Faculty of Medical Sciences

FMS Postdoc Society

FPoS

Athena SWAN Silver Award

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NUBI

3rd FMS Postdoc Symposium

15 min research talks from FMS postdocs
Flash poster talks
Best poster/talk and paper prize
Networking
Social Evening Drinks with FPoS team

All welcome!!
See you soon, FPoS team.

https://www.societies.ncl.ac.uk/fmspostdoccomm/

Professor Selina Wray

"Understanding mechanisms of Alzheimer's disease & Frontotemporal Dementia using patient-derived stem cells"

Professor of Molecular Neuroscience and Alzheimer's Research UK Senior Research Fellow at the UCL Queen Square Institute of Neurology

Tuesday 13th September 2022

9am-6pm

David Shaw Lecture Theatre – Medical School

Abstract Submission Deadline: 30th June 2022

Registration

Abstract and Best paper Submission


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investigate
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FMS Post Doc Symposium 2022

After the resounding success of the FMS Post-doc symposiums prior the pandemic (and after a two-year hiatus), we are finally back for the third FMS Post Doc Symposium to once again showcase the outstanding contributions and research of post-doctoral staff across all institutes. The symposium is organised by the Post-Doctoral Committee formed in 2017. We are delighted to have a keynote talk from **Prof. Selina Wray**, Professor of Molecular Neuroscience and Alzheimer's Research UK, Senior Research Fellow at the UCL Queen Square Institute of Neurology.

Our keynote speaker will also present a **prize for best paper (including a £150 Amazon voucher).** Prizes will also be awarded for **best talk** and for **best poster.**

We have secured generous sponsorship from PCR Biosystems, New England Biolabs, Starlab, ThermoFisher Scientific, Cambridge Research Biochemicals, Takara Bio, Merck, and Qkine who provided financial support for the symposium, material for the conference bags as well as sponsorship for the best paper, talk and poster prizes.

We hope you will enjoy the day!

Please use the symposium hashtag **#NUFMSPostDocSymp2022** to follow the event!

You can vote for the best poster! Go to: https://www.surveymonkey.co.uk/r/SBVBNWK, or scan the QR code below and input the poster number of your choice!

FMS Post-Doctoral Committee

Established in 2017, we are here to organise events, provide support and information, and represent post-doctoral researchers' views to the faculty. Here you will find a list of your local institute representatives on the committee, as well as upcoming events, and important links to faculty resources.

We are continuously eager to hear from PDRAs from all institutes of FMS and open to shape the role of this committee in response to your needs, so please share your opinion via your local institute representatives, by emailing: fmspostdoccomm@newcastle.ac.uk, or using the contact form on our website. If you have some ideas or would like to join us, please also get in touch. We are always welcoming to new faces and new ideas!

http://goo.gl/zeQmgN

@fms_postdoccomm ♯nclfpos

Website: https://www.societies.ncl.ac.uk/fmspostdoccomm/
Facebook: FMS Post-Doctoral Committee

FMS PostDoc Society Committee List		
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Theo Robert		
NEW RECRUITMENTS NEEDED – HAPPY TO JOIN??		

Acknowledgements

We would like to thank everyone who helped us to make this event happen.

- FMS and all sponsors for financial support of the symposium
- Kay Howes, Becky Burr, Trish Durham, Nicola Charlton, Stephanie Maughan, Jill McKenna and Judith Williams - for advice and help in organisation
- New England Biolabs, Cambridge Research Biochemicals and PCR Biosystems without whom the prizes would not be so generous
- All the academics who helped with the best paper selection: Dr James Connolly, Dr Adam Wollman, Dr Claudia Schneider, Dr Paul Donaghy, and Dr Lisa Russell
- Rui Chen for being our photographer

FMS PostDoc Symposium 2022

08:30-09:30 **Coffee and Registration**
09:30-09:40 **Welcome and Opening Remarks from the Dean Prof. Fiona Matthews**
09:40-10:40 **Morning Session**

Maria Emilia Dueñas (Biosciences): Developing and applying novel mass spectrometry approaches for drug discovery in idiopathic pulmonary fibrosis
Elizaveta Olkhova (NUTCRI): Characterisation of a novel mouse model recapitulating neurological features of mitochondrial disease
Eman Zied (PHSI): Spatial Determinants of Public Health: An Example from The Impact of The Environment and Pollution on Cognitive Health (EPOCH) Project
Florence Burte (Biosciences): Investigation of human uteroplacental tissue-specific protein signatures to assist in the development of targeted treatments for pregnancy complications

10:40-11:00 **Coffee Break in the Sponsors' Foyer**
11:00-12:00 **Midday Session**

Nicole Kattner (NUTCRI): Impact of cystic fibrosis on the endocrine compartment of the pancreas
Graham McLelland (PHSI): Development of an intervention to reduce ambulance on-scene times for suspected stroke patients
Marzena Kurzawa-Akanbi (Biosciences): Extracellular vesicles – a novel role as disease messengers in age-related macular degeneration
Laura Smith (NUTCRI): Dysfunction and degeneration of inhibitory interneurons underlying seizure activity in Alpers' syndrome

12:00-12:30 **Flash Poster Presentations: 2-Minutes Each**
12.30-12.45 Louise Wright: FMS Equality Project
12:45-13:00 PostDoc committee: Who are we and recruitment
13:00-14:00 **Lunch (Boardroom) and Poster Session (Sponsors' Foyer)**
14:00-15:00 **Keynote Speaker: Prof. Selina Wray, UCL, UK**
15:00-15:05 **Paper Prize Announcement**
15:05-15:30 **Best Post-Doc Paper Prize Talk**

Edward Fielder (Biosciences): Short senolytic or senostatic interventions rescue progression of radiation-induced frailty and premature ageing in mice

15:30-16:00 **Coffee Break**
16:00-17:00 **Afternoon Session**

Heather Moore (PHSI): Do parents and education professionals agree about the adaptive abilities of autistic children?
Javier Abellon-Ruiz (Biosciences): The hunger games, B12 uptake by gut microbiota
Marco Trevisan-Herraz (NUTCRI): A machine learning approach to analyse the relationship between mitochondria and the nuclear chromatin structure
Giang Nguyen (PHSI): Association between maternal adiposity measures and infant health outcomes: a systematic review and meta-analysis

17:00-17:30 **Poster and Talk Prizes & Closing Remarks**
17:30-19:00 **Drinks Reception**

Keynote Speaker- Prof. Selina Wray

"Understanding mechanisms of Alzheimer's disease and frontotemporal dementia using patient-derived stem cells"

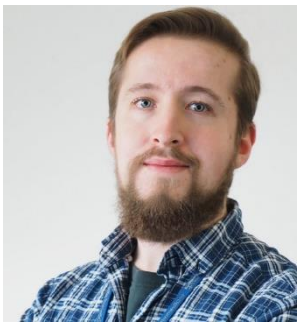
Selina Wray is a Professor of Molecular Neuroscience and Alzheimer's Research UK Senior Research Fellow in the Department of Neurodegenerative Disease at UCL Queen Square Institute of Neurology.

Selina received her degree in Biochemistry and Biological Chemistry from the University of Nottingham in 2004, before undertaking PhD training in Dr Diane Hanger's laboratory at the Institute of Psychiatry, Kings College London. Selina was awarded her PhD in 2008 and subsequently joined the laboratory of Professor John Hardy at UCL Institute of Neurology as an Alzheimer's Research UK Junior Research Fellow. Selina spent time as a visiting researcher in the groups of Tilo Kunath at the University of Edinburgh and Rick Livesey at the University of Cambridge. She was awarded an ARUK Senior Research Fellowship in 2017.

Selina's work is focussed on the use of induced pluripotent stem cell (iPSC) technology to model dementia, working closely with clinical colleagues to obtain samples from participants with rare, genetic forms of dementia and using these to understand the molecular basis of Alzheimer's Disease and Frontotemporal Dementia. In recognition of her research and public engagement, Selina was awarded the 2018 ARUK David Hague Early Career Investigator of the Year award and the 2014 Red Magazine Woman of the Year award in the Pioneer category.

Best Paper winner – Dr. Edward Fielder

(NUBI):



Short senolytic or senostatic interventions rescue progression of radiation-induced frailty and premature ageing in mice

(eLife 11:e75472)

DOI link: https://doi.org/10.7554/eLife.75492

Biography

Edward Fielder studied Biomedical Sciences here at Newcastle University, before working in the charity sector and as a senior constituency case-worker. He then started a Medical Research Council funded combined Masters/PhD at Newcastle, working on the contribution of senescence in neurons to brain ageing with Dr Diana Jurk and Dr João Passos. He obtained his PhD in 2018. Following this he worked with Dr Satomi Miwa & Professor Thomas von Zglinicki, and industrial partners, in developing assays to test novel compounds that specifically target senescent cells. He was the first ARROW fellow, and then worked for 2 years as a Knowledge Transfer Partnership associate. Since then he has continued his work in using these compounds to combat radiotherapy-induced frailty and accelerating ageing, funded by UK SPINE, NOVOS Labs, and now Procter & Gamble.

Paper Abstract

Cancer survivors suffer from progressive frailty, multimorbidity, and premature morbidity. We hypothesise that therapy-induced senescence and senescence progression via bystander effects are significant causes of this premature ageing phenotype. Accordingly, the study addresses the question whether a short anti-senescence intervention is able to block progression of radiation-induced frailty and disability in a pre-clinical setting. Male mice were sublethally irradiated at 5 months of age and treated (or not) with either a senolytic drug (Navitoclax or dasatinib + quer- cetin) for 10 days or with the senostatic metformin for 10 weeks. Follow-up was for 1 year. Treat- ments commencing within a month after irradiation effectively reduced frailty progression ($p<0.05$) and improved muscle ($p<0.01$) and liver ($p<0.05$) function as well as short-term memory ($p<0.05$) until advanced age with no need for repeated interventions. Senolytic interventions that started late, after radiation-induced premature frailty was manifest, still had beneficial effects on frailty ($p<0.05$) and short-term memory ($p<0.05$). Metformin was similarly effective as senolytics. At therapeutically achievable concentrations, metformin acted as a senostatic neither via inhibition of mitochondrial complex I, nor via improvement of mitophagy or mitochondrial function, but by reducing non- mitochondrial reactive oxygen species production via NADPH oxidase 4 inhibition in senescent cells. Our study suggests that the progression of adverse long-term health and quality-of-life effects of radiation exposure, as experienced by cancer survivors, might be rescued by short-term adjuvant anti-senescence interventions.

Talks

O1

Maria Emilia Dueñas
Biosciences

Developing and applying novel mass spectrometry approaches for drug discovery in idiopathic pulmonary fibrosis

MALDI-TOF mass spectrometry has become a powerful tool for high-throughput screening (HTS) approaches in drug discovery, overcoming the shortcomings of conventional fluorescence label-based technologies. Most of the MALDI-TOF based HTS approaches have focused on in vitro assays with simple readouts, and have been limited mainly to peptide/protein-centric activity assays. Although, phenotypic cellular assays using MALDI-TOF MS are possible using higher molecular masses, the capability of MALDI-TOF to detect compounds in the low mass range is generally considered limited due to interference peaks brought by the matrix. Metabolomics-based drug discovery presents therefore an exciting challenge for MS analysis as the system becomes inherently more complex. Herein, we apply this technology for cellular assays, specifically to detect metabolites and lipids in a comprehensive, untargeted, and unbiased HTS approach for drug discovery in idiopathic pulmonary fibrosis (IPF).

Primary human small airway epithelial cells were used to develop a cellular assay pipeline for untargeted metabolite phenotypic identification. Multiple IPF-relevant stimuli and inhibitors were tested to see if stimulation and inhibition could be distinguished in the assay. Next, different sample preparation conditions were investigated to ensure the most effective analysis for metabolites and lipids. All the parameters were optimised using liquid handling robot to allow a systematic screening of a large number of combinations to find the best conditions.

Preliminary testing revealed spectra that could be distinguished between the unstimulated, stimulated cells, and stimulated cells with inhibitor, in both the low and high mass region. Using principal component analysis, hierarchical clustering, and machine learning strategies, a subset of peaks was identified to be unique to each condition. These data suggests that it is possible to elucidate important metabolic features of cells in modelled pathophysiology. This approach has the potential to be further optimised as an automated HTS drug discovery assay in the industrial setting.

O2

Elizaveta Olkhova
NUTCRI

Characterisation of a novel mouse model recapitulating neurological features of mitochondrial disease

Introduction: Mitochondrial diseases comprise the largest group of inherited metabolic disorders. Neurological symptoms include epilepsy, stroke-like episodes, ataxia, and cognitive impairment. Previous post-mortem neuropathological studies implicated severe oxidative phosphorylation (OXPHOS) deficiencies in GABAergic inhibitory interneurons accompanied by neurodegeneration in mitochondrial disease. This study aims to model juvenile-onset neurological symptoms in mitochondrial disease using a novel murine model and to test the hypothesis that underlying hyperexcitability may arise due to neuronal network disinhibition and that metabolically demanding fast-spiking parvalbumin-expressing (PV+) interneurons are susceptible to mitochondrial dysfunction.

Methods: A novel mouse model of mitochondrial DNA (mtDNA) depletion selectively within the PV+ cells was generated by a conditional knockout of mitochondrial transcription factor A (Tfam) via cre-loxP system. Mice were characterised at behavioural, electrophysiological, neuropathological and molecular levels. A battery of behavioural tests was used to phenotype the mice: open field, rotarod, novel object recognition, elevated plus-maze, and visual cliff tests.

Results: Mutant mice exhibited a progressive phenotype, initiating at 8 weeks of age with tremor, cognitive impairment (novel object recognition test) and anxiety-like behaviour (elevated plus-maze test). Hyper-locomotion and stargazing (absence-like seizures) were detected at 10 weeks, with severe ataxia observed by 12-13 weeks. A downregulation of OXPHOS subunits of complexes I, III and IV was confirmed within the PV+ cells of the knockout mice with differential deficiency levels observed that were brain region dependent. PV+ interneurons demonstrated an upregulation of anaplerosis enzyme pyruvate carboxylase, demonstrating metabolic remodelling in response to OXPHOS deficiency. The most affected neurons were Purkinje neurons of the cerebellum, which showed a reduction in mtDNA copy number and neurodegeneration, which was accompanied by reactive microgliosis and astrogliosis. Mutant mice had reduced weight and severely shortened lifespan. The novel mouse model recapitulates key features of neurological mitochondrial disease phenotype and could be used as a powerful translational preclinical model.

O3

Eman Zied
PHSI

Spatial Determinants of Public Health: An Example from The Impact of The Environment and Pollution on Cognitive Health (EPOCH) Project

Where people live and what amenities they can access has been shown to influence their health throughout the life course. Due to this, spatial and social inequalities can often manifest as health inequalities. It is important to evaluate the built environment attributes of neighbourhoods (the immediate geographical areas surrounding individuals' homes) and their influences on population health. Although many studies have focused on specific features of the built environment such as points of interest (e.g., doctors, grocery, leisure) and green/blue spaces, accessibility of amenities can be determined by the street network, which affects people and their movement across different locations. A well-connected, walkable, dense neighbourhood with a wide range of land uses has been found to promote physical activity in older people and may also have a positive influence on their cognitive health.

This study focuses on the potential impact of the built environment on cognitive function, one of key aspects in healthy ageing. Using a population-based cohort study of over 7500 people aged 65 or above in the Cognitive Function and Ageing Study II (CFAS II), geographic information system (GIS) data were used to generate different measures of the built environment for each participant's immediate neighbourhood in Newcastle upon Tyne, Nottingham, and Cambridgeshire. These measures included street network connectivity and density, urban blocks size and shape, and land use mix. The associations between baseline cognitive function and the built environmental measures were examined using statistical modelling and adjusting for sociodemographic factors.

This work brings together geographic analysis and epidemiological cohort studies to understand how the built environment may support cognitive health in later life. It can potentially contribute to the body of knowledge in spatial and health inequalites and environmental determinants of health in ageing populations.

O4

Florence Burte
Biosciences

Investigation of human uteroplacental tissue-specific protein signatures to assist in the development of targeted treatments for pregnancy complications.

Pre-term birth, occurring before 37 gestational weeks, is a major pregnancy complication. WHO estimates there are 15 million preterm births per annum accounting for ~1 million neonatal deaths. Newborns that survive may suffer long-term physical or mental health issues and mothers are also at risk of post-partum complications. The aetiology of preterm birth is complex and encompasses a broad spectrum of uteroplacental dysfunctions, and ~50% of cases are due to premature uterine contractions with unknow origin. Current therapeutic strategies focus on the latter with the intent to delay labour initiation. However, there is no international consensus on the use of these tocolytics, effectiveness is limited and they are accompanied with a risk of maternal and fetal adverse effects due to vaso-cross-reactivity. We hypothesize that identification of the molecular signatures that define maternal and placental tissue-specific phenotypes could assist with directing future drug development strategies.

As part of the Newcastle Human Uteroplacental Tissue Bank (1616/NE/0167), we have collected three types of tissues from healthy pregnant women at the time of elective Caesarean sections: matched uterine smooth muscle, myometrial arteries and placental arteries. These were homogenised, tripsinized and processed for quantitative proteomics using LC-MS/MS acquired in data independent acquisition mode, followed by statistical and pathway enrichment analyses.

Amongst over 3,000 proteins quantified, the three tissue types showed distinct proteomic profiles, with placental artery profiles the most distinguishable from the other two. Proteins involved in electron transport chain, oxidative stress, extracellular matrix and regulation of transcription were differentially expressed between the three tissues. Also, myometrial and placental arterial tissues exhibited differences in expression of myofilament- and inflammatory-related processes.

This work points towards protein signatures underlining uteroplacental tissue-specific phenotypes that will increase our understanding of physiological remodelling events of pregnancy and possibly inform the development of more efficacious therapeutic strategies for pregnancy complications.

O5

Nicole Kattner
NUTCRI

Impact of cystic fibrosis on the endocrine compartment of the pancreas

Cystic fibrosis (CF) occurs due to a defect in the CF conductance regulator impairing ion transport across the channel and leading to mucus accumulation in epithelium of lungs, gut and the pancreas. This can lead to severe changes in pancreatic exocrine and endocrine compartments and ultimately to CF related diabetes in up to 50% of adults with CF. This is reported to be the most common co-morbidity in people with CF, however the pathogenesis is still poorly understood. We aim to investigate if the pancreatic disease progression in CF is impacting on the proportions of the hormone distribution as well as beta- and alpha-cell identity.

We are investigating a cohort of nine CF pancreata obtained post-mortem (age range: premature to 27 years; gender: 6/9 male). The progression of CF in the pancreas was classified into early, advanced and endstage disease according to morphology on H&E sections. Staining for the four predominantly present endocrine hormones insulin, glucagon, somatostatin and pancreatic polypeptide (PP) was assessed on immunohistochemistry double staining on five randomly selected hormone positive areas. The proportion of total measured endocrine area was calculated. Immunofluorescence staining for insulin, glucagon and vimentin was performed for more specific beta-/alpha-cell analysis.

Proportion of insulin, glucagon, or somatostatin expression was not significantly impacted by disease progression despite drastic changes to the exocrine compartment. Proportion of PP was higher in early disease compared to endstage, but the development stage and inclusion of the PP lobe has to be taken into consideration. Increased peri-islet fibrosis was associated with increase of an altered alpha-cell phenotype co-expressing glucagon and vimentin.

We conclude that not beta-cell loss, but possible changes in alpha-cell identity might be responsible for islet dysfunction observed in people with CF. Further phenotypic characterisation of the available tissue is ongoing.

O6

Graham McClelland
PHSI

Development of an intervention to reduce ambulance on-scene times for suspected stroke patients

Aims/objectives
Prehospital stroke care focusses on rapid access to specialist stroke units due to the time dependent nature of reperfusion therapies. One minute saved between onset and treatment can equate to 1.8 days of healthy life. However, regional and national data show increasing prehospital times for stroke patients. The aim of this study was to develop an intervention to reduce ambulance on-scene times (OST).

Methods
A convergent mixed methods approach was used. North East Ambulance Service (NEAS) clinicians were surveyed to collect data on stroke patient encounters, care interventions and timings. Survey responses were linked to corresponding electronic patient care records (EPCRs) to further describe pre-selected factors potentially contributing to ambulance OST. Interviews with ambulance clinicians used thematic analysis to explore decision making with stroke patients and factors contributing to OST.

Results/Findings
581 surveys were completed by 359 different clinicians, and linked with EPCRs, between July and December 2021. Median OST was 33 (IQR 26-41) minutes. Three modifiable activities were identified as contributors to extended OST. Advanced neurological assessments (median 34 vs 31 minutes, p=0.008); intravenous cannulation (median 35 vs 31 minutes, p=<0.001) and ECGs (median 35 vs 28 minutes, p=<0.001). Interviews were conducted with 13 paramedics between August and November 2021 and five themes contributing to OST were identified: Initial assessment and sources of information; Treatment and interventions; The environment; Hospital interactions; Changing practice. Survey, EPCR and interview data were used to develop an online training package to be delivered to NEAS staff.

Conclusions
This study identified multiple influences on clinician behaviour and OST. Three actions were strongly associated with longer OST for stroke patients in quantitative data and identified as potentially modifiable in qualitative data. These modifiable factors are being targeted in a training package intended to reduce prehospital OST for stroke patients.

O7

Marzena Kurzawa-Akanbi
Biosciences

Extracellular vesicles – a novel role as disease messengers in age-related macular degeneration

Background: Age-related macular degeneration (AMD) is a leading cause of blindness. Vision loss is caused by the progressive loss of the retinal pigment epithelium (RPE) and photoreceptors in the retina, and/or abnormal blood vessel growth damaging the retina. Drusen (extracellular debris) accumulation beneath the RPE is a characteristic feature of AMD. The RPE is the principal cell type affected in AMD and we hypothesized that signaling through RPE-produced extracellular vesicles (EVs) contributes to disease progression.

Methodology: We used AMD patient specific RPE with the Complement Factor H Y402H high-risk polymorphism to perform a comprehensive analysis of EVs, their cargo and role in AMD pathology. RPE EV secretion was assessed using state-of-the-art technologies. Analyses of total RNA, protein and lipids were used to identify the disease specific contents of EVs. Functional assays were employed to investigate the AMD RPE EV signaling to various cell types in the retina.

Results: Our analyses showed enhanced EV secretion in AMD RPE cells. We demonstrated that AMD RPE EVs carry RNA, proteins and lipids that reflect disease changes in the parental RPE and mediate key AMD pathological processes including oxidative stress, disruption of the cell architecture, and drusen accumulation. We showed that exposure of control RPE to AMD RPE EVs leads to the acquisition of key pathological AMD features, such as stress vacuoles, structural destabilization, and abnormalities in the morphology of the nucleus. Treatment of retinal organoids containing photoreceptor cells with AMD RPE EVs led to the disruption of morphology and features consistent with cytoprotective responses following injury. Furthermore, we showed that AMD RPE EVs stimulate vessel sprouting and can induce protein aggregation revealing their role in blood vessel growth and drusen formation, respectively.

Conclusions: Our findings indicate that AMD RPE EVs are potent inducers of AMD phenotype in the RPE and retinal cells.

O8

Laura Smith
NUTCRI

Dysfunction and degeneration of inhibitory interneurons underlying seizure activity in Alpers' syndrome

Introduction: Alpers' syndrome is a fatal paediatric mitochondrial disease resulting from depletion of mitochondrial DNA (mtDNA) and characterised by intractable epilepsy and severe neurodegeneration. Dysfunction and degeneration of inhibitory interneurons due to deficits in mitochondrial oxidative phosphorylation (OXPHOS) subunits is reported to underlie seizure-associated alterations in cortical activity within the occipital cortex in Alpers' syndrome. We hypothesised that parvalbumin-positive(+) interneurons, a neuronal class critical for inhibitory regulation of physiological cortical rhythms, would be particularly vulnerable in Alpers' syndrome due to the excessive energy demands necessary to sustain their fast-spiking activity.

Methods: We performed a quantitative neuropathological investigation of inhibitory interneuron subtypes (parvalbumin+, calretinin+, calbindin+, somatostatin interneurons+) in post-mortem brain tissues from fourteen patients with Alpers' syndrome, five sudden unexpected death in epilepsy (SUDEP) patients (to control for effects of epilepsy) and nine controls. A combination of immunohistochemistry and quadruple immunofluorescence assays were performed to quantify interneuron densities and OXPHOS subunit expression within individual interneurons.

Results: We identified a severe loss of parvalbumin+ interneurons and clear evidence of OXPHOS impairment in those that remained. Comparison of regional abundance of interneuron subtypes in control tissues demonstrated enrichment of parvalbumin+ interneurons in the occipital cortex, whilst other subtypes did not exhibit such topographic specificity.

Conclusions: These findings suggest the vulnerability of parvalbumin+ interneurons to OXPHOS deficits coupled with the high abundance of parvalbumin+ interneurons in the occipital cortex, are key factors in the aetiology of the occipital-predominant epilepsy that characterises Alpers' syndrome. These findings provide novel insights into Alpers' syndrome neuropathology, with important implications for the development of preclinical models and disease-modifying therapeutics.

O9

Heather Moore
PHSI

Do parents and education professionals agree about the adaptive abilities of autistic children?

Autistic children often experience adaptive functioning difficulties, i.e. difficulties with the practical, everyday skills that an individual needs to meet the demands of their environment. Traditionally, adaptive abilities of autistic children are measured through informant-report, often from parents. However, behaviour varies across settings, and context-specific reports should be considered. Limited and inconsistent results show low parent-education professional concordance, but no research has yet explored item level response variation, and research is limited on lower ability autistic children. Using a lower ability sample of autistic children, this study explored domain- and item-level concordance between parents and education professionals on the Vineland Adaptive Behaviour Scales-II (VABS-II), as well as patterns in reporting disparities. Data were available from 233 children, aged 3-11yrs, who took part in the Paediatric Autism Communication Trial-Generalised (PACT-G). Measures included parent and teacher VABS-II, as well as child nonverbal ability, language, and autism severity. Parents scored their children higher than education professionals on VABS-II ABC scores, as well as Socialisation and Motor Skills, but not Communication or DLS. Domain and item level agreement was low (~33% at the item level), but better on objectively measured behaviours. Higher child nonverbal ability improved concordance, predicting 10-35% of the variance in VABS-II scores. Autism severity and language were not significantly associated with discrepancy. Where disagreements occurred, education professionals identified emergent skills more and parents were more likely to rate present/absent. Parents and education professionals view the adaptive abilities of autistic children differently. They chose different item level rankings but it is not clear whether this is due to differences in perception/understanding, or context-dependent differences in performance, which future research should explore further. Multiple informant ratings are necessary to gain a full picture of a child's adaptive abilities when developing personalised interventions and support.

O10

Javier Abellon-Ruiz
Biosciences

The hunger games, B12 uptake by gut microbiota

The human gut microbiome is the highly complex community of microorganisms inhabiting the gastrointestinal tract that affects many aspects of human health. The vast majority of human commensal microbes is located in the distal gut with bacterial densities of ~1012/ml luminal content, making competition for resources likely severe. Therefore, bacterial fitness in the gut relies on the ability to compete for nutrients such as vitamins. To understand what makes a bacterium successful in the battle for these compounds, we need better insights into how the uptake of these nutrients occurs.

The Gram-negative bacterium Bacteroides thetaiotamicron (B. theta) is a prominent human gut symbiont which cannot synthesise vitamin B12 but possesses several B12-dependent enzymes making it an essential nutrient. Unlike the model organism E. coli, which has only one B12 uptake locus, B. theta has three vitamin B12 uptake loci, together containing ~25 proteins.

All three loci contain one copy each of the outer membrane transporter BtuB (BtuB1-3) as well as one copy of the lipoprotein BtuG (BtuG1-3), which is always located adjacent to BtuB on the genome. BtuG is absent in E. coli. Why does B. theta harbour this vast number of proteins? Are all of them involved in the vitamin uptake? What is the function of BtuG? Could this number explain the success of B. theta colonising the human gut?

Here we show that BtuG is a versatile corrinoid binder (including different forms of B12 and its precursors) which forms a stable complex with BtuB to uptake vitamin B12 in a cap-assisted mechanism. We propose that this lipoprotein-assisted active OM transport has evolved to increase competitiveness of bacteria in the human gut.

O11

Marco Trevisan-Herraz
NUTCRI

A machine learning approach to analyse the relationship between mitochondria and the nuclear chromatin structure

The data on the influence of mitochondria on nuclear chromatin architecture (and hence genome expression and regulation) is scarce and has only been considered globally, rather than gene specific. Understanding the correlation and influence of mitochondrial genome variants in the nuclear epigenome provides an additional key aspect of the mechanism of eukaryotic cells, as well as to the diseases where the energetic input provided by mitochondria plays an important role. In a previous project we developed Chromatinsight, a computational model that uses machine learning techniques to find consistent and statistically significant patterns in the epigenetic features of sets of samples. Here, we have adapted this model and its associated software to find which are the differential patterns in the nuclear chromatin that are specific to mitochondrial healthy variants.

To unveil these patterns, we have identified the mitochondrial variants in the Blueprint EpiVar ChIP-seq public datasets, which contain genome-wide information on the histone marks H3K27ac and H3K4me1 (key to find epigenetic features, such as active promoters and active enhancers) for three immune system cell types (neutrophils, monocytes and naive CD4 T cells) for approximately 200 healthy individuals.

Using Chromatinsight, we studied the epigenetic differential patterns between the main European mitochondrial variants (such as for mitochondrial haplogroups H, J, K, U, T, and the most frequent mtSNPs in the population), we have found four genomic regions (out of 14,858) containing differential patterns with 5% FDR, and nine genes (out of 46,257 gene definitions). Our results show that, although very subtle, there exist mtDNA variant specific chromatin patterns. Further developments in these computational techniques, along with more data, could help to reveal further differences. Our methodology is ready to analyse stronger perturbations in the chromatin architecture, such as the differences caused by pathogenic mitochondrial variants.

O12

Giang Nguyen
PHSI

Association between maternal adiposity measures and infant health outcomes: a systematic review and meta-analysis

Maternal obesity significantly increases risks of adverse infant health outcomes including large-/small-for-gestational-age (LGA/SGA), pre-/post-term delivery, infant morbidity/mortality and long-term obesity and diabetes development. All women with a body mass index (BMI) ≥30kg/m2 are considered to be at risk and offered additional antenatal care; approximately half will have uncomplicated pregnancies while some women with a BMI <30kg/m2 will develop complications. Evidence suggests body fat distribution might better predict individual risk, but there is a lack of robust evidence during pregnancy. We explored associations between maternal adiposity and infant health. Studies reporting associations between early pregnancy (<20 weeks) adiposity and pregnancy-related infant health outcomes were included. Searches included six databases (MEDLINE, EMBASE, PsycINFO, CINAHL (EBSCO), JBI Database of Systematic Reviews and Implementation Reports, and the Cochrane Library), references, citations and contacting authors. Screening and quality assessment were carried out by two authors independently. Random effects meta-analysis and narrative synthesis were conducted. We included 34 studies (n=40,143 pregnancies). Meta-analysis showed a significant association between maternal fat-free mass and birthweight (AE 18.07g, 95%CI 12.75, 23.38), but not fat mass (AE 8.76g, 95%CI -4.84, 22.36). Women with macrosomic infants had higher waist circumference than controls (MD 4.93cm, 95% CI 1.05, 8.82). There was no significant association between subcutaneous fat and large for gestational age (OR 1.06 95%CI 0.91, 1.25). Waist-hip-ratio, neck circumference, skinfolds and visceral fat were significantly associated with several infant outcomes including small for gestational age, preterm birth, neonatal morbidity and mortality, although meta-analysis was not possible for these variables. Our findings suggest that some measures of maternal adiposity may be useful for risk prediction of infant outcomes. Individual participant data meta-analysis could overcome some limitations in our ability to pool published data.

Selected flash poster presentations

P1 – Katja Menger: Two type I topoisomerases maintain DNA topology in human mitochondria

P3 - Hilmar Sigurdsson: Developing FDG-PET/MR imaging methodology to study gait in aging and neurodegenerative disease

P7 - Chun Chen: Deficiency of mitochondrial quality control proteins in Parkinson's: A post-mortem study using imaging mass cytometry

P18 - Marina Danilenko: Single-cell DNA-sequencing identifies risk-associated clonal complexity and evolutionary trajectories in childhood medulloblastoma development

P23 - Sara Pintar: Structure based targeting of the DNA replication process to tackle antimicrobial resistance

P26 - Maria Georgiou: Activation of autophagy reverses progressive and deleteriousprotein aggregation in PRPF31 patient-induced pluripotentstem cell-derived retinal pigment epithelium cells

P30 - Teresa Borrello: Harnessing the role of HDAC6 inhibitors in liver fibrosis

P33- Birthe Dorgau: Spatial transcriptomics of human pluripotent stem cell derived retinal organoids offers new insight in retinal development

Posters

P1

Katja Menger
Biosciences

Two type I topoisomerases maintain DNA topology in human mitochondria

Topoisomerases are a group of essential enzymes that play vital roles during DNA replication, transcription, and maintenance, by facilitating the reduction of topological tension and separating intertwined DNA molecules. Human mitochondrial DNA (mtDNA) is subjected to topological problems during transcription and DNA replication. Human mtDNA is replicated, transcribed and maintained independently from nuclear DNA by a dedicated protein machinery.

Human topoisomerases are nuclear-encoded and need to be localised to mitochondria to act upon mtDNA. Using molecular, genomic, biochemical and computational methods we first assessed the subcellular localisation of the 6 human topoisomerases and then delineated their roles in maintaining mtDNA topology. We found that only the two type I topoisomerases TOP1MT and TOP3A localise to mitochondria. Both TOP3A and TOP1MT contribute to mtDNA replication, as replication stalling occurs throughout the mitochondrial genome in TOP3A or TOP1MT knockdown cells, in addition to the previously reported involvement of TOP3A in decatenating replicated mtDNAs. We also find that the relaxation activity of TOP1MT is stimulated by mtSSB and repressed by TFAM in vitro, suggesting that its activity may be preferentially targeted towards replicating mtDNA. An analysis of mitochondrial gene expression upon the loss of either TOP3A or TOP1MT activity found a reduction in mitochondrial transcripts and reduced de novo transcription, with larger effects being observed upon promoter-distal transcripts. In vitro mitochondrial transcription is stimulated by recombinant TOP1MT or TOP3A. We did not find any evidence for mitochondrial localisation of the type II topoisomerases TOP2A or TOP2B, and the knockout of TOP2B had no effect on mtDNA maintenance or expression. Our results indicate that TOP3A and TOP1MT are sufficient for mtDNA topology control. These findings have important implications for the regulation of mitochondrial DNA replication, transcription and maintenance as well as on the role of topoisomerase inhibitors and their effect on mitochondrial function.

P2

Hilmar Sigurdsson
NUTCRI

The feasibility of non-invasive vagus nerve stimulation to improve gait in Parkinson's: a randomised sham-controlled trial

Gait and balance impairments appear early and are ubiquitous in Parkinson's disease (PD). They increase falls risk with negative consequences to quality of life and independence. As therapies to mitigate these are limited, novel interventions targeting gait impairments and their consequences urgently needed. Non-invasive vagus nerve stimulation (nVNS) is a neuromodulation technique targeting the cholinergic vagus nerve (VN). Recent work suggests that nVNS may reduce inflammation and improve gait variability in PD. Our aim to investigate the feasibility and safety of a multi-dose, multi-session nVNS in PD. This will be a single-site, double-blind sham-controlled randomised pilot and feasibility trial in 40 people with PD. Participants will be randomised to receive either active or sham treatment using covariate-adaptive randomisation. The sham device is identical in appearance and user interface but does not stimulate the VN. Participants will undergo a baseline assessment followed by a 12-week treatment phase with patient self-administering nVNS at home. Assessments will be repeated at 12 and 24 weeks. We will quantify both laboratory and free-living gait features in addition to the frequency of falls. The study will report on the proportion of eligible and enrolled patients, and rates of eligibility and reasons for ineligibility. We will report on the safety of the intervention and device tolerability in PD by recording all adverse events. Secondary outcome data will be summarised using descriptive statistics and include the median change in measures of discrete gait features that are likely to be underpinned by non-dopaminergic mechanisms pre- and post-treatment. These, in addition to changes in cognition, autonomic function and blood based cholinergic markers, will be analysed using non-parametric tests. This study aims to generate clinical, digital and laboratory data to assess the safety, tolerability, feasibility, and potential effectiveness of domiciliary multi-dose nVNS in PD.

P3

Hilmar Sigurdsson
NUTCRI

Developing FDG-PET/MR imaging methodology to study gait in aging and neurodegenerative disease

Studies suggest that hierarchically organised cerebral brain regions contribute to gait control. However, current understanding of discrete gait-brain networks is limited since monitoring of brain activity in real-time during walking is only possible with indirect methods that capture superficial cortical activity. The aim of this work was to develop a [18F]-2-fluoro-2-deoxyglucose (FDG)-Positron Emission Tomography (PET) and Magnetic Resonance (MR) imaging methodology to map real-time neural activation changes during walking from standing. We studied 15 healthy older adults (mean age: 65.7±3.9, 10 females). All participants received a bolus iv injection of FDG followed by 15-minute standing task and PET-MR brain imaging. Participants then received another FDG injection and completed a 15-minute walking task and another PET-MR scan. Gait characteristics were quantified using small, lightweight sensors according to a validated model of gait. To identify which regions of the brain were more active during walking relative to standing, the two FDG-PET images were coregistered, dose-corrected and then subtracted to generate a contrast image. Correlation between FDG metabolism and discrete gait characteristics was accomplished using a permutation approach. We additionally explored the cross-modality regional associations between FDG metabolism and grey matter volume, white matter and resting-state fMRI connectivity, using sparse canonical correlation analysis. Walking relative to standing elicited increased FDG metabolism in the supraspinal motor network (sensorimotor cortex, locomotor regions, thalamus, and the striatum), vestibular cortex and visual cortex. Greater swing time variability correlated positively with FDG metabolism in the midbrain locomotor region and lower limb region of the paracentral lobule. Here we have developed and successfully established the feasibility and safety of a functional protocol that robustly measures real-time neural substrates of gait and posture. This will allow us to detect brain network dysfunction in brain disease and test novel interventions to mitigate mobility decline and falls risk.

P4

Amy Tooke
Biosciences

Role of type VII secretion system substrate toxins in virulence and bacterial competition

Staphylococcus aureus is an opportunistic pathogen that colonises the skin and nose and is well known for the range of different diseases it can cause - from skin and soft tissue infections to endocarditis and chronic biofilm infections on medical implants - due to the wide range of toxins and virulence factors that it is able to produce. One way in which it can release toxins from the cell into its environment is via the type VII secretion system (T7SS). Cells produce antitoxins (immunity proteins) to prevent self-killing by the toxins they produce. S. aureus species compete with other bacteria to establish niches, such as in the nose. Some strains of S. aureus produce immunity proteins to different toxins but not the toxins themselves, suggesting that the toxins have a role in interbacterial killing rather than virulence (targeting the host). Previous work has demonstrated that the toxins EsaD and TspA confer a competitive advantage to S. aureus strains that can produce them, in in vivo interstrain competition experiments (1). I will use zebrafish larval infection models to investigate the role of further putative T7 substrate toxins in virulence, using a systemic bloodstream infection (2), or bacterial competition, by instigating a co-infection in the hindbrain ventricle (1), a sterile compartment that can be used to contain the bacteria. This will allow us to further elucidate the role of the T7SS in establishing S. aureus infection.

1. Ulhuq, F.R. et al., 2020. A membrane-depolarizing toxin substrate of the Staphylococcus aureus type VII secretion system mediates intraspecies competition. PNAS 117, 20836–20847.
2. Prajsnar, T.K., et al., 2012. A privileged intraphagocyte niche is responsible for disseminated infection of Staphylococcus aureus in a zebrafish model. Cellular Microbiology 14, 1600–1619.

P5

Alwin Gieselmann
Biosciences

Stimulus dependence of directed information exchange between cortical layers in macaque V1

Perception and cognition require the integration of feedforward sensory information with feedback signals. Using different sized stimuli, we isolate spectral signatures of feedforward and feedback signals, and their effect on communication between layers in primary visual cortex of male macaque monkeys. Small stimuli elicited gamma frequency oscillations predominantly in the superficial layers. These Granger-causally originated in upper layer 4 and lower supragranular layers. Unexpectedly, large stimuli generated strong narrow band gamma oscillatory activity across cortical layers. They Granger-causally arose in layer 5, were conveyed through layer six to superficial layers, and violated existing models of feedback spectral signatures. Equally surprising, with large stimuli, alpha band oscillatory activity arose predominantly in granular and supragranular layers and communicated in a feedforward direction. Thus, oscillations in specific frequency bands are dynamically modulated to serve feedback and feedforward communication and are not restricted to specific cortical layers in V1.

P6

Rachel Crossland
NUTCRI

MicroRNA Profiling of Low Concentration Extracellular Vesicle RNA utilizing NanoString nCounter Technology

Extracellular vesicles and the microRNAs that they contain are increasingly recognised as a rich source of informative biomarkers, reflecting pathological processes and fundamental biological pathways and responses. Their presence in biofluids makes them particularly attractive for biomarker identification. However, a frequent caveat in relation to clinical studies is low abundance of EV RNA content.

In this study we used NanoString nCounter technology to assess the microRNA profiles of n=64 EV low concentration RNA samples (180-49125 pg) isolated from serum and cell culture media using both precipitation reagent and ultracentrifugation. Data was subjected to robust quality control parameters based on 3 levels of limit of detection stringency, and differential microRNA expression analysis between biological subgroups. We report that RNA concentrations >100 times lower than the current NanoString recommendations can be successfully profiled using nCounter microRNA assays, demonstrating acceptable output ranges for imaging parameters, binding density, positive/negative controls, ligation controls and normalisation quality control. Furthermore, despite low levels of input RNA, high-level differential expression analysis between biological subgroups identified microRNAs of biological relevance.

Our results demonstrate that NanoString nCounter technology offers a sensitive approach for the detection and profiling of low abundance EV-derived microRNA, and may provide a solution for research studies that focus on limited samples material.

P7

Chun Chen
NUTCRI

Deficiency of mitochondrial quality control proteins in Parkinson's: A post-mortem study using imaging mass cytometry

Mitochondrial dysfunction has been suggested as a contributor to Parkinson's pathogenesis, though an understanding of the contribution of mitochondrial dysfunction to neurodegeneration remains elusive. Furthermore, the ability to perform pathway analysis and to simultaneously study multiple related signalling proteins within human tissue has previously been challenging. To overcome this, we employed imaging mass cytometry which detect multiple protein targets, with the retention of spatial information. We combined a previous panel of antibodies targeted to mitochondrial oxidative phosphorylation proteins, with additional targets to examine the consequences of mitochondrial deficiencies on several key signalling pathways important to neuronal function. We revealed a synergistic reduction in mitochondrial quality control proteins in Parkinson's neurons. This also showed a different correlation to neurons from mitochondrial disease patients. In Parkinson's neurons, significantly reduced abundance of PINK1, Parkin and phosphorylated ubiquitin, integral to the mitophagy machinery; mitochondrial chaperones, HSP60 and PHB1; regulators of mitochondrial protein synthesis and the unfolded protein response, SIRT3 and TFAM were revealed. We further found that mitochondrial respiratory chain deficient neurons showing an increased abundance of mitochondrial chaperones and proteases, PHB1, LonP and HTRA2 in mitochondrial disease, control and some Parkinson's cases. However, in neurons with deficiency in ATP synthase, SIRT3 abundance was increased in control neurons yet decreased in Parkinson's cases, such a decrease was not seen in mitochondrial disease. Our findings suggested an inability to turnover mitochondria and maintain mitochondrial proteostasis in Parkinson's neurons, which may exacerbate the impact of oxidative phosphorylation defects and aged related oxidative stress, leading to neuronal degeneration. In addition, we observed intraneuronal aggregation of phosphorylated ubiquitinSer65 alongside increased abundance of mitochondrial proteostasis proteins in Parkinson's neurons with Lewy body pathology, compared to those without. This provides preliminary evidence to support the hypothesis that Lewy pathology affect mitochondrial quality control through the disturbance of mitochondrial proteostasis.

P8

Muhammad Naeem Aamir
School of Pharmacy

Preparation, Characterization of Pregabalin and Withania coagulans Extract-Loaded Topical Gel, and Their Comparative Effect on Burn Injury

The current study depicts the comparative effects of nanogel using Withania coagulans extract, pregabalin alone, and a co-combination gel. The gels prepared were then analyzed for conductivity, viscosity, spread ability, globule size, zeta potential, polydispersity index, and TEM. The globule size of the co-combination gel, determined by zeta sizer, was found to be (329 ± 0.573 nm). FTIR analysis confirms the successful development of gel, without any interaction. Drug distribution at the molecular level was confirmed by XRD. DSC revealed no bigger thermal changes. TEM images revealed spherical molecules with sizes of 200 nm for the co-combination gel. In vivo studies were carried out by infliction of third degree burn wounds on rat skin, and they confirmed that pregabalin and Withania coagulans heals the wound more effectively, with a wound contraction rate of 89.95%, compared to remaining groups. Anti-inflammatory activity (IL-6 and TNF-α), determined by the ELISA technique, shows that the co-combination gel group reduces the maximum inflammation with TNF-α value (132.2 pg/mL), compared to the control (140.22 pg/mL). Similarly, the IL-6 value was found to be (78 pg/mL) for the co-combination gel and (81 pg/mL) in the case of the control. Histopathologically, the co-combination gel heals wounds more quickly, compared to individual gel. These outcomes depict that a co-combination gel using plant extracts and drugs can be successfully used to treat burn injury.

P9

Sabrina Mackinnon
Biosciences

Novel starting points for human glycolate oxidase inhibitors, revealed by crystallography-based fragment screening

Primary hyperoxaluria type I (PH1) is caused by AGXT gene mutations that decrease functional activity of alanine:glyoxylate aminotransferase. A build-up of the enzyme's substrate, glyoxylate, results in excessive deposition of calcium oxalate crystals in the renal tract, leading to debilitating renal failure. Oxidation of glycolate by glycolate oxidase (or hydroxyacid oxidase 1, HAO1) is a major cellular source of glyoxylate, and siRNA studies have shown phenotypic rescue of PH1 by knockdown of HAO1, representing a promising inhibitor target. Here, we report the discovery and optimization of six low-molecular-weight fragments, identified by crystallography-based fragment screening, that bind to two different sites on the HAO1 structure: at the active site and an allosteric pocket above the active site. The active site fragments expand known scaffolds for substrate-mimetic inhibitors to include more chemically attractive molecules. The allosteric fragments represent the first report of non-orthosteric inhibition of any hydroxyacid oxidase and hold significant promise for improving inhibitor selectivity. The fragment hits were verified to bind and inhibit HAO1 in solution by fluorescence-based activity assay and surface plasmon resonance. Further optimization cycle by crystallography and biophysical assays have generated two hit compounds of micromolar (44 and 158 μM) potency that do not compete with substrate and provide attractive starting points for the development of potent and selective HAO1 inhibitors.

P10

Ellen Lirani-Silva
NUTCRI

Circulating Ghrelin as a Dementia Biomarker

Background and aim: Although initially classified as a "hunger hormone", the hormone Ghrelin plays an important role in cognition and synaptic plasticity. The acylated form (AG) of circulating ghrelin has been linked to neurogenesis and enhancing memory function, while the unacylated form (UAG) has the opposite effect. Preliminary data in humans demonstrated reduced AG:UAG ratio in patients with Parkinson's disease dementia (PDD) compared to controls and PD without dementia, indicating the potential of ghrelin as a dementia biomarker. This study aims to determine if plasma AG:UAG ratio is reduced in PDD and, also, if it is changed in other dementias, including Alzheimer's disease (AD) and dementia with Lewy bodies (DLB).

Methods: Five subject groups are being recruited in two sites (Newcastle and Swansea) – 20 controls, 20 PDs, 20 PDDs, 20 DLBs and 20 ADs. The protocol is been conducted in two days: i) visit 1: consent form process and clinical and demographic data; ii) visit 2: blood samples collected in 4 moments: a baseline sample following 12 hours fasting period, and three samples following a standard meal test (5, 60 and 180 minutes after the meal). Samples will be processed and the plasma will be collected for analysis. Statistical analysis will include differences in fasting levels, magnitude of decrease post meal test and slope recovery. The area under the curve for AG:UAG ratios will be compared between groups and time point by ANOVAs.

Results: The recruitment process is ongoing. To date 5 participants have been recruited and completed the visits as per protocol (controls = 4, PD = 1).

Future perspectives: This study has potential not only to identify a potential biomarker for PDD, but also to investigate if AG:UAG ratio is able to differentiate dementia subtypes and may aid in shaping the development of new disease targets.

P11

Emma Lishman-Walker
Biosciences

Developing a kinase activity signature as a tool for personalised medicine in prostate cancer

The androgen receptor (AR) is crucial for normal prostate function, for example regulating cellular growth and enabling the production of proteins such as prostate specific antigen (PSA). When the normal function of AR is lost this can result in prostate cancer affecting 1 in 8 men. Current therapies target the AR to reduce its activity, and initially these effectively reduce tumour growth. However, over time the tumour evolves to become resistant to these therapies, rendering them ineffective. Therefore, there is a clear need to develop newer, more personalised therapies, particularly for men with resistant prostate cancer.

Protein kinases can attach markers to the AR to regulate function, for example to activate AR signalling resulting in production of proteins such as PSA. This relationship between the AR and kinases presents a new treatment window, targeting the kinases that activate the AR and drive prostate cancer. So far 10 kinases have been identified as potential treatment targets, and there are drugs available to target many of these. These drugs act differently to traditional chemotherapies and target specific proteins. Therefore, it is essential to identify which patients would benefit most from these treatments, however presently there is no diagnostic tool available for this.

To identify the most suitable treatment for each patient it will be essential to understand which kinases are present and how active they are. We will do this using imaging mass cytometry (IMC) – a method that allows simultaneous visualisation of up to 40 cellular markers in prostate cancer cell lines and patient tissue. Following this we will grow patient tissue within the lab and treat with drugs targeting the selected kinases to understand how this will impact cancer growth. Information from this study alongside clinical trial data will help us develop a tool for personalised prostate cancer treatment.

P12

Aurelie Guyet
Biosciences

Insights into the role of lipoteichoic acids in Bacillus subtilis- a new function for MprF

In Gram-positive bacterium, such as Bacillus subtilis and Staphylococcus aureus, the cell envelope is a structure that protects the cells from the environment but is also dynamic, in that is must be modified in a controlled way to allow cell growth. In our study, we show that the lipoteichoic acids (LTA), which are anionic polymers attached to the membrane, have a direct role in modulating the cellular abundance of cell wall degrading enzymes. We also find that the apparent length of the LTA is modulated by the activity of the enzyme MprF, previously implicated in the modification of the cell membrane leading to resistance to antimicrobial peptides. These findings are important contributions to our understanding of how bacteria balance cell wall synthesis and degradation to permit controlled growth and division. The results also have implications in the interpretation of antibiotic resistance particularly clinical treatment of Staphylococcus aureus MRSA infections with daptomycin.

P13

Svetlana Sarah Cherlin
PHSI

Cross-validated risk scores adaptive enrichment design

Adaptive enrichment clinical trial designs allow the trial to update the inclusion criteria based on the interim analysis. In the second stage, the entry is restricted to the subgroup of patients who are predicted to benefit from the treatment. This subgroup is often defined by a single binary or continuous biomarker which might not be known. With the recent advances in multi-omics technologies, an increasingly large numbers of biomarkers are becoming available. Several approaches that utilise high-dimensional data have been proposed, such as the risk scores approach that summarises the high-dimensional information into a single score for each patient. The risk scores are subsequently used for identifying a subgroup of patients who benefit from the treatment.

We propose a design that considers enriching the recruitment with patients who are predicted to benefit from the treatment, based on their high-dimensional baseline covariates. The sensitive group is identified using the risk score approach where each patient is assigned a score constructed from their baseline covariates. The design includes early stopping for futility if no promising treatment effect is identified in the sensitive group and also the difference between the arms in the overall trial population is not significant.

The new design, implemented in an R package, allows to narrow down the eligibility and also achieves this at a smaller expected sample size, in comparison to the non-enrichment alternative. For the null scenario, the design achieves a well-controlled type I error rate with a substantial reduction in the expected sample size. We illustrated our approach on a randomised clinical trial with publicly available high-dimensional gene expression data.

The new method shows a superior performance in terms of the power and the sample size in comparison to the non-enrichment approach. Further work could explore different distributions of outcomes, as well as multiple endpoints.

P14

Fiona Graham
PHSI

Effectiveness of shared medical appointments for long-term conditions: a systematic review of randomised controlled trials

Background: Shared medical appointments (SMAs) are a care delivery model that involves a group of patients with a shared long-term condition (LTC) meeting with their health professional for routine care, e.g. annual reviews. SMAs typically last between 60-120 minutes and are co-delivered by a physician (usually physician, nurse or pharmacist) and a facilitator (non-clinician). SMAs have the potential to address interlinked challenges of limited capacity in primary healthcare and rising prevalence of patients with multiple LTCs. This review aimed to examine the effectiveness of SMAs compared to one-to-one appointments in primary care at improving health outcomes and reducing demand on healthcare services.

Methods: We searched for randomised controlled trials (RCTs) of SMAs involving patients with LTCs in primary care across six databases (MEDLINE, EMBASE, Science Citation Index, Social Science Citation Index, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane Library including Central Register of Controlled Trials) from 2013 and added eligible papers identified from previous relevant reviews. Data were extracted and outcomes measures categorised into health outcomes, behavioural outcomes, and resource use. Data from studies that were sufficiently similar were combined and meta-analysed else were narratively synthesised.

Results: Twenty-three unique trials were included. Nineteen were conducted in United States, two in China and one each in Australia, Germany and UK. Studies were published between 2001 and 2020. SMA models varied in terms of components, mode of delivery and target population. Most trials recruited patients with a single LTC, mostly commonly diabetes (n=13), although eight trials selected patients with multiple LTCs. There was substantial heterogeneity in outcome measures reported. Meta-analysis showed that participants in SMA groups had lower diastolic blood pressure than those in usual care (d=-0.123, 95%CI = -0.22, -0.03, k=8). No statistically significant differences were found across other outcomes. Where individual studies showed significant differences (patient self-efficacy), these trended in favour of SMAs. Compared with usual care, SMAs had no significant effect on healthcare service use.

Conclusions: SMAs were at least as effective as usual care in terms of health outcomes and did not lead to increased healthcare service use in the short-term. They show some potential in improving self-efficacy which may boost self-care. To strengthen the evidence base, future studies should target standardised behavioural and health outcomes and clearly report SMA components so key behavioural ingredients can be identified. Similarly, transparent approaches to measuring costs would improve comparability between studies.

P15

Praveen Dhondurao Sudhindar
Biosciences

Human Precision Cut Liver Slices a model system to demonstrate antifibrotic effects of EZH2 inhibitors

Liver fibrosis is the common end point of almost all chronic liver diseases. The progression of fibrosis in chronic liver disease is dependent on the hepatic stellate cells transdifferentiating into myofibroblast like phenotype leading to accumulation of the extra cellular matrix (ECM). The HSC transdifferentiation process is regulated epigenetically by the enzymes that regulate the histone methylation. Hence targeting the HSC activation therapeutically using epigenetic small molecule inhibitors remains the key strategy to suppress or regress fibrosis progression. PPARγ is a key target gene in HSC's- a negative regulator of HSC transdifferentiation. EZH2 catalyses the trimethylation of lysine 27 on histone 3 on PPARγ gene which leads to its gene silencing. Small molecule inhibitors of EZH2 (DZNep, GSK126, Tazemetostat, EI1 and SAH-EZH2) have been examined in this study on human precision cut liver slices (PCLS) as an ex vivo culture model to test their potential anti-fibrotic effect. To confirm the anti-fibrotic activity of EZH2 inhibitors was associated with repression of H3K27me3 methylation mark we did a diagnostic staining for H3K27me3 in human PCLS and observed a loss of hepatic H3K27me3 in EZH2i treated PCLS relative to the control. Fibrosis was induced in human PCLS with transforming growth factor beta 1 (TGFβ1) and platelet derived growth factor (PDGFββ) stimulation in presence or absence of the EZH2i. Media samples were collected every day and the PCLS were harvested for biochemical, histological and gene expression analysis. Albumin and AST secretion was used as an indicator for PCLS hepatocellular health and function. We observed a significant increase in Col1a1 and Tissue Inhibitor of Metalloproteinase 1 (TIMP1) secretion after 72 hours of TGFβ1/PDGFββ stimulation, whereas the secretion was significantly attenuated in presence of EZH2i with DZNep and GSK126 showing increased efficacy. Expression of the pro-fibrotic genes Col1a1, TIMP1, αSMA and Fibronectin were significantly upregulated after 72 hours of TGFβ1/PDGFββ treatment and this was suppressed by addition of DZNep and GSK126. Picrosirius red staining showed increased deposition of collagen fibres in fibrosis stimulated PCLS whereas a significant attenuation was observed in DZNep and GSK126 treated slices. Similarly staining for αSMA revealed increased accumulation of scar forming myofibroblasts in fibrosis stimulated PCLS while DZNep and GSK126 treatment prevented the accumulation of myofibroblasts. In summary human PCLS were used as a model system in this study to demonstrate that progression of liver fibrosis can be manipulated therapeutically using small molecule inhibitors targeting EZH2.

P16

Amelia Lu
Biosciences

Mitochondrial changes in cholinergic neurons of the pedunculopontine nucleus (PPN) in Parkinson's disease

Parkinson’s disease (PD) is the second most common neurodegenerative disease. One of the places where neurons have highly degenerated and are highly related to the non-motor symptoms of the PD is called the Pedunculopontine Nucleus (PPN). PPN is a heterogeneous area in the midbrain containing various types of neurons (including cholinergic neurons). Previous research has suggested that cholinergic neurons are the main degenerating type, and the mitochondrial copy number (mtCN) is upregulated in PD. This research uses human post-mortem brain tissue to investigate the molecular changes in PPN cholinergic neurons. Single cholinergic neurons were laser microdissected before carrying to Illumina next-generation sequencing. Obtained BAM files went through a novel mtDNA deletion pipeline called eKLIPse (Goudenège et al., 2019), getting a list of mtDNA deletions in those neurons. PD neurons tend to accumulate more large deletions ($p = 0.0091$*) and have higher deletion load ($p = 0.012$*) comparing to aged control. Patients with severe Braak stage are more likely to expand deletions ($p = 0.034$*). No clear difference was observed between the PD and the control when analysing deletion breakpoints (GC contents, repeat length, types of breakpoints) in both PPN cholinergic neurons and in substantia nigra pars compacta (SNpc) dopaminergic neurons. We conclude that PD neurons are more likely to accumulate and expand large deletions compared to the aged control, and the clonal expansion of mtDNA deletion might correlate with the severity of the disease. The mechanism behind generating mtDNA deletions is the same in the PD cases. This research will look at the mtDNA point mutations and transcriptional and protein level changes for the next stage.

P17

Letizia Marchetti
Biosciences

Targeting hijacked super-enhancers in blood cancer

Haematological malignancies are a heterogeneous group of diseases caused by genetic aberrations, which include chromosomal aneuploidy, point mutations and chromosomal rearrangements.
In healthy cells, super-enhancers (SEs) are responsible for outstanding levels of expression of key genes involved in cell lineage, function and proliferation. In the case of B cells, a strong SE is associated with immunoglobulin heavy and light chain (IGH, IGL and IGK) loci, which together encode the necessary information to generate antibodies. In blood malignancies, and in particular the ones affecting the B-lymphoid lineage, the most common chromosomal translocations cause the juxtaposition of a SE next to a proto–oncogene, resulting in dysregulated overexpression of the latter. This is the case for translocations t(11;14) and t(8;14), where IGH SEs are juxtaposed next to CCND1 or MYC respectively, driving the expression of these oncogenes. By recruiting epigenetic remodellers and subsequently modifying the epigenomic neighbourhood, SEs ensure chromatin accessibility and high levels of expression of their target genes under normal conditions. In the event of chromosomal rearrangements involving SEs, not only the sequence, but the whole epigenomic machinery linked to SEs is moved next to the proto – oncogene, driving its overexpression and leading to the new concept of epigenetic translocation.
Our aim is to define the essential regions of the SEs that are driving the overexpression of the target proto–oncogene, by epigenetically silencing small regions predicted from 3D in silico simulations. Using a dCas9 – KRAB system guided by specific gRNAs, we want to confirm the efficient downregulation of the oncogene involved in the translocation when these specific SE regions are silenced. We are also interested in looking at the changes in the epigenomic landscape in response to the loss of the SE activity.
Preliminary analyses in cell lines carrying IGH rearrangements confirmed the epigenetic translocation at the proto – oncogenes, showing high signal of a histone modification associated to SE regulated genes, namely H3K4me3 broad domains. Optimisation of lentiviral transduction of dCAs9 – KRAB and gRNA nucleofection in cell lines of interest is laying down the basis for an extensive investigation around hijacked SEs in blood malignancies, opening the possibility to a new line of intervention for these complex diseases.

P18

Marina Danilenko
NUTCRI

Single-cell DNA-sequencing identifies risk-associated clonal complexity and evolutionary trajectories in childhood medulloblastoma development

Background
Medulloblastoma is one of the most aggressive childhood brain cancers, causing ~250 deaths/year in Europe. Recent studies involving immunohistochemistry and iFISH show that medulloblastomas possess high levels of intra-tumoral heterogeneity for key biomarkers. This is proposed to be a major cause of treatment failure. We hypothesised that the identification of biologically and clinically significant intra-tumoural heterogeneity and reconstruction of tumour evolution will improve the understanding of medulloblastoma progression, promoting the development of novel therapeutic strategies.

Methods
We applied single-cell whole-genome sequencing to 865 single nuclei extracted from 14 frozen medulloblastomas (2 tumour regions/patient). Single-cell copy number aberrations were assessed using the CNVKIT pipeline. Heterogeneity patterns were identified using hierarchical clustering approaches. Clonal evolution was reconstructed using SCICoNE.

Results
We found that medulloblastomas follow three distinct routes of clonal evolution. Favourable-risk disease sub-types (MBWNT and infant desmoplastic/nodular MBSHH) typically comprised a single clone with no evidence of further evolution. In contrast, highest-risk sub-classes (MYC-amplified MBGroup3 and TP53-mutated MBSHH) were most clonally-diverse and displayed gradual evolutionary trajectories. Standard-risk tumours appeared to evolve following punctuated evolution.
Key clinically-adopted biomarkers were typically early-clonal/initiating events, exploitable as targets for early-disease detection. A single tumour biopsy was sufficient to assess their status. Importantly, we also detected novel chromosomal aberrations, which arise later and sub-clonally and more commonly display spatial diversity. Their clinical significance and role in disease evolution post-diagnosis now require establishment.

Conclusion
These findings reveal diverse modes of tumour initiation and evolution in the major medulloblastoma sub-classes, with pathogenic relevance and clinical potential.

P19

Soren Nielsen
Biosciences

RNAP I utilises roadblock protein and backtracking to an RNA hairpin to terminate rDNA transcription

RNA polymerase I (RNAP I) synthesizes the majority of ribosomal RNA (rRNA) in eukaryotic cells and rely on efficient transcription termination to accomplish this task. RNAP I utilise a protein roadblock bound to rDNA to terminate transcription, but detailed mechanistic knowledge of the steps leading to termination are either lacking or controversial. During exponential growth in the model yeast Saccharomyces cerevisiae, one rRNA precursor is synthesized every 3-4 secs from each active ribosomal gene. Here we show that co-transcriptional cleavage of rRNA by an exoribonuclease, as proposed in the torpedo model of transcription termination, is not required. Instead, RNAP I pausing at the roadblock, while not terminating transcription per se, leads to unusual catalytic inactivation of RNAP I and promotes backtracking. This commits RNAP I to termination and transports it to the 3' proximal large RNA hairpin, which facilitates release of the nascent rRNA transcript. Our findings suggests that transcription termination by RNAP I follows an order of events, beginning with pausing, followed by catalytic inactivation, commitment to termination, backtracking and hairpin induced RNA release. The proposed mechanism is rapid and precise, accomplished in <10 sec and thus able to account for rRNA demand during exponential growth.

P20

Rob Atkinson
Biosciences

Elucidating the Mechanism of PRPF-linked Retinitis Pigmentosa

Purpose:
Splicing is essential for most basic cellular processes. It is not known why mutations in the largest and most conserved component of the spliceosome (PRPF8) cause Retinitis Pigmentosa (RP13). We hypothesised that mis-splicing affects those processes critical for vision such as retinal development and cilia function . This study investigates using iPSCs derived from four affected individuals and isogenic controls generated using CRISPR/Cas9. Retinal and non-retinal tissues were differentiated and analysed to make clear the tissue specific phenotype and identify similarities to other types of PRPF-linked RP.

Methods:
Fibroblasts from one de novo and three familial patients with RP13 were reprogrammed to iPSCs via RNA-based transduction. The missense mutation in PRPF8 was then edited using CRISPR/Cas9 to generate paired isogenic control lines (n = 8). All lines were differentiated in parallel to retinal pigment epithelium (RPE), retinal organoids (RO) and kidney organoids using published protocols. Isogenic pairs were cultured, collected, and processed in parallel for a range of experiments including qPCR, immunofluorescence microscopy, and electron microscopy. Statistical analysis was performed using two-tailed paired t test, results shown as mean ± SEM.

Results:
Expression of the mutant PRPF8 transcript was detected by qPCR in RP13 cells but not the RP13-Cas9 controls ($p < .001$ for all cell types). We investigated whether the mutation affects localisation to intranuclear domains enriched with splicing factors, known as nuclear speckles. The colocalization of PRPF8 and nuclear speckle marker SC35 was reduced in RP13 iPSCs, RPE and photoreceptors ($p < 0.05$ for all cell types). In addition, cilia of RP13 RPE were 10% longer ($p < 0.05$) and a fraction of these had swollen axonemes, but cilia frequency was unaffected. To gain insight into possible effects on tissue function we carried out a quantitative ultrastructural analysis. A significantly reduced number of mitochondria were found in RP13 RPE (10 ± 1 vs 17 ± 1, $p < 0.01$) and photoreceptors (57 ± 13 vs 107 ± 11, $p < 0.05$).

Conclusion:
These results suggest that retinal and non-retinal tissues exhibit a splicing phenotype in RP13. Retinal cells exhibit ciliary defects and a possible metabolic shift that warrant further investigation. The combined splicing and ciliary phenotype presented resembles that caused by PRPF31 mutations.

P21

José Luis Marín-Rubio
Biosciences

Role of deltex E3 ubiquitin ligase in interferon response during viral infection

The interferon response is one of the key defence mechanisms of the immune system and is typically employed to combat viral infections. Type I and type II interferons (IFN) induce the expression of interferon-stimulated genes (ISGs) through the Janus activated kinase (JAK)-signal transducers and activators of transcription (STAT) pathways, whose products contribute to the antiviral response. In infections caused by airborne viruses, the respiratory epithelium is the first line of defence, acting as a physical barrier to protect against infection. Consequently, the triggering of the interferon signalling pathways in airway epithelial cells is essential to controlling the early stages of infection and preventing viral spread.

In recent studies, it has been proposed that E3 ubiquitin ligases (and consequently ubiquitylation) are important regulators in the innate immune response as important mediators of the innate immune response. The specific role E3 ubiquitin ligases play in the interferon-mediated antiviral response is yet to be elucidated. In this study, we investigated the role of deltex E3 ubiquitin ligase (DTX3L) in adenocarcinoma human alveolar basal epithelial cells (A549) transduced with the human ACE2 and TMPRSS2 genes (which encode for the two proteins that mediate the entrance of SARS-CoV-2).

These cells were treated with IFN-α, IFN-γ, and IL-4 for 24h before cells lysis. These protein lysates were used for immunoblotting and for whole cell proteome analysis using mass spectrometry. DTX3L CRISPR knock-out cells exhibited a significant deregulation of the type I and II interferon signalling response. Indicating that DTX3L might regulate interferon signalling and viral activity during respiratory infections.

P22

Emma-Joy Holland
PHSI

Understanding patient and service provider views of the Recovery Navigator scheme for heavy drinkers

Background
North-East England suffers disproportionately from alcohol harms. People who come to emergency departments on a regular basis with alcohol problems have high levels of need, often due to their mental ill-health. Most are not getting the support they require from community services, which has a detrimental impact on their long-term recovery and results in significant cost to the NHS. A new role called a Recover Navigator is being introduced across the North East and North Cumbria (NENC) Integrated Care System (ICS) to help support individuals in their recovery journey from emergency care into the community.

Aim
To explore whether the introduction of Recovery Navigators NENC in secondary care makes a difference to the health and wellbeing (including mental health) of severely heavy drinkers, and/or the staff and services who care for them.

Methods
Qualitative interviews with approximately 20 staff and 20 patients based across six NHS Trusts within the NENC ICS. Staff interviews will focus on issues around implementation and uptake of the service, with participants to include Recovery Navigators from each Trust alongside representatives from associated services such as social care, mental health care, hospital care, and welfare support. Patients will be sampled purposively, to ensure the inclusion of a range of ages, genders and ethnicities who have been supported by Recovery Navigators. Interviews will explore patients' views on what has worked well or less well in the support they received from Recovery Navigators, and how the service could be improved.
All Interviews will be audio-recorded and transcribed in full. Data will be analysed using a two-stage approach. Firstly, reflexive thematic analysis will be used to generate themes inductively. Next, more deductive analysis will be applied, guided by Normalisation Process Theory constructs, a model often applied when exploring implementation work. Three Patient and Public Involvement (PPI) groups based across the NENC ICS region will work closely with the research team to support the analysis process.

Findings
The findings will shape future implementation of the Recovery Navigator role across and beyond the region and may lead to improvements in patient care.

Conclusion
This evaluation will provide an evidence-base for the use of Recovery Navigators in supporting adults with alcohol and mental health needs across the NENC ICS.

P23

Sara Pintar
NUTCRI

Structure based targeting of the DNA replication process to tackle antimicrobial resistance

The recent WHO report shows that antibacterial resistance is on the rise[1]. This year's report in The Lancet estimated global deaths related to antibiotic-resistant infections to 4.95 million in 2019[2]. New approaches to tackle antimicrobial resistance (AMR) are urgently needed. Structure-based drug design, where protein structures are used as a scaffold for the design of active compounds are used, is becoming an essential tool for faster, more cost-efficient and less resistance prone development of novel therapeutics. Fragment-based drug discovery uses small molecules to more efficiently probe the target protein and thus produce compounds with more favourable characteristics.

Each normal cell has a genome, hence a prerequisite for a successful cell division is genome replication. Bacterial DNA replication begins at specific chromosomal locus called the origin of replication (oriC), where conserved replication initiation proteins assemble and enable the loading of the replicative helicases and subsequent formation of replisome[3]. The bacterial DNA replication machinery is an attractive target, because the replication factors are distinct from their functional analogues in eukaryotes. No actives in current clinical use act directly on bacterial DNA replication initiation proteins, therefore novel compounds would constitute new class of medicines. We are combining bacterial genetics, infection models and fragment-based drug discovery to understand how best to interfere with DNA replication to develop new drugs to tackle AMR in ESKAPE pathogens and MTB.

[1]WHO, 2020 Antibacterial Agents in Clinical and Preclinical Development. 2021. [2]C. J. Murray et al., "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis," Lancet, vol. 6736, no. 21, 2022, doi: 10.1016/s0140-6736(21)02724-0. [3]T. T. Richardson, D. Stevens, S. Pelliciari, O. Harran, T. Sperlea, and H. Murray, "Identification of a basal system for unwinding a bacterial chromosome origin," EMBO J., vol. 38, no. 15, pp. 1–18, 2019, doi: 10.15252/embj.2019101649.

P24

Andy Frey
Biosciences

Exploring Ubiquitylation in the Macrophage Phagosome

Introduction
Ubiquitylation is a prevalent PTM, involved in all biological processes. This multifarious nature is due to ubiquitin's ability to covalently couple with amine groups of internal lysines and exposed N-termini of numerous proteins, including itself. The latter gives rise to polyubiquitin chains with the same-or-different linkage types (M1, K6, K11, K27, K29, K33, K48, K63). Besides PTM of target proteins, chain types also determine a protein's intracellular fate or function, with K48 polyubiquitin being well established in proteasomal targeting, while monoubiquitin and other polyubiquitin linkage types in different cellular niches is an emerging field of investigation. Here, we utilize specialized tools coupled with mass spectrometry to explore (poly)ubiquitylation, chain linkage types, and their functional significance in the macrophage phagosome.

Methods
Murine macrophage cell lines or primary bone marrow derived macrophages (BMDMs) were stimulated (with IFNy, IFNa, IL-4, LPS, or PAM3CSK4) to induce polarization or activate specific response states prior to harvesting, or their phagosomes isolated using polystyrene beads and gradient ultracentrifugation. Samples underwent one of several processing workflows. Methods included Tandem Ubiquitin Binding Entities (TUBEs) to enrich for ubiquitylated proteins, suspension trapping (S-trap) and tryptic digestion, and diGly (K-EPSILON-GG) pulldown for ubiquitylation site enrichment. Peptides were subjected to LC-MS/MS on tIMS-TOF Pro (Bruker) and QExactive HF (ThermoFisher Scientific) instruments. Polyubiquitin linkage types were quantified using the previously described Ubiquitin peptides for Absolute Quantification by Parallel Reaction Monitoring (Ub-AQUA-PRM) approach.

Preliminary data
Ub-AQUA-PRM revealed that total (poly)ubiquitylation in macrophages was substantially increased on exposure to IFNy. In terms of linkage type prevalence, the low abundance M1, K29, and K33 linkage types were increased by >1.5 fold in the presence of IFNy. The prevalence of other linkage types was also increased, with the exception of K48-linked polyubiquitin. DiGly pulldown permitted discovery of ubiquitylation sites on a number of membrane-associated innate immune signalling proteins, including Interferon induced transmembrane protein 3 (Ifitm3), H-2 class I histocompatibility antigen (MHC-Class I in humans), and an Ig-GAMMACHAIN receptor (Fcer1g). Given the role of Ifitm3 in anti-viral responses, and the role of the latter two in antigen presentation, the data provide further insight into the role of ubiquitylation in innate immunity.

Significance
Methods described can be applied to assess ubiquitylation in any niche, and provide insight into ubiquitylation during macrophage stimulation.

P25

Christopher Hurst
NUTCRI

Physical activity of older people living with frailty, multimorbidity and a recent deterioration in health

Introduction: Describing physical activity (PA) profiles of older people is important because of the relationship between PA and health status. Traditional methods to describe PA focus on time spent in specific categories such as moderate or vigorous activity. This risks marked floor effects in older people living with frailty, multiple long-term conditions (multimorbidity) and a recent deterioration in health, who often record little activity using these cut-points. Our aim was to use two novel data-driven metrics to describe the PA profiles of this group of older people.

Methods: As part of the LiLL-OPM (Lifestyle in Later Life: Older People's Medicine) study, twenty-four participants (18 female) aged 68-92 years were recruited from a Day Unit in Newcastle, UK. Participants wore a wrist-worn triaxial accelerometer (GENEActiv) for 7 days. PA was quantified using AccelAV, indicative of the volume of activity performed and IntensityGRAD, indicative of the intensity distribution of activity. Data were processed and analysed using the R-package GGIR.

Results: Mean AccelAV was 16.8 ± 5.6 mg which is consistent with low levels of PA and lower than that seen in other adult samples. Mean IntensityGRAD was -2.95 ± 0.26 indicating that participants were generally accumulating more time in low-to-mid range intensities with little time at higher intensity. AccelAV and IntensityGRAD were moderately correlated (r = 0.69).

Key conclusions: AccelAV and IntensityGRAD provide complementary information about PA in this group. Further work should examine (1) relationships between these metrics and health outcomes and (2) their potential utility as outcomes in intervention studies.

P26

Maria Georgiou
Biosciences

Activation of autophagy reverses progressive and deleteriousprotein aggregation in PRPF31 patient-induced pluripotentstem cell-derived retinal pigment epithelium cells

Introduction:
Mutations in pre-mRNA processing factor 31 (PRPF31) result in autosomal-dominant retinitis pigmentosa (adRP) known as RP11, characterised by global dysregulation of spliceosome in retinal cells and the adjacent retinal pigment epithelium (RPE). This study aims to investigate the disease pathomechanisms in Retinitis Pigmentosa caused by mutations in the PRPF31 gene.

Methods:
Human iPSCs from three patients with severe and very severe PRPF31-adRP, unaffected individuals and a CRISPR/Cas9-corrected isogenic control were used to generate RPE monolayers. To fully assess the impacts of PRPF31 mutations, quantitative proteomics analyses of control and RP11-RPE cells and biochemical assays were performed.

Results:
Quantitative proteomic analysis of control RPE cells showed RNA splicing, retinoid metabolism and visual perception, and protein folding (UPR) pathways to be affected in RP11-RPE cells. The patient-derived RPE cells were characterised by reduced amounts of tri-snRNPs, spliceosome activity, and the presence of insoluble aggregates containing the mutant PRPF31 and misfolded, ubiquitin conjugated proteins, which accumulated progressively with time. The waste disposal mechanisms via autophagy and proteasome mediated degradation were impaired, further exacerbating aggregate formation, which was closely linked with activation of cell death. Targeting the waste disposal mechanisms by activating the autophagy pathway using rapamycin resulted in reduction of these cytoplasmic aggregates in RP11-RPE cells and improved cell survival.

Conclusions:
Together these data indicate a vicious circle initiated by mutations in PRPF31, which lead to spliceosome dysregulation and accumulation of misfolded proteins in the form of insoluble cytoplasmic aggregates that affect RPE cell viability. Relieving the RPE cells from accumulation of these insoluble cytoplasmic aggregates presents a novel therapeutic strategy for RP11-patients.

P27

Kitty Cropmton
Biosciences

Developing Single-Molecule Fluorescence Microscopy Techniques to Investigate Cell Signal Transduction

Transcription factors (TFs) are proteins that are part of an important cell signal transduction process that regulates gene expression by turning genes on and off. A phenomenon known as transcription factor clustering may be a mechanism by which TFs increase their affinity and specificity. It may also be how they find their specific binding sites in an array of many random binding sites. TF research is important as these are involved in many cancers and inflammatory diseases. Research could help find methods to allieviate or prevent these. Single-molecule fluorescence microscopy can be used to observe TFs within the cell.

P28

Krutik Patel
NUTCRI

Using AI to decrypt expression patterns in Juvenile onset Huntington's Disease samples for biomarker discovery

Genetic testing on patients for the length of the CAG repeat found on the Huntingtin gene (HTT) being ≥60 indicates penetrance of developing Juvenile onset Huntington's disease (JHD). However, from numerous studies we know the length of the CAG repeats does not correlate with severity or age of onset of the disease. Given this, a growing field of interest is the identification of transcriptomic biomarkers which may indicate pathogenicity for JHD. microRNAs (miRNAs) are of particular interest here because they can circulate in bio-fluids such as blood plasma and cerebrospinal fluid, making them potential non-invasive tools to determine the health of the brain.

Given this, we set out to identify potential biomarkers for the development of JHD, with a particular interest in miRNAs. We downloaded, processed, batch corrected and normalised a dataset consisting of 112 RNA-seq and miRNA-seq experiments conducted on mouse hippocampus samples at ages: 2 (young) and 10 (aged) months. JHD samples were contrasted to WT samples using standard bioinformatic techniques such as differential expression analysis, however this proved to be a poor technique for the data, due to its extremely homogenic nature, and so we constructed a machine learning (ML) strategy.

Using this data, the core question we asked was, which set of genes gave us the best accuracy when classifying JHD samples from WT samples, but we asked this question in three ways: a) using aged data only to identify symptomatic markers, b) using young data only to identify pre-symptomatic markers, c) training on the aged data and testing on the young data to identify pre-disposition markers.

To limit bias, we contrased two feature selection techniques: recursive feature elimination and chi2 kbest feature selection. The selected best features were then examined by eight classifiers and the four best classifiers were hypertuned, and these were ADAboost, ExtraTrees, Random Forest and Gaussian Negative-Binomial. miRNAs and mRNAs were analysed separately, as generally miRNAs show lower variance than mRNAs, and keeping them together may have lead to feature selection methods ignoring the miRNAs. Best accuracies of all our training cases were between 75-95% and accuracies of all our testing cases were between 60-85%.

Note - Further optimization will be completed by September.

P29

Rachel Crossland
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Profiling Tissue and Biofluid miR-155-5p, miR-155*, and miR-146a-5p Expression in Graft vs. Host Disease

Acute graft vs. host disease (aGvHD) is a frequent complication following allogeneic haematopoeitic transplantation (HSCT). Despite recent advances, there are no universally accepted biomarkers to determine development of aGvHD. MicroRNAs miR-146a and miR-155 have been previously associated with aGvHD and show promise as clinically translatable biomarkers. In this study, we performed comprehensive expression profiling of miR-146a, miR-155, and miR-155* expression in aGvHD target tissue and biofluids and relate expression to post-HSCT outcomes. MicroRNA expression was assessed by qRT-PCR in gastrointestinal (n=31) and skin (n=31) biopsies as well as serum (exploratory cohort n=34, verification cohort n=81, diagnostic cohort n=65) and urine (exploratory cohort n=30, verification cohort n=56, diagnostic cohort n=20) biofluids, including extracellular vesicles (EV) (serum EV n=15, urine EV n=30). Expression was related to aGvHD incidence, severity and overall survival. In GI samples, expression of miR-155 (p=0.03) and miR-146a (p=0.03) was higher at aGvHD onset compared to patients with no GvHD. In skin biopsies, expression of miR-155 (p=0.004) was upregulated in aGvHD patients compared to normal control skin. Expression of miR-146a was higher in aGvHD compared to no aGvHD biopsies (p=0.002). In serum, miR-155 (p=0.03) and miR-146a (p=0.02) expression was higher at day 14 (D14), while in urine expression was elevated at D7 post-HSCT in patients who developed aGvHD compared to those disease-free. This was verified in an independent serum (miR-155 p=0.005, miR-146a p=0.003) and urine (miR-155 p=0.02, miR-146a p=0.04) cohort, where both microRNAs were also associated with aGvHD by ROC analysis. In serum and urine samples taken at the time of aGvHD symptoms, expression of miR-155 and miR-146a was also elevated (serum miR-155 p=0.03, miR-146a p<0.001; urine miR-155 p=0.02, miR-146a p=0.02). In contrast, miR-146a and miR-155 were downregulated at D14 in serum EVs and at D7 in urine EVs in patients who developed aGvHD compared to those that remained disease-free, in both an exploratory (serum miR-155 p=0.02, miR-146a p=0.06; urine miR-155 p=0.02, miR-146a p=0.07) and an independent cohort (serum miR-155 p=0.01, miR-146a p=0.02). These results further support a role for miR-155 and miR-146a as non-invasive, clinically relevant biomarkers for aGvHD. However, the link between their involvement in generalized inflammation and in specific pathophysiology requires further investigation at a systemic level.

P30

Teresa Borrello
Biosciences

Harnessing the role of HDAC6 inhibitors in liver fibrosis

Liver fibrosis is a medical condition leading to liver impairment and hepatocarcinoma. The limited pharmacological options make developing novel therapeutics for fibrosis an urgent medical issue. Liver fibrosis has been associated with aberrant HDAC activities and for many decades, HDACis have drawn widespread attention as therapeutic agents for different diseases. However, due to reports indicating several challenges encountered with the use of pan-HDACi and their off-target effects, there is a need to develop isoform-selective inhibitors. Based on these observations, further studies pointed out how only HDAC6 enzyme could be selectively targeted, mostly because of its cytoplasmic localization. HDAC6, a microtubule-associated HDAC, is significant in the pathogenesis and progression of fibrotic diseases. Selective HDAC6i have been examined in diverse fibrotic diseases but it appears that they have not been yet evaluated in liver fibrosis. We herein developed novel HDAC derivatives bearing an original chemical scaffold, which are highly selective for HDAC6, having low inhibitory potency over HDAC1 and HDAC8. Our preliminary results show that the compounds were capable of reducing primary rat Hepatic Stellate Cells activation at low dose (100 nM) and inhibiting their fibrogenic markers. The compounds were also able to reduce the expression of acetylated tubulin and α-sma expression, which are implicated in HSCs maturation and growth. Fibrotic lesions are associated with an aberrant expression of TGF-β1, which is a potent Epithelial to Mesenchymal inducer. Our HDAC inhibitors were able to reduce the TGF-β1-induced EMT markers and impaired the SMAD3 activation in response to TGF-β1 stimuli. Since SMAD3 is a core element for TGF-β1 signaling, its inactivation impairs HDAC6-dependent deacetylation of α-tubulin highlighting the role of HDAC6 in EMT through the TGF-β1-SMAD3 signaling pathway. The results highlighted in this work may pave the way for the identification of first-in-class molecules for the treatment of liver fibrosis.

P31

Lauren Walker
NUTCRI

Investigating the prevalence and severity of cerebral amyloid angiopathy in Lewy body disease.

Background. Dementia with Lewy bodies (DLB), Parkinson's disease dementia (PDD), and Parkinson's disease (PD) collectively known as Lewy body diseases (LBDs) are neuropathologically characterised by the presence of alpha-synuclein deposits termed Lewy bodies. However, LBDs also exhibit pathology commonly associated with Alzheimer's disease (AD) (i.e. hyperphosphorylated tau and amyloid β (Aβ). Aβ can be deposited in the walls of blood vessels in the brains of individuals with AD, termed cerebral amyloid angiopathy (CAA). The aim of this study was to investigate the type and distribution of CAA in DLB, PDD, and PD and if this differs from AD.

Methods. CAA type, severity, and topographical distribution was assessed in human post-mortem tissue from 94 AD, 30 DLB, 17 PDD, and 11 PD cases. The brain regions assessed included frontal, temporal, parietal and occipital cortices.

Results. Significantly more AD ($p<0.001$), DLB ($p<0.05$) and PDD ($p<0.01$) cases exhibited CAA (Type 1 or Type 2) compared to those without. There was no difference in PD cases with and without CAA ($p>0.05$). Type 1 CAA accounted for 37.2% of AD cases, 10% of DLB cases, and 5.9% of PDD cases. Type 1 CAA was not observed in PD cases. In all cases, occipital cortex was the most commonly affected region. There was a hierarchical topographical distribution in regions affected by CAA where AD and DLB displayed the same distribution pattern that differed from PDD and PD.

Conclusion. Topographical patterns and severity of CAA in DLB more closely resembled AD rather than PDD, and as Type 1 CAA is associated with clinical dementia in AD, further investigations are warranted into whether the increased presence of Type 1 CAA in DLB compared to PDD are related to the onset of cognitive symptoms and is a distinguishing factor between LBDs.

P32

Graham McClelland
PHSI

The impact of introducing feedback on ventilation rate and volume by paramedics treating cardiac arrests

Background
Adequate ventilation is an important aspect of high quality cardio pulmonary resuscitation (CPR). The European Resuscitation Council (ERC) recommends ventilating cardiac arrest patients at a rate of 10 per minute with a tidal volume of 500-600 ml and warns against hyperventilation. Research suggests rescuers frequently deliver excessive ventilations during CPR and that hyperventilation is associated with increased intrathoracic pressure, impaired haemodynamics and cerebral vasoconstriction which are known to be deleterious to survival. VANZ1, a manikin-based study ran in North East Ambulance Service (NEAS), demonstrated a significant improvement in compliance with ERC ventilation recommendations during CPR using the Zoll Accuvent feedback device.

Aim
The VANZ2 study aimed to determine whether introducing real time ventilation feedback improved compliance with ventilation recommendations in clinical practice during CPR.

Method
A stepped wedge, cluster randomised trial using three NEAS ambulance stations. Adult cardiac arrest patients who were resuscitated and where a study crew were first on-scene were eligible for recruitment. Traumatic cardiac arrests and suspected pregnancy were excluded. Patients were randomised to either ventilation with feedback or no feedback based on cluster and date. The primary outcome was % ventilations delivered in compliance with ERC recommendations. Secondary outcomes included survival to 30 days.

Results
Between 01/08/21 and 31/01/22 eighteen patients (mean age 58 years (SD 17), 50% male) were enrolled into the trial although only 14 provided ventilation data and only one patient survived to 30 days. The trial failed to recruit the anticipated number of patients so descriptive results are presented.

Table 1. VANZ2 results summary describing no feedback (n=11) vs feedback (n=3)

Mean ventilation rate (breaths per minute)	14 17
Ventilation rate in target (%)	18 46
Mean ventilation volume (mls)	467 563
Ventilation volume in target (%)	20 52
Ventilation rate & volume in target (%)	3 25

Conclusion
VANZ2 showed that ventilations are rarely delivered in line with guidelines and that feedback may improve compliance. The trial failed to recruit the necessary numbers of patients to demonstrate any statistical differences so needs to be interpreted with caution. The below

anticipated recruitment may be due to multiple factors including the small size of the study, issues keeping trial devices on trial stations and the immense background pressures due to COVID and winter that staff were dealing with at the time. The impact of delivering ventilations more compliant with guidelines, as part of an overall high-quality CPR package, needs further investigation.

P33

Birthe Dorgau
Biosciences

Spatial transcriptomics of human pluripotent stem cell derived retinal organoids offers new insight in retinal development

Single cell RNA-Seq (scRNA-Seq) enables identification of distinct and changing cell populations during retinal development, but the spatial resolution of those cell populations is missing. Spatial transcriptomics (ST) enables the visualisation and quantitative analysis of the transcriptome in spatial location, providing a novel tool for detailed studies of human retinal development. In addition to scRNA-Seq, this study performed for the first time ST on pluripotent stem cell derived retinal organoids (ROs) to examine the spatial resolution of retinal cell-types during development.
For this purpose, ROs were cultured until day210 of differentiation and collected at day10, 20, 35, 45, 60 ,90, 150 and 210 for ST and scRNA-Seq. experiments which were performed using either the 10xVisium Spatial Gene Expression Kit or the Chromium Single Cell 3' Library & Gel Bead Kit (10xGenomics).
Early retinal organoids (day10) were dominated by a cell cluster expressing eye-field marker genes whereas later stages (day20/35) were characterised by RPCs. Additionally, clusters of ocular surface epithelium and lens/cornea origin were found in retinal organoids at these stages. Retinal ganglion cells appeared at day35 and were detected throughout differentiation. Mid stages of retinal development were characterised by the reduction of the RPC pool and revealed photoreceptor precursor expression at day90, which matured to rods and cones in late developmental stages (day150/210). The comparison of ST and scRNA-Seq. data indicated a comparable cluster distribution in early and late development stages, but differences were apparent in the mid stage. This suggests that the mid developmental period is the most plasticity and important stage in retinal development.
In conclusion, ST provides a powerful tool to investigate the spatial resolution changes within a tissue during development, demonstrating here for the first time on human retinal organoids. Thus, ST offers significant insights in normal retinal developmental and/or disease pathological mechanisms.

P34

Valeria Di Leo
NUTCRI

The effects of resistance exercise training on mitochondrial myopathy patients

Patients with single, large-scale mitochondrial DNA (mtDNA) deletions often present with mitochondrial myopathy (MM) for which no treatment is available. Exercise intervention induces beneficial effects in mitochondrial myopathy. However, the mechanisms for this are not yet understood. The aim of this study is to investigate genetic, biochemical and molecular changes triggered by 16-week resistance exercise training for the development of exercise mimetic drugs for the cure of MM patients.

Quadriceps biopsies were taken from a cohort of single, large-scale mtDNA deletion patients before and after 16-week resistance exercise training. Biopsies were assessed for changes after exercise in oxidative phosphorylation (OXPHOS) staining for Laminin, VDAC1, Ndufb8 and COX1; fibre type distribution, size and contraction force staining for Myh7, Myh2 and Myh1; heteroplasmy using real-time PCR for MT-ND4, MT-CO1, MT-CO3 and MT-CYB genes; differentially expressed (DE) genes and related protein level using RNA-sequencing and imaging mass cytometry, respectively.

Myopathy score is a strong predictor of resistance exercise responsiveness. Compared to healthy controls, all patients present with a significantly lower contraction force of SKM fibres ($p=0.003$) and differential fibre type distribution with increased type 2a ($p<0.04$) and decreased fibre I ($p<0.0001$). After exercise, all cases show an increase in the proportion of fibres with higher level of COX1 ($p<0.0012$) and increase of Myh7+ fibre type size (all patients except P2, $p < 0.0001$). The majority of patients show a significant increase of mitochondrial mass that positively correlates with the increase of aerobic capability ($R=0.81$). Furthermore, 13,132 DE genes were identified between pre- and post-exercise cases, of which 40 DE genes were selected with $p<0.05$ and logarithmic fold change <1.5 or >1.5. Among these, 13 novel exercise-modulated targets were established with an increased protein level localising within the SKM fibres (e.g. Dock11, Edil3, Ikbkg, Gem). Further analysis will assess the protein level of targets with nuclear, perinuclear and extracellular localisation in SKM of MM patients.

In conclusion, resistance exercise training is effective in reducing the OXPHOS defects in MM patients. Importantly, resistance exercise training induces SKM remodelling by activating pathways that modulate the increase of mitochondrial mass and oxidative aerobic capacity, the fibre type determination, the activation of satellite cells and the repositioning of nuclei within SKM. The identification and characterisation of novel exercise-modulated targets may provide insight for the development of exercise mimetic drugs for the cure of MM patients.

P35

Valeria Di Leo
NUTCRI

Mitochondrial dysfunction in Myotonic Dystrophy type 1 patients

Myotonic dystrophy type 1 (DM1) is an inherited type of muscular dystrophy, for which no viable treatment exists. Resistance exercise intervention has been investigated as a potential way to improve skeletal muscle (SKM) weakness in DM1 patients. The aim of this study is to investigate the oxidative phosphorylation (OXPHOS) in SKM biopsies from a cohort of DM1 patients (n=10 males), who previously undertook a 12-week resistance exercise training.

A quadruple immunofluorescence staining was used to investigate Laminin (cell membrane marker), VDAC1 (mitochondrial mass marker), Ndufb8 (Complex I - CI - subunit), and COX1 (Complex IV - CIV - subunit). Using a linear model between VDAC1 and Ndufb8 or COX1, the 95% predictive interval of the fibres from the combined control population were used to classify patients fibres as being: i) normal when clustering in the predictive interval, ii) deficient/lower level than predicted (LLTP) or iii) higher level than predicted (HLTP). Data were resampled (n=5000) and changes between post- and pre-exercise were estimated by permutation test.

Before exercise, all patients, except P01, present with low or high CI deficiency, and some show additional CIV deficiency compared to controls. After exercise, seven patients out of 10 display a significant reduction in Ndufb8 LLTP fibres ($p<0.03$), and the majority of patients present a significant reduction in COX1 LLTP fibres ($p<0.02$) Furthermore, patients who do not show ameliorations in either CI or CIV deficiency, display a significant increase in HLTP fibres, especially for COX1 ($p<0.001$). Additionally, some patients have a significant increase in mitochondrial mass after exercise ($p<0.0001$).

Here, we demonstrate for the first time that DM1 patients may present with mitochondrial dysfunction in both CI and CIV in SKM tissue, confirming recent findings of mitochondrial dysfunction in DM1 fibroblast cell lines. Resistance exercise training rescues the OXPHOS defects both decreasing the deficiency and increasing CI and CIV protein levels.

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Sarah Charman
NUTCRI

Feasibility of the cardiac output response to stress test in suspected heart failure patients

Background
Diagnostic tools available to support general practitioners diagnose heart failure (HF) are limited.

Objectives
(i) Determine the feasibility of the novel cardiac output response to stress (CORS) test in suspected HF patients, and (ii) Identify differences in the CORS results between (a) confirmed HF patients from non-HF patients, and (b) HF reduced (HFrEF) vs HF preserved (HFpEF) ejection fraction.

Methods
Single centre, prospective, observational, feasibility study. Consecutive patients with suspected HF (N = 105; mean age: 72 ± 10 years) were recruited from specialized HF diagnostic clinics in secondary care. The consultant cardiologist confirmed or refuted a HF diagnosis. The patient completed the CORS but the researcher administering the test was blinded from the diagnosis. The CORS assessed cardiac function (stroke volume index, SVI) noninvasively using the bioreactance technology at rest-supine, challenge-standing, and stress-step exercise phases.

Results
A total of 38 patients were newly diagnosed with HF (HFrEF, n = 21) with 79% being able to complete all phases of the CORS (91% of non-HF patients). A 17% lower SVI was found in HF compared with non-HF patients at rest-supine (43 ± 15 vs 51 ± 16 mL/beat/m2, P = 0.02) and stress-step exercise phase (49 ± 16 vs 58 ± 17 mL/beat/m2, P = 0.02). HFrEF patients demonstrated a lower SVI at rest (39 ± 15 vs 48 ± 13 mL/beat/m2, P = 0.02) and challenge-standing phase (34 ± 9 vs 42 ± 12 mL/beat/m2, P = 0.03) than HFpEF patients.

Conclusion
The CORS is feasible and patients with HF responded differently to non-HF, and HFrEF from HFpEF. These findings provide further evidence for the potential use of the CORS to improve HF diagnostic and referral accuracy in primary care.

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Developing Single-Molecule Fluorescence Microscopy Techniques to Investigate Cell Signal Transduction

Transcription factors (TFs) are proteins that are part of an important cell signal transduction process that
regulates gene expression by turning genes on and off. A phenomenon known as transcription factor
clustering may be a mechanism by which TFs increase
their affinity and specificity. It may also be how they
find their specific binding sites in an array of many
random binding sites.
TF research is important as these are involved in many cancers and inflammatory diseases. Research could help find methods to allieviate or prevent these.
Single-molecule fluorescence microscopy can be used
to observe TFs within the cell.

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Cuing Bottom-Up Attention in Bumblebees (Bombus terrestis)

Attention allows animals to select relevant information from the constant sensory stimulation in their environment. In primates, attention can be willingly turned to specific stimuli (top-down attention) or captured by sudden or salient stimuli (bottom-up attention). Bottom-up attention has been shown to increase the perceived contrast of a target preceded by a cue. Here we investigated whether we can find similar processes in bumblebees in two different experiments. In a first experiment, bees were trained to drink a sugar reward at one of two artificial flowers situated on each side of green computer screen. The reward was indicated by a black circle displayed above the correct flower. Post training, bee attention was cued in a series of tests. In all tests with cuing conditions, a blue square cue was flashed either on the side of the target or the opposite side before the target appeared. A condition without a cue was used as a control. The target stimulus was presented after this cue with one of five different contrasts between a full contrast and zero contrast. We predicted that bees would detect the target at a lower contrast when the cue appeared on the same side compared to when the cue was on the other side or when it was not shown. In a second experiment, bees learned to choose a full contrast target instead of a distractor that could take various contrasts. During tests, a cue was presented as in the first experiment either on the side of the target, the side of the distractor or not displayed. Here, we predicted that the cue would hinder the bees' ability to discriminate the target from the distractor when it was on the side of the latter. We here present results for these two experiments and discuss the implications for attention-like processes in insects.

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Helen Griffin
NUTCRI

PROVIDING GENETIC DIAGNOSES FOR GENOMICS ENGLAND 100,000 GENOMES PROJECT PARTICIPANTS WITH INBORN ERRORS OF IMMUNITY

Genomics England (Gel) 100,000 Genomes Project sequenced whole genomes of 70 thousand individuals from UK families with rare diseases. We have reviewed clinical information and genetic variants from a heterogeneous set of 913 families with an inborn error of immunity (IEI) to identify pathogenic mutations. Within the Gel research environment, we developed an analysis pipeline to integrate per family phenotype data and genetic variants to perform a detailed interrogation of rare variants predicted to alter protein sequence in a virtual panel of 509 genes where mutations are known to cause an IEI. We used a case/control method to identify novel genetic causes of immune disorders where damaging variants cluster in specific genes in patients at a higher frequency compared to individuals without an IEI.

Genomic Medicine Centre Exit Questionnaires reported 42 families with a confirmed or likely genetic diagnosis and 68 families with a variant of unknown significance (VUS), requiring functional testing to establish pathogenicity. Our detailed gene centric and, to a lesser extent, case/control analyses revealed an additional 11 families with a likely genetic diagnosis and 66 families with a VUS in a gene compatible with the patient's phenotype, but where the variant had not previously been reported. Of genes outside the virtual IEI panel, identified as bearing an excess variant burden through the case/control analysis, 2 have since been confirmed and reported as novel disease genes. Detailed study of genomes from patients with IEI will ultimately help to improve clinical interpretation pipelines, providing faster and precise diagnoses.

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A stagewise response to mitochondrial dysfunction in mitochondrial myopathy

Mitochondrial DNA deletions clonally expand in skeletal muscle of patients with mtDNA maintenance disorders causing dysfunction of mitochondrial oxidative phosphorylation. Previously we have shown that these mtDNA deletions originally arise and accumulate in the perinuclear mitochondria before spreading through the muscle fibre. This leads to a mosaic pattern of mitochondrial dysfunction in adjacent muscle fibres and segmental deficiency along muscle fibres.

Here we use imaging mass cytometry in a cohort of mtDNA maintenance disorders to characterise the levels of mitochondrial complexes I-IV and ATP synthase alongside a mitochondrial mass marker. We then expand the panel to include markers of key signalling pathways to investigate the cellular response as mitochondrial dysfunction develops.
We find CI and CIV deficiency to be most common, with a smaller proportion of cells that are also CIII and/or CV deficient. Interestingly, we also note that in cells deficient for one or more complexes any unaffected complexes are commonly upregulated over and above the increase of mitochondrial mass expected in ragged red fibres. We further find that cellular response to mitochondrial dysfunction is stage wise and specific to the complexes affected with increases observed in PHB1, MTHFD2, HSP60, SIRT3, LONP1, PDH-E2 and TFAM.

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