THE UNIVERSITY OF MELBOURNE

27 & 28 OCTOBER, 2022

FACULTY OF MEDICINE, DENTISTRY AND HEALTH SCIENCES

ECA NETWORK SYMPOSIUM

THE UNIVERSITY OF MELBOURNE

We acknowledge the elders, families and descendants of the Wurundjeri people, the traditional owners of the land on which this Symposium is being held. We pay our respects to their Elders past, present and future, and to all Aboriginal or Torres Strait Islander people here today.

WELCOME

On behalf of the MDHS ECA Network, we are delighted to welcome you to the eighth edition of our Annual Network Symposium. After a challenging few years, it is our hope that the symposium will allow ECAs and MCAs from all Schools within the Faculty to connect and communicate in person. We will also host an exciting panel discussion featuring Prof. Kathryn Bowen, Dr Katie Greenaway and Dr Celia McMichael. Our three speakers are presenting on the impacts of climate change on health, and we look forward to learning from their research.

This symposium would not have been possible without the voluntary contributions of those in the Symposium Organising Committee and the Scientific Committee. We extend our thanks to both committees for their tireless commitment. Lastly, we would like to thank you, our attendees for joining our Annual Network Symposium. If you would like to learn more about how you could be involved in Network activities, please reach out to us.

MDHS ECA Network co-chairs, Dr Kelly Kirkland & Dr Alec Jamieson
https://mdhs.unimelb.edu.au/early-career-academic-network

Graeme Clark Institute

illumina®

Symposium Organising Committee

Dr Liam Sharkey

Dr George Taiaroa

Dr Sehrish Kanwal

Dr Rachael Moses

Dr Dinh Bui

Dr Lucas Calais Ferreira

Dr Catarina Almeida

With help from the Scientific Committee: *Tahnee Saunders, Michelle Wille, James Fernando, Huimeng Wang, Marcela de Lima Moreira, Abdou Hachani, Sushama Telwatte, Hyun Jae Lee, Danielle Ingle, Louise Rowntree, Belinda Lawford, Calum Walsh, Courtney Ierano, Jane Oliver, Judy Trevena, Marianne Coleman, Matt Lewis, Paul Garrett, Rachel Nelligan, Robyn Woodward-Kron, Ruby Biezen, Wenyi Li, Shurong Lu, Aimee Tan, Ash Porter, Bansari Shah, Leanne Teoh, Emma Bawden, Garth Cameron & Tom Fulford*

MDHS ECA Network symposium 2022

Program

Thursday October 27th

9:00	**9:30**	**MDHS ECA set up**
9:30	**9:45**	**Welcome & Housekeeping** MDHS ECA Chairs: Kelly Kirkland and Alec Jamieson
Session 1		**Chairs: Wenyi Li & Sehrish Kanwal**
9:45	**10:00**	Bridget Arman **Investigation of ticagrelor as a potential therapeutic to delay preterm birth using a pipeline of in vitro, ex vivo, and in vivo models of preterm birth**
10:00	**10:15**	Yi Yang **Inequalities in service utilisation in the National Disability Insurance Scheme (NDIS)**
10:15	**10:30**	Anna Harutyunyan **Kindling-induced expression of immediate early genes is associated with increased seizure severity and neuroinflammation in 5xFAD model of Alzheimer's Disease**
10:30	**10:45**	Leanne Teoh **Efficacy of oxycodone for post-operative dental pain: a systematic review and meta-analysis**
10:45	**11:00**	Session Questions (15 min)
11:00	**11:15**	**Morning tea**
Session 2		**Chairs: Shurong Lu and Aimee Tan**
11:15	**11:30**	Natalie Fini **Physical Activity Measurement After Stroke – Reaching International Consensus**
11:30	**11:45**	Pauline Coulon **Interplay between Quorum sensing and DNA methylation and their involvement in Burkholderia ambifaria phase variation**
11:45	**12:00**	
12:00	**12:10**	Session Questions (10 min)
12:10	**12:55**	**Lunch (45 min)**
Session 3		**Chairs: Ash Porter & Bansari Shah**
12:55	**1:10**	Carolien van de Sandt **Influenza-specific CD8+ T-cells across the human lifespan: a suboptimal reset for the elderly**
1:10	**1:25**	Rachael Moses **Developing a 3D chronic wound model using animal-free products**
1:25	**1:40**	Jacqui Morris **Hidden resistances: How routine whole genome sequencing uncovered an otherwise undetected blaNDM-1 gene in Vibrio alginolyticus isolated from imported seafood**
1:40	**1:55**	Natasha de Alwis **Phosphoglutamase-5 is dysregulated in pathological placenta and in models of placental dysfunction**
1:55	**2:10**	Session questions (15 min)
2:10	**2:30**	**Poster flash talks (4 min each)** Jieqiong Lou **Histone FRET microscopy reveals that genome architecture is differentially regulated by a HP1α monomer to dimer transition** Lucy Bartho **Circulating chemerin is increased in preeclampsia, preceding diagnosis of preeclampsia, and associated with placental hypoxia** Richard Rebello **SUPER-NEXT: employing comprehensive clinical genomics for cancer of unknown primary** Shuting Li **Assessing attention orienting in a mouse model of autism using a reverse-translated Posner task**
2:30	**3:00**	**Posters and afternoon tea (30 min)**
3:00	**4:30**	**MDHS ECA Panel discussion – The impacts of climate change on health** Prof. Kathryn Bowen, Dr Katie Greenaway and Dr Celia McMichael Moderated by Prof. John Wiseman and Ms Phoebe Quinn With questions encouraged from the audience

MDHS ECA Network symposium 2022

Friday October 28th

9:00	**9:30**	**MDHS ECA set up**
9:30	**9:45**	**Housekeeping**
Session 4		**Chairs: Leanne Teoh & Emma Bawden**
9:45	**10:00**	Yannick Alexandre **Splenic fibroblasts orchestrate immune responses during viral infection**
10:00	**10:15**	Charlie Higgs **Comparison of contemporary invasive and non-invasive Streptococcus pneumoniae reveals insights into circulating antimicrobial resistance**
10:15	**10:30**	Teralynn Ludwick and Marie Ishida **Witnessing Intimate Partner Violence Impacts Schooling and Labor Market Outcomes for Young Women in India**
10:30	**10:45**	Ali Mohammed **Orally Administered Hyaluronic Acid Ameliorates Chemotherapy-Induced Oral Mucositis in a Preclinical Model of Disease**
10:45	**11:00**	Session Questions (15 min)
11:00	**11:15**	**Morning tea**
Session 5		**Chairs: Sushama Telwate & Garth Cameron**
11:15	**11:30**	Adrianna Turner **Rifaximin causes resistance to the last-resort antibiotic daptomycin in Enterococcus faecium**
11:30	**11:45**	Marija Dinevska **Spatial analysis of the metastatic brain tumor immune and extracellular matrix microenvironment**
11:45	**12:00**	Emma Bawden **CD4+ T cells and the role of MHC class II expression in melanoma Immunosurveillance**
12:00	**12:10**	Session Questions (10 min)
12:10	**1:00**	**Lunch (50 min)**
Session 6		**Chairs: Hyun Jae Lee & Catarina Almeida**
1:00	**1:15**	Georga Bruechert **Relational anatomy of the tarsal tunnel**
1:15	**1:30**	Stephanie Neville **The structural basis of bacterial manganese import**
1:30	**1:45**	Sehrish Kanwal **Complementing Whole Genome Sequencing with Whole Transcriptome Sequencing in Precision Oncology**
1:45	**2:00**	**Session questions (10 min)**
2:00	**2:30**	**Poster flash talks (4 min each)** Jacob Paul **Numerosity tuning in human association cortices and local image contrast representations in early visual cortex** Meaghan Griffiths **Endometriotic chocolate cysts contain viable cells of endometrial origin: implications for endometriosis recurrence and infertility** Myrte Strik **Altered network topology in patients with visual snow syndrome: a resting-state 7 Tesla MRI study** Tom Fulford **A new paradigm in γδTCR recognition: butyrophilins 2A1 and 3A1 co-bind the Vγ9Vδ2+ T cell receptor**
2:30	**3:00**	**Posters and afternoon tea (30 min)**
Session 7		**Chairs: Calum Walsh & Tom Fulford**
3:00	**3:15**	Hue M. La **Defining the immune milieu of pancreatic adenocarcinoma using single-nucleus RNA sequencing**

3:15	**3:30**	Jennifer Jones **Frailty and Physical Function Recovery of Hospitalised Adults Receiving an Inpatient Rehabilitation Service**
3:30	**3:45**	Claire Gorrie **Public health investigation into a multi-facility, multi-species, antimicrobial-resistant plasmid outbreak**
3:45	**3:55**	**Session questions (10 min)**
3:55	**4:15**	**MDHS ECA overview and awards** MDHS ECA Chairs: Kelly Kirkland and Alec Jamieson

MDHS ECA Panel discussion:
The Impacts of Climate Change on Health

Plenary Presenters

Prof. Kathryn Bowen

Deputy Director, Melbourne Climate Futures | Professor in Environment, Climate & Global Health

A leading, internationally recognised expert on the science and policy of sustainability (particularly climate change) and global health issues, with 20 years' experience in original public health research, science assessment, capacity development and policy advice. Kathryn is regularly commissioned by international bilateral and multilateral agencies (e.g., WHO, UNEP, UNDP, ADB, GIZ, DFAT) to co-design solutions for sustainable futures.

Dr Katie Greenaway

Senior Lecturer in Psychology, Melbourne School of Psychological Sciences

Katie's research focuses on social functioning in three main domains: identity processes, emotion regulation, and human agency. At its heart, her research aims to understand the formation and consequences of social connections between people. Katie researches emotion, and has ran several projects examining the most effective interventions for climate change anxiety.

A/Prof. Celia McMichael

Associate Professor in Health Geography Geography, Earth and Atmospheric Sciences

Celia conducts research focused on health geography, international health, migrant and refugee health, and climate change-related migration and displacement. She has worked in Nepal, Fiji, Angola, Peru and Sri Lanka. Celia is currently working on research projects focusing on refugee resettlement in Australia, and climate change-related migration and relocation in Small Island States. Celia has experience in academic research, applied research consultancies and work in the international development sector (with WHO, IFRC, NGOs and UN agencies).

Moderated by:

Prof. John Wiseman

Honorary Professional Fellow, Melbourne Sustainable Society Institute; Adjunct Professor, Melbourne School of Population and Global Health

John's major research and policy contributions have been in the fields of social justice and citizen engagement, globalisation impacts, social wellbeing policy frameworks and indicators, sustainability transitions, and climate change risks and solutions. His current work focuses on the actions needed to accelerate the transition to a just and resilient post-carbon society.

Ms Phoebe Quinn

Research Fellow & PhD candidate, Child & Community Wellbeing Unit

Phoebe's research is exploring democratic innovations using digital technologies, and the role these can play in community decision-making around disasters and climate change. More broadly, her work includes research into disaster resilience, community wellbeing and social justice, and knowledge translation activities including the development of strengths-based resources relating to disasters and climate change.

Abstracts: *Long talks*

Yannick Alexandre

Splenic fibroblasts orchestrate immune responses during viral infection

Yannick Alexandre[1], Scott Mueller[1]

[1]Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

Introduction/Aim: The spleen is organised into distinct anatomical niches that support dedicated functions in blood filtration and initiation of immune responses against blood-borne pathogens. The splenic architecture is formed by networks of specialized fibroblastic stromal cells. These stromal cells are not simply the physical scaffolds but are critical for the maintenance of immune cells and for immune responses. Nonetheless, how these fibroblasts regulate immune responses is in the spleen is poorly understood.

Methods: We used a mouse model of viral infection combined with RNA sequencing and in vivo depletion model to investigate how fibroblasts respond to infection and orchestrate immune responses.

Results: We found that acute infection drives an extensive program of gene regulation in splenic fibroblasts characterised by the downregulation of genes associated with tissue homeostasis and up-regulation of genes associated with immune responses and interactions with immune cells. Following depletion of splenic fibroblasts in vivo, we found that the CD8 T cell immune response against impaired during viral infection. CD8 T cells had a decrease in their activation and production of cytokines involved in the killing of infected cells. Mechanistically we found that in the absence of fibroblasts, CD8 T cell were misplaced in the spleen and their interactions with other immune cells was impacted following viral infection.

Conclusions: Overall, our data indicate that viral infection reprogram fibroblasts to orchestrate adaptive immune responses in the spleen.

Bridget Arman

Investigation of ticagrelor as a potential therapeutic to delay preterm birth using a pipeline of *in vitro, ex vivo,* and *in vivo* models of preterm birth

Bridget Arman[1,2], Natalie Binder[1,2], Natasha de Alwis[1,2], Sally Beard[1,2], Stephen Tong[2,3], Tu'uhevaha Kaitu'u-Lino[2,3], and Natalie Hannan[1,2]

[1]Therapeutics Discovery and Vascular Function Group, Department of Obstetrics and Gynaecology, University of Melbourne, Mercy Hospital for Women, Heidelberg 3084, Victoria, Australia
[2]Mercy Perinatal, Mercy Hospital for Women, Heidelberg 3084, Australia
[3]Department of Obstetrics and Gynaecology, University of Melbourne, Mercy Hospital for Women, Heidelberg 3084, Victoria, Australia

Introduction/Aim: Preterm birth is the leading cause of death in infants; hence there is an urgent need to find new drugs to delay preterm delivery. One approach is repurposing existing drugs. Ticagrelor is an antiplatelet agent, but we have previously found that it can relax vascular smooth muscle. We hypothesised that ticagrelor may similarly relax the myometrium (outer uterine muscular wall). Here, we assessed the effect of ticagrelor on myometrial contractility and key inflammatory markers (central to preterm labour pathophysiology) in our pipeline of human and mouse models of preterm birth.

Methods: Human myometrial tissue (non-labouring; collected at caesarean-section) was used in tissue bath experiments to measure the effect of ticagrelor on spontaneous contractions (n=3). Cultured myometrial cells were treated with tumor necrosis factor and lipopolysaccharide (LPS) to evoke an inflammatory response and co-treated with ticagrelor (n=3); altered pro-inflammatory cytokine mRNA expression was measured via qPCR. Preterm birth was induced in pregnant dams using LPS to determine whether ticagrelor delayed delivery (20mg/kg, n=3; 40mg/kg, n=2).

Results: Ticagrelor did not inhibit *ex vivo* myometrial contractility (frequency, amplitude, duration) when compared with control. Ticagrelor did not reduce mRNA expression of interleukin *(IL)-1B, IL-6* and *CXCL8* in

cultured myometrial cells. Preliminary findings showed that ticagrelor could not delay LPS-induced preterm birth in mice.

Conclusions: Our pipeline of drug assessment rigorously evaluated ticagrelor and the data demonstrate that ticagrelor does not inhibit human myometrial contractions, reduce inflammation, or prevent preterm delivery in mice. This suggests ticagrelor is not a suitable preterm birth therapeutic candidate.

Emma Bawden

CD4⁺ T cells and the role of MHC class II expression in melanoma Immunosurveillance

Emma Bawden[1], Thomas Gebhardt[1]

[1] The Peter Doherty Institute, the University of Melbourne

Introduction/Aim: There is increasing evidence that CD4⁺ T cells play an important role in cancer immunosurveillance but considerable debate surrounds the underlying mechanisms. Our work seeks to characterize the CD4⁺ T cell response to melanoma using a recently developed, orthotopic murine model that closely mimics progression of human melanoma.

Methods: Through single-cell RNA sequencing analysis and intravital two-photon microscopy we performed in-depth spatial and phenotypic characterization of intratumoral CD4⁺ T cells. In addition, we generated genetically-modified melanoma cell lines to investigate components of the anti-tumoral response.

Results: In this model the adoptive transfer of naive or activated antigen-specific CD4+ T cells demonstrates remarkable protection against the development of melanoma. In addition to a classical "helper" function, CD4+ T cells act as peripheral anti-tumoral effector cells whereby they migrate into the skin and mediate local suppression of tumour development.
We identified several cytotoxic mechanisms by which CD4⁺ T cells can directly kill melanoma as well as indirect mechanisms involving the recruitment and activation of different immune cell types within the tumour microenvironment. We provide direct evidence that CD4⁺ T cells can bind to MHC-II expressed by melanoma cells or professional antigen-presenting cells and examine how the source of MHC-II can influence the ensuing anti-tumoral response.

Conclusions: This work provides novel insights into the complex immunosurveillance mechanisms employed by CD4⁺ T cells within the tumour microenvironment highlighting the potential for CD4⁺ T cells to revolutionize cancer immunotherapies.

Georga Bruechert

Relational anatomy of the tarsal tunnel.

G.K. Bruechert[1], C.G. Thorpe Lowis[1], W.H.B. Edwards[2], Q.A. Fogg[1]

[1]Department of Anatomy and Physiology, The University of Melbourne, Victoria, Australia
[2]Epworth Hospital, Richmond, Victoria, Australia

Introduction/Aim: It is predicted that, by 2035, a lower limb amputation will occur every 30 seconds globally, from diabetes alone. Complications associated with the diabetic foot may lead to an increase in tarsal tunnel syndromes, and ultimately, amputation. The tarsal tunnel is the primary conduit for the neurovasculature of the plantar foot, but there is no clear understanding of the surrounding soft tissue. This makes it difficult to accurately diagnose and treat associated disorders. The aim of this study was to develop a clear understanding of the spatial relations of the soft tissues of the tarsal tunnel.

Methods: Feet of embalmed Body Donor's (n=24; mean age=85.10±9.49; F=12; M=10) were analysed. They were dissected and modelled in virtual 3D space, or sectioned. The relations and dimensions of all tissues were measured.

Results: The tendons of the tarsal tunnel were all compartmentalised within separate tendinous sheaths. Forming the boundaries of these sheaths were septae that encapsulated the tendons, separating them from one another and the neurovascular bundle.

Conclusions: The tarsal tunnel is divided into at least four separate spaces by septae that contributed to the tendinous sheaths, which have not previously been described. These may all impinge the neurovascular structures, contributing to tarsal tunnel syndrome. These data strongly suggest that current surgical interventions are most likely to be ineffective and unpredictable, and will inform redesign of these approaches. For example, multiple 'releases' may be required for treatment, which may lead to better outcomes to reduce the occurrence of lower limb amputations.

Pauline Coulon

Interplay between Quorum sensing and DNA methylation and their involvement in Burkholderia ambifaria phase variation

Pauline M.L. Coulon[1], Marie-Christine Groleau[2], Abderrahman Hachani[1], Timothy P. Stinear[1] & Eric Déziel[2]

[1]Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne, Melbourne, Vic, Australia
[2]Centre Armand-Frappier Santé Biotechnologie, Institut National de la Recherche Scientifique (INRS), Laval, QC, Canada

Introduction/Aim: Phase variation is due to genomic variations, modulating gene expression or by epigenetic factors and has been observed in *Burkholderia*. In *B. cenocepacia*, phase variants lack their virulence megaplasmid (pc3) making them less virulent. Quorum sensing (QS) and some virulence factors are regulated by the *"shiny colony variant"* regulator (ShvR) while biofilm formation, and motility is controlled by DNA methylation. In this study, we showed that *B. ambifaria* generates two types of variants: losing or keeping their pc3. This discovery suggests that another mechanism is involved in colony morphotype variation in *B. ambifaria.*

Methods/Results: To further characterise colony morphotype variation phenotypes, we investigated the proteome of *B. ambifaria* CEP0996 WT and pc3-null variant, and *B. ambifaria* HSJ1 WT and pc3-positive variant. Proteomic results confirmed by assessing the production of virulence factors (EPS, siderophores, protease, motility and biofilm formation). As these virulence factors are controlled by the QS, homoserine lactones (AHLs) were quantified, and QS role in phase variation was determined. As *B. ambifaria* HSJ1 WT and variant assemblies do not have genomic variation, we have mutated *shvR* and DNA Methyltransferase (MTase) gene homologs in *B. ambifaria* to investigate their role in QS, virulence factors production and phase variation.

Conclusion: The modulation of virulence factors and phase variation in *B. ambifaria* HSJ1 was not due to a difference of AHL production. We showed that QS activates the apparition of variants, while one DNA MTase inhibits phase variation. Phase variation in *B. ambifaria* is controlled by both QS and DNA methylation – which could interplay.

Natasha de Alwis

Phosphoglutamase-5 is dysregulated in pathological placenta and in models of placental dysfunction

Natasha de Alwis[1,2,3], Sally Beard[1,2,3], Natalie K Binder[1,2], Natasha Pritchard[2,4], Tu'uhevaha J. Kaitu'u-Lino[2,5], Stephen Tong[2,4], Lisa Hui[2,3] and Natalie J Hannan[1,2,3]

[1]Therapeutics Discovery and Vascular Function Group, Department of Obstetrics & Gynaecology, The University of Melbourne, Heidelberg, Victoria, Australia
[2]Mercy Perinatal, Mercy Hospital for Women, Heidelberg, Victoria, Australia
[3]Department of Obstetrics & Gynaecology, Northern Health, The University of Melbourne, Epping, Victoria, Australia

[4]Translational Obstetrics Group, Department of Obstetrics & Gynaecology, The University of Melbourne, Heidelberg, Victoria, Australia
[5]Diagnostics Discovery and Reverse Translation in Pregnancy Group, Department of Obstetrics & Gynaecology, The University of Melbourne, Heidelberg, Victoria, Australia

Introduction/Aim: Placental dysfunction is a key driver of the serious pregnancy complications, preeclampsia and fetal growth restriction (FGR). We previously identified elevated phosphoglutamase-5 (PGM5) transcripts in the maternal circulation in cases of preterm FGR ± preeclampsia. However, its role in the placenta is little-known. Here, we investigated PGM5 levels and its potential role in the placenta and models of placental dysfunction.

Methods: PGM5 expression (qPCR) and protein (western blot) were measured in placental tissue collected from cases of preterm preeclampsia and FGR, and gestation-matched controls. PGM5 expression was assessed in placental explant tissue and isolated primary cytotrophoblast cells cultured under normoxic (8% O_2) and hypoxic (1% O_2) conditions (modelling placental dysfunction). Short interfering RNAs were used to silence cytotrophoblast PGM5 expression, with cell survival and expression of genes associated with angiogenesis, apoptosis, growth, and oxidative stress assessed.

Results: PGM5 mRNA, but not protein, was significantly reduced in placenta from cases of preterm preeclampsia and fetal growth restriction compared to controls. Conversely, PGM5 mRNA was significantly elevated in placental explants and cytotrophoblast cultured under hypoxia. Silencing PGM5 under hypoxia improved cytotrophoblast survival, decreased antiangiogenic factor sFLT-1 secretion, and increased expression of pro- and anti-apoptosis genes BAX and BCL2, growth genes EGFR and IGF2, and oxidative stress marker NOX4.

Conclusions: PGM5 expression is dysregulated in pathological placenta and models of placental dysfunction, suggesting it could act as a therapeutic target. Further studies are underway to establish whether changes in PGM5 levels are associated with canonical changes in the placental architecture and cytoskeleton.

Marija Dinevska

Spatial analysis of the metastatic brain tumor immune and extracellular matrix microenvironment

Marija Dinevska[1,6]*, Samuel S. Widodo[1,6]*, Lucero Cuzcano[1,6], Michael Papanicolaou[2,3], Thomas R. Cox[2,4], Stanley S. Stylli[1,5], Theo Mantamadiotis[1,6,7]. **equal contribution*

[1]Department of Surgery, Royal Melbourne Hospital, The University of Melbourne, Parkville, VIC, Australia
[2]The Garvan Institute of Medical Research and The Kinghorn Cancer Centre, Cancer Ecosystems Program, Sydney, NSW, Australia
[3]School of Life Sciences, Faculty of Science, University of Technology Sydney, Sydney, NSW, Australia
[4]School of Clinical Medicine, St Vincent's Healthcare Clinical Campus, Faculty of Medicine and Health, UNSW Sydney, Sydney, NSW, Australia
[5]Department of Neurosurgery, Royal Melbourne Hospital, Parkville, VIC, Australia
[6]Department of Microbiology and Immunology, The University of Melbourne, Melbourne, VIC, Australia
[7]Centre for Stem Cell Systems, The University of Melbourne, Parkville, VIC, Australia

Introduction/Aim: Metastatic cancer is responsible for the overwhelming majority of cancer-related deaths, and are the most common neoplasms affecting the central nervous system. One of the major factors regulating tumor biology is the tumor microenvironment. However, little is known about the cellular and non-cellular composition of metastatic brain tumors and how tumor cell ontogeny influences the metastatic brain tumor microenvironment (TME).

Methods: By integrating multiplex immunohistochemistry, histopathological staining, and spatial analysis of metastatic brain tumour tissue, we investigated the composition and the spatial relationship between neoplastic cells, immune cells, and the extracellular matrix (ECM).

Results: Metastatic brain tumors exhibit differences in ECM deposition compared to glioblastoma, including differences in collagen fibre density. Infiltrating macrophages and T-cells are the most common immune cells in metastatic brain tumors, with an enrichment of these cells within ECM-rich regions. The dominant macrophage subtype in metastatic brain tumors are immunosuppressive/anti-inflammatory macrophages, which preferentially localize to these ECM-rich regions.

Conclusions: Overall, our data shows that macrophages and T-cells are restricted within ECM-dense regions, which may prevent these cells from interacting with, and effectively killing neoplastic cells. We also identify tumor infiltrating macrophages as key players in establishing an immunosuppressive TME. The findings support the view that optimal therapy for patients with metastatic brain cancer would include drugs which modify the ECM, and drugs which target immunosuppressive macrophages, in addition to current cytotoxic therapies.

Natalie Fini

Physical Activity Measurement After Stroke – Reaching International Consensus

Natalie A. Fini[1,2], Dawn Simpson[2,3], Sarah A. Moore[4,5], Niruthikha Mahendran[2,6], Janice J. Eng[7,8] Karen Borschmann[2,9], David Moulaee Conradsson[10,11], Sebastien Chastin[12], Leonid Churilov[2,13], Coralie English[2,3,14]

[1] Physiotherapy Department, School of Health Sciences, The University of Melbourne, Parkville, Australia
[2] Centre for Research Excellence in Stroke Rehabilitation and Recovery, Florey Institute of Neuroscience, Melbourne Australia
[3] School of Health Sciences and Priority Research Centre for Stroke and Brain Injury, University of Newcastle, Newcastle, Australia
[4] Department of Sport, Exercise and Rehabilitation, Northumbria University, Newcastle, UK
[5] Stroke Research Group, Population Health Sciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne. NE2 4HH. UK
[6] Physiotherapy Division, School of health and Rehabilitation Sciences, The University of Queensland, Brisbane, Australia
[7] Department of Physical Therapy, University of British Columbia, Vancouver, Canada
[8] Rehabilitation Research Program, Vancouver Coastal Health Research Institute, Vancouver, Canada
[9] St Vincent's Hospital, Melbourne, Australia
[10] Division of Physiotherapy, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden
[11] Theme Women's Health and Allied Health Professional, Karolinska University Hospital, Stockholm, Sweden
[12] Institute of Applied Health Research, School of Health and Life Sciences, Glasgow Caledonian University, Glasgow, UK
[13] Melbourne Medical School, The University of Melbourne, Parkville, Australia
[14] Hunter Medical Research Institute, New Lambton Heights, Australia

Introduction: Consistency is lacking in stroke physical activity (PA) literature with many different outcomes and tools used.

Aim: To reach international consensus on recommendations of outcomes and tools used in post-stroke PA measurement.

Methods: An internationally recognised stroke researcher and clinician group participated in 3 survey rounds. Survey 1 identified tools used, key PA outcomes and measurement considerations. In Survey 2 participants ranked key outcomes and considerations.
The research team collated evidence on measurement tools from Survey 1 and aligned with key considerations according to ranked results of Survey 2. The research team arrived at consensus on recommended measurement tools based on evidence and experiential knowledge. Survey 3 provided participants with Survey 2's ranked results and evidence gathered and sought agreement on research team recommendations.

Results: Eighteen research and 17 clinician experts from six continents participated. Key considerations for measurement included ability to measure in real-world settings, across frequency, intensity, duration domains; ability to detect meaningful changes; and accessibility of measurement tools.
The highest-ranking outcomes were time in moderate-vigorous PA and step count.
The research team recommended use of the Actigraph, Actical and Activ8 devices for PA intensity; ActivPAL for duration and Step Activity Monitor for frequency; and the IPAQ and PASE questionnaires. Survey results indicated 100% agreement with device recommendations and 96% with questionnaire recommendations.

Conclusions: International consensus on post-stroke PA measurement was reached however selection of measurement tools will depend on resources, user-knowledge and measurement purpose. The use of both devices and questionnaires is recommended for comprehensive measurement.

Claire Gorrie

Public health investigation into a multi-facility, multi-species, antimicrobial-resistant plasmid outbreak

C. L. Gorrie[1,2], A. J. Turner[1], T. Thomson[3], J. Brett[4], M. Easton[3], D. Hennessy[3], D. Cameron[1,3], K. Stevens[1], W. Pitchers[1], M. Ivan[3], N. L. Sherry[1,2,5], C. R. Lane[1,2], B. P. Howden[1,2,5]

[1] Microbiological Diagnostic Unit Public Health Laboratory, The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
[2] Department of Microbiology & Immunology, The University of Melbourne at The Doherty Institute for Infection and Immunity, Melbourne, Australia
[3] Department of Health, Victoria, Australia
[4] VICNISS Healthcare Associated Infection Surveillance Coordinating Centre
[5] Department of Infectious Diseases, Austin Health, Melbourne, Australia

Introduction/Aim: Antimicrobial resistant (AMR) bacteria are a leading public health threat; among the most clinically concerning are the carbapenemase-producing Enterobacterales (CPE). In Victoria, CPE were historically associated with returned travellers, thought more recently an increase in CPE patients with no travel history, was detected. Among these were multiple CPE species with the same rare carbapenem resistance gene (*bla*$_{NDM-1}$), which suggested an outbreak of a mobile genetic element, such as a plasmid carrying the *bla*$_{NDM-1}$, rather than a single bacterial strain being transmitted. We aimed to investigate this possibility utilising a novel genomics approach to detect shared AMR plasmids.

Methods: Victorian *bla*$_{NDM-1}$ CPE (Jan-2012 to Oct-2021) underwent bespoke genomic investigation to identify putative AMR plasmids, combined with routinely collected epidemiological data, including travel and hospitalisation, to identify epidemiological links.

Results: 183 *bla*$_{NDM-1}$ CPE were identified in 114 patients. Among these, 36 patients had CPE carrying the same AMR plasmid, accounting for 59.6% without known high-risk travel. This AMR plasmid was also identified in returned travellers from 2017, increasing from July 2020. Preliminary analysis revealed 91.6% of these patients had been admitted to two specific hospitals with 10 transmission events identified.

Conclusions: AMR plasmid transmission is inadequately addressed by current surveillance and response protocols. This work provided the first example of near real-time detection and response to a multi-facility, multi-species AMR plasmid outbreak in Victoria, which will inform development of approaches to rapidly detect these events through routine genomic surveillance.

Anna Harutyunyan

Kindling-induced expression of immediate early genes is associated with increased seizure severity and neuroinflammation in 5xFAD model of Alzheimer's Disease

Anna Harutyunyan[1], Alison Anderson[1,2,3], Patrick Kwan[1,2,3], Nigel Jones[1,2,3]

[1]Department of Medicine, The Royal Melbourne Hospital, The University of Melbourne, Parkville, Victoria
[2]Department of Neuroscience, Central Clinical School, Monash University, Melbourne, Victoria
[3]Department of Neurology, Alfred Health, Melbourne, Victoria

Introduction/Aim: Alzheimer's disease (AD) is a neurodegenerative disease affecting 50 million people worldwide. There is increased prevalence of epilepsy in patients with AD, and the two diseases are thought to have a bi-directional association, however, the mechanism underlying this association remains unknown. This study aimed to investigate potential mechanisms of synergy between recurrent seizures and already-present amyloid pathology in a well-established model of AD.

Methods: Transgenic 5xFAD mice (N=20) and WT littermates (N=22) underwent electrical amygdala kindling to induce epilepsy phenotype or were treated as sham (no epilepsy). Kindling rate, seizure severity and cognitive behavioural performance were compared across the kindled and sham 5xFAD and WT mice. The transcriptome of the hippocampal tissue was examined through RNA sequencing and weighted gene coexpression network analysis (WGCNA).

Results: The 5xFAD mice showed significantly impaired spatial memory ($p<0.05$) and increased susceptibility to kindling-induced seizures ($p<0.001$) compared to WT littermates. Transcriptomic profiling and differential expression analysis revealed profound overexpression of genes involved in reactive gliosis and neuroinflammatory pathways in the kindled-5xFAD group compared to sham and WT groups. WGCNA identified a module of immediate early genes (IEG) showing significant ($p<0.00001$) correlation with kindled-5xFAD group, but not the shams. The regulatory hub genes of this module (Pcdh8, Nptx2) are involved in synapse formation and maintenance in homeostatic conditions and lead to loss of dendritic spine density if deregulated.

Conclusions: Our results suggest a significant synergistic interaction between AD pathology and recurrent seizures, which may potentially be mediated by activity-induced IEGs and reactive gliosis.

Charlie Higgs

Comparison of contemporary invasive and non-invasive *Streptococcus pneumoniae* reveals insights into circulating antimicrobial resistance

Charlie Higgs[1], Lamali Kumar[2], Kerrie Stevens[2], Janet Strachan[3], Norelle L. Sherry[1,2,4], Prof. Timothy P. Stinear[1], Claire L. Gorrie[1,2], Prof. Benjamin P. Howden[1,2,4]

[1] Department of Microbiology & Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Victoria, Australia.
[2] Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology & Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Victoria, Australia.
[3]Department of Health, Victoria, Australia
[4] Department of Infectious diseases, Austin Health, Melbourne, Victoria, Australia.

Introduction/Aim: *Streptococcus pneumoniae* is predominantly a community acquired bacterial pathogen that can cause a range of conditions from asymptomatic carriage to invasive pneumococcal disease (IPD). Non-invasive carriage isolates (those found in non-sterile sites such as the respiratory tract) are thought to act as a key source of invasive isolates (those found at normally sterile sites such as the bloodstream), as well as antimicrobial resistance (AMR) genes. Despite this, pneumococcal surveillance in Victoria has focussed exclusively on invasive isolates; we aimed to compare the invasive and non-invasive populations to understand how the two interact and gain insights into spread of resistance.

Methods: We compared the population structure and AMR profiles of IPD isolates (n=1,118) and non-invasive pneumococcal isolates (n=195), collected in Victoria between July 2018 and June 2022, using a combination of whole genome sequencing and phenotypic testing.

Results: We identified overlaps between the invasive and non-invasive populations with the two sharing multi-locus sequence types (MLSTs) and serotypes and no distinct phylogenetic clustering of the two populations. The non-invasive population had significantly higher levels antibiotic resistance to penicillin, ceftriaxone and azithromycin, erythromycin. The allelic diversity of the resistance determinants was also highly conserved between the two populations with more than 96% of alleles identified in the non-invasive population also being contained in the invasive population.

Conclusions: Our results show the importance of monitoring both invasive and non-invasive pneumococcal isolates for effective public health surveillance and the large potential of non-invasive isolates to act as a reservoir of AMR determinants.

Jennifer Jones

Frailty and Physical Function Recovery of Hospitalised Adults Receiving an Inpatient Rehabilitation Service

Jennifer Jones[1,2,3], Tessa O'Dea[2], Chris Michael[4], Talia Clohessy[2], Lucy Gao[1], Elena Gerstman[1,2], Mark Hindson[2], Rebekah McGaw[2], Rebecca Morris[2], Joleen Rose[2], Sharae Theisinger[2], Sue Berney[1,2], David Berlowitz[1,2,3]

[1]Physiotherapy Department, The University of Melbourne, Parkville, Australia
[2]Physiotherapy Department, Division of Allied Health, Austin Health, Heidelberg, Australia
[3]Institute of Breathing and Sleep, Heidelberg, Australia
[4]Clinical Analytics and Reporting, Austin Health, Heidelberg, Australia

Aim: Describe physical function recovery trajectories with the Clinical Frailty Scale (CFS) for hospitalised adults referred to the physiotherapy Early Rehabilitation service at Austin Health

Methods: A retrospective observational cohort study from January 1 2019 to December 31 2020. Physical function was assessed using the modified Iowa Level of Assessment Scale (mILOA), a 37-point scale with higher values representing greater physical disability. The mILOA was completed at admission (A-mILOA) and discharge (D-mILOA).

Results: We analysed 736 patients with a CFS score ranging from one to seven (CFS score = patients: 1=31, 2=99, 3 = 136, 4=141,5=157, 6=119, 7=53). A-mILOA scores were completed for 96% (n=708) of patients and physical disability increased with CFS score (CFS score = median (IQR) mILOA score: 1=18 (9, 27), 2=19 (11, 26), 3=20 (13, 27), 4=21 (16, 27), 5=23 (18, 29), 6=26 (21, 30), 7=31 (29, 32) p <0.001). D-mILOA scores were completed for 63% (n=466) of patients. The greatest improvement in mILOA scores from admission to discharge were observed in patients who were less frail and exceeded the minimal clinically important difference of 5.8 points (CFS score = change in mILOA score mean (SD): 1=-10(7), 2=-9 (8), 3=-8 (7), 4=-9 (7), 5=-7 (7), 6=-7 (6), 7=-3 (5) p < 0.001).

Conclusions: Hospitalised adults with less frailty had a better physical function recovery trajectory following a physiotherapy Early Rehabilitation program. These findings provide opportunity to predict recovery, explore rehabilitation dose-response and target health care resources in future research.

Sehrish Kanwal

Complementing Whole Genome Sequencing with Whole Transcriptome Sequencing in Precision Oncology

Sehrish Kanwal, Jacek Marzec, Joep Vissers, Oliver Hofmann, Sean Grimmond

The University of Melbourne Centre for Cancer Research

Introduction/Aim: Precision oncology is becoming a standard approach in cancer patients care, with cancer molecular characterisation through genome sequencing being the major focus. However, there is growing evidence showing that patients transcriptome profiling is useful in complementing genome-based findings and aids improving therapy recommendations and patient outcome. Interpreting the biological impact of WGS data alone can present ambiguities, particularly in predicting the impact of structural and splice-site variants, where reading frame may be unclear.

Methods: We have developed RNAsum https://github.com/umccr/RNAsum, a robust reporting tool capable of using RNA sequencing data from cancer patients to verify and complement DNA profiling results. Our approach incorporates transcriptome profiling into precision oncology framework by leveraging inhouse (UMCCR) and external (TCGA) RNAseq data, increasing confidence in clinical utility of findings. Depending on the tissue from which the patient's sample was taken, one of **33 cancer datasets** from TCGA can be used as a reference cohort for comparing expression changes in genes of interest in investigated sample. Additionally, RNAsum collates results with WGS based results, knowledge derived from in-house resources and public **databases** to provide evidence for clinical significance of altered genes, e.g. to flag variants with clinical significance or potential druggable targets.

Results:
RNAsum provides *quantitative* additional evidence for genome curation with:

- Expression data changes with respect to internal (UMCCR) and external (TCGA) data sets from 33 cancer types, can support genomic evidence for amplifications, homozygous deletions, and loss of heterozygosity.
- mRNA expression levels of mutated genes, copy number altered genes, homologous recombination deficiency (HRD) genes and genes considered to be immune markers.

RNAsum also offers *qualitative* information on the effects of:

- splice-site variants; for example, evidence of exon skipping, and intron retention.
- Prioritised fusion events; whether gene fusion is expressed, frame of fusion, and exons involved.

Conclusions: Analyses of tumour transcriptomes in parallel with genomic sequencing provide critical information to support interpretation of cancer patient genomes. Our current pipeline now routinely includes sequencing of both patient genome and transcriptome to inform our curation decisions.

Hue M. La

Defining the immune milieu of pancreatic adenocarcinoma using single-nucleus RNA sequencing

Hue M. La[1], Fernando J. Rossello[1], Luciano G. Martelotto[1,2], Jacek Marzec[1], Amber Jones[1], Anna Leichter[1], Sean M. Grimmond[1]

[1] University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Melbourne, 3000, VIC, AUSTRALIA.
[2] Adelaide Centre of Epigenetics, South Australian immunoGENomics Cancer Institute

Introduction/Aim: Pancreatic Adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers with a 5-year survival rate of 7%. Current treatments are limited and ineffective, partly due to complex cellular composition of tumour microenvironment that are largely undefined. Therefore, it is crucial to define the molecular pathology and oncogenesis of PDAC to improve treatment options and to develop novel therapeutic strategies. Here, we characterise the lymphoid and myeloid component of PDAC.

Methods: We performed single-nucleus RNA and ATAC high throughput sequencing, from the same sample preparation, of 30 extensively characterised PDAC - part of Australia ICGC (International Cancer Genome Consortium) program - using the 10X Genomics platform.

Results: Within lymphocytes, we identified distinct T cell subsets, including naïve CD4+ T, cytotoxic CD8+ T and CD4+ T-regulatory (T-reg) cells, along with natural killer (NK), B and plasma cells. Importantly, analysis of common immune checkpoint regulators, such as *PDCD1*, *CTLA4*, *TIGIT* and *LAG3,* revealed a strong expression of exhausted markers in NK, T-reg, and CD8+ T cells, suggesting that these lymphocytes are dysfunctional, unlikely to elicit immune responses to cancer cells. In contrast, the myeloid component is largely composed of macrophages. We identified and characterised 5 distinct macrophage subsets, many of which promote immune suppressive functions, such as SPP1+ tumour associated macrophages and C1QC+ macrophages with known roles in anti-inflammation.

Conclusions: These findings, *i.e.,* dysfunctional T cells and enrichment of tumour associated macrophages, indicate a strong immunosuppressive tumour microenvironment in PDAC that could play a role in both tumour growth and therapy resistant.

Teralynn Ludwick and Marie Ishida

Witnessing Intimate Partner Violence Impacts Schooling and Labor Market Outcomes for Young Women in India

Teralynn Ludwick[1], Marie Ishida[1] & Ajay Mahal[1]

[1]Melbourne School of Population and Global Health

Introduction/Aim: Intimate partner violence (IPV) affects ~30 percent of women globally. This study investigates the relationships through which witnessing IPV in adolescence may impact the labour force participation and employment prospects of women in early adulthood.

Methods: We developed a conceptual framework to hypothesize pathways through which exposure to IPV might influence labour market outcomes including through intermediate factors likely to influence employability skills, such as educational attainment, self-efficacy, mental health, and soft skills. We empirically tested these relationships (regression analysis) using a unique dataset from Bihar, India, which surveyed girls in adolescence and again nine years later.

Results: Exposure to IPV increased the likelihood of entering the labour force (including below the legal age of 14), but not the likelihood of being in paid employment or skilled occupations. Those exposed were 5-7% and 3-4% less likely to complete primary and secondary school respectively, with an average loss of 0.3–0.6 total years of schooling. Mental health outcomes were poorer.

Conclusions: Our findings do suggest lower yields (in terms of paid and skilled jobs) from labour force participation among women exposed to IPV as adolescents. Lost years of school translate into as much as 3-6% loss in hourly wages in India.

Ali Mohammed

Orally Administered Hyaluronic Acid Ameliorates Chemotherapy-Induced Oral Mucositis in a Preclinical Model of Disease

Ali I. Mohammed[1, 2*], Antonio Celentano[1], Rita Paolini[1], Jun T. Low[3], Lorraine A. O' Reilly[3,4], Michael McCullough[1], Nicola Cirillo[1]

[1] Melbourne Dental School, The University of Melbourne, 720 Swanston Street, 3053, Carlton, Victoria, Australia.
[2] College of Dentistry, The University of Tikrit, Tikrit, Iraq
[3] The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia
[4] Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia

Introduction/Aim: Oral mucositis (OM) is a highly debilitating inflammatory condition associated with cancer treatment and is characterized by erythema and painful ulcerative lesions. These side effects are so severe that may lead to discontinuation of chemo- and/or radiotherapy. Here we aimed to evaluate the protective effects of hyaluronic acid (HA) in a novel dual murine model of chemotherapy-induced OM.

Methods: Sixty C57BL/6 female mice received saline (control group) or 5-fluorouracil (5-FU) intravenously (50mg/kg, OM group, n=18) every 48 hours for 14 days. In the test group (n=18), high molecular weight HA (H-MW-HA) was also administered daily in the drinking water (0.01% w/v). OM was monitored clinically (daily) and histologically (selected timepoints). Animals were sacrificed on days 14, 16, and 19 and tongues were assessed at a macroscopic, histologic and molecular level. Pro-inflammatory cytokines and superoxide dismutase (SOD) serum levels were also evaluated.

Results: H-MW-HA supplementation significantly reduced the incidence (85.71% *vs.* 68.75%, $p<0.001$) and severity (0.46±0.32 *vs.* 0.14±0.17, $p<0.001$) of 5-FU-induced OM and abolished the severe histological damage induced by 5-FU treatment. At a molecular level, H-MW-HA supplementation prevented 5-FU-induced apoptotic damage to tongue epithelium, as demonstrated by a significant reduction in cleaved caspase-3 expression. In mice receiving both 5-FU and H-MW-HA, a significant reduction in the serum levels of IL-6 and chemokine CXCL1/KC was observed, compared to 5-FU-injected mice, while serum SOD antioxidant activity was elevated.

Conclusions: Overall, our data provide strong pre-clinical evidence that prophylactic use of HA is effective in reducing the onset and severity of OM.

Jacqui Morris

Hidden resistances: How routine whole genome sequencing uncovered an otherwise undetected *bla*$_{\text{NDM-1}}$ gene in *Vibrio alginolyticus* isolated from imported seafood.

Jacqueline M. Morris[1], Karolina Mercoulia[2], Mary Valcanis [2], Claire L. Gorrie [1], Norelle L. Sherry [1,2], and Benjamin P. Howden [1,2].

[1] Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia.
[2] Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia.

Introduction/Aim: This study aims to investigate and describe the antimicrobial resistance (AMR) of a carbapenemase-producing *Vibrio alginolyticus* isolate, identified by routine genomic surveillance from imported prawns.

Methods: Routine short-read sequencing was conducted for fourteen *V. alginolyticus* isolates identified at Microbiological Diagnostic Unit, Public Health Laboratory. Phylogenetic comparisons of the novel isolates were performed against a global *V. alginolyticus* dataset (n=109), and all isolates were screened for AMR genes using the abritAMR tool. The complete genome was assembled for the carbapenemase-producing isolate, AUSMDU00064140, using both long-read and short-read sequencing data. Phenotypic carbapenemase detection was performed using the carbapenemase inactivation method (CIM) test for AUSMDU00064140, in addition to antimicrobial susceptibility testing using a commercial broth microdilution panel.

Results: Phenotypic testing of AUSMDU00064140 showed evidence of carbapenemase activity (positive CIM test), despite low meropenem MICs (MIC ≤0.5 mg/L). Genomic analyses confirmed the presence of a full-length carbapenemase gene, $bla_{\text{NDM-1}}$, located on the chromosome, with ten additional acquired AMR genes (belonging to seven AMR classes) are co-located with the $bla_{\text{NDM-1}}$ gene. AUSMDU00064140 is phylogenetically distinct from all other *V. alginolyticus* isolates analysed and AMR genes were uncommon across the *V. alginolyticus* global dataset.

Conclusions: The presence of bla_{NDM-1} in *V. alginolyticus* is concerning due to the potential for gene transmission within hosts (gastrointestinal colonisation) and, between hosts and the environment. Phenotypic carbapenem MIC testing alone did not detect a carbapenemase gene in this isolate, demonstrating the value of genomics in uncovering hidden AMR determinants of public health significance.

Rachael Moses

Developing a 3D chronic wound model using animal-free products

Dr Rachael Moses[1], Professor Alastair Sloan[1]

[1]Melbourne Dental School, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Victoria, Australia.

Chronic wounds are scenarios where the acute wound healing response is impaired, inducing a burden to both the patient and the healthcare system. In order to replicate the human wound repair process, animal models are often used, to provide a greater wealth of information on the wound healing potential of novel therapies. These models are typically rodent models, including the diabetic mouse model, demonstrating chronic wound healing scenarios. However, these rodent models have limitations in their translation to human wound healing scenarios due to differences in healing manner. An alternative to animal models involves the use of 3D organotypic in vitro models, representing more closely the complex in vivo scenario the widely performed 2D monolayer cultures.

This 3D organotypic model comprises of human chronic wound derived fibroblasts and epidermal keratinocytes, cultured at the air-liquid interface, resulting in the differentiation of the various epidermal layers, including the protective cornified layer. This 3D organotypic model is cultured over 14 days, before subsequent H&E staining, along with immunostaining for key components of the epidermal and dermal layers, including keratin 10, keratin 14, involucrin and fibronectin. The majority of cell-based research utilises animal-derived products, whereas these models are cultured using synthetic, animal-free products, resulting in a better consistency in the studies, due to the batch variability associated with animal product use. Many industries and funders are already expressing an interest in replacing animal use in research, but a viable option is required to sufficiently replace these models. This 3D model will be a more cost-effective option than animal models and without the ethical considerations associated with their use.

Stephanie Neville

The structural basis of bacterial manganese import

Stephanie L. Neville[1], Jennie Sjöhamn[2], Jacinta A. Watts[1], Hugo MacDermott-Opeskin[3], Stephen J. Fairweather[3,4], Katherine Ganio[1], Alex Carey Hulyer[1], Aaron P. McGrath[2], Andrew J. Hayes[1], Tess R. Malcolm[5], Mark R. Davies[1], Norimichi Nomura[6], So Iwata[6], Megan L. O'Mara[3], Megan J. Maher[2,5], Christopher A. McDevitt[1].

[1]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Victoria, Australia.
[2]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria, Australia.
[3]Research School of Chemistry, Australian National University, Canberra, ACT, Australia.
[4]Research School of Biology, Australian National University, Canberra, ACT, Australia.
[5]School of Chemistry, The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, Victoria, Australia.
[6]Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Introduction/Aim: *Streptococcus pneumoniae* is a significant global pathogen, responsible for over one million deaths annually. As a host-adapted organism, the pneumococcus must acquire all nutrients directly from the host environment. Manganese is one such nutrient and is essential for virulence and viability. Consequently, the manganese uptake pathway, PsaBCA, has been investigated for therapeutic development in numerous studies. PsaBCA is a Type II ABC importer comprised of a manganese binding protein, PsaA, and transmembrane transporter, PsaBC. While the structure of PsaA has been previously determined, the structural and functional elements of PsaBC have remained undefined.

Results: To gain insight into the structural features of PsaBC, we solved the crystal structure to 2.9 Å in an open-inward conformation (Neville *et al*., 2021 *Science Advances*). However, structural analyses revealed substantial differences between PsaBC and previously characterised transporters, suggesting fundamental mechanistic differences in transporter function. We examined this further with molecular dynamics, which revealed the transporter was effectively closed to the extra-cytoplasmic environment, with specific extracellular gating residues acting to exclude water molecules and prevent reflux of manganese during transport. Below these residues, we observed a novel metal coordination site, not previously described for Type II ABC transporters. Through mutagenesis, we showed that these residues were essential for the unidirectional translocation of manganese and therefore, the viability of *S. pneumoniae*.

Conclusions: Collectively, our results define the structure of PsaBC, a key virulence factor for *S. pneumoniae,* and reveal essential structural features that may be appropriate for future rational drug design of novel antimicrobials.

Camille Short

Digitally-delivered models of exercise support for Haematological cancer survivors: A cross-sectional study exploring factors associated with likelihood of uptake

Camille E Short [1 2], Lara Edbrooke [3 4], Shaza Abo [3 4], Tamara Jones [1], Catherine Granger [3 5], Linda Denehy [3 4]

[1]Melbourne School of Psychological Sciences, Melbourne Centre for Behaviour Change , Parkville , VIC, Australia
[2]Melbourne School of Health Sciences, University of Melbourne , Parkville , VIC, Australia
[3]Department of Physiotherapy, Melbourne School of Health Sciences, University of Melbourne , Parkville , VIC, Australia
[4]Department of Health Services Research, The Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
[5]Department of Physiotherapy, The Royal Melbourne Hospital, Melbourne, VIC, Australia

Introduction/Aim: To explore factors associated with uptake of digitally-delivered exercise models to support treatment readiness and recovery among Haematological cancer survivors.

Methods: A survey study via Qualtrics was conducted (August 2021) among adults self-reporting a diagnosis of Haematological cancer within the last five years. Three models of digitally-delivered exercise support

(video teleconferencing, live coaching with remote monitoring, self-guided web-based program with minimal contact) were described. Participants were asked to report the likelihood of uptake for each model on a 5-point Likert scale (not at all likely to extremely likely). Response reasons were collected via an open-ended question. Demographic, health and exercise behaviours were also recorded. Ordinal regression was used to explore participant characteristics associated with likelihood of uptake of each model on a complete case basis. Open-ended responses were analysed thematically.

Results: Participants (N = 70) were generally representative of the target population. Though those meeting physical activity guidelines (67%) and those diagnosed with myeloma (63%) were overrepresented. Average time since diagnosis was 2.2 years (SD 1.7). Self-reported likelihood of uptake was highest for the remote monitoring model, followed by the web-based minimal contact model (65% versus 60% rating 'extremely or "quite a bit"). Video-teleconferencing was the lowest rated model (54%). Living outside of a major city and having a university degree were associated with higher likelihood ratings. English as a second language was associated with a lower likelihood rating for the minimal contact model. Participant open-ended responses highlighted both facilitators (e.g., flexibility) and barriers (e.g., lack of social connection, ill-health) for implementing the models.

Conclusions: This study provides insights into which patients may be less interested or able to participate in digitally-delivered models of exercise support, and subsequently for whom such programs may be most suitable and what barriers and facilitators should be considered.

Leanne Teoh

Efficacy of oxycodone for post-operative dental pain: a systematic review and meta-analysis

Leanne Teoh[1], Michael McCullough[1], Meng-Wong Taing[2]

[1]Melbourne Dental School, University of Melbourne
[2]School of Pharmacy, University of Queensland

Introduction/Aim: Dental pain is a commonly managed presentation in medicine and dentistry, where oxycodone is often prescribed. The aim of this systematic review and meta-analysis was to determine and quantify the effectiveness of oxycodone for acute dental pain.

Methods: Randomised controlled trials, controlled trials and comparative studies were included involving patients >12 years, where oxycodone was trialled for dental pain. Three databases were searched: Medline Ovid, Embase Ovid and Web of Science. Two authors independently screened title and abstracts for relevance, extracted data and performed bias assessments.

Results: Of 148 potentially relevant studies, 13 articles met the inclusion criteria for the systematic review and of the 13, nine studies were included in the meta-analysis. All studies were single-dose analgesia for surgical third molar extractions. Oxycodone produced more effective analgesia in combination with paracetamol. In the meta-analysis, monotherapy etoricoxib and rofecoxib showed significant pain relief compared to combination oxycodone/paracetamol (SPID6 mean difference=-2.13, CI=-3.29, -0.98; TOTPAR6 mean difference=-2.98, CI=-4.90, -1.06).

Conclusions: Non-steroidal anti-inflammatory drugs (NSAIDs) were more effective than oxycodone/paracetamol combinations, reinforcing the effectiveness of NSAIDs for acute post-operative dental pain. Oxycodone combined with NSAIDs offers limited therapeutic value and more adverse effects compared to NSAIDs alone.

Adrianna Turner

Rifaximin causes resistance to the last-resort antibiotic daptomycin in *Enterococcus faecium*

A.M. Turner[1], L. Li[1], I.R. Monk[1], J.Y.H. Lee[1,2], D.J. Ingle[1], S. Duchene[1], N.L. Sherry[1,3,4], T.P. Stinear[1], J.C. Kwong[1,4], C.L. Gorrie[1,3], B.P. Howden[1,3,4] and G.P. Carter[1]

[1]Department of Microbiology & Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, VIC, Australia
[2]Department of Infectious Diseases, Monash Health, Clayton, VIC, Australia
[3]Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia
[4]Department of Infectious Diseases, Austin Health, Melbourne, VIC, Australia

Introduction/Aim: Bacterial pathogens such as vancomycin-resistant *Enterococcus faecium* (VREfm) that resist almost all antibiotics are among the top threats to human health. Daptomycin is a new last-resort antibiotic for VREfm infections with a novel mode-of-action, but for which resistance has surprisingly been widely reported. The objective of this study was therefore to understand the daptomycin resistance mechanisms in VREfm.

Methods: Clinical VREfm (n=1000) collected during Australian surveillance studies underwent daptomycin susceptibility testing and whole-genome sequencing (WGS). Mutations associated with daptomycin resistance were identified using a genome-wide association study (GWAS) and confirmed with molecular mutagenesis.

Results: Daptomycin-susceptibility testing showed 18.9% of VREfm were daptomycinresistant, giving Australia one of the highest rates of resistance globally. A GWAS suggested daptomycin-resistant VREfm contained novel mutations within a characterised region of the *rpoB* gene typically associated with resistance to the antibiotic rifaximin. Construction of isogenic mutants containing the identified *rpoB* mutations indicated they conferred crossresistance to rifaximin and daptomycin. VREfm with these mutations were spread globally across at least 5 continents and 20 countries, with multiple co-circulating lineages identified. In a VREfm colonisation model, exposure to rifaximin resulted in the emergence of VREfm that were cross-resistant to rifaximin and daptomycin. Importantly, patients given rifaximin were significantly more likely to harbour daptomycin-resistant VREfm, containing RpoB mutations, than patients who didn't receive rifaximin.

Conclusions: Our study has identified a novel and globally prevalent daptomycin resistance mechanism in VREfm and highlights unanticipated and serious "collateral damage" that may arise in patients following use of rifaximin.

Carolien van de Sandt

Influenza-specific CD8$^+$ T-cells across the human lifespan: a suboptimal reset for the elderly

van de Sandt CE[1,2], Nguyen THO[1], Gherardin NA[1], Rizzetto S[3], Crawford JC[4], Samir J[3], Szeto C[5], Kaur J[6], Sonda S[6], Minervina AA[4], Pogorelyy MV[4], Redmond S[1], McQuilten HA[1], Menon T[1], Sant S[1], Jia X[1], Lim R[7,8], Clemens EB[1], Eltahla A[3], Crowe J[9], Lappas M[7,8], Flanagan K[6,10,11,12], Thomas PG[4], Rossjohn J[13,14], Godfrey DI[1], Gras S[5], Luciani F[3*] and Kedzierska K[1*]

[1] Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia
[2] Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
[3] School of Medical Sciences and The Kirby Institute, UNSW Sydney, Sydney, New South Wales 2052, Australia.
[4] Department of Immunology, St. Jude Children's Research Hospital, Memphis, USA
[5] Viral and Structural Immunology Laboratory, Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, School of Molecular Sciences, La Trobe University, Australia.
[6] School of Health Sciences and School of Medicine, University of Tasmania, Launceston, Australia
[7] Obstetrics, Nutrition and Endocrinology Group, Department of Obstetrics and Gynaecology, University of Melbourne, Australia
[8] Mercy Perinatal Research Centre, Mercy Hospital for Women, Heidelberg, Australia;
[9] Deepdene Surgery, Deepdene, Australia
[10] School of Health and Biomedical Science, RMIT University, Melbourne, Victoria 3000, Australia
[11] Tasmanian Vaccine Trial Centre, Clifford Craig Foundation, Launceston General Hospital, Launceston, Australia
[12] Department of Immunology and Pathology, Monash University, Commercial Road, Melbourne, Australia
[13] Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Australia

[14] Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff, United Kingdom

Introduction/Aim: Influenza viruses remain a constant global threat, causing morbidity and mortality. Age is a major factor in determining disease severity, but the underlying mechanisms that drive age-related differences are not well understood. $CD8^+$ T-cells play a key role in protection against novel influenza strains and subtypes. T-cell receptors (TCRs) can recognize conserved influenza proteins, providing broad cross-reactivity across distinct influenza viruses. This makes them an attractive target for novel influenza vaccine strategies. We investigated how TCR composition and diversity relate to $CD8^+$ T-cell functionality across immunologically-distinct phases of human life.

Methods: We combined *ex vivo* tetramer-associated magnetic enrichment with single-cell multiplex-nested RT-PCR to analyse paired TCRab repertoires specific for the most prominent influenza epitope, HLA-A*02:01-$M1_{58-66}$ ($A2^+M1_{58}$) in cord blood, children, adults and elderly individuals. We linked TCR clonotype dynamics to the magnitude, phenotype and functionality of $A2^+M1_{58}$-specific $CD8^+$ T-cells.

Results: Frequency and phenotype of the $A2^+M1_{58}$-specific $CD8^+$ T-cells changes across human lifetime. The optimal public TRAV27-TRBV19 clonotype dominates the TCRab repertoire in children and adults, is absent in cord blood and is replaced by private TCRab clonotypes characterized by broader usage of TRAV-TRBV gene segments with different binding-motifs in the elderly.

Conclusions: Overall, our study indicates that the changes in frequency and phenotype of the influenza virus-specific $CD8^+$ T-cells coincide with changes in the TCRab repertoire and overall strength of the $CD8^+$ T-cells. These findings suggest that priming T-cell compartments at different stages of life, might influence the clonal composition and diversity of responding TCR repertoires against viral infections.

Yi Yang

Inequalities in service utilisation in the National Disability Insurance Scheme (NDIS)

George Disney[1,2] *, Yi Yang[1,2] *, Peter Summers[1,2], Kirsten Deane[2], Alexandra Devine[1], Helen Dickinson[3], Bruce Bonyhady[2], Anne Kavanagh[1] ** Joint first author, contributed equally.*

[1] Disability and Health Unit, Centre for Health Equity, Melbourne School of Population and Global Health, The University of Melbourne

[2] Melbourne Disability Institute, The University of Melbourne

[3] Public Service Research Group, School of Business, University of New South Wales, Canberra, Australian Capital Territory, Australia

Introduction/Aim:
People with disability experience profound inequalities in health, largely driven by social exclusion, social disadvantage, and barriers to accessing public services and healthcare. It is argued that self-directed care and personalised budgets strengthen disabled peoples' choice and control over their support needs and lives. Australia has recently reformed disability services to this personalised and self-directed model through the NDIS. However, there is also concern that already existing social inequities could translate to inequitable use of individualised disability services. This is the first study using unit-record NDIS data to quantify potential inequalities in service utilisation experienced by disadvantaged groups.

Methods:
We estimated inequalities in support allocation and use between disadvantaged groups (First Nations participants and participants living in more socioeconomically disadvantaged areas) and their comparator groups (non-First Nations participants and participants not living in less socioeconomically disadvantaged areas). We estimated the plan size and spending differences standardised for the distribution of disability and sociodemographic characteristics using model-based standardisation.

Results:
Our analysis found that, overall, First Nations participants received on average larger plans than non-First Nations participants. However, the larger plans did not translate to higher spending. Participants living in socioeconomically disadvantaged areas received on average similar sized plans to their comparison group and spent similar amounts; proportion of plans spent were equally low in both groups.

Conclusions:
It appears that social inequality in plan allocation is less evident. The inequalities mainly arise when disadvantaged participants were unable to spend allocated funds to access the supports they need.

Abstracts: *Flash talks*

Lucy Bartho

Circulating chemerin is increased in preeclampsia, preceding diagnosis of preeclampsia, and associated with placental hypoxia

Lucy Bartho[1,2], Manju Kandel[1,2], Susan P Walker[1,2], Catherine A Cluver[3,4,5], Roxanne Hastie[1,2,3], Lina Bergman[3,4,5], Ping Cannon[1,2], Tuong-Vi Nguyen[1,2], Georgia P. Wong[1,2], Teresa M. MacDonald[1,2], Emerson Keenan[1,2], Natalie J. Hannan[1,2], Stephen Tong[1,2], Tu'uhevaha J. Kaitu'u-Lino[1,2]

[1]Translational Obstetrics Group, The Department of Obstetrics and Gynaecology, Mercy Hospital for Women, University of Melbourne, 163 Studley Road, Heidelberg 3084, Victoria, Australia.
[2] Mercy Perinatal, Mercy Hospital for Women, Victoria, Australia.
[3] Department of Obstetrics and Gynaecology, Stellenbosch University, Cape Town, South Africa
[4] Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
[5] Department of Obstetrics and Gynecology, Institute of clinical sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Introduction/Aim: Preeclampsia is a severe complication of pregnancy. Chemerin is an adipokine secreted from white adipose tissue and highly expressed in placenta. This study aimed to assess chemerin in several prospective cohorts and first trimester trophoblast stem cells.

Methods: Maternal plasma and placental samples were collected from cohorts of women with early-onset preeclampsia (<34 weeks' gestation); preeclampsia and eclampsia; or before diagnosis of preeclampsia (36 weeks' gestation). Human trophoblast stem cells were differentiated into syncytiotrophoblast or extravillous trophoblast cells over 96 hours. Cells were cultured in 1% O_2 (hypoxia) and 5% O_2 (normoxia). Chemerin was measured by ELISA and *RARRES2* (gene coding chemerin) was measured by RT-qPCR.

Results: Circulating chemerin was increased in 46 women with early-onset preeclampsia (<34 weeks' gestation) compared to 17 controls (P<0.0006). Chemerin levels were increased in 26 women with severe preeclampsia (P=0.01) and 34 with eclampsia (P=0.03) compared to 15 controls. Placental RARRES2 was reduced in 43 women with early-onset preeclampsia compared to 24 controls (P<0.0001).
In a predictive cohort collected at 36 weeks' gestation, circulating chemerin was increased in 23 women who later developed preeclampsia, versus 182 who did not (P<0.0001).
Functional studies revealed *RARRES2* expression was reduced following differentiation of cytotrophoblast cells into syncytiotrophoblast (P=0.005, n=5) or extravillous trophoblast cells (P<0.0001, n=5). Hypoxia (1% O2) increased RARRES2 expression in syncytiotrophoblast (P=0.01, n=5), but not cytotrophoblast cells (n=5).

Conclusions: Circulating chemerin was elevated in women with early-onset, established preeclampsia, eclampsia and preceding diagnosis of preeclampsia. *RARRES2* was dysregulated in preeclamptic placenta and may be regulated through hypoxia.

Thomas S. Fulford

A new paradigm in γδTCR recognition: butyrophilins 2A1 and 3A1 co-bind the Vγ9Vδ2$^+$ T cell receptor

Thomas S. Fulford[1], Caroline Soliman[1], Rebecca G. Castle[1], Marc Rigau[1,2], Zheng Ruan[1], Olan Dolezal[3], Rebecca Seneviratna[1], Hamish G. Brown[4], Eric Hanssen[4,5], Shihan Li[1], Samuel J. Redmond[1], Amy Chung[1], Michael A. Gorman[5], Andrew Hammet[6], Michael W. Parker[5,7], Onisha Patel[8,9], Thomas S. Peat[3], Janet Newman[3], Andreas Behren[10], Nicholas A. Gherardin[1], Dale I. Godfrey[1*], Adam P. Uldrich[1*]

[1]Department of Microbiology & Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, Victoria, 3010, Australia.
[2]University of Bonn, Bonn, Germany.
[3]Biomedical Manufacturing, Commonwealth Scientific and Industrial Research Organisation, 343 Royal Parade, Parkville, Victoria, 3052, Australia.

[4]Ian Holmes Imaging Centre, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, 3052, Australia.
[5]Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, 3052, Australia.
[6]CSL Limited, Bio21 Institute, Parkville, Victoria, Australia
[7]St. Vincent's Institute of Medical Research, Fitzroy, Victoria, 3065, Australia.
[8]The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, 3052, Australia.
[9]Department of Medical Biology, University of Melbourne, Parkville, Victoria, 3052, Australia.
[10]Olivia Newton-John Cancer Research Institute, Heidelberg, Victoria, 3084, Australia.

Introduction: Whilst MHC-Ag complexes drive αβT cell-mediated immunity, very little is known about the Ag-presentation elements of γδT cells. Recently, butyrophilin (BTN) molecules have emerged as key regulators of γδT cell immunity, however, how they activate the γδTCR is unknown.

Results: To determine how BTNs bind γδTCR, we solved the crystal structure of Vγ9Vδ2TCR in complex with BTN-member 2A1 (BTN2A1), revealing that BTN2A1 engages the side of the Vγ9Vδ2$^+$ TCR. Intriguingly, we also found that a second ligand, namely BTN3A1, can bind the exposed apical surface of Vγ9Vδ2$^+$ TCR alongside BTN2A1, but only following activation with an agonist anti-BTN3A mAb, or alternatively, mutation of the 'gatekeeper' residue, Lys53 of TCRδ. Unexpectedly, we found that BTN2A1 and BTN3A1 ectodomains also interact directly with each other in *cis*, forming heteromers, and that this BTN2A1–BTN3A1 interaction depends upon the same epitopes that BTN2A1/BTN3A1 each use to engage γδTCR. We propose that this heteromer represents a 'closed-state', which impairs the ability of γδTCR to bind the BTN-complex. Indeed, either forced separation or locking together of BTN2A1 and BTN3A1 resulted in enhanced or abrogated γδTCR reactivity, respectively. Finally, we demonstrate that the intracellular domains of BTN2A1 and BTN3A1 associate following pAg encounter, suggesting that pAg induces intracellular complex formation, which in turn causes a conformational change in the BTN ectodomains, thus enabling γδTCR to bind.

Conclusions: Our findings reveal a new paradigm in immune activation, whereby γδT cells recognise BTN-complexes following conversion of BTN epitopes from a closed-state into a permissive-state.

Meaghan Griffiths

Endometriotic chocolate cysts contain viable cells of endometrial origin: implications for endometriosis recurrence and infertility

Meaghan Griffiths[1], Brooke Backman[1], Fatima Figueiredo[1], Martin Healey[1,2], Keryn Harlow[1,2,3], Claudia Cheng[1,2], Charlotte Reddington[1,2], Georgie Prewer[1], Brodie Bilston[1], Peter Rogers[1], Jacqueline Donoghue[1]

[1] Department of Obstetrics and Gynaecology, University of Melbourne and Gynaecology Research Centre, Royal Women's Hospital, Parkville
[2] Gynaecology Endometriosis and Pelvic Pain Unit, Royal Women's Hospital, Parkville, Victoria, Australia
[3] Fertility Associates, Christchurch, New Zealand

Introduction/Aim: Endometriosis is a chronic, estrogen dependent disease affecting 11% of reproductive aged women. Ovarian endometrioma are a common subtype of endometriosis yet, our understanding of their origins is limited. Endometrioma contain chocolate brown fluid believed to arise from haemorrhaging endometrial tissue that lines the cyst. Their presence and growth are diagnosed by ultrasound or MRI and treated laparoscopically. Endometrioma recurrence is common (46% of patients within 3-years), and more severe than initial diagnosis. The origins of endometrioma fluid production are unknown but may contain live cells which contribute to recurrence in the event of cyst rupture. Elucidating the cellular make-up of endometrioma fluid is critical for furthering our understanding of endometrioma, and endometriosis-related infertility.

Methods: Cystic fluid was aspirated from endometriomas during surgery (n=7 patients) followed by PBS dilution and filtration through 100µm, 40µm, and 10µm filters. Filtrates were pelleted and cultured as monolayers or in 3D Matrigel™ domes.

Results: Cellular colonies and organoids appeared within 5-10 days. Immunophenotyping analysis confirmed vimentin-positive stromal cells, cytokeratin-positive epithelial cells, and CD68-positive macrophages were present in endometrioma fluid. Other cell types observed included myofibroblast-like cells, elongated cells with nerve-like projections and cobblestone colonies of endothelial-like cells.

Conclusions: Endometrioma fluid contains viable cells including cells that may be of endometrial origin. Future work to isolate cells from endometrioma fluid and cyst wall will determine cellular origin linkages using single cell transcriptomics. Uncovering the origins of endometrioma and their contents is critically important for enhancing our understanding of endometriosis and its impact on fertility.

Shuting Li

Assessing attention orienting in a mouse model of autism using a reverse-translated Posner task

Shuting Li[1], Carlos May[2], Terence Y. Pang[2,4], Leonid Churilov[3], Anthony J. Hannan[2,4], Katherine A. Johnson[1,*], Emma L. Burrows[2,*]

[1]Melbourne School of Psychological Sciences, The University of Melbourne, Parkville, VIC 3010, Australia
[2]Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC 3052, Australia
[3]Melbourne Medical School, The University of Melbourne, Parkville, VIC 3010, Australia
[4]Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, VIC 3010, Australia
*These authors jointly supervised this work.

Introduction/Aim: Atypical attention orienting is a potential early marker of autism spectrum disorder (ASD). To date, the neural mechanisms underlying atypical attention orienting in ASD remain unclear. Attention orienting involves exogenous (stimulus-driven) and endogenous (goal-directed) systems, which can be investigated using the Posner task. Our previous study successfully adapted the human Posner task for use in mice using touchscreen technology. With the availability of the mouse Posner (mPosner) task, we continued to investigate whether ASD-associated genetic mutations alter the attentional performance of mice and the effects of relevant attention-modulating drugs.

Methods: Twenty mice carrying the ASD-associated R451C mutation in neuroligin-3 (NL3) gene and twenty wild-type (WT) mice were trained to sustain their nose-poke to a central square until a validly or invalidly cued target was displayed. The cue was a peripheral non-predictive flash in the exogenous task and a central spatially-predictive image in the endogenous task. The effects of dopaminergic- and noradrenergic-modulating drugs, methylphenidate (MPH) and atomoxetine (ATX), on task performance were also assessed.

Results: On both tasks, mice were quicker and more accurate in the validly versus invalidly cued trials, consistent with results in humans. $NL3^{R451C}$ and WT mice were not significantly different in response times, accuracy, or attention orienting. Neither MPH nor ATX altered attention orienting but exerted differential effects between the genotypes.

Conclusions: Our study showed intact attention orienting in $NL3^{R451C}$ mice. Atypical responses to MPH and ATX in $NL3^{R451C}$ mice indicate that the mutation may alter specific attention networks, such as the dopaminergic-modulating executive attention network.

Jieqiong Lou

Histone FRET microscopy reveals that genome architecture is differentially regulated by a HP1α monomer to dimer transition

Jieqiong Lou[1,2], Qiji Deng[7], Xiaomeng Zhang[1], Charles C. Bell[7], Andrew B. Das[7], Naiara G. Bediaga[7], Prasad. N. Paradkar[3], Kieran F. Harvey[4,5,7], Mark A. Dawson[4,6,7], Elizabeth Hinde[1,2]

[1]School of Physics, University of Melbourne, Melbourne, VIC 3000, Australia
[2]Department of Biochemistry and Pharmacology Bio21 Institute, University of Melbourne, Melbourne, VIC 3000, Australia.
[3]CSIRO Health & Biosecurity, Australian Centre for Disease Preparedness, 5 Portarlington Road, Geelong 3220, Australia.
[4]Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, VIC 3010, Australia.

[5] Department of Anatomy and Developmental Biology and Biomedicine Discovery Institute, Monash University, Clayton, VIC 3168, Australia.
[6] Centre for Cancer Research, University of Melbourne, Melbourne, VIC 3000, Australia.
[7] Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, VIC 3000, Australia

Introduction/Aim: The fundamental feature of genome architecture that regulates DNA template access to the proteins that read, copy, and repair genetic information, is the nanometre spacing between nucleosomes along a chromatin fibre. However, direct measurement of nucleosome arrangement in a living cell is extremely challenging because this nanoscale aspect of chromatin structure occurs on a spatial scale well below the diffraction limit of optical microscopy

Methods: To investigate real time regulation of chromatin architecture, we modified the architectural protein heterochromatin protein 1 alpha (HP1α) and employed fluorescence lifetime imaging microscopy (FLIM) of Förster resonance energy transfer (FRET) between core histones to assess nucleosome proximity.

Results: Intriguingly, this super-resolved readout of nanoscale chromatin structure revealed nucleosome arrangement to be differentially regulated as a function of HP1α oligomeric state throughout the nucleus. Specifically, HP1α monomers imparted a previously undescribed global nucleosome spacing throughout the genome that was locally reduced upon HP1α dimer formation. Further, this dynamic nanoscale scaffold was impervious to sub-micron higher order chromatin structure and foci formation.

Conclusions: Collectively, these findings demonstrate that the macroscale features of genome architecture are dictated by different concentrations, but not configurations, of spaced and bridged nucleosomes, which collectively control the spatiotemporal kinetics of transcription, replication, and DNA repair.

Jacob Paul

Numerosity tuning in human association cortices and local image contrast representations in early visual cortex

Jacob M. Paul[1], Martijn van Ackooij[2],Tuomas C. ten Cate[2],Ben M. Harvey[2]

[1] Melbourne School of Psychological Sciences, University of Melbourne, Australia
[2] Department of Experimental Psychology, Helmholtz Institute, Utrecht University, Netherlands

Introduction/Aim: Humans and many animals perceive visual numerosity (object number) and neurons showing numerosity-tuned responses have been found in several species. However, it remains unclear how the brain estimates numerosity from visual images while disregarding object size and spacing. Recent results show that human early visual cortex responses monotonically increase following numerosity, regardless of object size or spacing. This is surprising because numerosity is typically considered a high-level visual or cognitive feature while early visual responses are normally thought to follow image contrast in the spatial frequency domain.

Methods: We therefore asked whether these early visual responses could be explained by the spatial frequency content of numerosity displays. Using 7 Tesla fMRI, we showed monotonic responses originate in primary visual cortex (V1) at the stimulus's retinotopic location. Responses here and in neural network models followed aggregate Fourier power more closely than numerosity.

Results: We found that aggregate Fourier power (contrast at all orientations and spatial frequencies) followed numerosity closely but nonlinearly, with little effect of object size, spacing or shape. This would allow straightforward numerosity estimation from spatial frequency domain image representations. Truly numerosity tuned responses emerged after lateral occipital cortex and were independent of retinotopic location.

Conclusions: We propose numerosity's straightforward perception and evolutionarily preserved neural responses may result from the pervasive spatial frequency analyses in early visual processing throughout the animal kingdom.

Richard Rebello

SUPER-NEXT: employing comprehensive clinical genomics for cancer of unknown primary

Richard Rebello[1,2*], Aidan Flynn[1,2], Clare Fedele[1,2], Tharani Sivakumaran[1,2,3], Samantha Webb[3], Georgia Scott[3], Shohei Waller[3], Atara Posner[1,2], Owen Prall[4], Catherine Mitchell[4], Nadia Trafacante[3,5], Camilla Mitchell[1,2], Joseph Vissers[1,2], Huiling Xu[4], Alex Caneborg[1,2] Shiva Balachander[1,2], Krista Fisher[3], Hui-Li Wong[3,5], Kym Sheehan, Chris Karapetis[6], Ian Collins[7], Mark Warren[8], Bo Gao[9], Madhu Singh[10], Christopher Steer[11], Desmond Yip[12], Pei Ding[13], Nicholas Wilcken[9], Stephen Quinn[14], Anna Defazio[15], Narayan Karanth[15], Anna Kuchel[16], Rachel Wong[17], Mark Shackleton[18], Zee Wan Wong[19], Louise Nott[20], Shamsudeen Padinharakam[21], Sarah Jane Dawson[1,3,5], Rodney Hicks[3,5], David Bowtell[3,5], Andrew Fellowes[4], Stephen Fox[4,5], Louisa Gordon[22], Oliver Hofmann[1,2], Sean Grimmond[1,2], Penny Schofield[3,23], Linda Mileshkin[3,5], Richard Tothill[1,2]

[1]University of Melbourne Centre for Cancer Research, The University of Melbourne, Melbourne, VIC
[2]Department of Clinical Pathology, The University of Melbourne, Melbourne, VIC
[3]Peter MacCallum Cancer Centre, Melbourne, VIC
[4]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, VIC
[5]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, VIC
[6]Flinders Cancer Centre for Innovation in Cancer, Flinders University, Adelaide, SA
[7]South West Healthcare, Warrnambool, VIC
[8]Bendigo Cancer Centre, Bendigo Health, VIC
[9]Westmead Private Hospital, Westmead, NSW
[10]Barwon Health Cancer Services, Barwon, VIC
[11]Border Medical Oncology, Albury Wodonga Regional Medical Centre, NSW
[12]Department of Medical Oncology, Canberra Hospital, Canberra, ACT
[13]Nepean Cancer Care Centre, Nepean Hospital, Kingswood, NSW
[14]Swinburne University of Technology, Department of Health Science and Biostatistics, VIC
[15]Medical Oncology Department, Alan Walker Cancer Centre, Royal Darwin Hospital, NT
[16]Department of Medical Oncology, The Royal Brisbane and Women's Hospital, Herston, QLD
[17]Box Hill Eastern Oncology, Box Hill, VIC
[18]Department of Medical Oncology, Alfred Health, Melbourne; Central Clinical School, Monash University, Melbourne, VIC
[19]Oncology Unit, Peninsula Health, Melbourne, VIC
[20]Royal Hobart Hospital, Hobart, TAS
[21]Launceston General Hospital, Launceston, TAS
[22]QIMR Berghofer Medical Research Institute, Population Health Department, Herston, Brisbane, QLD
[23]Department of Psychological Sciences, Swinburne University of Technology, Melbourne, VIC

Introduction/Aim: Cancer of unknown primary (CUP) is a heterogeneous group of many different cancer types and is the 6th most common cause of cancer-related death in Australia. CUP has a dismal prognosis with one and 5-year survival rates of 25% and 14%, respectively. Since cancer medicine is largely based on anatomical location, CUP significantly challenges the established care paradigms. Indeed, these patients have difficulties accessing targeted therapy as known tissue of origin (ToO) is often a requirement. A strong rationale therefore exists to develop better diagnostic methods for CUP to help resolve the primary origin of a patient's tumour to increase their access to therapies.

Methods: The SUPER-NEXT study offers clinical whole genome and transcriptome (WGTS) sequencing for CUP patients and measures the increased sensitivity of actionable/diagnostic gene variant detection over large gene panel-based approaches. Furthermore, the use of liquid biopsies to harvest circulating tumour DNA in CUP patient blood, is tested alongside, enabling more rapid turnaround of results and the potential for a non-invasive ToO classification.

Results: I will present the benefit achieved in a cohort of 100 patients, from 1 year of real-time prospective genomic profiling of CUP in Australia, including case examples where WGS and tissue of origin classification was able to provide helpful diagnostic or clinically actionable information in time to facilitate a change in management. I will also present the validation and real time use of a classifier (CUP Prediction Algorithm) as a supplement to molecular reporting, which utilizes WGS data to determine ToO for a CUP.

Conclusions: Tissue of Origin classification using Whole Genome Sequencing data of Cancer of Unknown primary has enormous transformative potential to step-change management for patients suffering with this disease.

Myrte Strik

Altered network topology in patients with visual snow syndrome: a resting-state 7 Tesla MRI study

M. Strik[1], M. Clough[2], E.J. Solly[2], R. Glarin[1], O.B. White[2], J. Fielding[2] and S.C Kolbe[2]

[1]Melbourne Brain Centre Imaging Unit, Department of Radiology, Melbourne Medical School, University of Melbourne, Melbourne, Australia.
[2]Department of Neuroscience, Central Clinical School, Monash University, Melbourne, Australia.

Introduction/Aim: Visual snow syndrome (VSS) is a neurological disorder characterized by a range of continuous visual disturbances. Little is known about the functional pathological mechanisms underlying VSS and their effect on brain network topology. The aim of this study was to characterize network dynamics in VSS patients using high-resolution resting-state (RS) 7T MRI.

Methods: Forty VSS patients and 60 controls underwent RS MRI. Functional connectivity matrices were calculated, and global efficiency (network integration), modularity (network segregation), local efficiency (connectedness neighbours) and eigen vector centrality (significance node in a network) were derived using a dynamic approach (temporal fluctuations during acquisition). Network measures were compared between groups, with regions of significant difference correlated with known aberrant ocular motor VSS metrics in VSS. Lastly, nodal co-modularity, a binary measure of node pairs belonging to the same module, was studied.

Results: VSS patients had lower modularity, supramarginal centrality and local efficiency dynamics of multiple (sub)cortical regions, centred around the occipital and parietal lobules. Local efficiency dynamics of the lateral occipital cortex correlated with shortened prosaccade latencies in VSS patients (p=0.041, r=0.353). Further, in VSS patients, occipital, parietal and motor nodes belonged more often to the same module and demonstrated lower nodal co-modularity with temporal and frontal regions.

Conclusions: This study revealed reduced dynamic variation in modularity and local efficiency strength in VSS patients, suggesting that brain network dynamics are less variable in terms of segregation and local clustering. Changes were widespread, but strongest effects were observed in occipital cortices, related to oculomotor motor processing.

Abstracts: *Posters*

Alaa Abdul-Ridha

Fragment screening against stabilized α_{1A}- and α_{1B}-adrenoceptors allows identification of novel sub-type selective ligands.

Alaa Abdul-Ridha [1], Andy Zhang [1], Ashenafi Haileyesus Betrie[1] Paul R. Gooley [2, 3], Ross A.D. Bathgate[1,2], & Daniel J. Scott[1,2]

[1]The Florey Institute of Neuroscience and Mental Health, 30 Royal Parade, The University of Melbourne, Parkville, Victoria 3052, Australia
[2] The Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, Victoria 3010, Australia
[3] The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia

α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (α_1-ARs) are members of the adrenoceptors G protein-coupled receptor (GPCR) family activated endogenously by epinephrine and norepinephrine. They are clinically targeted by non-subtype-selective antagonists, such prazosin and tamsulosin, for the treatment of hypertension and benign prostatic hyperplasia. Their abundant expression in the heart and CNS places them as potential clinical targets for the treatment of various cardiovascular and CNS disorders such as heart failure and Alzheimer's disease. However, understanding their physiological roles and involvement in disease has been hindered by the lack of subtype-selective tool compounds, especially for the α_{1B}-AR. GPCRs are known to undergo conformational changes upon ligand binding and signal transduction. This conformational flexibility represents a bottleneck in protein production and crystallographic studies, which open new routs for fragment-based and structure-based drug discovery. Our recent generation of solubilised, ultra-stable α_{1A}-AR and α_{1B}-AR has overcome this hurdle and allowed application of a fragment-based drug discovery screen. The screen identified a novel, selective α_{1B}-AR antagonist (Cpd3). Optimisation of Cpd3 into a higher affinity ligand will provide a useful laboratory tool or a clinical lead compound needed to probe the physiological roles of specific α_1-AR subtypes and examine their potential as targets for treating disease.

Sushma Anand

Vision restoration in glaucoma using direct reprogramming of fibroblasts.

Sushma Anand[1], Isabel Lopez Sanchez[1,] Ian Trounce[1]

[1]Centre For Eye Research Australia, Department of Surgery, The University of Melbourne

Introduction/Aim: Glaucoma ranks fourth among the leading causes of permanent blindness in the world according to the World Health Organization. According to Glaucoma Australia, 1 in 50 Australians will develop glaucoma in their lifetime and 50% of sufferers remain undiagnosed. Glaucoma is caused by the degeneration retinal ganglion cells (RGCs), resulting in optic nerve damage. RGCs are the eye neurons that carry information of "what we see" to the brain. The standard treatment for glaucoma is to reduce the fluid pressure within the eye, yet a significant fraction of patients develops vision loss despite treatment. Therefore, there is an urgent need to develop new treatments to prevent or reverse vision loss in glaucoma patients. Here, we are aiming to regenerate RGCs to prevent or reverse vision loss in glaucoma.

Methods: To achieve this aim we use a "direct cellular reprogramming" (DR) method. We will introduce genes that control neuronal development into human fibroblast cells. Forceful introduction of neuronal genes will reprogram fibroblasts into RGCs.

Results: This study will establish that DR is an efficient method to regenerate RGCs in a dish. Confocal microscopy images showed RGC^{+ve} like cells. The key measure of success of this glaucoma treatment

advancing research is the creation in the lab of patient cells that closely resemble the specific cells lost in glaucoma.

Conclusions: The insight gained from this research will drive innovation in the field of cell therapy disease that cause intractable vision loss.

Prabhathi Basnayake

Achieving Equity in Genomic Health (AEGH) for Indigenous Australians

Prabhathi Basnayake[1],Tala Mitchell[1],Claire Zammit[1], Angeline Ferdinand[1]

[1]Centre for Health Policy, Melbourne School of Population Health, University of Melbourne

Introduction/Aim: There is a significant lack in the equity in referral to and attendance at genetic health services among Indigenous Australians. The aim of the Achieving Equity in Genomic Health (AEGH) for Indigenous Australians project is to increase equity by improving the ability of the genetic services in NT, Queensland and WA to meet the needs of Indigenous patients and families and to improve access to follow up services. AEGH project aims to do this by the refinement of the recommendations made by the Better Indigenous Genetics (BIG) Health Services Project which previously explored the genetic services delivered in the three jurisdictions.

Methods: To refine the proposed recommendations by the BIG project, the AEGH team held co-design workshops with End User Groups, Project Reference Groups and Genetic Health Services in the three jurisdictions. From each of the co-design workshops specific jurisdictional priorities were set and logistics of the implementation are discussed with genetic services of the jurisdictions.

Results: During the co-design workshops several similarities were identified in the priorities set for the three jurisdictions. Conducting cultural capability audits and training in the genetic services, developing detailed administrative record systems to identify and follow up Indigenous patients effectively and establishing better communication with Indigenous specific organisations and support services were similar for all three jurisdictions. The co-design workshops also helped to identify important differences in genetic services in the three jurisdictions too.

Conclusions: These identified priorities are currently included in the implementation plans of the three jurisdictions and the logistics of their implementation are being discussed with the relevant genetic services.

David Chieng

Atrial fibrillation ablation for heart failure with preserved ejection fraction (HFpEF): a randomized controlled trial

David Chieng[1,2,3], Hariharan Sugumar[1,2,3], Louise Segan[1,2,3], Caleb Tan[2], Donna Vizi[1,2], Shane Nanayakkara[1,2], Ahmed Al-Kaisey[2,3,4], Joshua Hawson[3,4], Sandeep Prabhu[1,2,3], Aleksandr Voskoboinik[1,2,3], Sue Finch[3], Joseph B Morton[3,4], Geoffrey Fle[3,4], Justin Mariani[2], Andre La Gerche[1,2,3], Andrew J Taylor[1,2,3], Erin Howden[1], Peter M Kistler[1,2,3,5], Jonathan M Kalman[3,4,5], David M Kaye[1,2,5], Liang-Han Ling[1,2,3]

[1] The Baker Heart and Diabetes Research Institute, Melbourne, Australia
[2] The Alfred Hospital, Melbourne, Australia
[3] University of Melbourne, Melbourne, Australia
[4] Royal Melbourne Hospital, Melbourne, Australia
[5] Monash University, Melbourne, Australia

Introduction/Aim: Atrial fibrillation(AF) frequently accompanies heart failure with preserved ejection fraction(HFpEF). There is no randomised data examining the effects of rhythm control with catheter-based AF ablation on HFpEF outcomes. The aim of this study is to compare the effects of AF ablation versus usual medical therapy on markers of HFpEF severity, including exercise haemodynamics, natriuretic peptide levels and patient symptoms.

Methods: Patients with symptomatic AF and HFpEF underwent exercise right heart catheterization(RHC) and cardiopulmonary exercise testing(CPET). HFpEF was confirmed on exercise RHC based on pulmonary capillary wedge pressure (PCWP) of 15mmHg at rest or ≥25mmHg on exercise. Patients were randomised to AF ablation versus medical therapy, with investigations repeated at 6 months. The primary outcome was change in PCWP on follow-up

Results: 31 patients aged 66.1+/-7.5 years were randomized to AF ablation(16) versus medical therapy(15), with 51.6% female and 80.6% persistent AF. At 6 months, ablation reduced the primary outcome of peak PCWP (30.4±4.2 to 25.9±4.3 mmHg, p<0.01). Improvements were also seen in peak VO2 (1937.3±739.3 to 2216.3±861.9 mL/min, p<0.01), NT-pro BNP levels (771±703 to 167±66 ng/L, p=0.03), and Minnesota Living with Heart Failure (MLHF) score (51±-21.9 to 16.6±17.5, p<0.01). No differences were observed in the medical arm. Following ablation, 50% no longer met exercise RHC-based criteria for HFpEF versus 7% in medical arm (p=0.02).

Conclusions: AF ablation improves invasive exercise haemodynamic parameters, increases exercise capacity, and enhances quality of life in patients with concomitant AF and HFpEF. Successful AF ablation may reverse the clinical syndrome of HFpEF in a proportion of cases

Marianne Coleman

Awareness of and engagement with Faculty- and University-level diversity and inclusion events by MDHS early and mid career academics

Marianne Coleman[1], Kristin Diemer[2], Michelle Hall[3], Kate Hayward[3], Flora Hui[1], Rebecca Jarden[4], Elaina Kefalainos[5], Belinda Lawford[3], Gemma McKibbin[2]

[1]Department of Optometry and Vision Sciences, Melbourne School of Health Sciences
[2]Department of Social Work, Melbourne School of Health Sciences
[3]Department of Physiotherapy, Melbourne School of Health Sciences
[4]Department of Nursing, Melbourne School of Health Sciences
[5]Department of Audiology and Speech Pathology, Melbourne School of Health Sciences

Introduction/Aim: Within the MDHS Strategic Plan, "Beyond 2018", development of a Diversity and Inclusion Action Plan was proposed. The extent to which previous University- or Faculty-level D&I initiatives have reached or resonated with EMCAs, and fostered their inclusion, is yet to be explored. Providing this evidence base is likely to assist Faculty Diversity & Inclusion Action Plan development.

Methods: A Faculty-wide survey was circulated via MDHS ECR Network newsletter, Staff News, social media, Faculty/School newsletters and Faculty-wide emails. The survey listed diversity and inclusion events, funding schemes and other opportunities arising between January and December 2021, asking EMCAs the extent to which they were aware the event had been advertised, were/would have been interested, and had attended. A free text field was included to collect further comments and views. Content analysis was applied to free text. Descriptive statistics were reported for other data collected.

Results: Awareness of certain types of events was low, e.g. consultation opportunities advertised in Staff News. MDHS SWiM Stories events had greatest awareness and engagement. Key themes in free text responses included workload constraints precluding attendance, a sense of window-dressing, and missing information about events/opportunities due to tactics adopted to reduce email volume.

Conclusions: EMCAs experience barriers to engaging with Faculty- and University-level diversity and inclusion events and opportunities. Addressing workload challenges, providing events outwith Parkville campus and clearer indication of target/eligible audience would facilitate engagement. Use of multiple advertising avenues and clear, tailored advertising may also support increased awareness of events/opportunities.

Myra De Smet

SEZ6 IN PERIPHERAL NEURONS IS A MAJOR CONTRIBUTOR TO HEAT HYPERALGESIA IN AN INJURY-INDUCED NEUROPATHIC PAIN MODEL

Myra De Smet[1], Kathleen S-L Teng[1], Maja M Lovric[1], Alice McGovern[1], Jenny M Gunnersen[1]

[1] Department of Anatomy and Physiology, University of Melbourne, Australia

Introduction/Aim: Neuropathic pain is a debilitating condition and current therapies offer little relief for patients. Previous research in our lab demonstrated that Sez6 constitutive knock-out (KO) mice have significantly reduced heat hyperalgesia after sciatic nerve chronic constriction injury (CCI). The aim of this research was to test whether this was driven by peripheral or central nervous system Sez6 expression.

Methods: We generated an Advillin-Cre^{ERT2} Sez6$^{flox/flox}$ conditional KO (cKO) mouse model to delete Sez6 in DRG neurons. The expression pattern of Sez6 in dorsal root ganglion (DRG) neurons was established using immunohistochemistry and RNAscope. These mice were subjected to sham surgery or CCI and tested for heat and mechanical hypersensitivity.

Results: Sez6 expression varied from 16-27% in DRG neurons across vertebral levels, with most Sez6 expressing neurons identified as peptidergic c-fibres. After the successful deletion of Sez6, heat hyperalgesia after CCI was essentially abolished by 14-days post-injury (paw withdrawal time: Cre^{-ve}, 4.9±0.5s, n=13; Cre^{+ve}, 9.2±0.6, n=12; 2-way ANOVA [genotype vs surgery], $p<0.01$; Fisher's pairwise comparison, $p<0.01$). After CCI, Sez6 cKO mice exhibit a significantly reduced density of CD11b+ leukocytes at the site of injury compared to injured Cre^{-ve} control mice (Cre^{-ve}, 662±80 cells/mm^2; Cre^{+ve}, 1060±117 cells/mm^2, n= 6-8; mixed effect model [genotype], $p=0.025$; Tukey pairwise comparison, $p=0.017$), to a level that is comparable to the contralateral side (607±92 cells/mm^2; Fisher's pairwise comparison, $p=0.551$).

Conclusions: These data implicate Sez6 in sensory afferent neurons as a driver of heat hyperalgesia after peripheral nervous system injury.

Sarah Garnish

A common human *MLKL* polymorphism confers resistance to negative regulation by phosphorylation

Sarah E. Garnish[1 2], Katherine R. Martin[1 2], Maria Kauppi[1 2], Rebecca Ambrose[3 4], Vik Ven Eng[3 5], Shene Chiou[1 2], Andre L. Samson[1 2], Marcel Doerflinger[1 2], Vicki Athanasopoulos[6], Carola Vinuesa[6], Charlotte A. Slade[1 2 7], Jaclyn S. Pearson[3 4 5], John Silke[1 2], James M. Murphy[1 2] and Joanne M. Hildebrand[1 2]

[1]The Walter and Eliza Hall Institute, Parkville, VIC, 3052, Australia
[2]University of Melbourne, Department of Medical Biology, Parkville, 3052, Australia
[3]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, 3168, Australia
[4]Department of Molecular and Translational Science, Monash University, Clayton, VIC, 3168, Australia
[5]Department of Microbiology, Monash University, Clayton, 3168, VIC, Australia
[6]Department of Immunology and Infection, John Curtin School of Medical Research, Australian National University, ACT, Australia
[7]Department of Clinical Immunology & Allergy, Royal Melbourne Hospital, Parkville, VIC, 3052, Australia

Introduction/Aim: Across the globe, 2-3% of humans carry the *p.Ser132Pro* (S132P) single nucleotide polymorphism in *MLKL*, the terminal effector protein of the inflammatory form of programmed cell death, necroptosis. Previously, we had reported this variant to be enriched *in trans* with other common *MLKL* brace helix polymorphisms in a cohort of chronic recurrent multifocal osteomyelitis (CRMO) patients. In this study we aimed to investigate *MLKL* functional changes resulting from the S132P mutation and how this polymorphism may be predisposing carriers to disease.

Methods: To examine the potential human disease-causing effects of this *MLKL* variant, we exogenously expressed MLKL^{S132P} in human cells lines and introduced the mouse counterpart variant (*Mlkl*^{S131P}) into a genetically modified mouse model.

Results: We show that this substitution confers a gain in necroptotic function in human cells, with MLKL^{S132P} overriding pharmacological and endogenous inhibition of MLKL. In mouse cells, the equivalent *Mlkl S131P* mutation confers a gene dosage dependent reduction in sensitivity to TNF-induced necroptosis in both hematopoietic and non-hematopoietic cells, but enhanced sensitivity to IFN-β induced death in non-hematopoietic cells. *In vivo*, *Mlkl*^{S131P} homozygosity reduces the capacity to clear *Salmonella* from major organs and retards recovery of hematopoietic stem cells.

Conclusions: These observed phenotypes provide important insights into how this highly frequent human *MLKL* S132P polymorphism may contribute to the progression of complex disease.

Luca Godenzini

Learning dependent modulation of dendritic activity during auditory discrimination

Luca Godenzini and Lucy M. Palmer

Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC, Australia

Neural activity in the auditory cortex is strongly modulated by both learning and the behavioural state. Such modulation is believed to occur via top down projections, which target the apical tuft dendrites of cortical pyramidal neurons. Due to their non-linear properties, tuft dendrites can actively integrate different streams of information and are a great candidate for driving the dynamic changes required during learning. Here, we used two photon calcium imaging to investigate dendritic activity of layer 2/3 pyramidal neurons within the auditory cortex during learning of an auditory discrimination task. We found task-dependent activity in tuft dendrites following learning. Specifically, auditory-evoked responses were selectively increased during correct (HIT) performance in expert mice. Furthermore, we found that task engagement increased the proportion of dendrites with dampened activity, suggesting that the balance of excitation and inhibition is important during learning. Overall, our findings illustrate that apical tuft dendrites of cortical pyramidal neurons can flexibly encode task-relevant information, suggesting that dendrites can be primary drivers in modulating the cortical activity that is required during learning.

Amber Kennedy

School-age outcomes among IVF-conceived children: a causal inference analysis using linked population-wide data

Amber L Kennedy[1,2], Beverley J Vollenhoven[5,6,7], Richard J Hiscock[1,2], Catharyn J Stern[1,3,4], Susan P Walker[1,2], Jeanie L Y Cheong[1,8,9], Jon L Quach[1,8], Roxanne Hastie[1,2], David Wilkinson[2,10], John Mcbain[1,3,4], Lyle C Gurrin[11], Vivien Maclachlan[7], Franca Agresta[4], Susan P Baohm[7,10], Stephen Tong*[1,2], Anthea C Lindquist*[1]

[1] Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia,
[2] Mercy Perinatal, Mercy Hospital for Women, Heidelberg, VIC, Australia
[3] Reproductive Services Unit, The Royal Women's Hospital, Parkville, VIC, Australia

[4] Melbourne IVF, East Melbourne, VIC, Australia
[5] Department of Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia
[6] Department of Obstetrics and Gynaecology, Monash Health, Clayton, VIC, Australia
[7] Monash IVF, Clayton, VIC, Australia
[8] Murdoch Children's Research Institute, Parkville, VIC, Australia
[9]Department of Neonatology, The Royal Women's Hospital, Parkville, VIC, Australia
[10]City Fertility Centre, Melbourne, VIC, Australia
[11]Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Australia

Introduction/Aim: In-vitro fertilization (IVF) is a common mode of conception. This study aimed to determine the causal effect of IVF conception on school-age childhood developmental and educational outcomes, compared to spontaneous conception.

Methods: Causal inference methods were used to analyse observational data in a way that emulates a target randomised clinical trial. The study cohort comprised Victoria state-wide linked maternal and childhood data. Participants included singleton infants conceived spontaneously or via IVF, 2005-2014. Two outcome measures were assessed. First, childhood developmental vulnerability at school entry (age 4-6), was assessed using the Australian Early Developmental Census (AEDC) (n=173,200). Second, educational outcome at age 7-9, was assessed using National Assessment Program – Literacy and Numeracy (NAPLAN) data (n=342,311). Inverse probability weighting with regression adjustment was used to estimate population average causal effects.

Results: The study included 412,713 children across the two outcome cohorts. Linked records were available for 4,697 IVF-conceived cases and 168,503 controls for AEDC, and 8,976 cases and 333,335 controls for NAPLAN. There was no causal effect of IVF-conception on the risk of developmental vulnerability at school-entry compared with spontaneously conceived children (AEDC metric); with an adjusted risk difference of -0.3% (95% CI -3.7% to 3.1%) and an adjusted risk ratio of 0.97 (95% CI 0.77 to 1.25). There was no causal effect of IVF-conception on NAPLAN overall z-score, with an adjusted mean difference of 0.030 (95% CI -0.018 to 0.077) between IVF- and spontaneously conceived children.

Conclusions: The school-age developmental and educational outcomes for children conceived by IVF are equivalent to those of spontaneously conceived children.

Prasanti Kotagiri

Shared hypermutated disease specific B cell clones in Crohn's disease

Prasanti Kotagiri[1,2,3], William M. Rae[1,2,4], Laura Bergamaschi[1,2], Diana Pombal[2], Lukas W. Unger[1,2,5], Nurulamin M. Noor[2], Sofie H. Tolmeijer[2], Giulia Manferrari[2], Rachael J.M. Bashford-Rogers[2a], Anton Stift[5], James C. Lee[1,2,6,7], Kenneth G.C. Smith[1,2], Eoin F. McKinney[1,2], Paul A. Lyons[1,2]

[1]Cambridge Institute of Therapeutic Immunology and Infectious Disease, Jeffrey Cheah Biomedical Centre, University of Cambridge, Cambridge, UK
[2]Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, UK
[3]Department of Medicine, University of Melbourne, Melbourne, Australia
[4]Discovery Sciences, AstraZeneca, Cambridge Biomedical Campus, Cambridge, UK
[5]Division of Visceral Surgery, Department of General Surgery, Medical University of Vienna, Vienna, Austria
[6]The Francis Crick Institute, London, UK
[7]UCL Institute of Liver and Digestive Health, Division of Medicine, Royal Free Campus, London

Introduction/Aim: B cells play an important role in gut homeostasis and dysregulated B cell populations are frequently observed in patients with inflammatory bowel disease (IBD). How these B cell perturbations contribute to disease remains largely unknown.

Methods: We performed deep sequencing of the B cell receptor (BCR) repertoire in three cohorts of patients with Crohn's disease (CD) together with healthy and disease controls.

Results: We identified BCR clones that were shared between patients with CD but not found in healthy controls (HC) nor in patients with ulcerative colitis (UC), indicative of CD-specific B cell immune responses. Shared clones were present in both draining intestinal lymph nodes and circulating plasmablasts, suggesting the presence of common CD-associated antigens driving B cell responses.

Conclusions: Taken together, our data not only provides further support for the role of pathogenic B cells in CD but gives the opportunity – providing epitope-specific antibodies can be fully elucidated – for a diagnostic antibody test for CD.

Jane Oliver

Evaluating the impact of the Cohealth Health Concierge program in public housing communities during the COVID-19 pandemic in Melbourne, Victoria

Oliver J[1,2], Ferdinand A[3], Kaufman J[2], Allard N[4], Danchin M[2], Gibney KB[1]

[1] The Department of Infectious Diseases, Melbourne Medical School, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Victoria, Australia.
[2] Murdoch Children's Research Institute, Victoria, Australia.
[3] Centre for Health Policy, Melbourne School of Population and Global Health, University of Melbourne
[4] cohealth, Victoria, Australia.

Introduction/Aim: From July 2020 to June 2022, cohealth, a primary healthcare provider organisation in Melbourne, conducted a peer-to-peer Health Concierge (HC) health education program in high-rise public housing communities. HCs helped facilitate the pandemic response, including by promoting COVID-19 testing and vaccine uptake. Our aim was to determine the impact of the HC program on residents' engagement with public health.

Methods: Residents in four public housing communities were surveyed in April and May 2022. Qualitative interviews with residents, HCs, other cohealth staff and Government staff were conducted during March to May 2022.

Results: Of 301 surveyed residents, 71% were women and 38% spoke Somali at home. Three-quarters reported receiving information from a HCs about COVID-19 testing and vaccines; >90% thought this information was accurate. 76% reported having moderate/very-high trust in COVID-19 vaccines. While 94% reported having ≥2 COVID-19 vaccines doses, 46% reported having the recommended ≥3 doses. Data from 32 interviews indicated the HC program was valuable in facilitating rapidly changing public health responses to COVID-19. Unmet community needs around addressing poor mental health, language barriers and poverty were identified. A key strength of the program is the ability of HCs to become trusted information sources and share information in multiple languages.

Conclusions: The HC program may have increased residents' engagement with public health activities during the pandemic response. HCs valued their role and career opportunities stemming from it. In future, HCs could disseminate information about a wider range of health and support services with their communities.

Andrea Putica

Alexithymia and Treatment Non-Response For Prolonged Exposure Therapy: An Evaluation of Outcomes and Mechanisms

Andrea Putica[1], Nicholas T. Van Dam[2], Kim L. Felmingham[2], and Meaghan L. O'Donnell[1]

[1] Phoenix Australia Centre for Posttraumatic Mental Health, Department of Psychiatry, University of Melbourne, Parkville, VIC, Australia
[2] Melbourne School of Psychological Sciences, University of Melbourne, Parkville, VIC, Australia

Introduction/Aim: Prolonged exposure (PE) is the gold standard treatment for posttraumatic stress disorder (PTSD), relying on emotional clarity and awareness to process trauma stimuli. Those with alexithymia present unique deficits that may impede their ability to engage with emotions. We aimed to examine the impact of alexithymia on PTSD diagnosis, total symptom severity, and symptom cluster outcomes following a full course of PE. Further, we examined if emotional clarity and awareness moderated treatment outcomes.

Method: Participants (n = 68) with PTSD underwent 10 sessions of PE.

Results: Alexithymia was assessed via the Toronto Alexithymia Scale (TAS-20), as well as emotional clarity and awareness subscales of the Difficulties in Emotion Regulation Scale. Treatment outcomes were assessed via the PTSD Checklist and Clinician-Administered PTSD Scale for the DSM-5 at pre-treatment, post-treatment, and 6-month follow-up. Alexithymia was associated with PTSD diagnosis at post-treatment, $\chi^2_{(1)}$ = 5.20, p = .021, and follow-up, $\chi^2_{(1)}$ = 4.75, p < .001, with every unit increase in TAS-20 score associated with a 5.8% and 7.1% increase in odds of PTSD, respectively. Alexithymia was not correlated with symptom change as a function of treatment outcome. Alexithymia was found to inhibit responses across avoidance, $F_{(2, 66)}$ = 9.46, p = .001, d = 0.76., and negative alterations in cognition and mood, $F_{(2, 66)}$ = 3.40, p = .040, d = 0.65. Finally, emotional awareness, $F_{(1, 45)}$ = 5.89, p = 0.019, d = 0.72, but not clarity, moderated treatment outcomes.

Conclusion: Our results suggest that those with alexithymia may require treatment approaches that address deficits in emotional awareness.

Wilma Peters

Trauma-Focused Cognitive Behavioural Therapy for Symptomatic Young People Following Exposure to Interpersonal Trauma: Results from a Single Arm Pilot Study.

Wilma Peters[1], Simon Rice[1], Judith Cohen[2], Laura Murray[3], Carsten Schley[4], Mario Alvarez[1], Sarah Bendall[1]

[1]Orygen, University of Melbourne,, Melbourne, Australia
[2]Allegheny General Hospital, Pittsburgh, Pennsylvania, United States
[3]Department of International Health, John Hopkins University
[4]Headspace Sunshine, Melbourne, Australia

Background: Many young people attending community mental health care have been exposed to childhood interpersonal trauma (i.e., maltreatment, neglect, and abuse). These young people often experience poor mental health with anxiety, depression, problematic substance use, personality disorders and posttraumatic stress commonly noted. Clinical practice guidelines strongly recommend trauma-focused cognitive behavioral therapy (TFCBT, Cohen et al., 2017) for the treatment of PTSD and comorbidities in children and adolescents (up to age 18). However, TF-CBT has not yet been trialed in young people (up to age 25) or within an Australian community mental health facility, such as headspace.

Objective: To determine if TF-CBT is feasible, acceptable, safe, and potentially clinically effective for young people aged 15 to 25.

Method: We conducted an uncontrolled pilot study of TF-CBT with assessments at four time points. Participants with at least one symptom per PTSD diagnostic criteria received up to 20 sessions of TF-CBT.

Results: Eighteen participants (65% female, *M* age 19.5) completed an average of 15 sessions of TF-CBT. Most participants rated the intervention as helpful. A minority of participants reported a brief exacerbation in symptoms during stabilisation and directly after the trauma narrative. At the end of treatment, only 1 of the 16 participants with a baseline PTSD diagnosis still met diagnostic criteria. Significant improvements were also noted for self-report measures of PTSD, anxiety, and depression.

Conclusion: TF-CBT is a promising intervention for young people exposed to interpersonal trauma.

Sarah Florence Stuart

Interleukin 11 signalling promotes glioblastoma progression

Sarah Stuart[1], Rodney Luwor[1,2,3]

[1] Department of Surgery, The University of Melbourne, The Royal Melbourne Hospital, Parkville, Victoria 3050, Australia.
[2] Fiona Elsey Cancer Research Institute, Ballarat, Victoria 3350, Australia.
[3] Federation University, Ballarat, Victoria 3350, Australia.

Introduction/Aim: Glioblastoma is the most common and lethal brain tumour in adults with a mean survival rate of only 12-15 months with current treatment. The microenvironment of a tumour is considered to be essential to tumour pathogenesis. This includes the critical growth factors and cytokines that activate signalling pathways controlling many pro-oncogenic cellular functions. The IL-11 cytokine has become increasingly more important in the pathogenesis of a wide range of cancers, however, very little is known regarding its role in glioblastoma.

Methods: To study the role of IL-11 in glioblastoma, we first evaluated IL-11 and IL-11R expression in the TCGA database. We also stably transfected IL-11R into 2 patient derived primary glioblastoma cell lines (#20 and #28) to examine the effect of over-expression of IL-11R on Glioblastoma cell proliferation, migration, invasion and survival in glucose and glutamine-depleted conditions.

Results: Analysis of TCGA data identified that IL-11 and IL-11Ra expression correlates with tumour grade and glioblastoma patient survival. Expression of IL-11R also led to an increase in cell proliferation compared to matched control/un-transfected cells. Wound healing, transwell migration/invasion assays and the use of tumour spheroids also demonstrated that IL-11R transfected cell displayed significantly greater migratory ability than control/un-transfected cells. Finally, we demonstrated that cells transfected with the IL-11R could survival in media starved of either glucose or glutamine significantly better than control cells and this enhanced survival was due to reduced activation of cell apoptosis.

Conclusions: In conclusion, the data collected suggests interleukin-11 signalling plays a major role in glioblastoma proliferation, migration and survival in sub-optimal conditions.

William Turner

Predictive neural encoding of smoothly moving objects

William Turner[1], Philippa Johnson[1], Hinze Hogendoorn[1]

[1]Melbourne School of Psychological Sciences

Introduction/Aim: Because neural processing takes time, the brain never has access to real-time sensory information. This is a problem when trying to localise a moving object, as by the time its position has been determined it will have moved on. There is mounting evidence that the brain employs predictive

mechanisms to overcome this problem and accurately estimate the position of moving objects. Much of this evidence, however, comes from paradigms employing 'apparent motion' designs, where rapid sequences of spatially and temporally separated flashes generate the perception of coherent motion. As a result, it is relatively unclear how the human brain encodes the location of smoothly moving objects, which evoke continuous patterns of neural activation rather than distributed bursts.

Methods: In this study, we investigated this by asking participants to view a stimulus moving smoothly along a circular path, while EEG was recorded. Using multi-class LDA classification, we constructed probabilistic maps of the stimulus' location over time from participants' EEG recordings.

Results: We found clear evidence of 'representational overshoot' following the unexpected disappearance or reversal of the stimulus, indicative of predictive position encoding. Strikingly, by varying classifier training time, we found that temporally distinct stimulus representations were extrapolated by different amounts, with early representations showing greater extrapolation and more precise spatiotemporal localization.

Conclusions: These findings shed light on the neural encoding of smoothly moving objects, suggesting that the degree to which representations are extrapolated may vary along the processing stream.

Isabel Zbukvic

Using implementation science to inform workforce and service development in youth mental health: An Australian case study

Isabel Zbukvic[1,2], Jennifer Nicholas[1,2], Craig Hamilton[1], Paula Cruz-Manrique[1,2], Caroline Crlenjak[1], and Rosemary Purcell[1,2]

[1] Orygen, Parkville, Victoria, Australia
[2] Centre for Youth Mental Health, The University of Melbourne, Parkville, Victoria, Australia

Introduction/Aim: Globally, mental illness and substance use disorders are the leading cause of disability and disease burden for young people. Orygen is an Australian youth mental health organisation with a mission to reduce the impact of mental ill-health on young people, families and society, and one of only a few known research and clinical centres with a dedicated Knowledge Translation division. This paper provides a case study of the workforce development team within Orygen Knowledge Translation, outlining how implementation science informs their work and how the division has adapted its model of service support in the face of COVID-19.

Methods: Process data on training and resources developed and delivered by the workforce development team at Orygen over the period 2017 – 2021 was collated and synthesised with team reflections about the adaptations made by team in response to the COVID-19 pandemic.

Results: Since 2017, the team has delivered training to more than 4000 youth mental health workers across Australia, on the topics of trauma, psychosis, mood and anxiety disorders, brief interventions, cognition and other areas of youth mental health. The COVID-19 pandemic generated abrupt and dramatic changes to the delivery of workforce and service development initiatives in Australia due to significant restrictions to travel and in-person events. It also placed major delivery demands on youth mental health services.

Conclusions: The COVID-19 pandemic facilitated profound and rapid changes to service delivery and development in Australian youth mental health. Implementation science offers flexible models to support a changing system.