Faculty of Medical Sciences

FMS Postdoc Society

4th FMS Postdoc Symposium

15 min research talks from FMS postdocs & flash poster talks

Prizes for best poster, best talk and best paper

Networking & Social Evening Drinks

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

Newcastle University

Athena SWAN Silver Award

Keynote speaker - Dr Eric Hill

Reader in Science,
Department of Chemistry,
Loughborough University

"Determining the role of metabolic dysfunction in Alzheimer's Disease patient iPSC derived neuronal networks"

Friday 9th June 2023
9am-6pm
David Shaw Lecture Theatre – Medical School

Abstract Submission Deadline: 28th April 2023
Registration Deadline: 31st May 2023

Registration, abstract and best paper submission

FMS Post Doc Symposium 2023

After the resounding success of the FMS Post-doc symposium last year, we are back for the fourth 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 **Dr. Eric Hill**, Reader in Science at the Department of Chemistry, Loughborough University.

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, Proteintech, Starlab, Cambridge Research Biochemicals, Parse Biosciences** and **Merck** 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 **#NUFMSPostDocSymp2023** to follow the event!

You can vote for the best poster! Go to: https://www.menti.com/al2n2nfq1sg4


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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

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Website: https://www.societies.ncl.ac.uk/fmspostdoccomm/
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FMS Postdoc Society

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FMS PostDoc Society Committee List		
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Katja Menger		
Sara Luzzi		
Charles Winterhalter		
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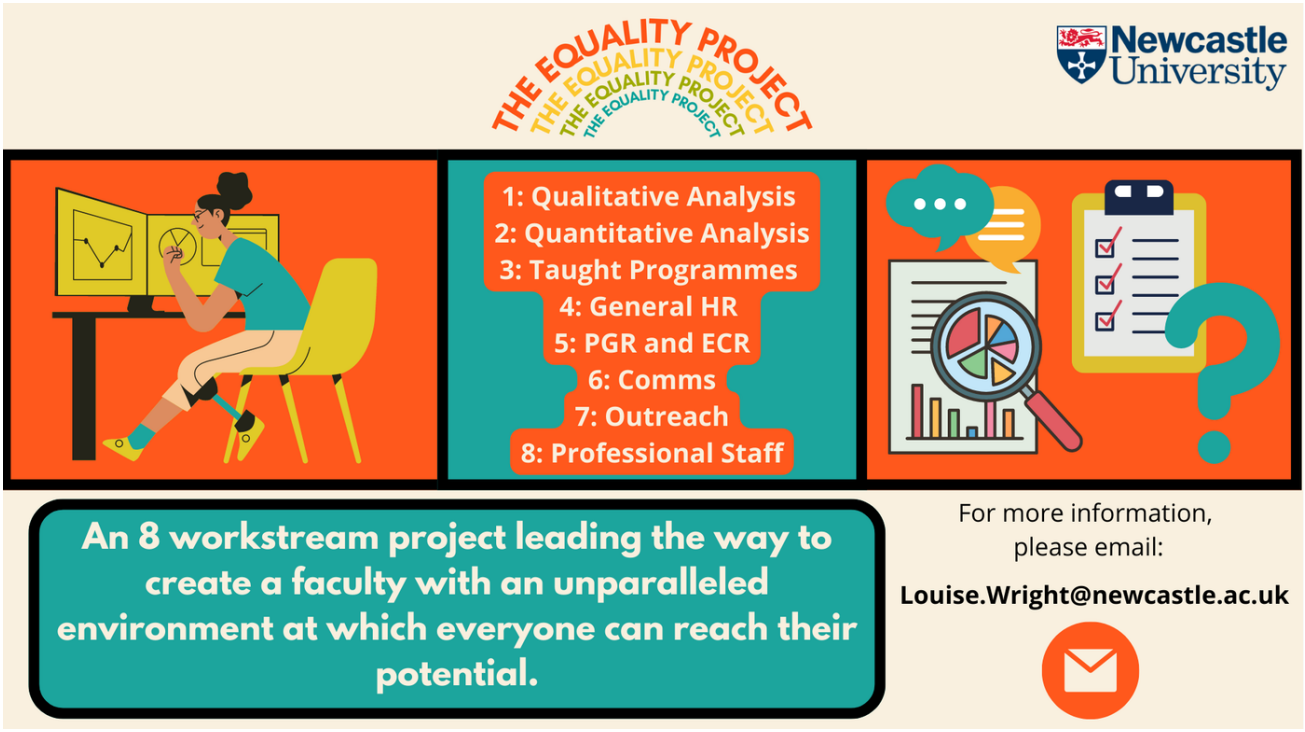
Theo Robert		
Letizia Marchetti		
NEW RECRUITS NEEDED – JOIN US!		

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 for advice and help in organisation,
- Amy Vincent for her contribution and inspiring work for the FMS post-doctoral committee over the years
- PCR Biosystems, Cambridge Research Biochemicals, Parse Biosciences and Merck without whom the prizes would not be so generous,
- All the academics who helped with the best paper selection: Dr Bernard Corfe, Dr Christopher Stewart, Dr James Connolly, Dr Kasia Mickiewicz, Dr Lisa Russell, Dr Elizabeth Veil, Dr Luke Gaughan, Dr Rachel Lawson, Dr Sarra Ryan, Dr Srikanth Ramaswamy and Dr Vivek Nityananda.



Programme

08:30-09:30 **Coffee and Registration**

09:30-09:40 **Welcome and Opening Remarks from Prof. David Burn, Pro-Vice-Chancellor**

09:40-10:40 **Morning Session**

David Koss (NUBI): Nuclear aSyn pathology, DNA damage and associated changes in the nuclear proteasome in Dementia with Lewy bodies
Chun Chen (NUTCRI): Application of imaging mass cytometry on post-mortem brain tissue to understand mitochondrial pathology on Parkinson's
Luke Ouma (NUPHSI): Enabling precision medicine using innovative designs and efficient statistical methods
Joint Best Post-Doc Paper Prize winner: Jenn Ross (NUBI): Discovery and characterisation of arabinofuranosidases which degrade mycobacterial arabinogalactan

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

11:00-12:00 **Midday Session**

Alina Goldberg Cavalleri (School of Dental Sciences): Does acetylcholine play a role in *Candida albicans* pathobiology
Elizaveta Olkhova (NUTCRI): Mitochondrial dysfunction in PV+ cells results in severe and progressive neurological disorder in vivo
Emma Lishman-Walker (NUBI): Developing a kinase activity signature as a tool for personalised medicine in prostate cancer?
Calum Hamilton (NUTCRI): Psychosis symptoms as an early manifestation of autopsy-confirmed Alzheimer's and Lewy body disease

12:00-12:30 **Flash Poster Presentations: 3-Minutes each**

12:30-12:40 **Nefeli Karataraki: Know your impact! Show your Impact!**

12:40-12:50 **Katherine Jacques: Voice: Public and Patient Involvement and Engagement (PPIE)**

12:50-13:00 **Richard McQuade and Emma Cockburn: Teaching opportunities for postdocs in FMS**

13:00-14:00 **Lunch (Boardroom) and Poster Session (Sponsors' Foyer)**

14:00-15:00 **Keynote Speaker: Dr Eric Hill, Reader in Science, Loughborough University**

Determining the role of metabolic dysfunction in Alzheimer's disease patient iPSC derived neuronal networks

15:05-15:30 **Joint Best Post-Doc Paper Prize Talks**

Merel Damen: The ESX-1 Substrate PPE68 Has a Key Function in ESX-1- Mediated Secretion in *Mycobacterium marinum*

Charles Winterhalter: The DNA replication initiation protein DnaD recognises a specific strand of the *Bacillus subtilis* chromosome origin

15:30-16:00 **Coffee Break**

16:00-17:00 **Afternoon Session**

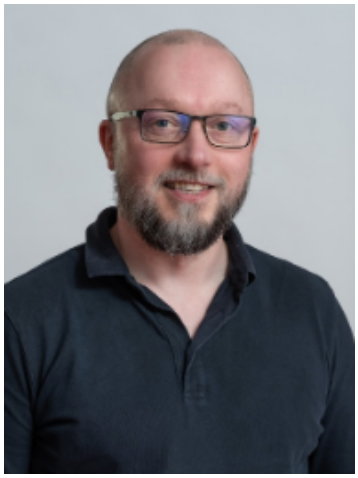
Ben Raymond (NUBI): Proteomic mapping of macrophage reprogramming in response to phagocytosis of apoptotic cells
Esther Fernández-Simón (NUTRCI): High-throughput screening of antifibrotic and antiadipogenic drugs using human FAP cells
Edward Fielder (NUBI): Synergistically Enhancing the Efficacy of Senolytics via Mitochondrial Uncoupling
Joe Inns (NUTCRI): Proteome-transcriptome coupled interrogation of CYLD cutaneous syndrome skin tumours

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

17:30-19:00 **Drinks Reception**

Keynote speaker: Dr Eric Hill

" Determining the role of metabolic dysfunction in Alzheimer's Disease patient iPSC derived neuronal networks"



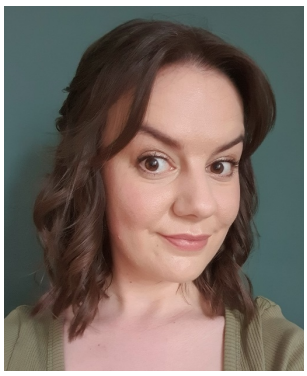
Dr Eric Hill was appointed as a Reader at Loughborough university in 2023 after spending 8 years developing his laboratory at Aston University.

Dr Hill received his Ph.D in Cell and molecular biology in 2004 investigating the secretion of Leukaemia Inhibitory factor in polarised cells.

In 2005 Dr Hill became a postdoctoral research fellow at the University of Aston developing 3D neuronal models for developmental neurotoxicity testing. Gaining experience in 3D neuronal culture, RNA analysis and confocal microscopy Dr Hill developed his research in Stem cell biology and in vitro neuronal model systems. In 2009 Dr Hill was awarded a Tenure tracked research fellowship to develop human in vitro models of Alzheimer's disease during which time he developed his interest in viral transduction methods to develop optogenetics, and calcium imaging methods in induced pluripotent stem cells. He was appointed a lectureship at Aston University in 2014 and promoted to Senior lecturer in 2018 and Reader in 2022.

He is currently engaged in developing tissue engineered stem cell derived models of the CNS in an attempt to study complex cellular interactions that occur in development and disease.

Paper prize winner



Dr Jennifer Ross

Identification of D-arabinan-degrading enzymes in mycobacteria
(Nat Comms; DOI: 10.1038/s41467-023-37839-5)

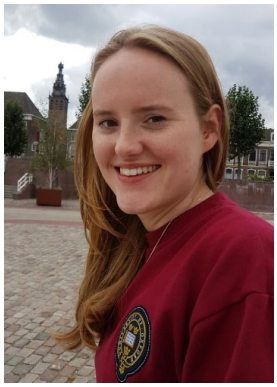
Biography

Jenn is a post-doctoral researcher in Elisabeth Lowe's lab in the Biosciences Institute, where she works on characterising the structure of enzymes which degrade rare sugars in mycobacteria.
Jenn has a history in biochemistry, completing her undergraduate degree with a Masters in Medicinal Chemistry at the University of Edinburgh. Her PhD focused on the structural characterisation of novel ferritin proteins, using techniques including mass spectrometry and cryo-EM. This PhD was a collaboration the University of Edinburgh and the Marles-Wright lab at Newcastle University.
After her PhD Jenn was a post-doc in Cullen lab at the University of Bristol where she continued her work in structural biology in the field of endosomal proteins.
In 2021, Jenn started her role in the Lowe lab and has since published in Nature Communications. The Lowe lab recently secured Wellcome and BBSRC funding to continue the work Jenn is conducting.

Abstract

Bacterial cell growth and division require the coordinated action of enzymes that synthesize and degrade cell wall polymers. Here, we identify enzymes that cleave the D-arabinan core of arabinogalactan, an unusual component of the cell wall of *Mycobacterium tuberculosis* and other mycobacteria. We screened 14 human gut-derived Bacteroidetes for arabinogalactan-degrading activities and identified four families of glycoside hydrolases with activity against the D-arabinan or D-galactan components of arabinogalactan. Using one of these isolates with exo-D-galactofuranosidase activity, we generated enriched D-arabinan and used it to identify a strain of *Dysgonomonas gadei* as a D-arabinan degrader. This enabled the discovery of endo- and exo-acting enzymes that cleave D-arabinan, including members of the DUF2961 family (GH172) and a family of glycoside hydrolases (DUF4185/GH183) that display endo-D-arabinofuranase activity and are conserved in mycobacteria and other microbes. Mycobacterial genomes encode two conserved endo-D-arabinanases with different preferences for the D-arabinan-containing cell wall components arabinogalactan and lipoarabinomannan, suggesting they are important for cell wall modification and/or degradation. The discovery of these enzymes will support future studies into the structure and function of the mycobacterial cell wall.

Paper prize winner



Dr Merel Damen

The ESX-1 Substrate PPE68 Has a Key Function in ESX-1-Mediated Secretion in *Mycobacterium marinum*

(mBIO; DOI: 10.1128/mbio.02819-22)

Biography

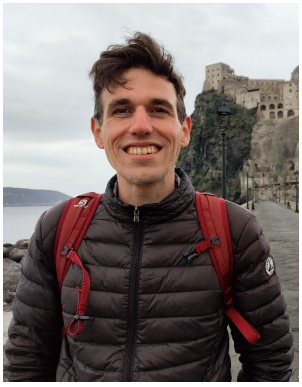
Merel Damen studied at the Vrije Universiteit in Amsterdam, the Netherlands. Since then, her education and training has focused on bacterial secretion systems. In 2018, she started a PhD candidacy in the group of Dr. Edith Houben, in the department of Professor Wilbert Bitter at the Vrije Universiteit in Amsterdam. Here she studied the Type VII secretion systems in pathogenic mycobacteria, such as *Mycobacterium tuberculosis*, the etiological agent of tuberculosis disease. She is now a postdoc in the research group of Professor Tracy Palmer at Newcastle University where she works on the Type VII secretion system of the human pathogen *Staphylococcus aureus*.

Abstract

Mycobacteria use specialized type VII secretion systems (T7SSs) to secrete proteins across their diderm cell envelope. One of the T7SS subtypes, named ESX-1, is a major virulence determinant in pathogenic species such as *Mycobacterium tuberculosis* and the fish pathogen *Mycobacterium marinum*. ESX-1 secretes a variety of substrates, called Esx, PE, PPE, and Esp proteins, at least some of which are folded heterodimers. Investigation into the functions of these substrates is problematic, because of the intricate network of codependent secretion between several ESX-1 substrates. Here, we describe the ESX-1 substrate PPE68 as essential for secretion of the highly immunogenic substrates EsxA and EspE via the ESX-1 system in *M. marinum*. While secreted PPE68 is processed on the cell surface, the majority of cell-associated PPE68 of *M. marinum* and *M. tuberculosis* is present in a cytosolic complex with its PE partner and the EspG1 chaperone. Interfering with the binding of EspG1 to PPE68 blocked its export and the secretion of EsxA and EspE. In contrast, esxA was not required for the secretion of PPE68, revealing a hierarchy in codependent secretion. Remarkably, the final 10 residues of PPE68, a negatively charged domain, seem essential for EspE secretion, but not for the secretion of EsxA and of PPE68 itself. This indicates that distinctive domains of PPE68 are involved in secretion of the different ESX-1 substrates. Based on these findings, we propose a mechanistic model for the central role of PPE68 in ESX-1-mediated secretion and substrate codependence.

Paper prize winner

Dr Charles Winterhalter



The DNA replication initiation protein DnaD recognises a specific strand of the *Bacillus subtilis* chromosome origin

(Nucleic Acids Res; DOI: 10.1093/nar/gkad277)

Biography

Charles completed his PhD in synthetic biology at Newcastle University in 2019 and has since worked as a postdoc in the Murray lab where he investigates bacterial DNA replication in *Bacillus subtilis*. He recently secured external funding to establish his own lab at Newcastle University and will soon focus on DNA damage repair as a research Fellow.

Abstract

Genome replication is a fundamental biological activity shared by all organisms. Chromosomal replication proceeds bidirectionally from origins, requiring the loading of two helicases, one for each replisome. However, the molecular mechanisms underpinning helicase loading at bacterial chromosome origins (*oriC*) are unclear. Here we investigated the essential DNA replication initiation protein DnaD in the model organism *Bacillus subtilis*. A set of DnaD residues required for ssDNA binding was identified, and photocrosslinking revealed that this ssDNA binding region interacts preferentially with one strand of *oriC*. Biochemical and genetic data support the model that DnaD recognizes a new single-stranded DNA (ssDNA) motif located in oriC, the DnaD Recognition Element (DRE). Considered with single particle cryoelectron microscopy (cryo-EM) imaging of DnaD, we propose that the location of the DRE within *oriC* orchestrates strand-specific recruitment of helicase during DNA replication initiation. These findings significantly advance our mechanistic understanding of bidirectional replication from a bacterial chromosome origin.

Abstracts selected for oral presentations

O1

David Koss

NUBI

Nuclear aSyn pathology, DNA damage and associated changes in the nuclear proteasome in Dementia with Lewy bodies

Dementia with Lewy bodies (DLB) is a neurodegenerative disease, typified by the cortical deposition of alpha-synuclein (aSyn) containing Lewy bodies(LBs) in the neuronal cytoplasm. The consequence of aSyn dysfunction and cause of LB formation is unknown.
In DLB, many cellular processes are impaired, which though diverse, are unified by the requirement of accurate gene expression. Genomic DNA damage has recently been highlighted as a prominent neurodegenerative mechanism and maybe associated with aSyn pathology in synucleinopathy models. As such, the delineation of disease dependent alterations to DNA damage and repair (DDR) may highlight novel therapeutic targets for DLB and related diseases.
Nuclear aSyn (aSynNuc), single strand (SSBs) and double strand DNA breaks (DSBs) in neuronal / non-neuronal populations were quantified in fixed sections of post-mortem human temporal cortex (Con and DLB, n=12 per group) via immunohistochemistry. Nuclear fractionates from frozen tissue were investigated for aSynNuc pathology and DNA damage via western blots and further investigated by quantitative proteomics.
aSynNuc was confirmed in control and DLB cases via immunohistochemistry, western blot and proteomic analysis. In DLB cases, aSynNuc oligomerisation and increased aSynNuc phosphorylation was evident. Strikingly, DSBs were upregulated in DLB cases, which correlated with the degree of pathological aSynNuc phosphorylation. Intriguingly, DSB derived DNA was observed in LBs (~90%) implicating damage mediated ectopic cytoplasmic DNA as faciliatory to aSyn aggregation. Via proteomic analysis, enrichment of the gene ontology biological function term “cellular response to DNA damage” was reported, with principle DSB repair effectors upregulated. Critically, elevated DSBs were confirmed in a pre-symptomatic aSyn mouse model, indicating the early-stage occurrence of DNA damage within disease progression and directly associates it with pathological aSyn.
The data support DNA damage and impaired DDR as contributing to synucleinopathies and implicates DNA damage as occurring alongside aSynNuc modification, whilst potentially also contributing to cytoplasmic aSyn aggregation.

O2

Chun Chen

NUTCRI

Application of imaging mass cytometry on post-mortem brain tissue to understand mitochondrial pathology on Parkinson's

Introduction

Mitochondrial dysfunction within neurons, particularly those of the substantia nigra, has been well-characterized in Parkinson's and associated with Parkinson's related neuronal loss. To further our current understanding of the nature of this dysfunction and its impact on the neuronal health, we utilised imaging mass cytometry (IMC) to perform complex proteomic profiling of mitochondrial and the associated proteins. IMC is a powerful technique that can simultaneously detect up to 47 proteins at a single cell level, through the use of lanthanide metal-conjugated antibodies. Here, we established and applied large antibody panels involving key components in varying key mitochondrial pathways alongside several different brain cell markers. This allows correlative analysis of changes in mitochondrial OXPHOS and regulatory mechanism of mitochondrial fatty acid metabolism and mitochondrial quality control in both neurons and astrocytes of Parkinson'smidbrain.

Methods

Post-mortem human brain sections (FFPE, 5um thickness) from Parkinson's cohorts (n=15) were subjected to IMC for the measurement of protein abundance in healthy controls (n=15). Statistical analysis was performed using R. Original signal intensity values for each targeted protein output from IMC were transformed into z score. Statistical differences between groups were described using Bayesian estimation, Mann-Whitney U test, and Wilcoxon signed-rank tests, alongside multiple linear regression modelling.

Results and Conclusions

Profiling of the mitochondrial protein abundance highlighted the heterogeneity between individuals within each group. We found evidence of deficiencies involving multiple respiratory chain subunit in both dopaminergic neurons (PMID: 33980828) and GFAP-positive reactive astrocytes (PMID: 34779538). Meanwhile, there is a synergistic decrease in the abundance of Parkin, PINK1, Phospho-Ub, HSP60, TFAM and SIRT3 in Parkinson's neurons, suggesting the regulatory machinery of mitochondrial quality control including mitophagy, mitochondrial proteases and biogenesis might be impaired. Whether or such changes also occur in Parkinson's astrocyte are still under investigation. Another analysis on progress is to measure a panel of key protein components in mitochondrial fatty acid oxidation pathways and their association with OXPHOS defects on Parkinson's brain. This dataset will allow us to explore whether Parkinson's cells are able to use fatty acid as a alternative energy source in response to OXPHOS defect.

Conclusion

Our studies highlighted the use of IMC in the assessment of mitochondrial protein expression in Parkinson's, providing important post-mortem evidence to support the complexity of impaired mitochondrial protein homeostasis in Parkinson's, which may increase the neuronal vulnerability to age-related oxidative stress and contribute to early neurodegeneration.

O3

Luke Ouma

NUPHSI

Enabling precision medicine using innovative designs and efficient statistical methods

Increased understanding of disease at the molecular level has underscored the need for a paradigm shift in drug development, from a one-size-fits-it-all approach – treatments that work for all patients, to developing targeted therapies that work for particular subgroups of patients.
Umbrella and basket trials are a vehicle for potentially efficient and faster drug development in the era of precision medicine by studying multiple diseases, multiple treatments or both under a single trial infrastructure and protocol. Umbrella trials investigate multiple therapies within a single disease setting, while basket studies investigate a single therapy in multiple diseases sharing a common characteristic.
Despite their operational advantages, umbrella and basket trials raise important statistical complexities in their attempt to answer multiple treatment-related questions.
However, by developing complex statistical methods, it is plausible to propose new trial designs and analysis techniques that lead to development of trials with fewer patients, shorter duration, lower costs and patients benefit early from the right treatment.
To enhance the efficiencies of these designs for faster and efficient drug development, we propose introducing two important efficiencies in their design and analysis; namely, the use of Bayesian methods that allow sharing of information across common treatment arms to i) inform trial adaption (adjusting allocation ratios) in favour of experimental treatments if they are superior to control; and ii) enable better approaches for the analysis of basket trials.
Our findings suggest that the use of Bayesian statistical methods to share information across common control treatments, clinicians can understand experimental therapies better. Further, our proposed approach for analysing basket trials shows the best performance for analysing basket trials with small sample sizes compared to existing methods.
Novel statistical methodology presents a useful framework for introducing considerable efficiencies in trial design in the era of precision medicine and revolutionise drug development.

O4

Jenn Ross

NUBI

Discovery and characterisation of arabinofuranosidases which degrade mycobacterial arabinogalactan

Complex polysaccharides from a range of sources can be broken down by multiple members of the human gut microbiota. We exploited the diversity of microbiota to find enzymes capable of degrading the elaborate cell walls of acid-fast organisms, such as Mycobacterium tuberculosis. Mycobacterial cell walls contain a complex glycan called arabinogalactan (AG), with no known enzymes known to cleave its D-arabinan component.

We have discovered members of a glycoside hydrolase family (GH172) from gut bacteria which have exo-D-arabinofuranosidase activity on AG. We have characterised three GH172 enzymes from the gut Bacteroidetes species *Dysgonomonas gadei*, and used a combination of X-ray crystallography and cryo-EM to gain insight into their structure. This reveals an unexpected diversity of oligomerization states within the family, with trimeric, hexameric and dodecameric structures being observed.
Additionally, we have shown enzymes from this family are also found in *Nocardia brasiliensis*, *M. avium paratuberculosis*, and parasitic bacteria, suggesting that the ability to degrade mycobacterial glycans plays an important role in the biology of diverse organisms.

O5

Alina Goldberg Cavalleri

School of Dental Sciences

Does acetylcholine play a role in Candida albicans pathobiology

Candida albicans is a common member of the human microbiota. However, in some environmental contexts, such as microbial dysbiosis or when the host immune system is compromised, *C. albicans* can become pathogenic. This can cause invasive damage to mucosal surfaces and if it becomes systemic it can cause life-threatening infections. Key features of candida pathogenesis are the ability to switch from the yeast to a filamentous invasive form (hypha) and to produce biofilms. Since biofilms are known to be resistant to antifungal drugs and provide a reservoir for future infections, drugs that affect morphogenesis and biofilm formation could represent and important addition to the therapeutic armoury against candidiasis.
The human muscarinic receptor agonist, pilocarpine hydrochloride (PHCl), can reduce filamentation and biofilm formation in vitro in a dose dependent manner in *C. albicans* clinical isolates. The inhibitory effect was also observed using different hypha-inducing media. Comparative transcriptomic analysis of cells treated with PHCl showed that, compared to untreated controls, expression of virulence factors like adhesins (e.g. Als3), proteases (e.g. SAP5-7) and the toxin, candidalisyn (ECE1), were significantly down regulated by PHCl. Intriguingly, the 7 transmembrane receptor RTA3, which was reported to be involved in biofilm formation, was found to be up regulated. To begin to elucidate the molecular mechanisms and signalling pathways underlying the effect of PHCl on *C. albicans*, we assessed the effect of PHCI on biofilm formation in mutants deficient in transcriptional regulators and genes from signalling pathways involved in morphological switching and virulence. Additionally, we generated and assessed a RTA3 mutant using CRISPR/Cas9 technology. Preliminary data indicate that mutants of key transcriptional regulators and signalling mediators are recalcitrant to the effects of PHCl offering clues to the potential regulatory pathways in *C. albicans* by which PHCl modulates the yeast-hypha transition and biofilm formation

O6

Elizaveta Olkhova

NUTCRI

Mitochondrial dysfunction in PV+ cells results in severe and progressive neurological disorder in vivo

Introduction:
Mitochondrial diseases comprise the largest group of inherited metabolic disorders. Neurological symptoms include epilepsy, ataxia, and cognitive impairment. Previous studies implicated severe oxidative phosphorylation (OXPHOS) deficiencies in GABAergic inhibitory neurons accompanied by neurodegeneration in mitochondrial disease. This study aims to test the hypothesis that metabolically demanding fast-spiking parvalbumin-expressing (PV+) neurons are highly 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) gene via cre-loxP system. Mice were characterised at behavioural, neuropathological, and molecular levels. A human post-mortem neuropathological study was conducted in primary visual cortex tissues from 11 patients with primary mitochondrial disease with epilepsy and/or cognitive impairment and/or ataxia and compared to 16 neurologically-normal controls.

Results and conclusions:
In control human post-mortem tissues, we detected an increased expression of OXPHOS machinery in PV+ cells vs. non-PV cells, and PV+ neuronal loss in patients with primary mitochondrial disease in the occipital lobe, concomitant with OXPHOS defects in remaining PV+ neurons. Mutant mice exhibited a progressive phenotype, initiating at 8 weeks of age with tremor, and neuropsychiatric features including cognitive impairment and anxiety-like behaviour. Hyper-locomotion and stargazing (absence-like seizures) were detected at 10 weeks, with severe ataxia observed by 12 weeks. Knockout mice had reduced weight and severely shortened lifespan. OXPHOS complexes I and IV within the PV+ cells of the knockout mice had differential deficiency levels which were brain region dependent. PV+ neurons demonstrated an upregulation of anaplerosis enzyme pyruvate carboxylase. PV+ Purkinje neurons showed a reduction in mtDNA copy number and modest cell loss. Cerebellum exhibited reactive microgliosis and astrogliosis. The novel mouse model recapitulates key features of neurological phenotype associated with mitochondrial dysfunction and could be used as a powerful translational model.

O7

Emma Lishman-Walker

NUBI

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

Androgen receptor (AR) signalling is essential for the normal development and function of the prostate. Prostate cancer (PCa) is characterised by aberrant AR signalling. Hence, first-line intervention is androgen deprivation therapy (ADT) to target this pathway. Whilst initially effective, resistance to ADT commonly arises highlighting the clinical need for newer personalised therapies to be developed for PCa.
Protein kinases post-translationally modify the AR to regulate activity. This relationship between kinases and the AR represents a potential therapeutic window, through inhibiting the kinases that activate the AR we propose to indirectly modulate AR signalling. Through a kinome-wide siRNA screen we identified a panel of protein kinases that regulate AR transcriptional activity, that can be targeted with small molecule inhibitors. We have developed a multiplex antibody cocktail for imaging mass cytometry (IMC) to visualise the expression of our chosen kinases within PCa tissue. Furthermore, additional antibodies will be incorporated to assess kinase activity using downstream targets, components of the AR signalling cascade and structural markers to provide cellular context. Using IMC we will evaluate the activity and protein level of our kinases in PCa. This will first be assessed in our cell line tissue microarray which includes kinase inhibitor and siRNA-treated samples to understand crosstalk between the AR and our chosen kinases before transitioning into valuable patient samples. Furthermore, we will use our ex vivo cultured tissue slice model to determine kinase activities both pre- and post-treatment with kinase inhibitor to determine whether our signature of kinase activities can predict response.
In the future we hope this tool will assist in the delivery of personalised medicine for PCa patients, and help to stratify patients for clinical trials to increase the likelihood of success for kinase inhibitors.

O8

Calum Hamilton

NUTCRI

Psychosis symptoms as an early manifestation of autopsy-confirmed Alzheimer's and Lewy body disease

Background
There have been several case reports of neurodegenerative dementias characterised initially by symptoms of psychosis, but these have not been widely examined. Psychosis-related symptoms - hallucinations and delusions - are particularly important, causing additional burden, predicting poor outcomes, and possibly indicating underlying Lewy body disease, which is often missed in clinical practice.
We therefore examined the reported history of psychosis symptoms in brain tissue donors prior to known dementia to examine whether these symptoms were indicative of underlying neurodegenerative pathology including Lewy body disease.

Methods
Brain tissue donors were drawn from six UK brain banks through the Brains for Dementia Research collaboration. Informants provided information on the presence or absence of psychiatric symptoms including psychosis in the years prior to donation. Presence of dementia at each time point was assessed through clinical dementia rating. Lewy body and Alzheimer's pathologies were examined with standardised neuropathological assessments.
Death-age adjusted models examined the associations between psychosis symptoms in people initially free from dementia, and eventual pathological findings after brain tissue donation.

Results
In people not yet known to have dementia (n = 332), psychosis symptoms were infrequent (n = 35), but associated with significantly greater likelihood of dementia-like neurodegenerative pathology being present at eventual autopsy (Risk Ratio = 3.13, $p < 0.001$). This effect included both Alzheimer's disease (Risk Ratio = 3.91, $p < 0.001$) and limbic or neocortical Lewy body pathology (Risk Ratio = 3.32, $p < 0.001$), the two commonest causes of dementia.
Delusional beliefs were associated with both major pathologies, while presence of hallucinations in cognitively healthy people were strongly indicative of eventually confirmed of Lewy body pathology (Risk Ratio = 5.60, $p < 0.001$).

Conclusions
Dementia-free individuals with late-onset psychosis should be investigated for possible unrecognised neurodegeneration, and may be at risk of future dementia.

O9

Ben Raymond

NUBI

Proteomic mapping of macrophage reprogramming in response to phagocytosis of apoptotic cells

In the human body, millions of cells regularly undergo apoptosis. To maintain tissue homeostasis, these apoptotic cells are removed via a process referred to as efferocytosis, or the phagocytosis of apoptotic bodies. This process triggers a cascade of reprogramming events in the phagocytic cell, namely a shift towards a pro-resolving phenotype that promotes wound healing. Impairment of this process is detrimental to the body, with an accumulation of secondary necrotic cells, leading to heightened inflammation, and development of disease states such as autoimmunity. To date, no studies have interrogated global changes in the macrophage proteome following efferocytosis. Our aim was to thus gain a deeper understanding of the molecular mechanisms underpinning macrophage efferocytosis, which could provide insight to novel regulators of this crucial process. To do so, we developed a robust protocol for the investigation of proteome changes in immortalised and primary murine macrophages following efferocytosis of apoptotic Jurkat cells using stable isotope labelling by amino acids in cell culture (SILAC) combined with data-dependent acquisition mass spectrometry. With this method, we show that upon efferocytosis, macrophages undergo significant proteomic changes, indicative of complete reprogramming of the cell. As expected, we found numerous pathways associated with efferocytosis to be upregulated, such as phagocytosis, phagosome maturation, oxidative phosphorylation, and lipid metabolism. Collectively, these findings validate the ability of our methodology to map macrophage reprogramming and plasticity. Our data also uncovered numerous proteins that may have a yet undiscovered role in efferocytosis, such as the lipopolysaccharide co-receptor CD14. Supporting this, using flow cytometry and confocal microscopy, we found that antibody blocking of CD14 impaired efferocytosis. Using our novel proteomic approach, our study successfully mapped proteomic changes that occur in macrophages following efferocytosis. This has potential widespread applications including the study of ex vivo samples from patients suffering from diseases associated with defective efferocytosis.

O10

Esther Fernández-Simón

NUTCRI

High-throughput screening of antifibrotic and antiadipogenic drugs using human FAP cells

Background:
Fibroadipogenic precursor cells (FAPs) are the main cells involved in the expansion of fibro-fatty tissue in muscles of patients with muscular dystrophies. FAPs can differentiate into fibroblast or adipocytes leading to the increase of fibrotic and adipogenic tissue in muscle. Growing studies have shown that FAP regulation in muscular dystrophies is altered, contributing to muscle degeneration, defective muscle regeneration and also reducing the effective delivery of potential drugs into the muscle tissue. There are different pathways governing this process, however, the precise mechanisms underlying FAP differentiation in muscular dystrophies remains not fully understood. Many food and drug administration (FDA) approved drugs can modulate signaling pathways involved in that differentiation, suggesting that modulation of FAPs with pharmacological agonists or antagonists may provide a potential therapy to slow down the progression of muscular dystrophies. This suggestion raises an attractive issue on drug discovery for exploring chemical modulators of FAP regulation.

Aims:
Our principal aim is to obtain a high throughput method that allow us to screen large libraries of FDA approved drugs to find therapeutical approaches able to modulate FAPs in muscular dystrophies. Methods: Herein, we provide an application to measure the rate of differentiation of FAPs into fibroblast or adipocytes after drug treatment. The method is based on human FAPs differentiated in vitro and the differentiation rate is quantified by the detection of specific proteins of fibroblast and adipocytes by in-cell wester assay.

Results:
This protocol has allowed us to set-up a throughput drug screening using batteries of a total of 526 drugs with antiadipogenic/antifibrotic effect. A final group of 8 drugs were selected to have a reduced fibrotic and adipogenic differentiation in human FAPs in in vitro experiments.

Conclusion:
The high throughput screening is a powerful tool for drug discovery that may accelerate the screening of specific FAP modulators to identify drugs that effectively reduce fibrofatty expansion in vitro. Therapies that modify the differentiation of FAPs could influence disease's progression in muscular dystrophies.

O11

Edward Fielder

NUBI

Synergistically Enhancing the Efficacy of Senolytics via Mitochondrial Uncoupling

The accumulation of senescent cells is one of the primary drivers of ageing, and age-associated diseases. This can be exacerbated by insults such as cancer therapy, resulting in premature aging. An emerging class of drugs, senolytics, aim to eliminate these cells. However, first-generation senolytics are often repurposed anti-cancer drugs, which have low therapeutic indices and serious side effects. Our study identifies a specific vulnerability in senescent cells that allows for improved efficacy and reduced doses.

We demonstrate that senescent cells exhibit a lower mitochondrial membrane potential compared to young cells, enabling preferential targeting through mitochondrial uncoupling. When combined with existing senolytics that target anti-apoptotic Bcl-2 proteins, such as Navitoclax, this synergistic approach promotes apoptosis and reduces the effective senolytic dose by over an order of magnitude, with minimal impact on proliferating cells.

Mitochondrial uncouplers, including BAM15, have previously shown promise in treating metabolic disorders. Our findings indicate that short-term BAM15 treatment modestly delays radiotherapy-induced frailty and cognitive deficits in mice. This effect is substantially enhanced by combining it with extremely low doses of Navitoclax. This yields outcomes comparable to the higher doses of Navitoclax previously employed to treat radiotherapy-induced premature aging.

O12

Joe Inns

NUTCRI

Proteome-transcriptome coupled interrogation of CYLD cutaneous syndrome skin tumours

CYLD encodes a de-ubiquitinase targeting K63 and M1-linked ubiquitin chains, regulating a variety of cellular processes. Patients with CYLD cutaneous syndrome (CCS) carry germline heterozygous pathogenic variants in CYLD, typically resulting in predicted truncation of the catalytic domain. CCS skin tumours arise following loss of the remaining wild-type CYLD allele, associated with loss of functional CYLD, making CCS tumours an intriguing model to understand CYLD's role as a de-ubiquitinase in human skin.

We used unbiased proteomics and transcriptomics to study CCS tumour and control skin samples. Snap frozen tissue was homogenised, and prepared for LC-MS/MS proteomics analysis (n=12) or fresh tissue was flow sorted into CD45+/- fractions for transcriptome analysis between CD45- CCS tumour cells and healthy skin (n=10). We investigated which proteins/genes could be dysregulated due to lack of CYLD de-ubiquitination activity by comparing dysregulated proteins/genes to UbiBrowser 2.0 predicted de-ubiquitination substrates of CYLD.

Proteomics analysis identified 5243 proteins of which 1568 were differentially expressed in CCS whole lysate samples, while transcriptome analysis found 2183 genes differentially expressed in CD45- CCS tumour cells, both compared to healthy skin (FC>2, adjusted p-value <0.05). Alignment of proteome and transcriptome data revealed 228 genes dysregulated at both protein and transcript level, and 216 dysregulated genes which are predicted de-ubiquitination targets of CYLD. Ontology analysis revealed enrichment of protein phosphorylation and cell signalling pathways, delineation of which may give insight into druggable targets.

List of poster flash talks

Laura Wright
Aberrant connectivity in resting-state brain networks underlying neuropsychiatric symptoms in prodromal Lewy body disease.

Nicole Mietrach
A role for the ESX4 Type VII secretion system in bacterial competition in *Mycobacterium abscessus*

Chloe Hinchliffe
Identification of Fatigue and Sleepiness in Immune and Neurodegenerative Disorders from Measures of Real-World Gait Variability

Letizia Marchetti
Targeting hijacked super-enhancers in blood cancer

Ellen Silva
Falls in Mild Cognitive Impairment with Lewy bodies (MCI-LB)

Lauren Walker
Investigating the effect of mixed pathologies in dementia with Lewy bodies

Esther Fernández-Simón
Analysing the effect of human fibro-adipogenic progenitor cells from DMD on myogenic differentiation in vitro

Poster abstracts

P1

Laura Wright

NUTCRI

Aberrant connectivity in resting-state brain networks underlying neuropsychiatric symptoms in prodromal Lewy body disease.

Neuropsychiatric symptoms (NPS) are common and occur early in Lewy body dementias (LBD). However, research into their neurobiological underpinnings has been limited. This study investigated associations between NPS and brain functional connectivity (FC) in prodromal LBD using resting state functional MRI.

Forty-seven participants were included with mild cognitive impairment relating to dementia with Lewy bodies (MCI-LB, n=18) or Parkinson's disease (PD-MCI, n=29). Twelve cortical seeds assessed FC in five resting state networks (RSNs): default mode network (DMN), dorsal attention network (DAN), salience network (SN), temporoparietal network (TPN), limbic network (LN) and visual network (VN). Seed-based connectivity maps were entered into general linear models to determine associations with affective disorder, anxiety and psychosis.

In PD-MCI, affective disorder was associated with reduced FC within the LN and SN and increased FC between subcallosal cingulate and multiple RSNs. The LN also demonstrated increased and decreased connectivity with cerebellum and brainstem, respectively. Similar FC patterns were associated with anxiety. The LN and DAN demonstrated increased FC with occipital, cerebellar and frontal regions related to psychosis in PD-MCI. Increased FC within the DMN and between VN and DMN also related to psychosis, as did reduced connectivity between SN and frontal pole. In MCI-LB, affective disorder related to increased FC between right posterior temporal lobes and all networks except for the VN. Psychosis related to increased FC between DAN and right lateral occipital cortex.

Mechanisms of NPS in prodromal LBD are heterogeneous. Although FC within subcallosal cingulate, an area associated with major depression, significantly related to affective disorders in PD-MCI, temporal regions showed greater involvement in MCI-LB. Similarly, aberrant connectivity associated with psychosis, in DMN and attention networks, in PD-MCI was not reflected in MCI-LB. Further research is needed to establish how disease specific alterations in neurotransmission contribute to functional changes associated with NPS in LBD.

P2

Nicole Mietrach

NUBI

A role for the ESX4 Type VII secretion system in bacterial competition in *Mycobacterium abscessus*

The Type VII secretion system (T7SS) was discovered in *Mycobacterium tuberculosis* and linked to pathogenicity of the bacterium. In following years, it was shown that not only Actinobacteria like *Mycobacterium abscessus* but also Firmicutes encode the system. Firmicutes like *Streptococcus intermedius* and *Staphylococcus aureus* use the system for bacterial warfare. They encode for anti-bacterial toxins with adjacent immunity genes. These toxins are transported by the T7SS.

By contrast, the T7SS in Actinobacteria has not been associated with bacterial competition. It is heavily studied in pathogenic Mycobacteria, which can encode up to five of these systems, designated ESX1 to ESX5. ESX1, ESX3 and ESX5 play major roles for virulence and nutrient uptake. However, not much is known about the roles of ESX2 and ESX4.

M. abscessus is a fast-growing non-tuberculous Mycobacterium that is an opportunistic pathogen. The bacterium is being detected in infections of patients with respiratory pathologies, particularly with cystic fibrosis. It rapidly becomes a dominant pathogen within its niche, overcoming well-established bacteria. Little is known about the mechanisms that *M. abscessus* uses for competitive colonisation. It encodes two T7SS; ESX3 and ESX4. At present there is limited knowledge about the role of either of these systems in *M. abscessus*. Preliminary genomic analysis of theT7SS in *M. abscessus* identified a large family of proteins containing a T7SS-targeting domain combined with a toxin domain, and a potential immunity protein encoded adjacently. These proteins appear to be genetically linked to ESX4. Across *M. abscessus* sequenced genomes 40 different toxins have been found. Therefore, I propose that *M. abscessus* uses its ESX4 for antibacterial warfare. To test my hypothesis, I am characterizing three potential toxin/immunity proteins and detect their secretion via the T7SS. This study may ultimately identify the mycobacterial T7SS as an apparatus for bacterial competition assigning an unexpected role to the ESX-systems.

P3

Chloe Hinchliffe

NUTCRI

Identification of Fatigue and Sleepiness in Immune and Neurodegenerative Disorders from Measures of Real-World Gait Variability

Current assessments of fatigue and sleepiness rely on patient reported outcomes (PROs), which are subjective and prone to recall bias. The current study investigated the use of gait variability in the “real world" to identify patient fatigue and daytime sleepiness. Inertial measurement units were worn on the lower backs of 159 participants (117 with six different immune and neurodegenerative disorders and 42 healthy controls) for up to 20 days, whom completed regular PROs. To address walking bouts that were short and sparse, four feature groups were considered: sequence-independent variability (SIV), sequence-dependant variability (SDV), padded SDV (PSDV), and typical gait variability (TGV) measures. These gait variability measures were extracted from step, stride, stance, and swing time, step length, and step velocity. These different approaches were compared using correlations and four machine learning classifiers to separate low/high fatigue and sleepiness.

Most balanced accuracies were above 50\%, the highest was 57.04\% from TGV measures. The strongest correlation was 0.262 from an SDV feature against sleepiness. Overall, TGV measures had lower correlations and classification accuracies.

Identifying fatigue or sleepiness from gait variability is extremely complex and requires more investigation with a larger data set, but these measures have shown performances that could contribute to a larger feature set.

Clinical relevance— Gait variability has been repeatedly used to assess fatigue in the lab. The current study, however, explores gait variability for fatigue and daytime sleepiness in real-world scenarios with multiple gait-impacted disorders.

P4

Letizia Marchetti

NUBI

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 genes involved in cell lineage and proliferation, by recruiting epigenetic remodellers and modifying the epigenomic neighbourhood to ensure chromatin accessibility. In healthy B cells, strong SEs are associated with immunoglobulin heavy and light chain (IGH, IGL and IGK) loci, which together encode the necessary information to produce antibodies. In blood malignancies, and in particular the ones affecting the B-lymphoid lineage, 5-10% of chromosomal translocations result in super-enhancer hijacking, where the juxtaposition of a translocated IG SE next to a proto–oncogene results in its activation. Previous data in our lab helped defining the new concept of epigenomic translocation, where not only the sequence, but the whole epigenomic machinery linked to SEs is moved next to the proto – oncogene in the case of genomic rearrangements, driving cancer development.

Our aim is to confirm the interaction between SEs and proto-oncogenes and to define the essential regions within SEs required for the overexpression of the activated oncogenes; based on 3D in silico simulations, we designed specific gRNAs to direct a dCas9 – KRAB system to epigenetically silence the SE regions predicted to be important for the interaction with the proto-oncogenes. We want to assess the efficient downregulation of the oncogenes involved in the translocation when these specific SE regions are silenced, but we are also interested in the changes in the epigenomic landscape in response to the loss of the SE activity.

Cell lines of interest expressing dCas9 – KRAB were generated by lentiviral transduction and nucleofection protocols were optimised for efficient delivery of the gRNAs. Automated Western Blot (WES) and CUT&RUN were performed as readouts of the silencing, for expression levels and changes to the epigenome respectively. Preliminary data suggest that our line of research 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.

P5

Ellen Silva

NUTCRI

Falls in Mild Cognitive Impairment with Lewy bodies (MCI-LB)

Background: Falls occurrence in MCI patients can be twice that of cognitively intact individuals. Falls consequences can be more serious in this population and MCI fallers are more likely to be institutionalized than non-fallers. The literature has advanced in understanding falls in MCI, however, most of the studies did not determine MCI subtype, concentrating largely on amnestic groups, or focused on those already diagnosed with dementia. Repeated falls are reported as one of the clinical characteristics in dementia with LB (DLB), however, little is known regarding the clinical-specific characteristics of fallers and non-fallers in prodromal DLB.

Objective: to identify clinical, physical activity and mobility measures that differentiate MCI-LB patients fallers and non-fallers from healthy controls.

Methods: As part of the SUPErB study (22 MCI-LB and 13 healthy controls), MCI-LB patients were classified as fallers (at least one fall; n=13) or non-fallers (n=9) based on retrospective report over 12 months. Demographic, disease-specific and cognitive measures were collected. In addition, gait speed was measured through a 4-meters walking test, completed in usual speed whist being timed. Patients were also asked about the participation frequency in vigorous, moderate, and light physical activities. Variables were compared between groups using ANOVAs and nonparametric tests.

Results: MCI-LB fallers walked more slowly ($p<0.001$) and took part in fewer light physical activities ($p<0.001$) compared to healthy controls and MCI-LB non-fallers. MCI-LB fallers and non-fallers were similar for all other measures.

Conclusions: To the best of our knowledge, this is the first study to examine differences in fall status in those with MCI-LB. Even at early stage of disease, there are characteristics that define fallers among people with MCI-LB, including slower gait speed and reported reduction in physical activity. The results highlight the importance of falls prevention/management to prevent and delay the consequences of falls in LB disease.

P6

Lauren Walker

NUTCRI

Investigating the effect of mixed pathologies in dementia with Lewy bodies

Objective: Dementia with Lewy bodies (DLB) is neuropathologically defined by inclusions of α-synuclein (α-syn). However, concomitant Alzheimer's disease (AD) pathologies, hyperphosphorylated tau (HP-*T*) and β amyloid, are observed frequently at *post-mortem* examination, with 50-70% of DLB cases found to have medium to high- level of AD neuropathologic change. Mixed pathologies are associated with an accelerated cognitive decline, which can make diagnosis challenging. An increased burden of all three pathologies in end-stage dementia suggests a potential synergistic interaction between these proteins and is supported by studies that demonstrate α-syn and HP-*T* are co-localised. Proteins can undergo numerous alterations, which can affect their structure and enhance toxicity, however little is known regarding which post-translational modifications (PTMs) of α-syn and tau are co-localised, and are associated with specific clinical symptoms.

Methods: Using tissue microarray (TMA) slides that incorporate 15 anatomically distinct brain regions we investigated if a number of PTMs of α-syn and tau (i.e asyn phosphorylated at serine 129, MC1, CP13, Alz 50 and PHF-1) are frequently co-localised and how they affect protein clearance mechanisms in DLB by using human *post-mortem* brain tissue.

Results: Co-localisations between a-syn pS129 and all tau markers were observed in multiple brain regions, in particular the amygdala and protein clearance mechanisms were impaired in DLB cases with high levels of concomitant AD related neuropathology.

Conclusions: It seems paramount that we understand the interplay between these two pathological proteins, as presently there are no combination therapies to target both pathologies in clinical trials.

P7

Esther Fernández-Simón

NUTCRI

Analysing the effect of human fibro-adipogenic progenitor cells from DMD on myogenic differentiation in vitro

Background: Fibroadipogenic precursor cells (FAPs) are the main cells involved in the expansion of fibro-fatty tissue in muscles of patients with muscular dystrophies. FAPs can differentiate into fibroblast or adipocytes leading to the increase of fibrotic and adipogenic tissue in muscle. Growing studies have shown that FAP regulation in muscular dystrophies is altered, contributing to muscle degeneration, defective muscle regeneration and also reducing the effective delivery of potential drugs into the muscle tissue. There are different pathways governing this process, however, the precise mechanisms underlying FAP differentiation in muscular dystrophies remains not fully understood. Many food and drug administration (FDA) approved drugs can modulate signaling pathways involved in that differentiation, suggesting that modulation of FAPs with pharmacological agonists or antagonists may provide a potential therapy to slow down the progression of muscular dystrophies. This suggestion raises an attractive issue on drug discovery for exploring chemical modulators of FAP regulation.

Aims: Our principal aim is to obtain a high throughput method that allow us to screen large libraries of FDA approved drugs to find therapeutical approaches able to modulate FAPs in muscular dystrophies.
Methods: Herein, we provide an application to measure the rate of differentiation of FAPs into fibroblast or adipocytes after drug treatment. The method is based on human FAPs differentiated in vitro and the differentiation rate is quantified by the detection of specific proteins of fibroblast and adipocytes by in-cell wester assay.

Results: This protocol has allowed us to set-up a throughput drug screening using batteries of a total of 526 drugs with antiadipogenic/antifibrotic effect. A final group of 8 drugs were selected to have a reduced fibrotic and adipogenic differentiation in human FAPs in in vitro experiments.

Conclusion: The high throughput screening is a powerful tool for drug discovery that may accelerate the screening of specific FAP modulators to identify drugs that effectively reduce fibrofatty expansion in vitro. Therapies that modify the differentiation of FAPs could influence disease's progression in muscular dystrophies.

P8

Laura Cordova Rivera

NUTCRI

The prevalence of sarcopenia in an international Parkinson's disease cohort

Background: Sarcopenia (reduced skeletal muscle-mass and strength) appears to be increased in Parkinson's Disease (PD) and is associated with poorer outcomes. However, estimates of its prevalence according to definitions by the revised European Working Group on Sarcopenia in Older People (EWGSOP2) guidelines and its association with disease outcomes have been sparsely reported.
We aimed to evaluate the prevalence of probable and confirmed sarcopenia in an international PD cohort, and to determine its associations with measures of disease severity.

Methods: A longitudinal cohort study of 602 PD participants with mild-to-moderate disease severity is underway in five international sites (Mobilise-D consortium). The EWGSOP2 guidance was used to evaluate the prevalence of probable [assessed by grip-strength or 5 sit-to-stand test] and confirmed [skeletal muscle mass (SMM) by bioimpedance analysis] sarcopenia. Descriptive statistics were used to determine associations between sarcopenia and disease severity [Movement Disorders Society Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS III)] and retrospective falls.

Results: 602 participants [64.8% men; mean(sd) 65.7(9.5) years; mean MDS-UPDRS III score 26.7(12.6)] were recruited at baseline assessment. Data on grip-strength [mean(sd) 33.8(11.2) kg] and SMM [mean(sd) 27.9(7.4) kg] were collected in 596 and 220 participants. Probable and confirmed sarcopenia was observed in 40.1% and 32.3% of participants, respectively. Mean MDS UPDRS III score was 30.1 in those with probable sarcopenia versus 24.5 in those without probable sarcopenia ($p<0.001$), while the mean difference between participants with confirmed versus nonsarcopenic participants was 4.3 points ($p<0.05$). 45.2% participants with probable sarcopenia reported to have fallen in the last 12-months versus 28.9% without sarcopenia ($p<0.001$). Participants with confirmed sarcopenia tended to fall more, but there was no significant differences compared to people without confirmed sarcopenia.

Conclusion: Probable and confirmed sarcopenia was common in a large and representative international PD cohort and was associated with measures of disease severity. This is of relevance as sarcopenia is potentially treatable and associated with adverse outcomes.

P9

Soren Nielsen

NUBI

Mechanisms of transcription termination by Pol I

Transcription by Pol I is stopped by a protein Reb1 roadblock, but the mechanism that leads to termination, i.e. destruction of elongation complex, is controversial. Here, by using nuclear lysates and purified proteins, we found a factor-independent mechanism of termination that solves the current controversies. We show that Pol I paused at Reb1 roadblock undergoes deep backtracking that brings Pol I to a conserved RNA hairpin of the pre-rRNA, where termination takes place. We show that it is encounter with Reb1 roadblock that modifies Pol I so that it loses its intrinsic RNA hydrolytic activity, making the backtracking irreversible. Furthermore, Reb1 makes Pol I vulnerable to termination upon reaching the RNA hairpin. RNA hairpin is strictly required for termination. A mere stopping of Pol I in the absence of Reb1 does not change catalytic properties of Pol I, nor does it leads to termination at the hairpin. The discovered mechanism is rapid and precise and can account for termination demand during exponential growth. We propose that previously reported mechanisms of Pol I termination are fail-safe cover for the factor-independent termination.

P10

Praveen Dhondurao Sudhindar

NUTCRI

Small molecule drug treatments for inherited kidney disease: Nephronophthisis

Renal ciliopathies are a heterogeneous class of disorders caused by dysfunctions of the primary cilia. They are often multisystem disorders characterized by extensive genetic heterogeneity and clinical variability with high levels of lethality and there is marked phenotypic overlap among distinct ciliopathy syndromes. Nephronophthisis (NPHP) is a typical renal ciliopathy phenotype that causes kidney failure often within early childhood, for which there are no curative treatments beyond dialysis and transplantation.

To identify novel therapeutics for NPHP, we designed a high-throughput ciliary phenotype-driven screening strategy to interrogate the TOCRIS library of 1120 biologically active compounds, making use of the Operetta high-content imaging system with Harmony/Columbus software. Initially, 33 compounds were identified that restored ciliary phenotype in renal epithelial cells derived from Cep290 mutant mice. These compounds were subjected to a secondary screen using NPHP patient fibroblasts (P-BB), carrying compound heterozygous CEP290 mutations, including an allele that we had previously shown to be amenable to Anti-sense Oligonucleotide (ASO) mediated exon skipping. In this screen, 12 compounds either restored ciliogenesis or corrected cilia length defects.

A tertiary phenotypic screen of the 12 TOCRIS compounds was then carried out in human urine-derived renal epithelial cells (hURECs) from a NPHP patient (P-HB) carrying CEP290 mutation (p. (Thr832Asnfs*12) and p.(Gly1890*)) along with control hURECs from an unaffected sibling. The initial hUREC phenotypic screen of P-HB cells displayed a ciliogenesis defect compared to the unaffected sibling who had normal cilia. Two of the TOCRIS chemical compounds- an entirely novel inhibitor of p-selectin mediated cell adhesion and an EGFR kinase inhibitor rescued the ciliary phenotype in patient (P-RB). We will employ RNAseq to identify the underlying molecular pathways using the TOCRIS compounds which may reveal novel insights into the mechanisms underlying NPHP secondary to CEP290 mutations and explore this in additional genetic causes.

P11

Rachel Crossland

NUTCRI

Extracellular Vesicles Contribute to the Mechanism of Action of Autologous Serum Eye Drops as a Treatment Modality for Chronic Graft Versus Host Disease

Chronic graft versus host disease (cGvHD) is a late complication of allogeneic stem cell transplantation. It is a multisystemic alloimmune disorder that occurs in 30-70% of transplanted patients. Eyes are affected in >50% of patients, resulting in eye irritation, inflammation and infiltration with immune cells, leading to keratoconjunctivitis sicca. Autologous serum eye drops (ASED) are a possible treatment option, however, the mechanism of action is poorly understood. Extracellular vesicles (EVs) act as communication molecules in the circulation and have been shown to play a role in transplantation and immunology. They show huge potential as novel GvHD therapeutics, and as clinically translatable biomarkers.

This pilot study aimed to evaluate circulatory extracellular molecular biomarkers in ASED from cGvHD patients, compared to healthy population serums, which may inform on the mechanism of action (MOA).

EVs were isolated by precipitation from the serum of cGvHD patients during ASED processing (N=6) and healthy blood donors (N=6). EV size and concentration were assessed by nanoparticle tracking analysis (NTA). EV microRNA cargo (n=800) was evaluated using NanoString technology. Signature microRNA targets were identified using in silico algorithms.

Isolated EVs demonstrated characteristic cup-shaped morphology and expressed EV-specific markers Alix and Flotillin. ASED EVs demonstrated significantly larger modal size ($p=0.02$), but significantly lower RNA yield ($p=0.02$) compared to control serum. Assessing EV microRNA cargo, ASED EVs expressed an overall higher average number of microRNAs than controls ($p=0.01$). There were 98 microRNAs expressed above the lower limit of detection (LOD) in >3 samples per analysis group. Unsupervised hierarchical clustering showed that ASED EVs clustered distinctly from control EVs. A signature of 62 microRNAs were significantly differentially expressed (DE) in ASED EV compared to controls after FDR correction, of which 33 were upregulated and 29 were downregulated (FC range -6.04-3.35, p-value range $p=<0.001$-0.047). Target enrichment, focusing on the top 20 DE microRNAs, identified 48 genes (TargetScan) associated with 15 microRNAs. Interestingly, these mapped to KEGG (TGF-B signaling) and REACTOME (retinoid cycle, diseases of visual transduction) pathways aligned to vision or ocular GvHD therapy associated MOA.

This preliminary study indicates that EVs in the ASED of patients with ocular GvHD may be in part responsible for the therapeutic mechanism of action. EVs show potential for further investigation as a potent ocular GvHD therapy.

P12

Paul Norton

NUBI

Synchrotron Micro Computed X-ray Tomography and Spatial Resolution of a Whole Human Embryo

X-ray computed tomography (CT), is a powerful, non-destructive technique which provides unrivalled metrics of internal structures for biological specimens and inert materials. Frequently used within hospital settings for routine medical diagnostics to provide detailed cross sections of bone and organs for rapid detection of disease, this technology has been expanded upon in recent years. The UKs national synchrotron particle accelerator, based at Harwell Diamond, Oxford, is a light-based synchrotron. Near-light speed colliding subatomic particles produces a light source 10 billion times brighter than our own Sun. The light-source is harnessed and distributed across 40+ beamlines. The I13-1 Manchester beamline is the world's longest beamline at the time of construction standing at 250m and produces X-rays 1 million times more powerful than conventional hospital CT scans and allows for the imaging of specimens to a microstructural degree.

The human cell atlas (HCA) and the developmental HCA initiative is a global effort which charts all the cells of a human body from development to adulthood. In recent decades, powerful next-generation single cell RNA sequencing and spatial transcriptome technologies have been introduced. To complement these technologies, this project has aimed to include another modality and perform whole human embryo (Carnegie stage 18, where most organs begin to develop) synchrotron imaging at single cell resolution with the reconstructed 3D imaging volume mapped with spatial genomic data output which will directly correlate organ level information to genetic level information.

A whole human embryo, Carnegie stage 18, was ethanol dehydrated and fixed in 4% paraformaldehyde and subsequently embedded in 100% epoxy resin. The embryo was successfully imaged on both a non-synchrotron-based Nikon 225 XTH ST laboratory source CT scanner, for a more immediate reference image and the synchrotron I13-1 Manchester beamline. The Nikon CT scanner saw reconstructed volumes of 2000 x 2000 x 1350 at 14.422µm voxel size resulting in a 10-hour scan time with a raw data output of 9.83GB, visualised and processed using Avizo 3D software. In comparison, the synchrotron experiment saw a 1.625µm voxel size and a 16-hour scan time, 1.25 objective, 4.5TB raw data and 1.6TB reconstructed data resulting in 7601 reconstructed 2D TIFS.

P13

Sarah Wigham

NUPHSI

Developing a trauma screening tool for people with a learning disability in primary care, and a trauma training resource for community support and service providers

Background: Research suggests exposure to trauma in people with learning disabilities is high; this may affect their mental health and may result in trauma-related mental health conditions (Byrne, 2022: https://doi.org/10.1177/1524838020960). However, resources to identify and support people with learning disabilities affected by trauma are scarce (McNally et al, 2021: https://doi.org/10.1111/jar.12872) and this can be a barrier to accessing appropriate mental health services. This study builds on work developing a measure of trauma for people with learning disabilities in clinical settings (Wigham et al, 2021: https://doi.org/10.1016/j.ridd.2021.103914).

Objectives: (1) to develop a trauma screening questionnaire for people with learning disabilities in primary care and (2) gather data to inform development of an online trauma training resource for providers of community services for people with learning disabilities.

Method: The study is qualitative with data collected using surveys, focus groups and interviews. A local advisory group of people with learning disabilities are consultants to the study advising on study design, data collection, analysis and dissemination of findings.

Results: A summary of findings will be presented covering the format and content of the trauma screening questionnaire and the trauma training resource and views on barriers and facilitators to implementing them.

Conclusions: The primary care trauma screening tool and trauma training resource will contribute to supporting people with learning disabilities with trauma-related mental health conditions in community settings. The work aligns with UK NHS priorities including early intervention, prevention, and recognition of the impact of trauma on mental health.

P14

Amy Tooke

NUBI

Role of the *Staphylococcus aureus* Type VII Secretion System

Staphylococcus aureus is an opportunistic pathogen that colonises the skin and nose, well known for the range of different diseases it can cause, due to the diverse variety of toxins and virulence factors that it produces. One mechanism by which it releases toxins from the cell into its environment is the type VII secretion system (T7SS). Strains produce antitoxins (immunity proteins) to prevent self-killing by the toxins they produce. *S. aureus* competes 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 in in vivo interstrain competition experiments. We investigate whether the T7SS plays a role in colonisation and bacterial competition using animal models. Currently we utilise infected zebrafish larvae to carry out pairwise competition experiments to investigate whether specific toxins confer a competitive advantage to attacker strains against mutants lacking T7SS machinery and cognate immunity proteins. Future work will involve using a cotton rat nasal colonisation model of *S. aureus* by comparing the extent of colonisation by a WT *S. aureus* strain and a T7SS deletion mutant. Then we will identify species targeted by the *S. aureus* T7SS by comparing the nasal microbiome of cotton rats colonised WT *S. aureus* strain to those with a T7SS deletion mutant.

P15

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DnaA-boxes distant from the unwinding site promote helicase loader recruitment in a bipartite chromosome origin

The duplication of genetic material is essential to life. In bacteria DNA replication begins with the master initiator DnaA binding to DnaA-box sequences in the chromosomal origin, followed by DNA strand separation and replicative helicase loading. Despite the universal presence of multiple DnaA-boxes in bacterial origins, the functional and spatial requirements underlying their number and activity is not understood. This is especially true for bipartite origins such as found in *Bacillus subtilis*, where an additional cluster of 17 DnaA-boxes (*incAB*) is essential for replication despite being spatially separated (~1300bp) from the unwinding site (*incC*, 7 boxes). We genetically investigated the spatial and functional relationship between *incC* and *incAB in vivo*. Surprisingly, we find that *incAB* still promotes replication initiation when transposed 2.1 megabase pairs away from *incC*. Following DnaA, in *B. subtilis*, the essential auxiliary initiators DnaD and DnaB are recruited to the origin for helicase loading. Using ChIP we show that the deletion of *incAB* results in a decreased enrichment of auxiliary initiators at *incC*, suggesting their delivery to the unwinding site by *incAB*. Fittingly, we identify a mutation in *dnaB*, increasing the initiator's affinity for DNA and DnaD, to suppress the deletion of *incAB*. Finally, we show that the *dnaB* suppressor allows the extreme minimization of the origin to just three DnaA-boxes as opposed to the native 24. Altogether, our results uncover surprising flexibility in the spatial layout of a bacterial origin and provide new insight into *oriC* function and minimal sequence requirements for bacterial DNA replication initiation.

P16

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A novel mouse model of ciliopathy syndromes reveals roles for Cep164 in development and homeostasis.

Ciliopathies are a group of rare, genetic disorders caused by defects in the biogenesis, functioning or maintenance of the ciliary complex, found on nearly every mammalian cell. Accordingly, ciliopathies are associated with a plethora of overlapping, multi-system, clinical manifestations, of which there are currently no curative treatments. Recently, recessive mutations in CEP164 (NPHP15), a distal appendage centrosomal protein vital for primary cilia biogenesis, have been identified in families with nephronophthisis-related ciliopathies (NPHP-RC). These patients present with NPHP, a fibrotic cortico-medullary cystic kidney disease which is a major contributor of juvenile end-stage renal disease. Other associated phenotypes include retinal degeneration, neurological defects, and skeletal abnormalities however, knowledge of CEP164 NPHP-RC disease progression and pathogenic mechanisms are poor, which is further confounded by extensive genotype-phenotype heterogeneity.

In this study, a novel Cep164 conditional knockdown mouse model was generated and utilised for global tamoxifen-mediated (temporal) inactivation of Cep164, circumventing Cep164-mediated embryonic lethality, to characterise ciliary-driven phenotypes. Cep164 inactivation in postnatal (juvenile) and adult mice were compared to uncouple CEP164's role in development and adult tissue maintenance/homeostasis.

Our data demonstrates a novel mouse model which accurately recapitulates patient CEP164-NPHP RC phenotype, confirming CEP164 inactivation causes an expanding multi-system phenotype, and showing for the first time a combination of both primary and motile ciliary-driven defects. Data established specific roles for CEP164 in organ development and an ongoing requirement in adult tissue homeostasis. Together, this offers new insights into CEP164 NPHP-RC disease, which can be used to guide further mechanistic and therapeutic target research.