Queen Mary University of London
Barts & The London

WILLIAM HARVEY RESEARCH INSTITUTE
Annual Review 2021

Wednesday 23 June 2020
Microsoft Teams
Click here to join the meeting
Click here for the poster session

PROGRAMME

9.30am	**WELCOME by Prof Panos Deloukas**
Session 1	***Chaired by Dr Mathieu-Benoit Voisin***
9.35am	**Prof Qingbo Xu** *"Impact of CD34+ Cells in Vascular Remodelling"*
9.50am	**Dr Sammi El-Mansi** (CMR) *"F-actin and septins regulate the exocytosis of a von Willebrand factor from endothelial cells"*
10.00am	**Dr Christophe Cleese** (CP) *"Who may benefit from the repurpose of immunotherapies to treat depression in Chronic Kidney Disease?"*
10.10am	**Dr Cristina Perez-Ternero** (CP) *"C-type naturetic peptide is a master regulator of metabolic homeostasis"*
10.20am	**Dr Sasha Howard** (Endo) *"The role of NOS1AP in GnRH processing; a regulator of pubertal timing?"*
10.30am	**Dr Claudio Raimondi** (CVDM) *"Role of endothelial Neuropilin-1 in inflammation"*
10.45am	**BREAK**
11.00am	**Derek Willoughby Award Lecture:** **Prof Christopher Buckley** (*University of Birmingham) "The implications of the human cell atlas for targeting Fibroblasts in Immune Mediated Inflammatory Diseases"* *Introduction and prize award by* ***Prof Mauro Perretti***
12.00pm	**Public and Patient Advisory Group Presentation** **Dr David Collier; Prof Vernon Trafford**
12.15pm	**LUNCH**
1.00pm	**Online Poster Session**
1.45pm	**Alex Prestage** *Head of Equality, Diversity & Inclusion "Fostering a Culture of Inclusion: Sensitively discussing and addressing issues of racism in Higher Education"*
Session 2	***Chaired by Dr Eirini Marouli***
2.05pm	**Dr Suzanne Eldridge** (EMR) *"Agrin induces long-term osteochondral regeneration by supporting repair morphogenesis"*
2.20pm	**Dr Valentina Cipriani** (CTB) "Beyond Factor H: the influence of genetic variation associated with age-related macular degeneration on circulating FHL-1 and FHR protein levels"
2.30pm	**Dr Julia Ramirez** (CP) *"Prediction of Coronary Artery Disease and Major Adverse Cardiovascular Events using Traditional and Genetic Risk Scores for Cardiovascular Risk Factors"*
2.40pm	**Dr Trini Montero-Melendez** (BP) *"MC1-induced senescence: Opportunities for Pathway-centred and Pharmacogenetics Approaches"*
2.50pm	**Dr Elisabetta Sciacca** (EMR) *"Linking network analysis with gene-gene interaction analysis yields gene pairs*

predictive of response in rheumatoid arthritis"

3.00pm **Dr Dunja Akesentijevic** (BP) *"Metabolic remodelling in heart failure"*

3.15pm **BREAK**

3.30pm **Outstanding Contribution to Science Award Lecture:**

Prof Sekar Kathiresan (*Verve Therapeutics*) "*From reading the genome for risk to rewriting it for cardiovascular health"*

Introduction and prize award given by ***Prof Panos Deloukas***

4.30pm **WHRF Presentation**
Mr Jeremy Tigue, Chair WHRF
Lay Communications and Travel Awards

4.35pm **Prizes**
Presented by Prof Panos Deloukas
Young Investigators Award (less than 5yrs postdoc experience)
Young Investigators Award (more than 5yrs postdoc experience)
Best poster

4.45pm **CLOSING REMARKS**
Prof Panos Deloukas

Abstracts from selected oral presentations

F-actin and septins regulate the exocytosis of a von Willebrand factor from endothelial cells

El-Mansi S[1], Robinson C, Nightingale T.D[1].
Centre of Microvascular Research

Weibel-Palade bodies (WPBs) are rod-shaped, endothelial-specific storage organelles. Their main content is the ultra-large, pro-haemostatic von Willebrand factor (VWF). Exocytosis of WPBs results in the unfurling of coiled VWF multimers into long, platelet-catching 'strings'. This process underpins the endothelium's ability to instigate a haemostatic response to injury. Excessive levels of VWF are associated with thrombotic pathologies, including myocardial infarction and ischaemic stroke.

VWF is the largest soluble protein found in the blood and represents an unprecedented burden on the secretory machinery. Endothelial cells employ an additional piece of machinery in the form of an actomyosin ring to aid the release of VWF. This contractile ring is thought to “squeeze” the largest multimers out the cell[1]. Here we reveal, septin rings are a novel piece of this exocytic machinery.

An APEX-2 proximity proteomics approach was employed to reveal molecular machinery related to actomyosin ring formation and function. Subsequently, a siRNA sub-screen of actin interacting “hits” was performed. siRNA depletion of Septin-7 (SEPT7) reduced VWF release from Human Umbilical Vein Endothelial Cells (HUVEC). Furthermore, siRNA depletion of septins reduced the proportion of VWF exit sites > 2 μm^2 (a read out of actin ring recruitment).
Immunofluorescence studies demonstrated that septins form hetero-oligomer rings around fused WPBs and disruption of ring formation by SEPT7 knockdown reduced the number and length of VWF strings but had no effect on WPB fusion or actin ring recruitment. Next, the actin poison cytochalasin E was used to demonstrate that septin ring formation is independent of F-actin. Live cell imaging of PMA stimulated HUVEC transiently expressing SEPT6-GFP clearly demonstrated the formation of a ring shaped structure during WPBs exocytosis (at a time point that could overlap with the actomyosin ring). Finally we showcase a novel model to study WPB exocytosis *in vivo* using intravital confocal microscopy of the murine cremaster microvasculature.

Taken together these data demonstrate that the septin cytoskeleton is necessary for regulated VWF release and carries important implications for cardiovascular and haematological conditions.

References:
1) Nightingale TD, White IJ, Doyle EL, et al. Actomyosin II contractility expels von Willebrand factor from Weibel-Palade bodies during exocytosis. J Cell Biol. 2011;194(4):613-629. doi:10.1083/jcb.201011119

Who may benefit from the repurpose of immunotherapies to treat depression in Chronic Kidney Disease?

Clesse C., Bhui, K., Yaqoob M. & Carvalho L.A.
William Harvey Research Institute, QMUL

Abstract

Background: Evidence for a likely causal association between inflammation and depression among patients with chronic physical illnesses such as Chronic Kidney Disease led to clinical trials using immunotherapies. However, the clinical phenotypes of those who may benefit from immunotherapies have been rarely studied.

Methods: We used the UK Biobank data to investigate clinical phenotypic characteristics of depressed (currently depressed and lifetime depression) Chronic Kidney Disease patients grouped as those with higher (CRP≥3mg/L) or lower (CRP<3mg/L) inflammation. Phenotypes investigated included sociodemographic variables (age, sex, ethnicity), socioeconomic characteristics (Townsend deprivation index), adverse health behaviours (BMI, Waist to Height ratio (WHtR), alcohol consumption, smoking), main CKD comorbidities (Cardiovascular Diseases, Diabetes, Chronic Obstructive Pulmonary Disease, Glomerular Diseases), and treatment (antidepressants, antipsychotics, and anti-inflammatory drugs).

Results: Inflammation in CKD currently depressed patients was associated with higher WHtR (t=4.35 p<0.000), higher BMI (t=4.41, p<0.000) and higher prevalence of Diabetes (X^2=8.09, p<0.004). We did not find an association for Age, Sex, Ethnicity, Townsend deprivation index, Smoke and Alcohol consumption, Cardiovascular diseases, COPD and current medication.

Conclusion: Using a large dataset, this research highlights the clinical phenotype of those who may benefit from the repurpose of immunotherapy for depression in patients with CKD.

C-TYPE NATRIURETIC PEPTIDE IS A MASTER REGULATOR OF METABOLIC HOMEOSTASIS

[1]Cristina Perez-Ternero, [1]Aisah A Aubdool, [2]Raj Makwana, [2]Gareth J Sanger, [3]Roland H Stimson, [1]Li F Chan, [1]Amie J Moyes & [1]Adrian J Hobbs

Background

Thermogenesis and adipogenesis are tightly regulated mechanisms that maintain lipid homeostasis and energy balance; dysfunction of these critical processes underpins obesity and contributes to the cardiometabolic syndrome. C-type natriuretic peptide (CNP) fulfils a multimodal protective role in the cardiovascular system, governing local blood flow, angiogenesis, cardiac function and immune cell reactivity. Herein, we investigated a parallel, preservative function for CNP in coordinating lipid handling, thermogenic programming, and metabolic balance.

Methods

In vivo thermogenic and adipogenic effects of CNP were explored in wild type, global inducible CNP knockout (gbCNP$^{-/-}$) mice, and global constitutive natriuretic peptide receptor NPR-C null animals. Calorimetric studies were performed in a metabolic phenotyping unit at or below thermoneutrality. Glucose clearance was investigated using standard glucose or insulin tolerance tests. Adipocytes differentiated from murine stromal vascular fractions were used to investigate the signaling underlying the metabolic effects of CNP *in vitro*.

Results

Global deletion of CNP results in reduced body weight gain, lower adiposity, higher core body temperature, and greater resting energy expenditure *in vivo*. This thermogenic phenotype was associated with increased expression of uncoupling protein (UCP)-1 and peroxisome proliferator-activated receptor γ coactivator (PGC)-1α and underpinned, in part, by preferential utilization of lipid by mitochondria; a switch corroborated by a parallel diminution of insulin secretion and glucose clearance. Studies in primary murine adipocytes *in vitro* demonstrate that CNP mediates these metabolic regulatory actions in an autocrine manner, inhibiting sympathetic thermogenic programming via cognate G_i-coupled natriuretic peptide receptor (NPR)-C and reducing PGC-1α expression, whilst concomitantly driving adipogenesis via a NPR-B/protein kinase G pathway.

Conclusions

These findings establish a pivotal physiological role for CNP as a metabolic switch to balance energy homeostasis. The data also highlight a novel reciprocal engagement between guanylyl cyclase-coupled NPR-B and G_i-coupled NPR-C in regulating thermogenesis, adipogenesis and glucose handling. Pharmacological targeting of these receptors may therefore offer therapeutic **utility in the metabolic syndrome and consequent cardiovascular disease.**

The role of NOS1AP in GnRH processing; a regulator of pubertal timing?
Sasha Howard

Puberty is a fascinating transition period in the mammalian lifespan, but the biological control of pubertal timing remains poorly understood. The onset of puberty is driven by a coordinated increase in frequency and amplitude of the pulsatile release of gonadotrophin-releasing hormone (GnRH) from the anterior hypothalamus. Nitric oxide signalling is known to be important for synchronous GnRH pulsatility, in conjunction with kisspeptin. We hypothesised that defects in the neuronal nitric oxide pathway may lead to pubertal disorders and be identifiable by next generation sequencing in patients with delayed puberty.

We identified a candidate of interest in the nitric oxide pathway from whole exome sequencing analysis in a large cohort with familial delayed puberty. Nitric Oxide Synthase Adaptor Protein 1 is coded for by the *NOS1AP* gene, in which we found an in-frame deletion in 7 individuals from 2 families. This rare variant lies in the NOS1AP protein domain responsible for interaction with carboxypeptidase E (CPE). CPE is a vital enzyme for processing of GnRH from pro-GnRH, and *CPE* knockout mice are infertile. RNAscope analysis of adult mouse hypothalamus showed co-expression of *NOS1AP* and *CPE* mRNA within GnRH neurons of the MePO. In the ventromedial hypothalamus *CPE* and *NOS1AP* appeared to colocalise in nitric oxide synthase 1 positive neurons. Co-immunoprecipitation experiments demonstrated that mutant NOS1AP displays an abnormal interaction with CPE. GnRH secretion by stimulated GT1-7 (mature, hypothalamic immortalised GnRH-like) cells overexpressing variant *NOS1AP* was reduced by 80% ($p<0.001$) compared to WT-overexpressing cells.

Thus, pathogenic variants of *NOS1AP* may inhibit CPE function, resulting in abnormal GnRH processing, which in turn leads to impaired GnRH secretion and ultimately the phenotypic manifestations of delayed puberty. Discovery of key regulators of pubertal disorders enables the potential for both diagnostic testing and novel therapies, and sheds light on the physiological control of puberty.

Beyond Factor H: the influence of genetic variation associated with age-related macular degeneration on circulating FHL-1 and FHR protein levels

(revised manuscript is currently under review in the AJHG for back-to-back publication with second, independent group with similar findings)

Valentina Cipriani

Age-related macular degeneration (AMD) is a leading cause of vision loss, with a strong genetic susceptibility at the complement factor H (*CFH*) locus. This locus encodes a series of complement regulators: factor H (FH), a splice variant factor H-like 1 (FHL-1), and five factor H-related proteins (FHR-1 to FHR-5), all involved in the regulation of complement factor C3b turnover. Little is known about the influence of AMD associated variants at this locus on FHL-1 and FHR protein levels. We have used a bespoke targeted mass spectrometry assay to measure the circulating levels of all seven complement regulators, and demonstrated elevated levels in AMD for all FHR proteins (FHR-1, *P*=2.4x10-10; FHR-2, *P*=6.0x10-10; FHR-3, *P*=1.5x10-5; FHR-4, *P*=1.3x10-3; FHR-5, *P*=1.9x10-4) and FHL-1 (*P*=4.9x10-4), while no difference was seen for FH (P=0.94). Genome-wide association analyses revealed genome-wide significant signals at the *CFH* locus for all five FHR proteins, and Mendelian randomization analyses provided strong statistical support for a causal role of FHR-1, FHR-2, FHR-4 and FHR-5 in AMD susceptibility. These findings provide a strong biochemical explanation for how genetically-driven alterations in circulating FHR proteins could be major drivers of AMD and highlight the need for research into FHR protein modulation as a viable therapeutic avenue for AMD.

Prediction of Coronary Artery Disease and Major Adverse Cardiovascular Events using Traditional and Genetic Risk Scores for Cardiovascular Risk Factors

Julia Ramírez, Stefan van Duijvenboden, William J Young, Andrew Tinker, Pier D Lambiase, Michele Orini, Patricia B Munroe

Background: Coronary artery disease (CAD) and major adverse cardiovascular events (MACE) are leading causes of death in the general population, but risk stratification remains suboptimal. Genetic risk scores (GRSs) for CAD predict risk independently from traditional risk factors. We assessed if there was improvement in risk stratification when including GRSs for multiple cardiovascular traits.

Methods: We used data from 379,581 participants in the UK Biobank without known cardiovascular conditions (median follow-up 11.5 years, 2.9% CAD cases, 4.5% MACE cases). In a training subset (50%), we built four scores using risk factors associated with each endpoint in Multivariable Cox analyses. Score s1 included sex and age, s2 included s1 and traditional risk factors, s3 included s2 and a GRS for CAD and s4 included s3 and multiple GRSs for cardiovascular traits. In an independent test subset (50%), we evaluated their performance using the area under the curve (AUC), hazard ratios (HRs) and net reclassification index (NRI).

Results: For both CAD and MACE, score s4 had a higher AUC than score s3 (0.753 versus 0.747, and 0.742 versus 0.733). The HR (95% confidence interval) for individuals in the top versus bottom 20% of the s4 distribution was 22.1 (18.7 – 26.2), versus 20.2 (17.1 – 23.8) for s3 for CAD, and 17.6 (15.6 – 20.0) versus 17.1 (15.1 – 19.3) for s3 for MACE. The overall mean NRI for s4 versus s3 was 1.8% for CAD and 2.4% for MACE. Score s4 reclassifies 1,757 individuals as ≥10% CAD risk (2,845 for MACE), of whom 168 would have a CAD event (340 for MACE) within the follow-up period.

Conclusions: In individuals without known cardiovascular disease, adding GRSs for multiple cardiovascular traits to a score integrating traditional risk factors and GRS for CAD improves risk stratification, identifying individuals who may benefit the most from early primary prevention measures.

MC_1-induced senescence: Opportunities for Pathway-centred and Pharmacogenetics Approaches.

Trinidad Montero-Melendez

Rheumatoid arthritis (RA) affects individuals commonly during the most productive years of adulthood. Poor response rates and high costs associated with treatment mandate the search for new therapies. Here we show that targeting a specific G-protein coupled receptor called melanocortin receptor type 1 (MC_1) promotes a senescence phenotype in synovial fibroblasts, enabling amelioration of joint inflammation. Following activation of MC_1, synovial fibroblasts acquire a senescence phenotype characterized by arrested proliferation, metabolic re-programming and marked gene alteration resembling the remodelling phase of wound healing, with increased matrix metalloproteinase expression and reduced collagen production. These later findings strongly suggest the potential application of this MC_1-mediated senescence in other conditions such as fibrosis, characterised, indeed, by the opposite profile: excess collagen production and reduced remodelling. Using dermal fibroblasts stimulated with TGFβ we demonstrated that MC_1 activation was also able to recapitulate the same senescence phenotype observed in RA fibroblasts and to reduce signs of fibrosis *in vitro*. Hence, we propose the exploitation of this new mechanism using a pathway-centred approach to disease based on targeting the "pathophenotype" (i.e. aberrantly activated fibroblasts), rather than the traditional approach to disease based on targeting the anatomical location (joints, skin, lungs, etc). MC_1 targeting can also benefit from a Pharmacogenetics approach. We observed that ~45% of individuals (UK population) carry a loss-of-function mutation on *MC1R* gene, and we demonstrated that the variant D298H prevented the pro-senescence effect of MC_1 stimulation. Hence, pharmacogenetics may add an extra layer of precision to this new pathway-centred approach based on MC_1-induced senescence in pathogenic fibroblasts.

Linking network analysis with gene-gene interaction analysis yields gene pairs predictive of response in rheumatoid arthritis

Elisabetta Sciacca1*, Anna E.A. Surace1, Salvatore Alaimo2, Vito Latora1, Felice Rivellese1, Alfredo Pulvirenti2, Alfredo Ferro2, Myles J. Lewis1†, Costantino Pitzalis1

Background: Differential gene expression analysis is a common starting point for many gene expression studies aimed at comparing groups of samples within a cohort. However, this only reveals differences at the single gene level. Analysing differential interactions between genes can give much greater understanding of biological processes, and details of functional mechanisms active in the tissue.

Methods: We utilised the information of four well curated pathway repositories obtaining 10,537 experimentally evaluated interactions. We extracted specific gene-gene interactions in synovial RNA-Seq to characterise rheumatoid arthritis (RA) pathotypes and leverage these to predict response to csDMARDs in the Pathobiology of Early Arthritis Cohort (PEAC). Differential interactions of each network were statistically evaluated through a robust linear model. Significant response to csDMARD treatment was further tested by receiver operating characteristic (ROC) curve analysis.

Results: Analysis comparing different histological pathotypes showed a coherent molecular signature matching the histological changes and highlighting novel pathotype-specific gene interactions and mechanisms. Analysis of responders vs non-responders revealed higher expression of apoptosis regulating gene-gene interactions in patients with good response to csDMARDs. Detailed analysis of interactions between pairs of network-linked genes identified a negative correlation between SOCS2 and STAT2 as measured by the ratio SOCS2/STAT2 as predictive of treatment success, improving ROC area under curve (AUC) from 0.62 to 0.778. Furthermore, we identified a key role for angiogenesis: there were significant statistical interactions between NOS3 and both CAMK1 and AKT3 (known to activate NOS3) when comparing responders and non-responders. The ratio of CAMKD2/NOS3 enhanced a prediction model of response improving ROC AUC from 0.624 to 0.726.

Conclusion: In summary we demonstrate a novel, powerful method which harnesses gene interaction networks for leveraging biologically relevant gene-gene interactions leading to improved models for predicting treatment response.

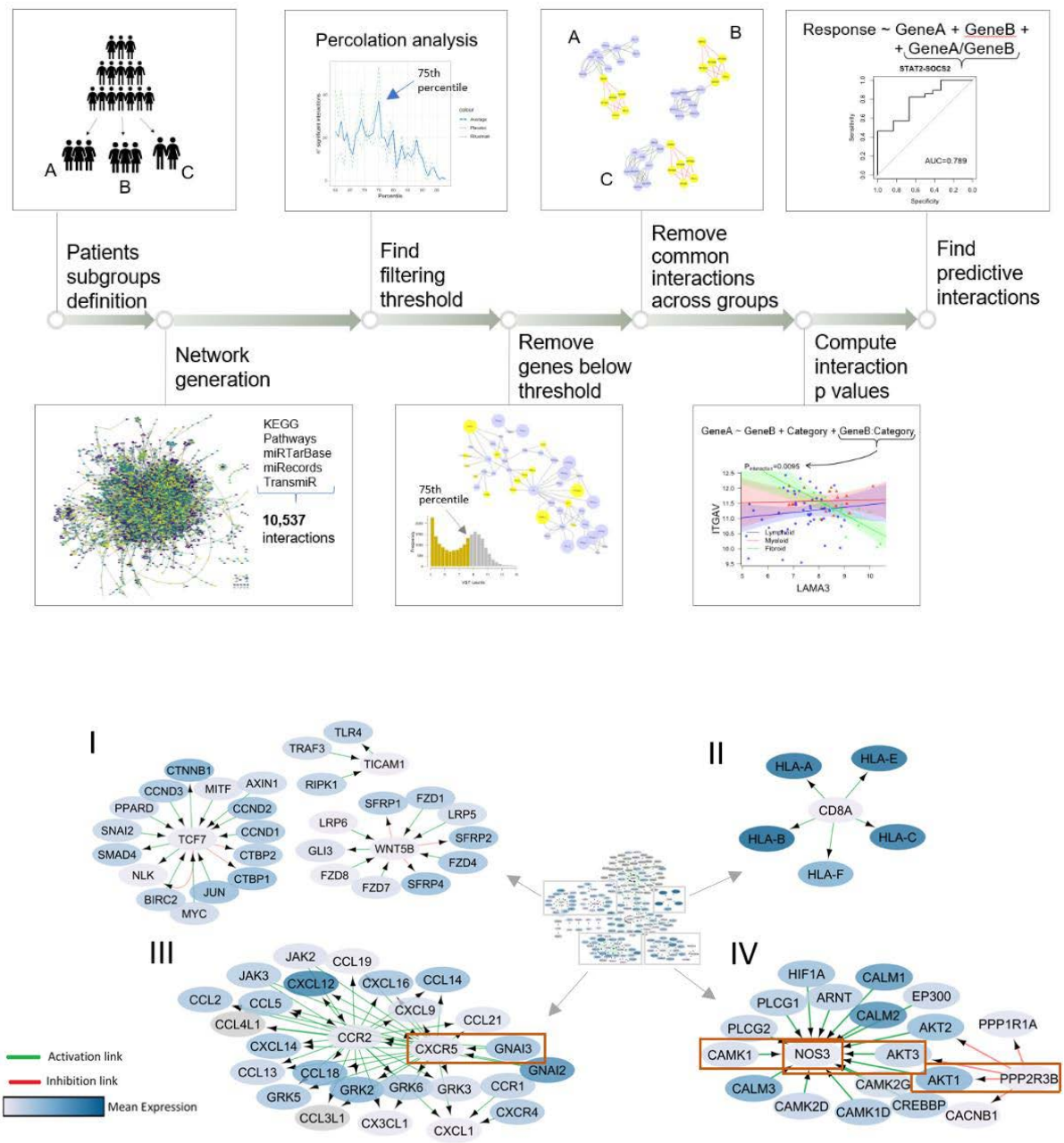

Figure 1 (A) Analytical pipeline using network approach and robust linear regression with interaction term to extract predictive gene ratios. (B) Gene interactions associated with csDMARD non responders.

The mechanisms of PI3K-p110δ signalling in dendritic cell-mediated control of gut immunity

Luiz Da Costa Vasconcellos

Phosphatidylinositol-3-OH kinases (PI3Ks) control pattern recognition receptor (PRR) signalling and coordinate phagosome dynamics. Deficiency in the PI3K-p110δ isoform leads to colitis due to antimicrobial immunity defects, though the mechanisms of p110δ protective role in intestinal immunity are not fully characterised. Herein, we show intestinal inflammation, instigated by genetic or chemical inactivation of p110δ involving enteric bacteria-reactive intestinal dendritic cells (DCs) associated with T cell immunity imbalance highlighted by T regulatory cell defects and (Th)1/Th17 overreaction. PRR-engaged cargo activates p110δ promoting NOX2-generated ROS, which influence antimicrobial control and antigen presentation. PI3K-p110δ deficiency causes perturbation of phagosome dynamics, marked by hyperacidification and extensive protein degradation affecting MHCII antigen presentation. Moreover, p110δ deficiency in DCs phenocopies functional loss of NOX2, tipping innate reactions towards inflammasomes, marked by caspase-1 hyperprocessing and enhanced IL-1β secretion. Our findings reveal that p110δ unites microbial sensing with RAC2-NOX2 activities on DC phagosomes to fine-tune intestinal immunity.

EXPLORING EXTRACELLULAR VESICLES IN SELF-RESOLVING MODELS OF ARTHRITIS

Miss Shani Austin-Williams[1], Dr. Lucy Norling[1], Dr. Silvia Oggero[1], Dr. Dianne Cooper[1]

Extracellular vesicles (EVs) are small packages of information, released by all cell-types including those of bacteria, parasites and fungi. Cells in our body release EVs as a method of communication between cells. These messages are fundamental to life, but can be detrimental to human biology. In rheumatoid arthritis (RA), a number of these EV messages have been described specifically messages that are pathological including platelet-EVs that are upregulated in the RA synovium and have been shown to drive pathology.
Here we have employed the serum transfer K/BxN model of arthritis; this model displays a number of characteristics that are similar to human disease. One advantage of studying this animal model is that the inflammation abates naturally.

We profiled both blood-derived EVs and their parent cell using flow cytometry and imaging flow cytometry, creating a time-course (D0, D8, D12) showing both peak inflammation as well as resolution. Interestingly, total EV concentration nor size, was affected over the time-course as analysed by Nanosight. Whilst platelet-EVs were significantly reduced at D8 whilst their parent cell was not significantly altered in this acute model. Double positive CD11b+Ly6G+ (Neutrophil-derived) EVs increased at D8 and returned to baseline at D12, and could therefore represent an early indication of resolution in arthritis. EVs and specifically Ly6G+CD11b+ EVs and CD41+ EVs could be explored as biomarkers for drug-free remission in patients.

Defining how skin fibroblast lineage identity and specificity is maintained *ex vivo*

Thomas Kirk[1], John Connelly[2] and Emanuel Rognoni[1]
[1]*Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London*
[2]*Blizard Institute, Queen Mary University of London*

The skin is our most important protective barrier and its repair requires coordinated function of two layers, the outer epidermis and underlying dermis. In the dermis the most common cells are called fibroblasts that synthesise the connective tissue and maintain the structural integrity. During skin development fibroblasts differentiate into distinct subpopulations (lineages) with specific functions that give rise to the dermal sublayers, including the papillary and reticular dermis. Despite the fundamental role of fibroblasts in tissue maintenance and diseases such as fibrosis, the molecular biology controlling dermal fibroblast subpopulation specific functions are largely unknown. A major challenge in the field is that fibroblast subpopulations quickly lose these outside their natural environment, hindering their analysis in culture and the development of therapeutic applications.

Here we focus on the two main skin fibroblast populations, papillary and reticular fibroblasts and unravel how different expansion methods influence their ability to regenerate different dermal skin structures in vivo. By combining ATAC-seq and RNA-seq, we will compare the fibroblast genome organisation and gene expression in the different cultures and reveal the genetic changes underlying the loss of lineage specific functions. The identified molecular regulators will be manipulated genetically or chemically to understand the mechanism that modulates and maintains papillary and reticular fibroblasts.

Our goal is to develop strategies to maintain, control and manipulate different fibroblast lineages outside their natural environment. This will open up the path for new cell therapies and support the development of regenerative medicine applications (e.g. scaffold cultures) in the future.

Cannabinoids as a combination therapy in HER2+ breast cancer

Nicolas Roth, Richard Grose and Peter McCormick
William Harvey Research Institute, Queen Mary University London

Breast cancer is a heterogenous disease that is a leading cause of cancer-related deaths among women. Identification of different breast cancer subtypes has permitted the stratification of patients and the development of targeted therapies. The HER2+ subtype of breast cancer is defined by the overexpression of the ERBB-family member, HER2. Signalling by HER2 results in increased proliferation, invasion and metastasis and is associated with worse patient outcomes, compared to HER2 negative breast cancers. Expression of HER2 allows selective treatments to target and abrogate its protumoural signalling, such as the monoclonal antibody trastuzumab, and the tyrosine kinase inhibitor, lapatinib. The use of such therapies has increased overall survival of HER2+ patients, yet many patients continue to not respond to HER2 inhibition, or go on to develop resistance. To overcome these challenges, combination therapy has become increasingly used in the clinic. The identification of suitable targets for combination therapy would result in increased treatment responsiveness and a decreased rate of resistance. One potential target is cannabinoid receptor 2 (CB2), a G-protein coupled receptor of the endocannabinoid system. CB2 expression is correlated with HER2 expression in breast cancer and relates to a more aggressive phenotype, whilst activation of CB2 with cannabinoids has been shown to promote anti-cancer signalling. To understand the role of CB2 in the promotion of tumourigenesis and its applicability as a target for therapy, we designed a CB2 expression system in the HER2+ breast cancer cell lines. Using this system, we aim to elucidate CB2-mediated effects on gene expression, metabolism and invasion, and the potential of CB2-targeting therapies as part of a combination therapy in HER2+ breast cancer.

Pancreatic cancer chemotherapy is potentiated by induction of tertiary lymphoid structures in mice

Francesca R Delvecchio[1,4], Rachel E A Fincham[1], Sarah Spear[2], Andrew Clear[3], Marina Roy-Luzarraga[1], Frances R Balkwill[2], John G Gribben[3], Michele Bombardieri[4], Kairbaan Hodivala-Dilke[1], Melania Capasso[2,5], Hemant M Kocher[1].

Background and aims Pancreatic cancer, the most lethal gastrointestinal cancer, is an immunologically cold malignancy with immunotherapy showing limited success. Tertiary lymphoid structures (TLS) are sites of ectopic immune cell maturation and have been described in several cancers. Cancer-associated TLS may confer survival benefit to a sub-group of patients with pancreatic ductal adenocarcinoma (PDAC). The role of TLS in PDAC is not yet fully known. We investigated the structure and role of TLS in human and murine pancreatic cancer.

Methods TLS presence was assessed by multicolour immunofluorescence in human and murine (genetically engineered murine model – KPC: *Kras*^{G12D}, *p53*^{R172H}, *Pdx-1-Cre* – and orthotopic tumour implantation model) pancreatic cancer. An orthotopic murine model of TLS development was generated to assess the effect of the combined chemotherapy (gemcitabine) and immunotherapy on tumour growth.

Results Presence of mature and functional TLS correlates with anti-tumour immune microenvironment. TLS are present, albeit not ubiquitous, in human PDAC and KPC murine cancers but absent in the orthotopic tumour implantation murine model. In the latter, TLS formation could be induced by intra-tumoral injection of lymphoid chemokines (CXCL13/CCL21). Furthermore, co-administration of systemic chemotherapy (gemcitabine) and intra-tumoral lymphoid chemokines into orthotopic tumours re-shaped the tumour immune-microenvironment facilitating TLS formation and potentiated anti-tumour activity of chemotherapy leading to significant tumour reduction, not achieved by either treatment alone. TLS-associated B cells support anti-tumour immunity through dendritic cell activation.

Conclusion TLS induction facilitates the intra-tumoral immune response and potentiates chemotherapy efficacy in a murine model of PDAC. A detailed understanding of cancer-associated TLS formation may help design personalised cancer immunotherapeutic approaches.

Pancreatic cancer chemotherapy is potentiated by induction of tertiary lymphoid structures in mice

Francesca R Delvecchio[1,4], Rachel E A Fincham[1], Sarah Spear[2], Andrew Clear[3], Marina Roy-Luzarraga[1], Frances R Balkwill[2], John G Gribben[3], Michele Bombardieri[4], Kairbaan Hodivala-Dilke[1], Melania Capasso[2,5], Hemant M Kocher[1].

Background and aims Pancreatic cancer, the most lethal gastrointestinal cancer, is an immunologically cold malignancy with immunotherapy showing limited success. Tertiary lymphoid structures (TLS) are sites of ectopic immune cell maturation and have been described in several cancers. Cancer-associated TLS may confer survival benefit to a sub-group of patients with pancreatic ductal adenocarcinoma (PDAC). The role of TLS in PDAC is not yet fully known. We investigated the structure and role of TLS in human and murine pancreatic cancer.

Methods TLS presence was assessed by multicolour immunofluorescence in human and murine (genetically engineered murine model – KPC: *Kras*^{G12D}, *p53*^{R172H}, *Pdx-1-Cre* – and orthotopic tumour implantation model) pancreatic cancer. An orthotopic murine model of TLS development was generated to assess the effect of the combined chemotherapy (gemcitabine) and immunotherapy on tumour growth.

Results Presence of mature and functional TLS correlates with anti-tumour immune microenvironment. TLS are present, albeit not ubiquitous, in human PDAC and KPC murine cancers but absent in the orthotopic tumour implantation murine model. In the latter, TLS formation could be induced by intra-tumoral injection of lymphoid chemokines (CXCL13/CCL21). Furthermore, co-administration of systemic chemotherapy (gemcitabine) and intra-tumoral lymphoid chemokines into orthotopic tumours re-shaped the tumour immune-microenvironment facilitating TLS formation and potentiated anti-tumour activity of chemotherapy leading to significant tumour reduction, not achieved by either treatment alone. TLS-associated B cells support anti-tumour immunity through dendritic cell activation.

Conclusion TLS induction facilitates the intra-tumoral immune response and potentiates chemotherapy efficacy in a murine model of PDAC. A detailed understanding of cancer-associated TLS formation may help design personalised cancer immunotherapeutic approaches.

Impact of Erythroid ACKR1 on Neutrophil Host Defence Functions

Anderson C.A.[1]*, Bianchini M.[2], Gutjahr J.C.[1], Samus M.[1], Saleeb, R.[1], Bidzhekov, K[2], Duchene J.[2], Rot, A.[1,3]

[1]*Centre for Microvascular Research, William Harvey Research Institute, Queen Mary University of London, UK*

[2]*Institute for Cardiovascular Prevention, Ludwig-Maximilians University, Munich, Germany*

[3]*Centre for Inflammation and Therapeutic Innovation, Queen Mary University of London, UK*

**caroline.anderson@qmul.ac.uk*

Duffy-negative individuals homozygous for an allelic variant of *ACKR1* carry a single nucleotide polymorphism (SNP) in GATA1 binding region of the ACKR1 gene that causes a lack of ACKR1 expression selectively in the erythroid lineage, but not in endothelial cells. This SNP is 100% prevalent in West Africa and confers resistance to *P. vivax* malaria. However, Duffy-negative individuals are also more likely to reject kidney transplants, have pre-eclampsia and have an increased mortality risk from acute respiratory distress syndrome. Previously, our group described differences in neutrophil phenotype between ACKR1 deficient and WT mice as well as Duffy-negative and Duffy-positive individuals (Duchene et al, *Nat Immunol*. 2017). Whether these differences influence neutrophil effector functions, including NETosis, phagocytosis and ROS production remained unknown.

To address this, we investigated the impact of erythroid ACKR1 on NETosis of neutrophils isolated from age- and sex- matched healthy Duffy-positive and Duffy-negative volunteers by negative immuno-magnetic selection. Neutrophils from Duffy-negative donors spontaneously formed NETs in the absence of stimulation, while those from Duffy-positive donors did not. Following stimulation with PMA, CXCL8 and CCL7, neutrophils from both donor groups formed NETs. In response to stimulation by PMA, but not CXCL8 or CCL7, the NET-associated myeloperoxidase/DNA area was significantly higher in Duffy-negative samples as compared with those from Duffy-positive donors.

To study more complex *in vivo* correlates, a new mouse model of the human *ACKR1* SNP, $ACKR1^{GATA1\text{-}G}$, was generated using CRISPR-Cas9 to introduce a nucleotide substitution identical to the human SNP. Using this model, we found increased cell surface expression of CD11b and decreased expression of CD62L in peripheral blood neutrophils from $ACKR1^{GATA1\text{-}G}$ mice compared with wildtype mice, suggestive of an activated phenotype. Phagocytosis assays using IgG-opsonised pHrodo Green *S. aureus* bioparticles showed no overall differences in the percentage of pHrodo Green+ cells between wildtype and $ACKR1^{GATA1\text{-}G}$ mice. However, median fluorescence intensity data and imaging flow cytometry indicated that pHrodo Green+ classical monocytes from $ACKR1^{GATA1\text{-}G}$ mice phagocytosed a greater number of particles per cell than those from wildtype mice.

In summary, our results suggest that neutrophils that develop in the absence of erythroid ACKR1 have an activated phenotype with a greater propensity to form NETs spontaneously. This might be beneficial in the context of innate immune challenges yet may contribute to the altered incidences and severities of inflammatory and autoimmune diseases observed in Duffy-negative individuals.

Elevated Tissue-resident Memory T cells in the Epicardial Adipose Tissue of Atrial Fibrillation patients

Vishal Vyas *(Longhi Laboratory, Biochemical Pharmacology)*

Background: A wealth of imaging data has established epicardial adipose tissue (EAT) as an independent risk factor for all forms of atrial fibrillation (AF). However, the immune profile of EAT remains incomplete with human tissue studies sparse and patients typically poorly matched for baseline clinical characteristics. This study sought to define the immunological signature of EAT in a propensity-matched cohort of patients who remained in sinus rhythm and those with a prior history of AF.

Methods: Adult patients with pre-existing AF and those with no prior history of AF undergoing elective cardiac surgery were recruited to undergo EAT sampling. The tissue samples were immediately taken to the laboratory for immune cell isolation, flow cytometry and quantitative polymerase chain reaction (qPCR) analysis. Patients were then propensity-matched to ensure baseline clinical variables were similar across the groups. In a small cohort of patients, atrial myocardial tissue was also obtained for initial histological analysis.

Results: In the 30 patient propensity-matched cohort of patients, flow cytometry analysis did not demonstrate differences in EAT immune cell numbers between the two groups of patients. Additionally, qPCR analysis of inflammatory mediator expression in EAT showed similar expression levels between the groups. Further immune cell subset analysis in a cohort of 18 propensity-matched patients revealed a highly significant increase ($p<0.005$) in both EAT-resident CD4+ and CD8+ memory T cell populations in pre-existing AF patients. Immune cells were seen at the atrial tissue-EAT borderzone on histological analysis.

Conclusions and Implications: Pre-existing AF patients demonstrate increased tissue-resident CD4+ and CD8+ T cell populations locally in the EAT. Histological analysis suggests immune cells migrate from the EAT to the underlying atrial tissue reiterating the importance of EAT's as the immune cell depot of the heart. Further characterisation of this EAT-resident T cell population may provide a novel paradigm in the management of the inflammatory components of AF genesis.

MC1-mediated pro-senescence therapy for the treatment of systemic sclerosis

Camilla SA Davan-Wetton, Mauro Perretti1, David Abraham2, Trinidad Montero Melendez1

The melanocortin 1 receptor (MC1) is a G-protein coupled receptor (GPCR) best described for its role in melanogenesis and UV protection, and in more recent years, for its function in inflammation and immune regulation. However, recent work from our lab has highlighted a novel biological outcome of MC1 receptor agonism: induction of senescence in synovial fibroblasts, resulting in reduced aggressive phenotype and amelioration of arthritis. We therefore investigated if this phenomenon was reproducible in other fibroblast-driven diseases like fibrosis. Healthy dermal fibroblasts (HDFs), pre-treated with transforming growth factor beta (TGFβ) to model the fibrotic phenotype, were treated with BMS-470539, a selective MC1 drug, and assessed for markers of senescence. BMS-treated HDFs exhibited a number of hallmarks of senescence including reduced proliferation rates with increased metabolic activity, prominent beta-galactosidase staining, increased cell size and lysosomal expansion, thereby demonstrating this mechanism of senescence induction to be functional in distinct fibroblast populations. Interestingly, no markers of DNA damage measured by γH2AX and 53BP1 immunofluorescence were detected, making this type of GPCR-induced senescence distinct from that induced by the pro-fibrotic compound bleomycin. In addition, preliminary data suggest that the pro-fibrotic phenotype can been reversed by BMS administration, observed by reduced alpha smooth muscle actin (αSMA) expression, increased interleukin (IL)-6 secretion, and reduced proliferation. In summary, our data show therapeutic potential for MC1-induced senescence for the treatment of diseases mediated byaberrantly activated fibroblasts. Ongoing experiments will assess the anti-fibrotic effects of senescence induction in fibroblasts from systemic sclerosis patients, as well as the contribution of senescence to disease amelioration by using senolytic drugs.

CMR radiomics analysis can detect subtle cardiac alterations associated with cardiac and cerebral ischaemia: results from the UK Biobank

Elisa Rauseo[1,2], Cristian Izquierdo Morcillo[3], Zahra Raisi-Estabragh[1,2], Polyxeni Gkontra[3], Karim Lekadir[3], Steffen E. Petersen[1,2]

(1) William Harvey Research Institute, NIHR Barts Biomedical Research Centre, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ, UK.
(2) Barts Heart Centre, St Bartholomew's Hospital, Barts Health NHS Trust, EC1A 7BE, London, UK.
(3) Departament de Matematiques i Informatica, Universitat de Barcelona, Artificial Intelligence in Medicine Lab (BCN-AIM), Barcelona, Spain.

Background: Ischaemic heart disease (IHD) and cerebrovascular disease are two distinct but closely interrelated clinical entities. Cardiovascular magnetic resonance (CMR) radiomics may capture a wide variety of subtle cardiac changes associated with these two diseases providing new insights into such interactions.

Aim: To define the CMR radiomic signatures for IHD and cerebrovascular disease and study their incremental value for disease discrimination over conventional CMR indices.

Methods: Participants from the UK Biobank with pre-existing IHD, ischaemic cerebrovascular disease, myocardial infarction (MI), and ischaemic stroke (IS) were included in the study (n= 781, n= 360, n= 542, n= 119, respectively). Each disease group was compared with an equal number of healthy controls. We extracted 641 shape, first-order, and texture CMR radiomics features from three regions of interest (right ventricle, left ventricle, left ventricular myocardium) in end-diastole and end-systole. Systematic feature selection combined with machine learning (ML) algorithms and ten-fold cross-validation tests were used to build the radiomics signature for each condition. Finally, we compared the discriminatory power of the radiomic models vs conventional CMR indices.

Results: In all the study groups, radiomic signature provided a significantly better disease discrimination than conventional indices, as suggested by AUC (IHD:0.82 vs 0.75; cerebrovascular disease: 0.79 vs 0.77; MI: 0.87 vs 0.79, IS: 0.81vs 0.72). For IHD and MI, shape and texture features equally contributed to the radiomic signature, indicating global cardiac remodelling when ischaemia primarily affects the heart. For IS and cerebrovascular disease, texture features were the most informative, suggesting more subtle myocardial alterations associated with cerebral ischaemia.

Conclusions: Radiomic models provided incremental value over conventional indices in detecting subtle cardiac alterations associated with cardiac and cerebral ischaemia, even in the presence of more heterogeneous clinical pictures. These findings suggest the potential utility of radiomics in improving our understanding of the mechanisms underlying brain-heart interactions.

Trans-ancestry GWAS of 252,730 Individuals Identifies 114 Novel Loci Associated with the QT Interval

William J Young[1], Aaron Isaacs[2], Najim Lahrouchi[3], Christopher Newton-Cheh[4], Nona Sotoodehnia[5], The CHARGE EKG working group, Pier D Lambiase[6], Borbala Mifsud[7], Patricia B Munroe

Background: The QT interval, a marker of ventricular repolarization, is a heritable, independent predictor of risk for ventricular arrhythmias and sudden cardiac death (SCD). Previous genome-wide association studies (GWAS) of QT interval highlighted pathways regulating cardiac ion channels, calcium signaling and myocyte internal structure. A large proportion of the heritability remains unexplained, suggesting additional mechanisms remain undiscovered.

Methods: We performed the largest trans-ancestry GWAS meta-analysis of QT to date, using 35 studies imputed with 1000G or HRC reference panels, comprising a total sample of 252,730 individuals (84% European, 7.7% Hispanic and 6.7% African ancestry/ethnicity). Candidate gene prioritisation and gene-set enrichment analyses were performed using DEPICT.

Results: We identified 177 independent loci (114 novel) associated with QT. SNP-based heritability in European ancestry UK-Biobank participants was 29.3%. The variance explained by lead and conditionally independent variants was 14.6%. Across all loci, the top 30 gene-ontology terms highlighted by DEPICT included processes involved in either muscle cell differentiation, tissue development, insulin receptor signaling or regulation of gene expression. At one locus (*CD36*), the association was driven by studies of African ancestry only. This gene encodes an immune-metabolic receptor necessary for appropriate myocardial substrate utilisation. Another locus (*FAM9B*) was identified in male-stratified X-chromosome analyses. The lead variant is strongly correlated ($r^2 > 0.9$) with other variants associated with testosterone levels and male-pattern baldness. Other candidate genes highlighted include cardiac Z-disk proteins (*C10orf71*), enzymes with cardioprotective roles in oxidative stress (*PON2*), cardiomyocyte glucose transporters (*GLUT4*) and regulators of cell morphology and cytoskeleton organization (*BRWD1*). A QT polygenic risk score was significantly associated with atrial fibrillation, cardiac conduction disease, and in females only, SCD.

Conclusions: Our analyses highlight novel genes and pathways associated with the QT interval that may expose new mechanisms which contribute to arrhythmogenesis and SCD and could serve as new therapeutic targets.

Investigating the effects of loss of S-palmitoylation on Dopamine1 receptor a G Protein–Coupled Receptors.

Authors: G.Chalhoub ;P.McCormick;S.Lefrancois;E.Savinaud;R.Irannejad;N.Puri.

Abstract

G protein–coupled receptors (GPCRs) play a key role in the signal transduction of various biological phenomena, hence serving to modulate at a molecular level many elements of human biology. S-palmitoylation is a key frequent post-translational modification (PTM) found on proteins. It is involved in membrane association, protein sorting and many other activities. Many GPCRs have been reported to undergo S-palmitoylation. Through its reversibility, S-palmitoylation also provides mechanisms to regulate the functional activities of GPCRs and in the case of our study the Dopamine 1 receptor (D1DR). The mechanisms involved in palmitoylation are poorly understood. The relevant enzymes (zDHHCs) are poorly characterised. To understand the role of S-palmitoylation on GPCRs we studied the effects of loss S-palmitoylation on D1DR using a wide array of techniques to uncover its effect on receptor trafficking, signalling and G protein coupling using Bioluminescence resonance energy transfer (BRET) taking advantage of a wide library of tagged proteins, microscopy, immunohistochemistry techniques (Western blotting, staining), signalling with cAMP biosensors and phosphorylation detection kits. Our data provide information on how S-palmitoylation modulates signalling and trafficking when and which enzymes mediate it. These data provide a fundamental understanding for the paradigm of GPCR palmitoylation.

Key words: G protein–coupled receptors; Dopamine1 receptor; Palmitoylation; zDHHCs; Bioluminescence resonance

Targeting the G protein-coupled receptor, CB2, towards the development of novel anticancer therapeutics.

Zara Farooq[1,2], Lesley A. Howell[2] and Peter J. McCormick[1]

G protein-coupled receptors (GPCRs) are the largest class of membrane proteins consisting of ~800 members with ~30% of currently marketed drugs targeting them. The cannabinoid receptors, CB1 and CB2 are two GPCRs involved in many important physiological processes. Emerging evidence has demonstrated that CB2 is involved in a number of diseases, including neurodegenerative disorders and various cancers, which make it an attractive pharmacological target. Classically, a protein active site or orthosteric binding site where the endogenous ligand binds to, is used as a target for the design of most small molecule drugs. This can become a problem when it comes to biologically similar proteins, such as the cannabinoid receptors as evolutionarily, their orthosteric binding sites are similar. An alternative option is to target sites that are unique to the receptor that still impact receptor function, termed allosteric sites. These sites are distinct between similar receptors like CB1 and CB2. Targeting allosteric sites is akin to picking the lock of these hidden grooves within the CB2 protein and is a crucial step towards selectivity and specificity for drug design and development. With the recent establishment of the human cannabinoid receptor 2 X-ray crystal structure, this has aided the prospective discovery of a CB2 allosteric site using computational software. In vitro signalling assays using known allosteric modulators and CB2 agonists have been used to verify the in-silico data. This potential identification provides promising outcomes for the development of selective and specific CB2 ligands for anticancer therapeutics.

Functional effects of PD-1/PD-L1 interactions on endothelial cells

Anitha S Nair, *Guosu Wang, Federica Marelli-Berg*

Programmed death-1 (PD-1) receptor and its ligand PD-L1 are key components of the T-cell co-inhibitory pathway. PD-1 is expresssed on activated T-cells, while PD-L1 is expressed on both T-cells and antigen presenting cells. Together they play a critical role in restraining T-cell activity in the event of an overwhelming alloimmune response. PD-L1 is constitutively expressed by endothelial cells (ECs) at basal levels and is upregulated in response to inflammatory stimuli like IFN-γ. In solid organ transplantation, ECs are the first target cells encountered by the recipient immune system, but the role of PD-1/PD-L1 interactions during allorecognition of the endothelium has only been elucidated in T-cells. The resultant effect on EC is not yet known as PD-L1 was thought to not contain any intracellular signalling domains. However, It has been recently discovered that PD-L1 contains signalling motifs capable of transducing intracellular signals upon activation. Therefore, the overall goal of this project is to study the functional and metabolic effects mediated by PD-1/PD-L1 engagement during cognate and non-cognate T-cell interactions with EC.

To study this *in vitro*, we used recombinant PD-1 to stimulate the PD-L1 pathway in IFN-γ activated mouse lung microvascular ECs. We found PD-L1 signalling to adversely affect key EC functions such as migration, proliferation and viability by targeting mitochondrial function and glycolysis. Interestingly, we also found chronic PD-L1 signalling induced EC senescence resulting in the release of pro-inflammatory mediators. *In vivo* studies showed PD-L1 gene deficiency and PD-L1 blockade in mice had a protective effect against bacterial superantigen mediated vascular leakage, which indicates PD-L1 signalling to have a negative effect on EC barrier function. Altogether, the current findings indicate, PD-L1 signalling may lead to endothelial dysfunction in chronic T-cell mediated conditions like chronic graft rejection.

GROWTH HORMONE RECEPTOR 6Ω PSEUDOEXON ACTIVATION: A NOVEL CAUSE OF SEVERE GROWTH HORMONE INSENSITIVITY

Emily Cottrell,[1] Avinaash Maharaj,[1] Jack Williams[1], Sumana Chatterjee,[1] Grazia Cirillo,[2] Emanuele Miraglia del Giudice,[2] Adalgisa Festa,[2] Stefania Palumbo,[2] Donatella Capalbo,[3] Mariacarolina Salerno,[4] Claudio Pignata,[4] Martin O. Savage,[1] Katharina Schilbach,[5] Martin Bidlingmaier,[5] Vivian Hwa,[6] Louise A Metherell,[1] Anna Grandone,[2] Helen L Storr[1]

Context: Severe forms of Growth Hormone Insensitivity (GHI) are characterized by extreme short stature, dysmorphism and metabolic anomalies. They are classically caused by homozygous or compound heterozygous mutations of the Growth Hormone Receptor (*GHR*).

Objective: Identification of the genetic cause of growth failure in 3 'classical' GHI subjects.

Design: A novel intronic *GHR* variant was identified using our GHI targeted whole genome custom gene panel. *In vitro* splicing assays were performed to confirm aberrant splicing. Patient fibroblast analysis confirmed the presence of GHR pseudoexon in cDNA transcripts. A 6Ω pseudoexon *GHR* vector created by Gibson assembly enabled us to assess the functional consequence of the novel pseudoexon inclusion.

Results: We identified a novel homozygous intronic *GHR* variant (g.5:42700940T>G, c.618+836T> G), 44bp downstream of the previously recognized intronic 6Ψ *GHR* pseudoexon mutation, in the index patient. In the second kindred, two siblings were found to harbour this novel intronic 6Ω pseudoexon *GHR* variant in compound heterozygosity with the known *GHR* c.181C>T (R43X) mutation. *In* vitro splicing analysis confirmed inclusion of a 151bp mutant 6Ω pseudoexon not identified in wild-type constructs. Inclusion of the 6Ω pseudoexon causes a frameshift resulting in a non-functional truncated GHR lacking the transmembrane and intracellular domains. Our experiments using the truncated 6Ω pseudoexon protein demonstrated extracellular accumulation and diminished activation of STAT5B signalling following growth hormone stimulation.

Conclusion: Novel *GHR* 6Ω pseudoexon inclusion results in loss of GHR function consistent with a severe GHI phenotype. This represents a novel mechanism of Laron syndrome and is the first deep intronic variant identified causing severe postnatal growth failure. The two kindreds originate from the same town in Campania, Southern Italy, implying common ancestry. Our findings highlight the importance of studying variation in deep intronic regions as a cause of monogenic disorders.

Investigating the genetic interplay between adult height and cardio-metabolic traits and disease

Areti Papadopoulou, Sridharan Raghavan3,4,5, Elizabeth Litkowski6,7, Kari North8, Panos Deloukas1,2,9, Eirini Marouli1,2

Abstract

We are conducting a large-scale meta-analysis (GWAS) for anthropometric traits including height, BMI and Waist to Hip Ratio (WHR) in up to 4M individuals under the Genetics of Anthropometric Traits – _GIANT- consortium.

In a parallel study stemming from the above, we aim to identify traits and disease related outcomes that are affected by genetically determined height. We employed Phenome Wide Association Studies (PheWAS) analysis, using as exposure a Predictive Risk Score (PRS) for height. The score is based on 6,772 conditionally independent height variants, based on a transethnic meta-analysis in up to 2,427,800 individuals, explaining 52.29% of phenotypic variance for height in the UK Biobank. We examined health-related outcomes from Hospital Episode Statistics (HES) and General Practice data from the UK Biobank (N= 311,195) and the Million Veteran Project (MVP) (N=217,225). We also performed a meta-analysis of the data obtained from the two cohorts including 1,421 phecodes.

The meta-analysis results from UK Biobank and Million Veteran Project (MVP) HES data yielded 162 significant signals. Height PRS was positively associated with the majority of circulatory diseases like atrial fibrillation and flutter (OR = 1.17, 95% CI [1.15, 1.18], pvalue=5.22E-183), varicose veins (OR = 1.16, 95% CI [1.14, 1.17], pvalue=5.89E-97) and cardiac dysrhythmias (OR = 1.07, 95% CI [1.06, 1.08], pvalue=5.08E-65). The score was negatively associated with most of endocrine/metabolic diseases like hyperlipidaemia (OR = 0.93, 95% CI [0.92, 0.94], pvalue=2.53E-76) and hypercholesterolemia (OR = 0.94, 95% CI [0.93, 0.95], pvalue=6.27E-50). Height PRS was positively associated with the majority of musculoskeletal diseases such as osteomyelitis (OR = 1.17, 95% CI [1.14, 1.21], pvalue=5.89E-97) and arthropathy (OR = 1.36, 95% CI [1.27, 1.46], pvalue=2.60E-19). The score was negatively associated with obstructive chronic bronchitis (OR = 0.95, 95% CI [0.94, 0.97], pvalue=4.18E-09). We will further interrogate these signals through Mendelian Randomisation and explore the mediatory pathways.

Targeting purinergic signalling in pancreatic cancer

Elena Tomas Bort[1,2], Peter McCormick[2], Richard Grose[1]

Pancreatic cancer is a deadly disease with limited responses to new and conventional anti-cancer treatments, in part due to its characteristically dense fibrosis. There is a need to understand the cancer-protective mechanisms of fibrosis to discover possible therapeutic targets. One pathway known to be cytoprotective in highly hypoxic environments is purinergic signalling, but little is known about its effects in pancreatic cancer. This pathway is highly druggable and affects relevant biological processes, including immune cell behaviour, cell proliferation and invasion. The expression of key purinergic signalling genes in pancreatic cancer patients was analysed using public databases. Gene expression was correlated to clinical data to identify possible targets, with the purinergic receptor P2Y2 showing a significant decrease in survival when expressed. The significance of this receptor has been explored using pharmacological and gene knockdown approaches in a 3D multicellular spheroid model of pancreatic ductal adenocarcinoma. P2Y2 was shown to have a link to cytoskeletal changes and cancer cell invasion. Further research is needed to understand the mechanisms underlying the role of P2Y2 in pancreatic cancer invasion.

A murine model of OA that reproduces clinical features of joint degeneration and their sex differences

Sabah Barde, Shafaq Sikandar

Pain is the leading cause of disability in the degenerative joint disease osteoarthritis (OA), but functional correlations between structural integrity of the joint and pain remain poorly understood. Preclinical models that can mimic the progressive nature of joint degeneration and the temporal delay in the development of chronic pain is necessary to improve translational success of new drug targets for pain in OA[1,2]. Moreover, several studies show a higher prevalence of chronic pain in the female OA population[3,4,5] and sex differences that can give rise to distinct pain mechanisms in male and female rodents with OA[6,7,8].

We aim to define the correlations between structural and functional outcomes in a murine model of surgically-induced OA with meniscal/ligamentous injury (MLI). We characterised temporal changes in neuronal hypersensitivity and joint integrity using a combination of behaviour, *in vivo* electrophysiology, joint histology and quantification of gene expression in sensory neurones. Male and female mice were studied to identify possible sex differences in OA pain.

Our data provides functional and structural phenotyping of the knee joint, as well as quantification of the plasticity of nociceptive neurones in a surgically-induced OA model. PCA analysis identified key structural outcomes that highly correlate with altered nociceptive function. Our results validate the use of MLI surgery as a clinically relevant mouse model for OA pain. Additionally, the severity and onset of pain behaviours differed between male and female MLI mice, which may be attributed to differences in the molecular response to joint destabilisation.

Probe Confined Dynamic Mapping for GPCR Allosteric Site Prediction
Amandeep Kaur Gill

Identifying drugs that are able to bind to the allosteric site and activate specific G Protein-Coupled Receptors (GPCRs), with a reduced likelihood of unwanted off-target side effects, is advantageous when treating a variety of diseases. This is due to the fact that orthosteric sites tend to be highly conserved amongst receptor subtypes, leading to significant side effects. The challenge with targeting allosteric sites is they are not readily identifiable on any given receptor and often, there are more than one. The advancements in structural biology have enabled identification of allosteric sites, as seen in the publications of various crystal structures of GPCRs with the allosteric ligand bound. However, identifying *all* plausible allosteric sites of GPCRs has been challenging. In this study, we tested a recent algorithm developed in conjunction with our co-authors that can predict allosteric sites of GPCRs on the extracellular side, intracellular side and receptor-lipid interface. The algorithm was applied to predict the allosteric site of the UCB compound at the D_2 receptor. The D_2 receptor is a validated drug target in both neurology and psychiatry. Selectively targeting the D_2 receptor could open up a plethora of opportunities for designing selective allosteric modulators that could be used in the treatment of CNS disorders. Key residues believed to constitute the allosteric site were first identified by the algorithm. Site-directed mutagenesis studies were then performed, following by *in vitro* cAMP signalling assays to validate this prediction. The results showed that the predicted site by the algorithm was indeed correct. Therefore, this novel algorithm is an efficient, useful and fast method by which putative allosteric sites of GPCRs can be identified and can be used to guide drug design of allosteric drugs.

Neutrophil elastase is A Novel Therapeutic for Aortic Dissection

Xinmiao Zhou, Mei Yang, Stuart Pearce, Jun Luo, Wei Wu, Kaiyuan Niu, Chenxin Liu, Guanmei Wen, Qingzhong Xiao

Background and Purpose: Thoracic aortic dissection (TAD) is a lethal aortic pathology with acute onset, minimal prior symptoms, and a poor prognosis for long term survivors. Treatment of this fatal vascular disease needs to be achieved by timely interventional therapy and surgical repair, but medical treatment has not yet achieved good clinical results. The fundamental reason for a lack of effective treatment is that the underlying pathological mechanisms responsible for AD remain elusive. Neutrophil elastase (NE) has potent proteolytic activity on various extracellular matrix (ECM) proteins, as well as a variety of non-matrix proteins. It has been suggested that NE plays a central role in many human diseases including atherosclerosis. In this study, we investigated the causal role of NE in TAD, and further explored the therapeutic potential of targeting NE in TAD.

Methods: NE knockout (NE_KO: $NE^{-/-}$/ $ApoE^{-/-}$) and control (WT: $NE^{+/+}$/ $ApoE^{-/-}$) mice were used to investigate the direct causal role of NE in TAD development, and NE pharmacological inhibitor GW311616A was used to demonstrate the therapeutic potential of NE inhibition in TAD. Proteomic analysis was performed to identify potential NE targets in TAD. RT-qPCR, Western Blot and immunostaining analyses were performed to examine gene/protein expression in human and murine TAD aortas.

Results: TAD was successfully induced by BAPN administration. We found that NE gene expression and activity was significantly increased in aortic tissues and plasma in mice treated with BAPN compared with mice received saline treatment. Importantly, compared to the WT mice, a significant decrease of TAD incidence, mortality, and elastin breaks was observed in NE_KO mice. Similar phenomenon was observed with GW311616A treatment in WT mice. Aortic proteomic and Western blot analysis showed increased levels of F-box-like/WD repeat-containing protein TBL1X, but decreased levels of immunity-related GTPase family M protein 1 (IRGM1) and leukotriene A-4 hydrolase (LTA4H or LKHA4) in the NE_KO aorta treated with BAPN. Moreover, aortic gene expression data showed that the expression levels of Irgm1, Lkha4, IL1β, IL6, and Ccl2, but not Tbl1x, were significantly decreased in NE_KO aorta.

Conclusion: We provide new insight into the molecular mechanisms underlying TAD and identify NE as a potential therapeutic for TAD.

Disrupted resolution mechanisms contribute to altered phagocyte responses in COVID-19

Duco S. Koenis[1], Issa Beegun[1], Gabriel Amador Aguirre[2], Patricia R. Souza[1], Charlotte Jouvene[1], Maria Gonzalez-Nunez[1], Lucy Ly[1], Kimberly Pistorius[1], Hemant M. Kocher[2], William

ABSTRACT

Background: Resolution mechanisms play a central role in both tissue maintenance and the return to homeostasis following injury and/or infections. Specialized pro-resolving lipid mediators (SPM) are essential fatty acid-derived signalling molecules that modulate immune cell responses to promote inflammatory resolution and limit disease severity. Notably, little is known about the relationship between the expression and activity of SPM pathways, circulating phagocyte function, and disease severity in patients infected with novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) leading to coronavirus disease 2019 (COVID-19). Here, we investigated the link between circulating SPM concentrations and phagocyte activation status and function in COVID-19 patients compared with healthy volunteers.

Methods and Results: Lipid mediator profiling demonstrated an activation of SPM pathways in patients with COVID-19 that becomes dysregulated with increasing disease severity. Plasma SPM concentrations were correlated with both circulating phagocyte activation status and function. Perturbations in plasma SPM concentrations and phagocyte activation were retained after the resolution of COVID-19 clinical symptoms. Treatment of patients with dexamethasone upregulated both the expression of SPM biosynthetic enzymes in circulating phagocytes as well as plasma concentrations of these mediators. Furthermore, incubation of phagocytes from COVID-19 patients with SPM rectified their phenotype and function. This included a downregulation in the expression of activation markers, a decrease in pro-coagulation factor and inflammatory cytokine expression, and an upregulation of bacterial phagocytosis.

Conclusions: Taken together, these findings highlight the role of altered resolution mechanisms in the disruption of phagocyte responses and the propagation of systemic inflammation in COVID-19.

Revealing the key players involved in achieving the satiety state via the activation mechanism of the MC4 receptor

Vidicha Chunilal, Peter McCormick

Obesity is a global epidemic that continues to contribute to chronic disease and disability with over 40% of the adult population classified as overweight or obese worldwide. The melanocortin receptor 4 (MC_4R) is known to be a central player in regulating appetite and energy homeostasis, and loss or gain-of-function mutations have been linked to obesity. Thus, MC_4R is recognised to be a major target for anti-obesity drugs. Our work has revealed the mechanism of MC_4R activation with the revealing of a 3 Å resolution cryo-EM structure of the human MC_4R G_s-coupled complex bound to a cyclic peptide setmelanotide (Imcivree): a recently approved FDA drug for the treatment of genetically triggered obesity. Our findings suggest that Ca^{2+} is an essential ion, that is required to achieve full agonist efficacy, but not in the case of antagonists at MC_4R. Three acidic residues on MC_4R were recognised to be co-coordinating with a Ca^{2+} ion, and mutagenesis studies further confirmed the importance of these residues for achieving full receptor activation. Interestingly, our results have also identified a unique molecular switch in the brain that can initiate satiation. Overall, our data will not only help identify patients that are likely to benefit from the current therapy but will also provide a strong base for future work revolving around the development of novel therapeutics to treat obesity and potentially other disorders linked to the MC_4R.

Inhibition of Bruton's tyrosine kinase reduces organ injury and dysfunction in a rat model of severe haemorrhage

Authors: Nikita Mayur Patel[1], Filipe Rodolfo Moreira Borges Oliveira[2], Hanna Pillmann Ramos[2], Eleonora Aimaretti[3], Gustavo Ferreira Alves[3], Massimo Collino[3], Regina Sordi[2], Christoph Thiemermann[1]

Background: Trauma and/or haemorrhagic shock (HS) drive an excessive systemic inflammatory response, which contributes to multiorgan failure (MOF), and is the main cause of death in the late post-injury phase. There is no specific therapy for MOF. Bruton's tyrosine kinase (BTK) is known to play a role in the activation of the NLRP3 inflammasome which is a key component of the innate inflammatory response. However, its role in trauma-haemorrhage is unknown. BTK activity can be blocked by acalabrutinib (irreversible) and fenebrutinib (reversible). We hypothesised that inhibition of the effects of BTK would reduce MOF in two rat models of HS.

Methods: Male Wistar rats were subjected to HS by withdrawal of blood from the carotid (acute model) or femoral (chronic model) artery to maintain MAP at 35±5mmHg for 90min. Resuscitation was initiated by rapid infusion of shed blood plus Ringer's lactate. Animals received acalabrutinib (3mg/kg), fenebrutinib (3mg/kg) or the vehicle (5% DMSO, 95% Ringer's lactate). At 4h (acute model) or 24h (chronic model) after resuscitation, organ injury and dysfunction were evaluated by measuring creatinine, urea (renal dysfunction), ALT, AST (liver injury) and LDH (general organ injury). Pulmonary and hepatic myeloperoxidase activity were determined as an indicator of neutrophil infiltration. The activation of BTK, NF-κB and NLRP3 pathways were analysed by western blot. Statistical analysis: One-way ANOVA followed by Bonferroni's *post-hoc* test; level of significance $p<0.05$.

Results: When compared to sham, HS resulted in significant increases in organ damage, hypotension (post resuscitation), myeloperoxidase activity and activation of BTK, NF-κB and NLRP3 pathways. Treatment with either acalabrutinib or fenebrutinib significantly attenuated these rises ($p<0.05$).

Conclusion: The results point to a role of BTK in the pathophysiology of organ injury and dysfunction caused by trauma-haemorrhage and indicate that BTK inhibitors may be a potential therapeutic approach for MOF after trauma and/or haemorrhage.

Defining the adaptive immune response in Autoimmune Myocarditis.

Silvia Fanti, Prof Federica Marelli-Berg

Background:

Myocarditis is a potentially life-threatening disorder that is challenging to diagnose and treat. T cell-mediated autoimmunity is now recognized as a key mechanism in myocarditis. Under physiologic conditions, regulatory T cells maintain peripheral tolerance and prevent spontaneous myocarditis development, but damaged tissue and activation of the innate immune system promotes CD4+ effector T cell expansion and myocarditis progression. Understanding the specific roles of these T cell populations at different stages of the disease might provide a key for the development of successful therapeutic strategies. Our group has previously identified a subset of memory T cells, called cardiotropic T cells (cT cells), which is characterised by the expression of the hepatocyte growth factor receptor, c-Met, and selectively localise to the inflamed heart muscle. Circulating cT cells are present in murine models of heart allograft rejection and in patients with acute myocarditis. In this study, we have investigated the phenotype and functional changes of cT cells during the development of murine experimental autoimmune myocarditis, which recapitulates human disease.

Aims:

The overall goal of this study is to set up a model of myocarditis which can be used to investigate the autoimmune response leading to dilated cardiomyopathy in autoimmune myocarditis (AM) and to validate new immunomodulatory therapies.
The specific aims are:
1. To characterise and define the immune response mediated by cT cells during the progression of the disease.
2. To test the therapeutic potential of targeting cardiotropic T cells.

Results and conclusions:

Our investigation on recirculating cMet+ memory cT cells shows that they are implicated in the pathogenesis of cardiac autoimmunity. We observed that cMet+ T-cells are present in the blood and inflammatory infiltrates of murine heart tissue. cMet+ T-cells respond to the autoantigen cardiac myosin and produce a cytokine pattern similar to that observed in human myocarditis.
cMet+ T cells display a unique functional phenotype that includes co-production of cytokines such as IL17, IL13, and IL22.
Our experiments also show increased IFNγ production by cMet-negative T cells in AM. The role of this cytokine in myocarditis is still unclear.
We found that following immunisation with the autoantigen, cardiac myosin, effector cT cells in the treatment with PHA-665752 a selective c-Met inhibitor, inhibited the autoimmune response, leading to a reduction of fibrous tissue and myocarditis regression.

Overall, our data suggest that cMet+ cT cells are key mediators in the development of murine experimental acute myocarditis and that they have the potential to be utilized as a novel therapeutic target for myocarditis in humans.