads to increased chromatin accessibility over the ζ-globin gene and ζ-globin expression in mouse and human definitive erythroid cells. Together, these data provide insights into the regulation of ζ-globin which may be exploited to develop novel treatments for severe α-thalassaemia.

POSTER NO: 21

Arginine starvation disrupts chromatin remodelling and metabolic reprogramming associated with T-cell activation

Nicholas T. Crump[1]^, Andreas V. Hadjinicolaou[2]^, Meng Xia[2], Uzi Gileadi[2], John Walsby-Tickle[3], Jili Chen[2], Mashiko Setshedi[2], Lars R. Olsen[4], I-Jun Lau[1], Laura Godfrey[1], Lynn Quek[1], Zhanru Yu[5], Erica Ballabio[1], Mike B. Barnkob[2], Giorgio Napolitani[2], Mariolina Salio[2], Hashem Koohy[2], Benedikt M. Kessler[5], Stephen Taylor[6], Paresh Vyas[1], James S.O. McCullagh[3], Thomas A. Milne[1]*, Vincenzo Cerundolo[2]*

[1]*MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, NIHR Oxford Biomedical Research Centre Haematology Theme, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK.*
[3] *Chemistry Research Laboratory, Department of Chemistry, University of Oxford, UK.*
[4] *Section for Bioinformatics, DTU Health Technology, Technical University of Denmark, Denmark.*
[5] *Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, UK.*
[6] *MRC WIMM Centre for Computational Biology, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
^These authors contributed equally;
*These senior authors contributed equally;
Correspondence: thomas.milne@imm.ox.ac.uk



Depleting the microenvironment of important nutrients such as arginine is a key strategy for immune evasion by cancer cells. We use high-throughput metabolomic and genomic techniques to show that T-cell activation is impaired in arginine-depleted conditions, with a significant metabolic perturbation linked to impaired global epigenetic reprogramming, widespread changes in chromatin accessibility, altered transcription factor binding and downregulation of key genes. We find that arginine synthesis is impaired in T-cells, as *ASS1* (argininosuccinate synthetase, required for arginine biosynthesis), is epigenetically repressed in T-cells. In contrast, we identify an intragenic enhancer within *ASS1* that is active in cancer cells and facilitates *ASS1* expression, explaining their ability to tolerate arginine starvation. Strikingly, engineered expression of *ASS1* in T-cells rescues their ability to grow without arginine, supporting the use of this strategy for adoptive T-cell transfer in cancer patients. Our results highlight a physiological change of T-cell metabolism mediated by nutritional stress-induced epigenetic changes, exploited by cancer cells to enable immune evasion.

POSTER NO: 22

Nitrate induced vasoplegia depletes cardiac energy reserves: a model for sepsis cardiomyopathy?

Watson WD[1], Green PG[1], Chamley RR[1], Burrage MK[1], Lewis AJ[1], Neubauer S[1], Rider OJ[1]

[1]*Oxford Centre for Clinical Magnetic Resonance Research, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*

Research Rationale

The healthy heart is at its most efficient when it is filled and stretched with preload. We hypothesized that in vasoplegia, loss of preload (due to dilation of capacitance veins) and compensatory rises in heart rate and contractility would compromise the energetic efficiency of the heart.

Methodology

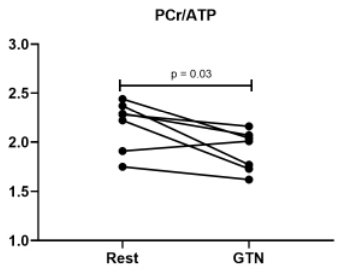
We recruited 7 healthy volunteers and measured their baseline cardiac volumes and function and cardiac energetics using cardiac magnetic resonance imaging and magnetic resonance spectroscopy. At the same visit, vasoplegia was induced with a glyceryl trinitrate (GTN) infusion and measurements were repeated.

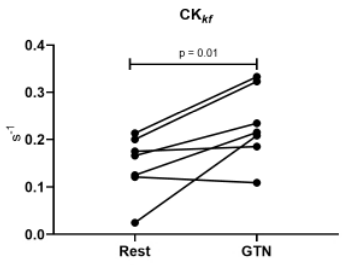
Results

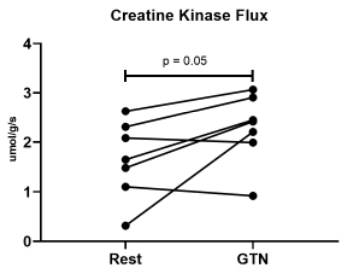
See figure below. The GTN infusion brought about a fall in mean arterial pressure (from a baseline of 79 ± 7mmHg to 64 ± 7 mmHg, $p < 0.0001$), a fall in LV end diastolic volume (169 ± 57 ml vs 148 ± 58 ml, $p = 0.003$) indicating a reduction in preload with rises in heart rate (61 ± 7 bpm vs 69 ± 10bpm, $p = 0.0005$) and ejection fraction (62 ± 3 % vs 67 ± 3 %, $p = 0.001$), however cardiac output remained unchanged (6.72 ± 1.49 L/min vs 6.68 ± 1.48 L/min, $p = 0.87$). Cardiac work (calculated as stroke volume x MAP x heart rate) fell (477 ± 123 vs 424 ± 119 L.mmHg/min, $p = 0.03$). There was a fall in PCr/ATP ratio on GTN (2.18 ± 0.25 vs 1.91 ± 0.2, p 0.03) while CKkf more than doubled (0.14 ± 0.06 s-1 vs 0.23 ± 0.08 s-1, $p = 0.02$) and creatine kinase flux also showed a significant increase (1.65 ± 0.78 µmol/g/s vs 2.28 ± 0.71 µmol/g/s, $p = 0.05$).

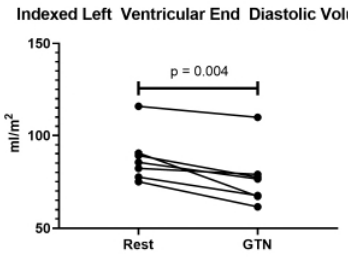
Conclusions

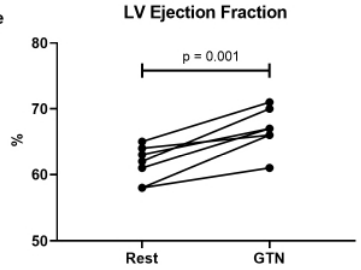
What is novel here is that we show a fall in PCr/ATP ratio: as ATP concentrations in the cell are strictly maintained, this suggests phosphocreatine pool depletion occurs when preload is lost and cardiac output is maintained by an increase in inotropy and chronotropy. The rise in CKkf and CK flux confirm the increased energy demand. Progressive energetic depletion during high demand may give rise to contractile dysfunction over time as the heart is unable to keep up with increased requirements for ATP, which could be a mechanism of cardiac dysfunction in septic shock and other states of vasoplegia.

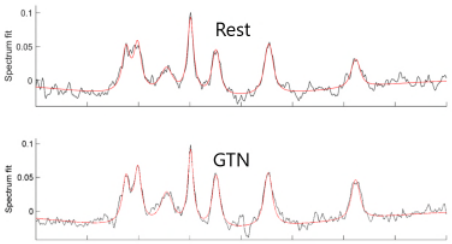

PCr/ATP
p = 0.03
3.0
2.5
2.0
1.5
1.0
Rest
GTN


CK$_{kf}$
p = 0.01
0.4
0.3
0.2
0.1
0.0
s^{-1}
Rest
GTN


Creatine Kinase Flux
p = 0.05
4
3
2
1
0
umol/g/s
Rest
GTN


Indexed Left Ventricular End Diastolic Volume
p = 0.004
150
100
50
ml/m^2
Rest
GTN


LV Ejection Fraction
p = 0.001
80
70
60
50
%
Rest
GTN


Rest
GTN
Spectrum fit
0.1
0.05
0

POSTER NO: 23

Playing hide and seek with glioblastoma: Using epigenetic modulators to increase cancer testis and neoantigen expression

Ruichong Ma[1,2], Margarida Rei[1], Isaac Woodhouse[1], Katherine Ferris[1], Hashem Koohy[1], Puneet Plaha[2,3], Vincenzo Cerundolo[1]

[1]*MRC Human Immunology Unit, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.*
[2]*Department of Neurosurgery, Oxford University Hospitals NHS Foundation Trust, Oxford, UK.*
[3]*Nuffield Division of Clinical Neurosciences, University of Oxford, Oxford, UK.*

Background

Glioblastoma (GBM) is the most common primary brain tumour in adults. Despite maximal treatment the median survival is still only 14-24 months. Progression of glioblastoma is thought to be facilitated in part by an immunosuppressive microenvironment. However, clinical response in GBM patients to immunomodulatory drugs, including checkpoint inhibitors, is modest. This is in part due to the low number of mutations seen in GBM. Epigenetic regulation of tumour cells is becoming recognised as an important factor in tumour immune escape. Here, I examine the effect of a decitabine (DAC), a DNA methyl-transferase inhibitor, on the expression of both cancer testis antigens (CTA) and neoantigens (NAg) in GBM.

Methods

Neoantigens from primary and U87MG cell lines were predicted using whole exome sequencing data. Differential expression of NAg/CTA caused by 1uM DAC treatment was determined using RNA sequencing. Peptide specific T cells were isolated from PBMC of autologous and healthy donors. T cell functionality was tested through intracellular cytokine staining and/or LDH release killing assay.

Results

6/9 potential neoantigen-encoding mutations were significantly upregulated following DAC treatment in U87MG and further 19 in the primary patient cell lines. In addition, a wide range of CTA were upregulated across all primary cell lines. Peptide specific T cells were isolated from both patient and healthy donors. All T cell clones respond to their specific peptide in a concentration dependent manner. Within these, there are 5 clones true NAg specific clones which are able to recognise and kill the relevant cell lines in a TCR-MHC dependent fashion. Furthermore, upregulation of NAgs/CTA by DAC leads to increased activation and killing.

Conclusions

Here I show for the first time that a large spectrum of CTAs and several NAgs are upregulated following DAC treatment. This increase in expression leads to increased T cell mediated recognition and killing. These results support the use of DAC to increase immunogenicity of an otherwise ‘cold’ tumour.

POSTER NO: 24

Participants with Diabetes have Less Augmentation in Cardiac Function and Energetics in Response to Increased Supply of Fatty Acid

Green PG[1,2], Watson WD[1], Herring N[2], Neubauer S[1], Rider OJ[1]

[1]*Oxford Centre for Clinical Magnetic Resonance Research, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Burdon Sanderson Cardiac Science Centre, Department of Physiology, Anatomy and Genetics, University of Oxford, UK.*

Research Rationale

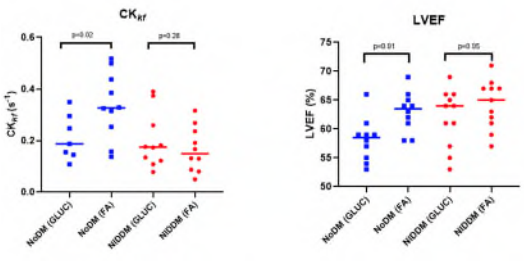
Measurement of the Phosphocreatine (PCR)/ATP ratio and the Creatine Kinase rate constant (CK_{kf}) provides a sensitive measure of the heart's energy status, and allows calculation of ATP delivery rate through the creatine kinase shuttle (CK flux). The normal heart is metabolically flexible, and so should maintain energetics and function regardless of the substrate available (fat or glucose). This flexibility may be impaired in diabetes mellitus (DM). It is unknown to what extent flexibility can be influenced by artificially altering the substrate available for metabolism. We aimed to compare heart function and energetics between diabetic and non-diabetic participants clamped on either fatty acid (FA) or glucose metabolism.

Methodology

Participants with non-insulin dependent diabetic mellitus (NIDDM) and without DM (NoDM) were recruited and received IV infusions of either 20% fat emulsion (60ml/hr) or insulin/dextrose 20% (GLUC, variable rate) at 2 visits, before undergoing cardiac MRI. Cardiac volume and function, PCR/ATP ratio and CK_{kf} (s^{-1}) were assessed. CK flux was calculated.

Results

Ten NoDM participants (3 male, age 41.3 ± 19.7 years) and 11 NIDDM participants (10 male, age 59.2 ± 6.8 years) were recruited. Left ventricular ejection fraction (LVEF) was higher on FA in both groups (NoDM FA 63.0 ± 3.4 %; GLUC 58.1 ± 3.8 %, p=0.01; NIDDM FA 64.3 ± 4.2 %; GLUC 61.9 ± 5.0 %, p=0.05) but the increase in absolute terms was less in the NIDDM group (2.4 % vs 4.9 %). NoDM participants had a significantly higher CK_{kf} on FA than GLUC (FA 0.31 ± 0.10 s^{-1}; GLUC 0.21 ± 0.09 s^{-1}, p=0.02), which did not occur in NIDDM participants (FA 0.15 ± 0.07 s^{-1}; GLUC 0.18 ± 0.09 s^{-1}, p= 0.28). This was associated with a trend towards an increase in CK flux in the NoDM group which did not reach statistical significance (FA 3.50 ± 0.99 µmol (g wet weight)$^{-1}$ s^{-1}; GLUC 2.61 ± 1.01 µmol (g wet weight)$^{-1}$ s^{-1}, p=0.06; NIDDM FA 1.60 ± 0.79 µmol (g wet weight)$^{-1}$ s^{-1}; GLUC 1.85 ± 0.90 µmol (g wet weight)$^{-1}$ s^{-1}, p=0.32).



Conclusion

Increasing FA supply results in an increase in LVEF, but this is lower in absolute terms in diabetic participants. In non-diabetic participants this is associated with an increase in CK_{kf} and a trend towards increased CK flux, but not in diabetic participants. This may reflect maximal baseline FA metabolism in diabetes and so impaired flexibility and an inability for further upregulation.

POSTER NO: 25

LRP5 promotes adipocyte insulin sensitivity

Nellie Y. Loh[1], Senthil K. Vasan[1], Matt J. Neville[1,2], Clive Osmond[3], Celia L. Gregson[4], Fredrik Karpe[1,2], Constantinos Christodoulides[1]

[1]Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, UK.
[2]NIHR Oxford Biomedical Research Centre, Oxford University Hospital Foundation Trust, UK.
[3]MRC Lifecourse Epidemiology Unit, Southampton General Hospital, University of Southampton, Tremona Road, Southampton, UK.
[4]Musculoskeletal Research Unit, Translational Health Sciences, University of Bristol, Southmead Hospital, Bristol, UK.

WNT signalling is important in post-natal bone accrual. We previously showed that, in addition to exhibiting extreme high bone mass (HBM), subjects with rare gain-of-function mutations in the WNT co-receptor LRP5 have increased lower-body fat mass. Here, we examined the effects of LRP5 on systemic metabolism using human physiological studies and glucose-uptake assays in human adipocytes.

We studied 6 HBM-LRP5 individuals from 3 pedigrees. Each case was age- and BMI-matched to 10 healthy volunteers. Compared to controls, HBM-LRP5 subjects had lower fasting glucose ($p=0.02$), reduced fasting insulin ($p=0.02$), decreased HOMA-IR ($p=0.02$) and lower adipose tissue insulin resistance (Adipo-IR) ($p=0.001$). Furthermore, HBM-LRP5 cases had lower non-esterified fatty acid levels post-OGTT *versus* controls ($n=8$) (AUC $p=0.005$). To test whether LRP5 directly regulates adipocyte insulin-sensitivity, we used the Tet-On system to express scrambled control or LRP5 shRNAs in *in vitro* differentiated abdominal and gluteal adipocytes. Forty-eight-hour doxycyline-treatment led to >90% LRP5-knockdown in both cell-types, without affecting *PPARG* and *CEBPA* expression or lipid accumulation. However, mRNA levels of the insulin-sensitising hormone adiponectin (*ADIPOQ*) were decreased ($p<0.01$). LRP5-knockdown was associated with reduced basal glucose-uptake in both abdominal ($p<0.05$) and gluteal adipocytes ($p<0.01$), and impaired insulin-stimulated glucose-uptake in abdominal cells ($p<0.01$). Consistently, insulin-stimulated AKT-phosphorylation was blunted in LRP5-knockdown abdominal adipocytes. *ADIPOQ* was more highly expressed in adipocytes from HBM-LRP5 cases *versus* controls ($p<0.01$) and correlated positively with *LRP5* expression in abdominal and gluteal adipocytes (*rho*>0.4, $p<0.001$). We conclude that LRP5 has beneficial effects on metabolic health, partly through enhanced adipocyte insulin sensitivity.

POSTER NO: 26

Coupling cellular mechanics and protein translation

Adam Keen[1], Lisa Simpson[1], Luke Payne[1], Alistair Rice[2], Armando del Rio Hernandez[2], John Reader[1], Ellie Tzima[1]

[1] *Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Department of Bioengineeing, Imperial College London, UK.*

Vascular endothelial cells are highly mechanosensitive. Cellular mechanosensitivity is conferred via cytoskeleton: it responds to externally applied forces and also generates its own forces that play a central role in several fundamental cellular processes. Protein synthesis (or translation) is a highly complex process that requires the coordinated functions of various players, including ribosomes and their regulation by numerous translation factors. Since the discovery that specific mRNAs associate with the cytoskeleton, there has been interest in the complex cross-talk between these two systems. One of the less studied members of the eukaryotic initiation factor family is eIF6. eIF6 binds to the large ribosomal subunit (60S) and prevents its binding to the small ribosomal subunit (40S) in the cytoplasm, therefore regulating the formation of an active 80S ribosome capable of protein translation. We found that eIF6 associates with the cytoskeleton and regulates cellular mechanobiology in a protein translation-autonomous manner. Cells deficient in eIF6 show cytoskeletal and focal adhesion defects accompanied by reduced stiffness and traction force generation. Tensional force experiments reveal that eIF6 tunes cellular responses to external mechanical tension. Mechanistically, loss of eIF6 is not associated with defects in protein synthesis or protein expression; instead, eIF6 regulates the spatio-temporal activation of cellular signalling pathways critical for force transduction and focal adhesion growth. Furthermore, we identify a novel, eIF6-dependent mechano-complex composed of regulators of protein translation and cytoskeletal proteins. These results reveal an extra-ribosomal function for eIF6 and a novel paradigm for how mechanotransduction, the cytoskeleton and protein translation constituents are linked.

POSTER NO: 27

Cooption of normal stromal fibroblasts and tumour cells is associated with chemokine stimulation and metabolic pathway regulation

Dan Jiang[1], Zhipeng Wang[1], Sylvia Raftopoulou[1], Tomoyashi Soga[1], David Kerr[1], Shijie Cai[1]

[1]Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, UK.

Background

Stromal fibroblast is the most abundant cellular component of the tumour microenvironmment. Activation by tumour, it is differentiated into myofibroblast, in turn, supporting tumour initiation, progression and protecting tumour from chemocytotoxicity. However, mechanisms of the cooption between fibroblast and tumour remain elusive. Our present study sought to investigate the role of chemokine and metabolism in normal fibroblasts that might set the stage for colorectal cancer (CRC) growth.

Methods

Coculture of fibroblast and colorectal cancer (CRC) was a model used to study the interaction; incucyte to monitor cell proliferation, scratch wound healing/invasion in live; cytokine/chemokine antibody arrays to screen the secretion and ELISA for the validation; metabolomics to analyse metabolic pathways; qPCR and immunoblotting to study gene and protein expression.

Results

In functional study, the normal human fibroblast (CCD18co) conditioned medium (CM) stimulated HCT116 CRC proliferation, migration and invasion; The HCT116-derived CM also promoted CCD18co proliferation and differentiation to myofibroblast. In transwell cocultures, CCD18co facilitated HCT116 migration and epithelial to mesenchymal transition (EMT). Several cytokines/chemokines were detected in the coculture medium, but only CCD18co-derived chemokines were differentially elevated by HCT116 - CXCL1 at 6 hours and CXCL3 at 48 hours. Metabolic pathways including Warburg effect, glycolysis, gluconeogenesis, and amino acid synthesis were activated in cocultures of HCT116 and CCD18co. Essentially, CCD18co functionally protected HCT116 from 5-fluorouracil (5-FU) induced cytotoxicity. These findings were validated in murine NIH3T3 fibroblasts and MC38 CRC.

Conclusions

The reciprocal communication between normal colorectal fibroblasts and CRC results in CXCL1/CXCL3 secretion and metabolism alteration, driving the tumour growth.

POSTER NO: 28

Using genetics to assess coronary artery disease risk in type 2 diabetics

Chris Grace[1], Anuj Goel[1], Hugh Watkins[1], Martin Farrall[1]

[1]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, Oxford, UK.*

Rationale:
Coronary artery disease (CAD) and type 2 diabetes (T2D) are common complex diseases with substantial polygenic components that have been examined in numerous genome-wide association studies (GWAS); the most recent published large-scale GWAS meta-analyses reported 161 genome-wide significant loci associated with CAD[1] and 243 variants for T2D[2]. Such data provide opportunities to probe for shared heritable, and thus potentially causal, links between diseases. For instance, based on earlier data, Bulik-Sullivan *et al.* reported a substantial genetic correlation of 45% between T2D and CAD in a linkage disequilibrium (LD) score regression analysis[3].

Method:
We analysed genetic association summary statistics with a series of complementary statistical genetic methods, including a Mendelian randomization (MR) analysis that takes into account pleiotropic confounders and a locus-by-locus assessment of genetic overlap between T2D and CAD.

Results:
We observed a highly significant, potentially causal link between T2D and CAD heritable risk (OR 1.13 for CAD per log odds unit of T2D risk [1.11–1.14]; $p=2.78x10^{-71}$) when all available GWAS-significant ($p<5x10^{-8}$) instrumental variables were simultaneously analysed by MR. When pleiotropic variants were removed from the MR analysis, we observed a slightly weaker signal (OR 1.098 for CAD per log odds unit of T2D [1.07–1.12]; $p=6.48x10^{-19}$). We identified 40 regions in the genome where variants were significantly ($p<5x10^{-8}$) associated with both CAD & T2D within a +/- 1cM boundary; a GWAS-PW analysis detected 6 of these regions with a posterior probability of >50% that the same causal variant was associated with the two diseases.

Conclusions:
We present an up-to-date re-evaluation of the genetic burden of coronary artery disease in T2D patients. We found limited genetic evidence of same causal variant driving the association in both T2D and CAD and an overall burden of under 12% suggesting that in common diseases like CAD & T2D, pleiotropy plays an important role and not taking it into account can lead to misleading inflated estimates.

References:

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POSTER NO: 29

TherapeuTick: from tick saliva to anti-inflammatory peptides

B. Darlot[1], J.R.O. Eaton[1,2], GK. Yakala[2], G. Davies[2], K. Karuppanan[2], L. Geis[1], C.V. Robinson[1], A. Kawamura[1,2], S. Bhattacharya[2]

[1]*Department of Chemistry, University of Oxford, UK.*
[2]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*

Rationale: Inflammation is a major pathologic mechanism that adversely affects organ function in diseases such as myocarditis, myocardial infarction and atherosclerosis. Chemokines[1] bind to GPCRs expressed on leucocytes and cause directed migration or chemotaxis of leucocytes to sites of inflammation. The high total chemokine load in disease, promiscuity of receptor expression and chemokine-receptor interactions and feedforward loops render the chemokine network robust, and targeting individual chemokines or receptors has repeatedly failed as a pharmacological strategy in inflammatory disease. Ticks inject chemokine-binding proteins called evasins at the site of the bite. These evasins promiscuously bind multiple chemokines, effectively inhibiting inflammation, and allowing the tick to feed for days to weeks. Evasins are potently active in inflammatory disease models, and have potential as biological therapeutics[2]. Key limitations of biologicals are the possibility of immunogenicity and the requirement for parenteral delivery, which hinder their application in chronic diseases. These limitations have been overcome in several instances by the development of peptidomimetic therapeutics. Here we use the tick evasin P672[3], identified previously by our group and shown to bind 13 CC chemokines, to develop anti-inflammatory peptides that possess promiscuous chemokine-binding properties, providing a way for peptidomimetic development.

Methodology: Recombinant proteins were expressed in *E.coli* (chemokine CCL8) or HEK293 cells (evasin P672 and variants) and purified using affinity chromatography. Evasin-chemokines interactions were studied using hydrogen-deuterium exchange mass-spectrometry (HDX-MS) and biolayer interferometry (BLI). Peptides were synthesised using solid phase peptide synthesis (SPPS), validated by mass spectrometry (MS) and studied using biochemical assays (fluorescent polarisation (FP) and AlphaScreen), Boyden chamber THP1 monocyte chemotaxis assays, and efficacy tested *in vivo* using a mouse air-pouch zymosan-induced inflammation model.

Results: HDX-MS identified a 16-mer region of P672 that binds CCL8. Transfer of this region to EVA-1 (an evasin that doesn't bind CCL8) followed by BLI analysis confirmed that this 16-mer region possessed CCL8 binding activity. A synthetic peptide based on this 16-mer region (BK1.1) was characterised using FP and shown to bind multiple chemokines (CCL8, CCL7, CCL18). BK1.1 inhibited CCL8-induced cell migration in vitro. Derivatives of BK1.1 were synthesised and characterised using Alphascreen and FP for binding affinity and chemotaxis assays for functional inhibition. This led to remarkable improvements in chemokine binding and neutralization activity. The most potent peptide, BK1.3 was then studied using a short-term mouse air-pouch zymosan-induced inflammation model, and shown to potently block the recruitment of neutrophils, eosinophils, monocytes and T-cells in this model.

Conclusions: This work shows that through the characterisation of an evasin-chemokine interaction it is possible to design peptides that retain promiscuous CC-chemokine binding properties mimicking the parent evasin. We demonstrate the efficacy of a modified evasin-derived peptide in inhibiting inflammation *in vivo*. These studies pave the way for developing new peptidomimetic therapeutics that target the chemokine network in inflammatory disease.

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POSTER NO: 30

Does homoarginine regulate cardiac metabolism?

Sevasti Zervou[1], Hannah A. Lake[1], Debra J. McAndrew[1], Helen E. Lockstone[2], David Hauton[3], Dorothee Atzler[4], Stefan Neubauer[1], Craig A. Lygate[1]

[1]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.
[2]Core Genomics Group, Wellcome Centre for Human Genetics, University of Oxford, UK.
[3]Chemistry Research Laboratory, Department of Chemistry, University of Oxford, UK.
[4]Walther Straub Institute of Pharmacology & Toxicology and Institute for Cardiovascular Prevention, Ludwig-Maximilians-Universität (LMU), Munich, Germany.

Research rationale. L-Homoarginine (L-HA) is a non-proteinogenic amino acid, structurally similar to L-arginine and synthesised by the enzyme L-Arginine:Glycine Amidinotransferase (AGAT) in the kidney. Low plasma levels are an independent risk factor for stroke and cardiovascular disease, while we showed that L-HA supplementation preserves cardiac function in a murine model with heart failure[1]. Here we employed a non-biased approach to elucidate the potential mechanisms of L-HA action on gene expression and metabolic profile in the murine heart.

Methods and Results. A cohort of C57BL/6 mice were treated with 14mg/l L-HA daily, for 4 weeks and were compared to untreated controls (n=6 each). For RNA sequencing experiments, total RNA was extracted from mouse left ventricular tissue, PolyA enriched libraries were prepared, then sequenced 75 paired-end in an Illumina Hiseq4000. In total 13,908 genes were detected, 661 differentially expressed, 450 up- and 252 down-regulated by L-HA, including genes involved in fatty acid and other metabolic pathways (P<0.05 and 30% cut-off). Metabolite analysis using mass spectrometry and ion exchange chromatography confirmed a 4-fold increase in L-HA levels in the treated group vs untreated (P<0.001), unchanged arginine, glutamine and citrulline. L-HA significantly decreased acyl carnitines, specifically O-decanoyl, L-palmitoyl, tetradecanoyl and dodecanoyl carnitine (P<0.05). Verification work showed that L-HA causes transcriptional changes in genes involved in substrate utilisation, namely a trend for lower pyruvate dehydrogenase kinase (PDK4) and increased glucose transporter 4 (GLUT4; P=0.01).

Conclusions. These previously unreported effects of L-HA are being further investigated towards discovering potential molecular targets in cardiac physiology.

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POSTER NO: 31

Bud23 modulates mitochondrial function by reprogramming the ribosome

Maria Voronkov[1], Matthew Baxter[1], Rebecca Northeast[2], Toryn Poolman[1], Leanne Hodson[1], David Bechtold[2], David Ray[1]

[1]Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, UK.
[2]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK.

Bud23 is a ribosomal methyltransferase which is known to be involved in both ribosomal development and modification. In humans, it was originally characterised for its role in Williams-Beuren syndrome, a rare developmental disorder resulting from the haploinsufficiency of approximately 27 genes, including Bud23. Symptoms include diabetes mellitus, stunted growth, premature aging, and congenital heart disease. We have investigated Bud23 and attempted to further elucidate its biological role. One of the major functions of Bud23 is the deposition of a methyl mark on a key residue of the small ribosomal subunit. Ribosomal modifications have been described to regulate protein translation. Interestingly, we identified a novel mechanism linking together protein translation and mitochondrial function.

We developed murine models that lack Bud23 in two mitochondrially rich tissues – adipose and cardiac muscle. The muscle creatine kinase (MCK) driven loss of Bud23 in hearts causes decreased mitochondrial content and reduced mitochondrial oxidative potential, resulting in extensive cardiac remodelling that culminates in the early death of the animals. Proteomic analysis reveals a selective reduction of mitochondrial protein mass, but shows no evidence of a global translation defect. We also performed selective knockdown of Bud23 in adipose tissue using an adiponectin-Cre driver, which resulted in a loss of thermogenic potential in brown adipose, a process which is entirely dependent on mitochondrial function. Brown adipose was unresponsive to beta-3 agonists, which normally stimulate heat production by disrupting the mitochondrial protein gradient. This identifies a novel mechanism that highlights the extensive cross-talk between translation and mitochondrial function. We hypothesise that Bud23 reprograms the ribosome to enable the efficient translation and processing of mitochondrial proteins.

This work is funded by the Medical Research Council, UK.

POSTER NO: 32

Bestrophin 4 as a novel suppressor in colorectal tumour development

Zijing Wang[1,2], Jin Wang[1], Yi Long[1], Xian Zhang[1], Li Yang[1],Miao Zhang[2], David Kerr[2], Shijie Cai[2], Jinlin Yang[1]

[1]West China Hospital, Department of Gastroenterology, Sichuan University, China.
[2]Nuffield Division of Clinical Laboratory Science, Radcliffe Department of Medicine, University of Oxford, UK.

Background: Bestrophin (BEST) is a gene family encoding calcium-sensitive chloride channels; the expression of isoform 4 (BEST4) is mainly defined in human colon absorptive cells. Single cell genomic analysis defines that an absorptive subpopulation expresses high levels of BEST4 in conjunction with Notch2 and HES4 genes. Since high Notch2 is implicated in better outcomes of colorectal cancer (CRC), we sought out to investigate BEST4 expression in the patients and its role in regulating CRC development.

Methods: RNA sequencing was performed to analyze gene expression in the tumour and paired adjacent non-tumour tissues; cell proliferation, scratch wound healing, and colonogenesis in vitro and xenograft implantation were used for functional assays. Analysis of gene and protein expression was evaluated by qPCR and immunoblotting.

Results: Normal colon tissue expressed higher the BEST4 gene than the CRC. Low BEST4 was significantly correlated with larger tumours, and node positive and worse overall survival. In CRC cultures, BEST4 – overexpression inhibited HCT116 and Caco2 proliferation, invasion/migration, and colonogenesis. The knockout by the CRISPR/Cas9 increased HCT15 cell proliferation. In implants, BEST4 inhibited HCT116 tumor growth. Mechanically, BEST4 suppressed HCT116 and Caco2 epithelial-mesenchymal transition (EMT). It was induced by Notch2 or HES4 transiently transfected HCT116 or Caco2. Furthermore, reciprocally pulled down by their antibodies against BEST4 or HES4, suggests a direct interaction between them.

Conclusion: Our study demonstrates an inhibitory effect of BEST4 in CRC growth in vitro and in vivo. It inhibits EMT induction and responds to Notch2-Hes4 expression, thus representing a novel tumour suppressor for CRC growth.

POSTER NO: 33

Investigating a novel familial cardiac arrhythmia syndrome with ST-segment depression on the ECG

Carin de Villiers[1], Elizabeth Ormondroyd[1], Alistair Pagnamenta[2], Rob Hastings[1], Svanhild Nornes[3,4], Damien Downes[5], Anuj Goel[1,2], Alexander Sparrow[1], Silvia Salatino[2], Stefano Lise[2], Ron Schwessinger[5], Martin Farrall[1,2], Sanjay Sharma[6], Pier Lambiase[7], Jim Hughes[5], Sarah De Val[3,4], Alan Graham Stuart[8], Hugh Watkins[1,2]

[1]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Wellcome Centre for Human Genetics, University of Oxford, UK.*
[3]*Ludwig Institute for Cancer Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK.*
[4]*Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK.*
[5]*MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.*
[6]*St George's University of London, London, UK.*
[7]*Institute of Cardiovascular Science, University College London, London, UK.*
[8]*Bristol Heart Institute, University Hospital Bristol NHS Foundation Trust, Bristol, UK.*

Rationale — Cardiac repolarisation abnormalities are associated with arrhythmias and cardiac events that can lead to devastating outcomes. To date, several classic hereditary repolarisation disorders recognisable from the 12 lead ECG have been described and knowledge of their underlying genetic basis has added value to clinical management and guidance. This study outlines a novel repolarisation syndrome characterised by widespread ST depression on the ECG.[1] We investigate three ST syndrome families to determine the primary disease-causing variant and disease mechanisms involved.

Methodology —Genetic analysis was performed by whole genome sequencing and single nucleotide polymorphism (SNP) arrays. A combination of bioinformatic tools were used to determine linkage regions, define segments identical by decent, and prioritise plausible disease-causing variants. An interesting non-coding variant was further investigated by immunofluorescence microscopy in transgenic zebrafish embryos generated by the Tol2 system.

Results — A plausible causal-variant, a non-coding deletion/insertion, was identified in a linkage region. It is located in a region of high sequence conservation and computational analysis predicts this variant to produce an open chromatin structure leading to enhanced expression. Consistently, compared to the wild type, zebra fish with this variant show increased green fluorescent protein expression in the heart.

Conclusion — This study describes a novel repolarisation abnormality with ST depression on the ECG. The non-coding causal-variant detected interrupts a cardiac enhancer site. Understanding the downstream effect of this enhancer could help tailor future treatment strategies for patients with this disorder.

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POSTER NO: 34

Lost in translation: endothelial cytoskeleton 'out of line'

Lisa J Simpson[1], Adam Keen[1], John S Reader[1], Ellie Tzima[1]

[1]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*

Haemodynamic forces such as fluid shear stress generated by blood flow are critical determinants of vascular function in health and disease. Endothelial cells lining straight regions of the vasculature experience laminar shear stress which induces cytoskeletal alignment parallel to the direction of blood flow. This hallmark shear stress response promotes a protective, anti-inflammatory endothelial phenotype regulated by transcription and translation. Eukaryotic translation initiation factors (eIFs) are key components of the protein translation machinery which have been shown to have a close association with the cytoskeleton. However, the importance of this interaction in regulating the endothelial phenotype and the relationship between haemodynamics and translational control are largely unexplored.

Using siRNA-mediated knockdown *in vitro* or inducible deletion in endothelial cells of mice *in vivo*, we depleted cells of endogenous levels of this eIF and evaluated their responses to mechanical forces. Bespoke shear stress apparatus was used to apply force to endothelial cell monolayers to investigate mechanosignalling pathways in the absence of this eIF.

Here, we show an eIF co-localising with the endothelial cytoskeleton in aortic endothelial cells. Loss of this initiation factor *in vitro* or *in vivo* leads to dramatic impairment of the endothelial cytoskeleton and disrupts alignment of cells in response to laminar shear stress. Mechanistic experiments show that shear stress-mediated activation of key cytoskeletal signalling pathways become disrupted in the absence of this eIF. Interestingly, cytoskeletal protein levels remain unaffected, suggesting it is only their dynamic activation in response to shear stress which is reduced. Absence of this eIF also does not impair protein translation signalling pathways such as mTOR or nascent protein synthesis in response to shear stress *in vitro*.

Our working model demonstrates a crucial role for protein translation initiation factors in the endothelial cell response to fluid shear stress.

POSTER NO: 35

TCF7L2 regulates human adipose progenitor biology which may alter the genetic susceptibility to type 2 diabetes

Manu Verma[1], Nellie Y. Loh[1], Senthil K. Vasan[1], Andrea D. van Dam[1], Marijana Todorčević[1], Matt J. Neville[1], Fredrik Karpe[1,2], Constantinos Christodoulides[1]

[1]Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, UK.
[2]NIHR-Oxford Biomedical Research Centre, Oxford University Hospital Foundation Trust, Oxford, UK.

Non-coding genetic variation at *TCF7L2* is the strongest genetic determinant of type 2 diabetes (T2D) risk in humans. TCF7L2 is a transcription factor which functions as the key nuclear effector of WNT signalling, a developmental pathway, which plays a central role in adipose tissue (AT) biology. Here we mapped the expression of *TCF7L2* in human AT and investigated its role in adipose progenitor (AP) biology and T2D susceptibility using *ex vivo* expression studies, *in vitro* loss- and gain-of-function experiments, RNA-sequencing in TCF7L2 knockdown (KD) APs and AT phenotyping of carriers of an intronic variant (rs7903146) at *TCF7L2* that is the strongest genetic determinant of T2D risk. *TCF7L2* is highly expressed across multiple AT depots and its expression levels in subcutaneous abdominal AT are reduced in obesity. In fractionated AT, APs exhibited the highest *TCF7L2* mRNA abundance *vs.* mature adipocytes and adipose-derived endothelial cells. Furthermore, *TCF7L2* expression (positively) correlated with donor BMI specifically in abdominal APs. TCF7L2 KD in abdominal APs led to impaired proliferation, dose-dependent activation of WNT/β-catenin signalling, and dose-dependent effects on adipogenesis. Whilst partial KD enhanced adipocyte differentiation, complete KD impaired lipid accumulation and adipogenic gene expression. Overexpression of TCF7L2 accelerated adipogenesis. Transcriptome-wide profiling revealed that TCF7L2 can modulate multiple aspects of AP biology including extracellular matrix secretion, immune signalling and apoptosis. Finally, the T2D-risk allele (rs7903146) was associated with reduced AP TCF7L2 expression and enhanced AT insulin sensitivity. Our study highlights a complex role for TCF7L2 in AP biology and suggests that in addition to regulating pancreatic insulin secretion, genetic variation at *TCF7L2* may also influence T2D risk by modulating AP function.

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POSTER NO: 36

Mapping testis development at single-cell resolution

Maria Giebler[1], Jingtao Guo[2], Xichen Nie[2], Hana Mlcochova[1], Yueqi Wang[3], Edward J. Grow[2], Robin Kim[4], Melissa Tharmalingam[5], Gabriele Matilionyte[5], Cecilia Lindskog[6], Douglas T. Carrell[2], Rod T. Mitchell[5], James M. Hotaling[2], Bradley R. Cairns[2], Anne Goriely[1]

[1]MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.
[2]Huntsman Cancer Institute, University of Utah School of Medicine, USA.
[3]Department of Computer Science, Columbia University, USA.
[4]Section of Transplantation, University of Utah School of Medicine, USA.
[5]MRC Centre for Reproductive Health, University of Edinburgh, UK.
[6]Science for Life Laboratory, Uppsala University, Sweden.

Unusually, the human testis is one of the few organs that defines most of its cell types and function after birth. During puberty, the human testis undergoes dramatic developmental and morphological changes, including proliferation and maturation of germ cells and their supporting niche cells to initiate spermatogenesis. To characterize this under-studied process, in collaboration with the group of Brad Cairns we have profiled the single-cell transcriptomes of testicular cells from infants, boys spanning puberty and adults. With the onset of puberty, undifferentiated spermatogonial stem cells deploy a specific transcriptional programme driving a sequential amplification, prior to differentiation and the initiation of gametogenesis. Furthermore, we identified pre-pubertal progenitors of niche cells and delineated the key lineage-specific pathways controlling their pubertal differentiation. To validate these results, we performed immuno-fluorescence using stage-specific markers. These data provide new insights into the key molecular factors regulating germ cell proliferation and niche development and allows us to track the morphogenetic changes occurring during testis maturation. To broaden our understanding of the human testis ontogeny, we will exploit the multiplex power of the Hyperion System (Imaging Mass Cytometry) to describe the spatial distribution of a large number of cell-specific markers identified in our study. We aim to generate a spatial map of the developing and adult testis at single-cell resolution. This basic knowledge will pave the way to developing strategies for spermatogonial stem cell transplantation/regeneration that can be offered to male cancer patients affected by the sterilising effects of their treatments or to infertile men.

POSTER NO: 37

Genetic and Functional Insights into CDA-I Prevalence and Pathogenesis

Aude-Anaïs Olijnik[1^], Noémi Roy[1,2,3^], Caroline Scott[1], Joseph A Marsh[4], Jill Brown[1], Karin Lauschke[5,6], Katrine Ask[5,7], Nigel Roberts[1], Damien J. Downes[1], Sanja Brolih[8], Errin Johnson[9], Barbara Xella[1], Melanie Proven[10], Ria Hipkiss[10], Kate Ryan[11], Per Frisk[12], Johan Mäkk[13], Evalena Stattin[14], Nandini Sadasivam[11], Louisa McIlwaine[15], Quentin A Hill[16], Raffaele Renella[17], Jim R. Hughes[1], Richard Gibbons[1], Anja Groth[5], Peter J. McHugh[8], Douglas R Higgs[1], Veronica J Buckle[1], Christian Babbs*[1]

[1]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK.
[2]Department of Haematology, Oxford University Hospitals NHS Foundation Trust, UK.
[3]BRC Blood Theme and BRC/NHS Translational Molecular Diagnostics Centre, John Radcliffe Hospital, Oxford, UK.
[4]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, UK.
[5]Biotech Research and Innovation Institute (BRIC) and Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark.
[6]current address: National Food Institute, Technical University of Denmark, Kemitorvet building 202, 2800 Kongens Lyngby, Denmark.
[7]current address: Eli Lilly Danmark A/S Lyskær 3E, 2.tv 2730 Herlev Denmark.
[8]Department of Oncology, MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK.
[9]Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, UK.
[10]Molecular Haematology Laboratory, Oxford University Hospitals NHS Foundation Trust, Oxford, UK.
[11]Haematology Department, Manchester University NHS Foundation Trust, UK.
[12]Department of Women's and Children's Health, Uppsala University and Uppsala University Childrens' Hospital, Sweden.

Background: Congenital Dyserythropoietic Anaemia type I (CDA-I) is a hereditary anaemia caused by biallelic mutations in the widely expressed genes *CDAN1* and *C15orf41*. Little is understood about either protein and it is unclear in which cellular pathways they participate.

Methods: Genetic analysis of a cohort of CDA-I patients identifies novel pathogenic variants in both known causative genes. We analyse the mutation distribution and the predicted structural positioning of amino acids affected in Codanin-1, the protein encoded by *CDAN1*. Using western blotting, immunoprecipitation and immunofluorescence we determine the effect of particular mutations on both proteins and interrogate protein interaction, stability and sub-cellular localisation.

Results: We identify five novel *CDAN1* mutations and one novel mutation in *C15orf41* and uncover evidence of further genetic heterogeneity in CDA-I. Additionally, population genetics suggests CDA-I is more common that currently predicted. Mutations are enriched in six clusters in Codanin-1 and tend to affect buried residues. Many missense and in-frame mutations do not destabilise the entire protein. Rather C15orf41 relies on Codanin-1 for stability and these proteins, that are found in the nucleolus,

interact to form an obligate complex in cells. We show this interaction is affected by a patient mutation present in the largest cluster.

Conclusion: Stability and interaction data suggest C15orf41 may be the key determinant of CDA-I and offer insight into the mechanism underlying this disease. Both proteins share a common pathway likely to be present in a wide variety of cell types, however, nucleolar enrichment may provide a clue as to the erythroid specific nature of CDA-I. The surprisingly high predicted incidence of CDA-I suggests better ascertainment would lead to improved patient care.

POSTER NO: 38

Increased myocardial fat supply improves cardiac energetics and function in heart failure

Watson WD[1], Green PG[1], Neubauer S[1], Rider OJ[1]

[1]*Oxford Centre for Magnetic Resonance Research, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*

Introduction

The failing heart is starved of energy, in part accounting for its contractile dysfunction, with reduced uptake of the fat and sugar required to produce energy an important causative factor. Altering metabolism of glucose and/or fat within the myocardium is therefore attractive as a therapeutic strategy for heart failure.

Methodology

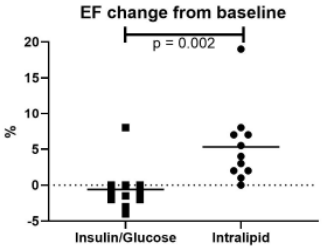
11 patients with clinical diagnosis of heart failure and nonischaemic cardiomyopathy were recruited. They were randomised to receive either an infusion of fat emulsion or a hyperinsulinaemic euglycaemic clamp. Following an hour of infusion, CMR was repeated followed by 31P cardiac magnetic resonance spectroscopy, then a dobutamine stress sequence targeting 65% of maximum heart rate. At a second visit at least 7 days later, they received the alternate infusion.

Results

Mean baseline ejection fraction was 37 ± 9 %. PCr/ATP ratio was greater with the fat infusion compared to the euglycaemic clamp (1.82 ± 0.26 vs 1.68 ± 0.24, p = 0.04). Fat emulsion infusion also brought about an ejection fraction increase over the baseline, compared to the euglycaemic clamp in which there was little difference (+5.3 ± 5.3 % vs -0.6 ± 3.1 %, p = 0.004) and calculated cardiac work was greater in the fat infusion group than the Insulin/glucose group (682 ± 156 L.mmHg/min vs 581 ± 85 L.mmHg/min, p = 0.009). The PCr/ATP ratio showed positive correlation with the stress ejection fraction (R2 = 0.656, p = 0.001), but not with resting ejection fraction.

Conclusions

Increased supply of fat to the myocardium brought about improved contractility and cardiac energetics in these heart failure patients compared to an increased glucose supply. The increase in PCr/ATP ratio would imply that (given ATP concentrations remain relatively constant in the myocardium), there is a greater availability of phosphocreatine, suggesting relatively improved mitochondrial ATP synthesis. These data suggest targeting myocardial fat metabolism may provide novel treatments for cardiac dysfunction.


EF change from baseline
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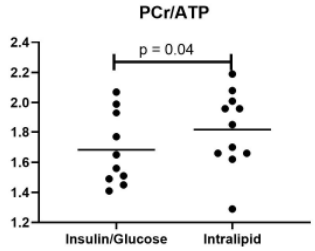
%
20
15
10
5
0
-5
Insulin/Glucose
Intralipid


PCr/ATP
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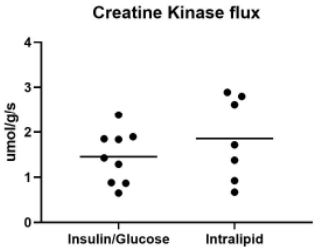
1.6
1.4
1.2
Insulin/Glucose
Intralipid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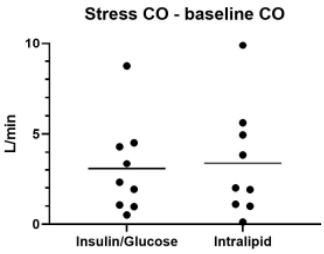
Insulin/Glucose
Intralipid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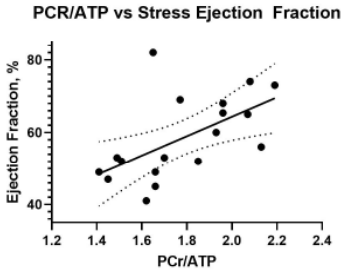
0
Insulin/Glucose
Intralipid


PCR/ATP vs Stress Ejection Fraction
Ejection Fraction, %
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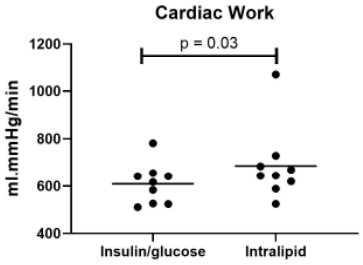
1.4
1.6
1.8
2.0
2.2
2.4
PCr/ATP


Cardiac Work
p = 0.03
ml.mmHg/min
1200
1000
800
600
400
Insulin/glucose
Intralipid

POSTER NO: 39

New insights into cranial suture development from single-cell RNA sequencing

Hana Mlcochova[1], Yan Zhou[1], Nils Koelling[1], Neil Ashley[2], Andrew O. M. Wilkie[1], Stephen R. F. Twigg[1]

[1]*Clinical Genetics Group, MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK.*
[2]*Single Cell Facility, MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK.*

Craniosynostosis is a rare condition which occurs in 1 in 2200 births and is characterised by the premature fusion of the calvarial sutures. If untreated it can lead to serious physical and intellectual disability. Both environmental and genetic factors contribute to craniosynostosis development, and monogenic causes (~60 known genes) can be identified in around a quarter of new patients with the coronal suture most commonly affected in these cases.

To help understand the pathological mechanisms underlying coronal suture fusion we have performed single-cell RNA sequencing in the mouse to define the cellular structure of developing sutures. To date, we have analysed the transcriptomes of supraorbital mesenchyme (SOM; E12.5), an organising centre which includes future suture cells, as well as actively growing sutures isolated at E17.5. Immunohistochemistry/immunofluorescence analysis at E17.5 with cluster-specific markers has provided new insights into the cellular organisation of the suture, particularly in the cell types that line the bone within the periosteum, pointing at a more complex picture of the “sutural niche” than previously appreciated. Our data from the E12.5 SOM correlate with the literature suggesting that earlier stages of sutural development are mainly characterized by genes involved in canonical WNT signaling and transcription factors involved in cartilage development. Comparison of the two datasets has shed light on the identity of some of the unassigned clusters in the E17.5 data, and additional transcriptomic analyses of intervening embryonic stages are planned to understand the cellular trajectories of sutural populations across development.

Our ongoing studies will allow us to define the key molecular events and cell populations in suture development, including their relationships and spatial organisation, and will lead to the generation of a valuable reference data resource for the scientific community. This will form a framework for understanding normal development and pave the way for deciphering the pathophysiology of premature suture fusion.

POSTER NO: 40

The MYB oncogene initiates enhancer function in leukemogenesis

I-Jun Lau[1], Yale Michaels[2], Alexander Thomas[1], Joe Harman[1], Nicholas Crump[1], Laura Godfrey[1], Simona Valletta[1], Phillip North[1], Jonathan Kerry[1], Claus Nerlov[1], Benjamin Davies[3], Tudor Fulga[2], Paresh Vyas[1], Thomas Milne[1]

[1]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, NIHR Oxford Biomedical Research Centre Haematology Theme, Radcliffe Department of Medicine, University of Oxford, UK.
[2]MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.
[3]Wellcome Centre for Human Genetics, Henry Wellcome Building of Genomic Medicine, University of Oxford, UK.

MYB is a DNA-binding transcription factor with a critical role in the establishment of definitive haematopoiesis and is commonly overexpressed in multiple hematological malignancies. While MYB inhibition is able to attenuate leukaemogenesis, its specific requirement in the processes of leukaemia initiation and transformation on a molecular level is still unclear. Recent evidence from disease models suggests that MYB functions through establishing aberrant enhancer activity, however this hypothesis has yet to be rigorously tested. To investigate the requirement for MYB in leukaemia initiation, we engineered a conditional *miRNA* mediated *Myb* repression circuit in a mouse model system. *Myb* repression during leukaemia initiation was correlated with a trend in decreased clonogenic potential *in vitro*, as well as a reduction in leukaemia fitness *in vivo*, suggesting that a threshold level of Myb is required to propagate an aggressive leukaemia phenotype. Using a novel chromatin recruitment system as well as analysis at endogenous loci, we also found that MYB is sufficient to generate an enhancer-like element and induce transcription at distal loci, suggesting that MYB may be competent to initiate enhancer activity *in vivo*. Importantly, MYB dependent gene activation can be disrupted with minimal changes in enhancer-promoter interaction frequency, suggesting that these are functionally and temporally separable events. Understanding the mechanism of MYB function in detail could have implications for therapeutic strategies designed to target MYB in leukaemogenesis.

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A novel approach using hand-held echocardiography to guide the diagnosis of heart failure in pregnant women in a low-resource setting; Maternal and Perinatal Health Research Collaboration, India (MaatHRI)

Maryam Alsharqi[1, 2*], Linda Arnold[1*], MaatHRI collaborators[3], Cameron Dockerill[1], Colin Baigent[4], Paul Leeson[1*], Manisha Nair[4*]

[1]*Cardiovascular Clinical Research Facility, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Department of Cardiac Technology, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia.*
[3]*Site collaborators from 10 MaatHRI collaborating hospitals in India.*
[4]*Nuffield Department of Population Health, University of Oxford, Oxford, UK.*
*Contributed equally

Background: Point of care imaging devices are promising tools for cardiovascular imaging in low-resource settings.

Purpose: Our aim was to determine whether hand-held echocardiography scans performed by obstetricians can help to identify the cardiac phenotypes in pregnant women with heart failure in India.

Methods: In November 2018, eighteen obstetricians from 10 hospitals across the states of Assam, Meghalaya and Uttar Pradesh were given 2 days of hands-on training in image acquisition using Philips Lumify devices. Obstetricians were asked to follow a simplified protocol of image acquisition and optimisation designed by the Oxford Cardiovascular Clinical Research Facility team. The protocol includes 3 key echocardiography views; parasternal long axis, parasternal short axis, and apical four chamber. Remote supervision and constructive feedback on performance was provided to improve image quality. Echocardiographic images of 269 pregnant women (with and without suspected heart failure) were acquired by the trained obstetricians, between February 2018 and January 2020. The first 147 scans were transferred with end-to-end encryption to the University of Oxford Research Echocardiography Core Laboratory (ORECL). Image interpretation and formal quality assessment was performed by 2 experienced echocardiographers.

Results: Parasternal long axis image quality was assessed as good in 78.3%, and was superior to both the parasternal short axis view (76.5%) and the apical 4-chamber view (61.9%). Image depth and gain optimisation was the main reason for loss of quality. The acquisition quality was related to reported clinical disease severity, and advanced pregnancy status. Left ventricular systolic function was impaired in 32 participants (21.9%), and 23.3% of the cohort had a dilated left atrium. Rheumatic heart disease was found in 12 participants (8.3%), in which the mitral valve was stenotic; 10 severe and 1 moderate. Mitral regurgitation was reported in 29 cases (20.8%); 6 severe, 7 moderate and 16 mild. Tricuspid and aortic valve abnormalities were also detected. Pericardial effusion was reported in 45 participants (30.8%).

Conclusion: Obstetricians with supervised training, using hand-held echocardiography have demonstrated acceptable image acquisition quality which could be assessed through core laboratory analysis to detect cardiac abnormalities. Such an approach could be useful to guide the diagnosis of heart failure in pregnant

women in low-resource settings. Further training for the obstetricians and image quality assurance have been implemented in the ongoing MaatHRI study with plans to conduct a validation analysis.

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A recruit-by genotype approach to investigate the biological function of GWAS hits in human adipose cell populations

Andrea van Dam[1], Enrique M. Toledo[2], Nellie Loh[1], Matt Neville[1], Katherine Pinnick[1], Maja Todorčević[1], Tim Walker[2], Diana Mantripp[1], Jane Cheeseman[1], Kerry McLaughlin[1], Amy Barrett[1], Manu Verma[1], Anne Clark[1], Richard Dumbill[3], Fredrik Karpe[1], Costas Christodoulides[1]

[1]*Oxford Centre for Diabetes Endocrinology and Metabolism, Churchill Hospital, Radcliffe Department of Medicine, University of Oxford, UK.*
[2]*Novo Nordisk Research Centre Oxford, UK.*
[3]*General Surgery, Oxford University Hospitals NHS Foundation Trust, Oxford, UK.*

Research rationale

Body fat distribution is an important determinant of metabolic health. Recent GWAS meta-analyses revealed that at least 2 independent signals at the hedgehog interacting protein (*HHIP*) locus (rs1812175, rs13146972) are associated with hip circumference adjusted for BMI (HIP_{adjBMI}). Our aim was to unravel the mechanisms by which variants at this locus promote lower body fat accrual.

Methodology and results

Carriers of the HIP_{adjBMI} increasing allele have larger adipocytes in both the abdominal and gluteal depots compared to BMI- and age-matched controls. The linkage disequilibrium block containing rs1812175 and rs13146972 lies within a topologically associated domain containing HHIP, ANAPC10, and ABCE1 and overlaps histone marks in the HHIP promoter.

HHIP expression was low in whole adipose tissue and mature adipocytes. Within the stromal vascular fraction of adipose tissue, *HHIP* transcript levels were highest in pre-adipocytes. Comparison of single-cell RNA sequencing data from carriers of the HIP_{adjBMI} increasing allele and controls will provide insight into whether there are differences in fat depot-specific *HHIP* expression and cell population specific eQTLs.

Two variants that are in high linkage disequilibrium with the primary signal rs1812175 (rs1355603 and rs13106087) lie within the *HHIP* promoter. In *HHIP* promoter reporter assays the HIP_{adjBMI} increasing allele at rs1355603 displayed lower activity than the major allele. Knockdown of HHIP in primary gluteal APs promoted lipid accumulation and increased expression of adipogenic markers.

Conclusions

Our data suggest that HIP_{adjBMI} increasing variants at the HHIP locus reduce HHIP expression in APs thereby promoting adipogenesis.