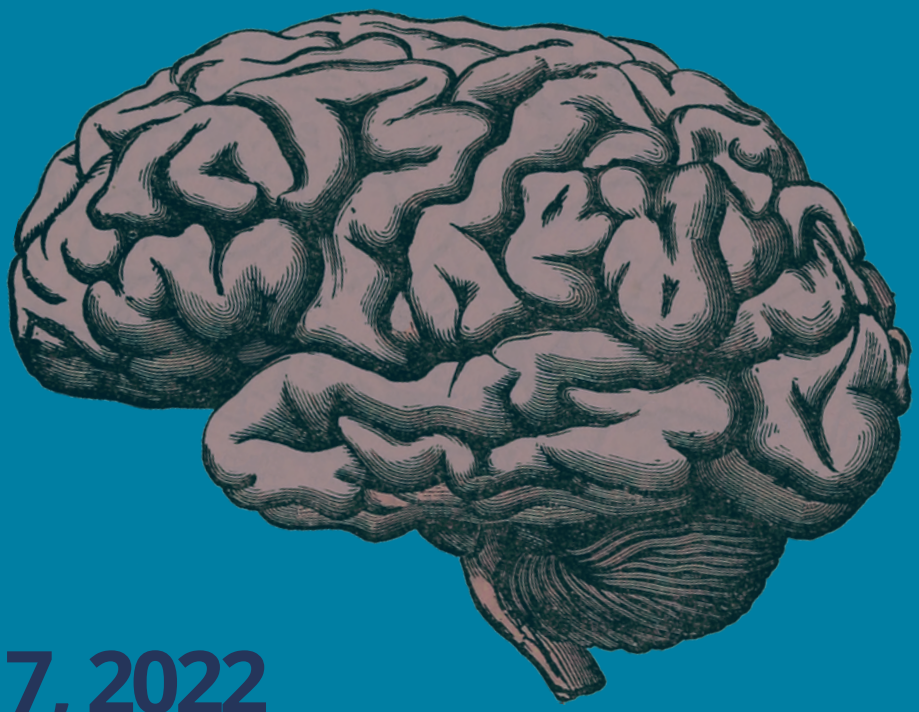
Institute of Medical Science
UNIVERSITY OF TORONTO

IMS SCIENTIFIC DAY:

Alan Wu Poster Competition Abstract Booklet



MAY 17, 2022

HART HOUSE

Temerty Medicine

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8:00 – 8:45 AM – Registration and Breakfast

8:45 – 10:15 AM – Alan Wu Poster Competition

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11:30 – 11:45 AM – Coffee Break

11:45 – 12:45 PM – Laidlaw Competition

12:45 – 2:00 PM – Lunch Break

2:00 – 3:15 PM – Research Panels (Concurrent)

3:15 – 3:30 PM – Break

3:30 – 4:00 PM – Awards Ceremony

4:00 – 5:00 PM – Reception

Poster Judges

Group	Judge 1	Judge 2	Theme	Location
A	Dr. Gelareh Zadeh	Dr. Amit Singnurkar	Neuroscience-Brain Health	Great Hall
B	Dr. Stefan Kloiber	Dr. Nadia Minian	Neuroscience-Brain Health	Great Hall
C	Dr. David Mikulis	Dr. John Griffiths	Neuroscience-Brain Health	Great Hall
D	Dr. Graham Trope	Dr. Karen Gordon	Neuroscience-Brain Health	Great Hall
E	Dr. Katharine Dunlop	Dr. Tony George	Neuroscience-Brain Health	Great Hall
F	Dr. Mohammad Akbari	Dr. Sruthi Alahari	Neuroscience-Brain Health	Great Hall
G	Dr. Michael Milosevic	Dr. Lori Holden	Cancer	East Common
H	Dr. Raghu Singh	Dr. Iska Moxon-Emre	Infection-Immunology	Music
I	Dr. Indra Narang	Dr. Joel Fish	Cardiovascular-Respiratory-Musculoskeletal	East Common
J	Dr. Sharmistha Mishra	Dr. Korryn Bodner	Population-Health-Education	East Common
K	Dr. Thulasi Madanagopal	Dr. Eno Hysi	Population-Health-Education	East Common
L	Dr. Rupert Kaul	Dr. Kevin Gilmore	Regenerative Medicine Development	East Common
M	Dr. Isabella Caniggia	Dr. Lucie Malbeteau	Endocrine/Gastroenterology/ Other	Music
N	Dr. Michael Tang	Dr. Tahani Baakdhah	Cancer	East Common
O	Dr. Nikolaus Wolter	Dr. Mansoor Husain	Cardiovascular-Respiratory-Musculoskeletal	East Common
P	Dr. Alastair Flint	Dr. Xue Xia	Neuroscience-Brain Health	Great Hall
Q	Dr. Liang Zhang	Dr. Sandra Pereira	Neuroscience-Brain Health	Great Hall
R	Dr. Theodore Brown	Dr. Laura Best	Infection-Immunology / Other	Music

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Group A: Neuroscience-Brain Health

EFFECT OF AGITATION AND AGGRESSION ON QUALITY OF LIFE IN PATIENTS WITH DEMENTIA

STUDENT: SAMIRA CHOUDHURY
SUPERVISORS: DR. AMER M. BURHAN, DR. SANJEEV KUMAR

Background: Behavioral and psychological symptoms of dementia (BPSD) are highly prevalent in patients with dementia. Among BPSD symptoms, agitation and aggression occur commonly and are a source of distress for both the patients and their caregivers. In this study, we investigated the impact of agitation and aggression on Quality of Life (QoL) in patients with dementia living in long-term care homes or admitted into inpatient psychiatric units.

Purpose: Considerable research has been done to determine factors effecting QoL in dementia, however, less focus has been placed on examining the relationship between agitation and aggression, and QoL in patients with dementia. The objective of this study was to examine the effect of agitation and aggression on QoL in patients with dementia using an informant rated measure of QoL-the Alzheimer's Disease Related Quality of Life (ADRQL).

Hypothesis: The severity of agitation and aggression as assessed by the Neuropsychiatric Inventory-Clinician Rating Scale (NPI-C) will be independently associated with a lower QoL as assessed by the ADRQL (after controlling for stage of illness, medical burden, age, sex, and current residence). Agitation and aggression as assessed using and Cohen Mansfield Agitation Inventory (CMAI) will be a significant predictor of lower QoL.

Method: Data were obtained from the Standardizing Care for Neuropsychiatric Symptoms and Quality of Life in Dementia (StaN) study, a multisite trial that was conducted in Canada. Agitation and aggression were assessed with the CMAI and NPI-C; QoL with the informant-rated Alzheimer's Disease Related Quality of Life (ADRQL) tool; stage of dementia with the Functional Assessment Staging Tool (FAST); and burden of physical illness with the Cumulative Illness Rating Scale-Geriatric (CIRS-G). Regressions were performed to investigate the associations between agitation and aggression and QoL controlling for demographic factors, FAST score, and CIRS-G score.

Result: 179 participants were included (52.5% female; mean (SD) age=80.79 (9.45) years. The ADRQL score was correlated with: (1) CMAI total frequency score (Pearson's r=-.333, p<.001); (2) NPI-C agitation score (Pearson's r=-.209, p=.005); and (3) NPI-C aggression score (Pearson's r = -.300, p<.001). The associations remained significant for the CMAI total frequency score (β=-.235, SE B=0.054, p<.001), and NPI-C agitation and aggression composite score (β =-.288, SE B=1.254, p<.001) after controlling for age, sex, current residence, FAST score, CIRS-G score.

Conclusion: QoL is related to agitation and aggression in patients with dementia. The relationship is independent of demographic factors, stage of dementia, and burden of comorbid physical illness. This suggests that better management of agitation and aggression may be an important factor in improving QoL in patients with dementia.

GREATER INDIVIDUAL VARIABILITY IN FUNCTIONAL BRAIN ACTIVITY DURING WORKING MEMORY PERFORMANCE IN SCHIZOPHRENIA SPECTRUM DISORDERS (SSD)

STUDENT: JULIA GALLUCCI
SUPERVISOR: DR. COLIN HAWCO

Background Heterogeneity has been a persistent challenge in understanding Schizophrenia Spectrum Disorders (SSD). Traditional studies make use of case-control comparisons or linear brain-behaviour statistical approaches, which tend to show variable results and may not reflect individuals. A growing body of evidence suggests heterogeneity in brain function is the norm rather than the exception in healthy populations; this is likely especially true in SSD. In order to better characterize brain heterogeneity underlying behaviour and psychiatric disorders, there is a need for a shift away from group aggregate averages and consider individual metrics that can best characterize variability. We examined individual variability in functional brain activity in SSD and typically developing controls (TDC) during a working memory (WM) task that is known to be impaired in SSD.

Methods Neuroimaging and behavioural data were extracted from age and gender-matched groups of 34 TDC and 56 individuals with SSD (n=90) from two datasets originating from outpatient clinics at the Centre for Addiction and Mental Health (CAMH) in Toronto, Canada. Functional activity in response to an N-Back WM task (3-Back vs 1-Back) was examined between and within groups. Through the use of a recently developed novel metric, individual variability was quantified via the correlational distance of fMRI activity maps between participants; the mean correlational distance from one participant to all others was defined as a 'variability score'.

Results Mean correlational distance of functional brain activity across SSD (0.63 ± 0.043) was significantly higher than TDC (0.60 ± 0.034) ($F(2,84)=7.62$, $p=0.00090$); TDC showed patterns of task-related activation more similar to the overall group whereas SSD had more idiosyncratic brain responses. At the group level, a case-control comparison suggested SSD had reduced activity in task-positive and task-negative networks. However, when individuals with SSD were separated by median individual variability (0.62) into equal subgroups of high and low variability, the low variability group showed no differences relative to TDC while the high variability group showed little activity at the group level. In SSD but not TDC, variability was also related to cognitive performance ($F(1,84)=5.25$, $p=0.024$); response accuracy during the N-Back task was not significantly different between the low variability group and TDC ($t=1.46$, $p=0.15$) whereas the high variability group performed significantly worse than TDC ($t=2.80$, $p=0.0065$).

Conclusion Our results demonstrate a subset of low variability individuals with SSD have similar functional brain activations and behavioural outcomes to controls, suggesting a normative range of cognitive abilities and function despite diagnosis. This is important as it implies diagnostic-based group differences between SSD and TDC are being driven by a subset of individuals with atypical activity patterns, whereas many show normal brain function. By validating the use of within-group heterogeneity as a measure of interest, we encourage future studies to move away from a group-average comparison approach. Enhancing the field's understanding of neurobiological diversity in individuals with SSD may have implications for individualized treatment and targeted intervention; setting the stage for more personalized approaches.

EXAMINATION OF NUCLEAR-ENCODED MITOCHONDRIAL GENES IN AUTISM SPECTRUM DISORDER

STUDENT: STAVROULA GIANNOULIS
SUPERVISOR: DR. VANESSA GONCALVES
CO-SUPERVISOR: DR. JAMES KENNEDY

Background: The global prevalence of autism spectrum disorder (ASD) is estimated to be ~1% and appears to be increasing. The pathophysiology of this heterogeneous neurodevelopmental disorder remains unknown. Mitochondrial dysfunction is considered to play a role in the etiology of ASD, due to extensive evidence indicating disrupted oxidative phosphorylation and increased oxidative stress in individuals with ASD. Therefore, variation within mitochondrial genes may contribute to ASD risk

Purpose: To examine whether nuclear-encoded mitochondrial genes, single nucleotide polymorphisms (SNPs), and pathways are associated with ASD risk and clinical symptomology. Hypothesis: We hypothesize mitochondrial gene variants found within the nuclear genome are associated with ASD risk and symptom severity.

Methods: The Adolescent Brain Cognitive Development study was used to select N = 192 ASD 'cases' and 426 controls of European ancestry for analysis. Linear and logistic regression were used to analyze 20, 191 nuclear mitochondrial SNPs with ASD clinical measure (Parent Short Social Responsiveness Scale; PSSRS) and risk, respectively. 1781 nuclear mitochondrial genes and all eight mitochondrial pathways were analyzed using MAGMA.

Results: Regression and MAGMA gene analyses identified no significant associations for mitochondrial genes and SNPs. MAGMA pathway analysis revealed the mitochondrial dynamics pathway is significantly associated with PSSRS score ($p = 0.003$), after multiple testing corrections.

Conclusions: These preliminary findings suggest alterations of the mitochondrial dynamics pathway, which includes mitochondrial fusion, fission, and trafficking, may contribute to the severity of ASD symptoms. Replication of these findings in a larger and ancestrally diverse sample is required.

YOUTH EXTERNALIZING BEHAVIOUR AND COMT VAL158MET: EVIDENCE OF DEVELOPMENTAL CHANGE FROM THE ADOLESCENT BRAIN COGNITIVE DEVELOPMENT EUROPEAN SUBSAMPLE

STUDENT: TUANA (TIA) KANT
SUPERVISOR: DR. JAMES L. KENNEDY

Background: Youth externalizing problems is one of the leading risk factors for violence and death in youth. Previous studies demonstrating changing effects catechol-o-methyltransferase (*COMT*) Val158Met on executive functioning starting from age 12 suggests that the role of Val158Met may change through development with increasing COMT enzyme levels.

Purpose and Hypothesis: The purpose of this study is to analyze the possible changing role of Val158Met in youth externalizing behaviours, by analyzing the association at two different time points of development in a large sample of youth. It is hypothesized that Val158Met will be associated with psychopathic traits in youth, however, will exhibit a change with development.

Methods: Participants were 4098 children (2185M:1913F) of European ancestry, confirmed genetically using ancestry PCA, recruited longitudinally as part of the Adolescent Brain Cognitive Development (ABCD) study. Val158Met (rs4680) genotypes were obtained from the Smokescreen® Genotyping Array. Externalizing Problems were analyzed using the externalizing behaviour scores from Child Behavior Checklist collected at baseline (age=9.92,SD=0.62) and 3-year-follow-up (age=12.88,SD=0.63). Data analyses were performed using PLINK and R. Kruskal-Wallis test was conducted using the number of Met allele as the independent variable.

Results: Association between Val158Met and externalizing scores were not significant at baseline. On the other hand, at 3-year-follow up, youth with Val/Val genotype were more susceptible to exhibit externalizing problems than Met carriers (p=0.043).

Conclusions: To our knowledge, this is the first study to demonstrate the changing effects of Val158Met on externalizing problems in children and adolescents. Results propose that Val158Met has a differential effect on externalizing behaviours during development, and may significantly influence the behaviours starting from adolescence. This emphasizes the importance of developmental stages in Val158Met-externalizing behaviour interaction, and may serve as a base for developing novel personalized prevention and treatment techniques depending on development stages.

GLUCOSE DYSREGULATION IN ANTIPSYCHOTIC-NAÏVE FIRST EPISODE PSYCHOSIS PATIENTS: IN SILICO EXPLORATION OF GENE EXPRESSION SIGNATURES

STUDENT: JIWON LEE
SUPERVISOR: DR. MARGARET HAHN

Background: Psychosis spectrum disorders (PSDs) including schizophrenia may confer an intrinsic risk for developing glucose dysregulation. Indeed, individuals who are in the earliest stages of the illness (first episode psychosis or FEP) and are antipsychotic (AP)-naive demonstrate prediabetic traits or early dysglycemia that are not fully explained by extrinsic factors such as illness duration and AP use. Nevertheless, putative biological links tying PSDs and dysglycemia together remain largely unknown.
Purpose: We aimed to examine whether PSDs and glucose dysregulation disorders share an overlap at the gene expression level by comparing peripheral transcriptomic signatures of AP-naïve FEP patients to individuals with early dysglycemia, as well as identify potential mechanisms and drug treatments for intrinsic dysglycemia in PSDs.
Hypothesis: We hypothesized that AP-naïve FEP patients and early dysglycemia patients share an overlap at the gene expression level.
Methods: Based on pre-specified inclusion criteria, we systematically compiled: 1) peripheral transcriptomic studies of AP-naïve FEP patients from Ovid PsychINFO, EMBASE, and MEDLINE databases, and 2) peripheral transcriptomic datasets of individuals with early dysglycemia from Gene Expression Omnibus (GEO) database. Differential expression (DE) datasets were extracted from the AP-naïve FEP studies and pre-processed. Raw gene expression data from GEO were preprocessed and subject to DE analysis. Then, adaptively weighted Fisher's method (AW-Fisher) was used to meta-analyze AP-naïve FEP DE datasets together and early dysglycemia DE datasets together. The resulting DE datasets of AP-naïve FEP and early dysglycemia were meta-analyzed together again through AW-Fisher to identify common or overlapping differentially expressed genes (DEGs) with $p<0.05$. The common DEGs were subject to pathway analysis via Metascape. Finally, the common DEGs were analyzed using Integrated Library of Integrated Network-Based Cellular Signatures (iLINCS) to identify FDA-approved drugs with signatures that are discordant (reverse) to the common DEGs and may represent potential pharmacological treatments.
Results: We included 5 AP-naïve FEP studies and 2 early dysglycemia datasets. AP-naïve FEP and early dysglycemia shared 338 common transcriptomic signatures. The top pathways were mainly involved in endoplasmic reticulum stress (protein n-linked glycosylation, ubiquitin-mediated proteolysis, protein folding, and ubiquitin-dependent endoplasmic reticulum associated protein degradation (ERAD)), mitochondrial dysfunction (negative regulation of ATP and cellular carbohydrate metabolic process), and lipid metabolism (lipid biosynthetic process and its regulation). Several drugs were identified to have signatures that are discordant to the common DEGs between AP-naïve FEP and early dysglycemia, most notably the antidiabetic agent metformin and lipid lowering agent simvastatin.
Conclusion: Our findings suggest that PSDs and glucose dysregulation disorders share common gene expression changes. These changes may mediate intrinsic glucose dysregulation in PSDs through endoplasmic reticulum stress, mitochondrial dysfunction, and dysregulated lipid metabolism. Further, metformin and simvastatin represent potential treatment options for intrinsic metabolic dysfunction, which could hold important implications for improving cardiometabolic outcomes in PSDs.

EFFECTS OF SYSTEMIC SNCA GENE SILENCERS ON ENTERIC ALPHA-SYNUCLEIN

STUDENT: MARC DANZELL LOPEZ
SUPERVISOR: ANURAG TANDON

Background: Parkinson's disease (PD) is characterized by the misfolding of alpha-synuclein (a-syn, encoded by SNCA) into toxic oligomers which can spread between neurons in a prion-like mechanism. Clinically, a significant number of PD patients develop gastrointestinal (GI) symptoms many years before their motor deficits manifest. The finding of a-syn in the gut and the recent characterization of a body-first model of PD suggest that synucleinopathy may begin in the GI tract and implicate enteric a-syn as a possible target for enteric PD therapeutics.

Objectives: We aim to test whether systemically delivered SNCA silencers 1) can reduce enteric a-syn in mice and 2) delay the gut-to-brain spread of synucleinopathy.

Methods: Mice received intravenous injections of AAV9-SNCA-shRNA or a scrambled control (AAV9-scr-shRNA) tagged with turboGFP. In a previous study in our lab, these mice received shRNA in their brains via focused ultrasound and showed significant reduction of a-syn in the targeted regions. The GI organs were analyzed to determine shRNA expression and a-syn knockdown via immunohistochemistry and western blot analyses, respectively.

Results: TurboGFP immunostaining of the GI tract confirmed that leftover shRNA is expressed in enteric neurons while western blot quantification revealed a trend towards decreased a-syn in the stomach but not in the colon.

Conclusions: Intravenous injections of SNCA silencers can reduce enteric a-syn. Preparations are being made for future experiments that will test the prophylactic ability of enteric a-syn knockdown in mice which will receive synucleinopathy triggers in the GI tract. Together, these can potentially translate to a novel, non-invasive, and relatively compliable neuroprotective agent for PD.

INVESTIGATING ENDOCANNABINOID METABOLISM IN SOCIAL ANXIETY DISORDER WITH THE POSITRON EMISSION TOMOGRAPHY (PET) TRACER [^{11}C]CURB

STUDENT: CHRISTINA F. PEREIRA
SUPERVISORS: ISABELLE BOILEAU, STEFAN KLOIBER

Background: Social anxiety disorder (SAD) affects 2-3 million Canadians per year. Recently, research has implicated the endocannabinoid system (ECS) in the regulation and experience of anxiety. In particular, the enzyme fatty acid amide hydrolase (FAAH) may be elevated in anxiety disorders, causing a decrease in its substrate, the ECS neurotransmitter anandamide.

Purpose: Unfortunately, many people with SAD are unable to achieve symptom relief from current treatments, highlighting the need for novel interventions. With recent interest in exploring ECS targets for treatment of SAD, the current study aims to investigate if FAAH levels are higher in the brains of individuals with SAD compared to healthy controls (HC) using the positron emission tomography (PET) radioligand [^{11}C]CURB.

Hypotheses: Hypothesis one is that FAAH levels will be higher in the brains of individuals with SAD relative to HCs. The secondary hypothesis is that higher FAAH levels will be associated with greater SAD symptom severity as indicated by questionnaire scores.

Methods: Individuals with SAD (n=16, M/F = 3/13) were invited to undergo a PET scan using the FAAH probe [^{11}C]CURB and complete clinical assessments. Blood samples were taken to assess for genetic variability of FAAH. This preliminary analysis was conducted to compare FAAH levels in 8 regions of interest (ROI) to 34 (M/F = 14/20) demographically-matched controls using a repeated-measures ANOVA, without controlling for genetic variability.

Results: Whole-brain FAAH levels were marginally higher (7%) in individuals with SAD compared to controls ($F(1,48) = 2.34$, $p=0.132$). Differences ranged from 6% - 12% across ROIs. The most prominent effect was noted in the dorsal striatum (12%, $p = 0.03$).

Conclusion: The results from this analysis suggest that FAAH levels are marginally elevated in individuals with SAD. Investigating how the ECS is altered can aid in understanding the pathophysiology of SAD and inform the development of future treatments.

ACT-COG: COGNITIVE ASSESSMENTS IN THE ALTEPLASE COMPARED TO TENECTEPLASE (AcT) TRIAL

STUDENT: SAJEEVAN SUJANTHAN
SUPERVISOR: DR. RICHARD SWARTZ

Background: Stroke is a leading cause of morbidity and mortality in Canada, and there is an urgent need to identify interventions that can improve cognitive outcomes after stroke. The use of remote assessments have potential to improve access (especially in pandemic times), can test across multiple cognitive domains, and can be scaled to multiple centers. To study the importance of cognitive outcomes, we will be leveraging the AcT clinical trial (Alteplase compared to Tenecteplase)

Objectives: To assess uptake & biases, relevance and utility of cognitive assessments as a potential solution to effectively monitor cognitive change in stroke patients

Methods: All patients with acute ischemic stroke who meet criteria for intravenous alteplase are eligible for the AcT trial. Eligible patients from the ACT trial will be invited to complete several screening questions (T-MoCA, computer proficiency etc.) and an online cognitive assessment using the Cambridge Brain Sciences (CBS) platform (~30 mins) to assess cognition.

Results: As an ongoing trial, we anticipate that >80% of eligible participants will complete the online testing. In addition, better cognitive scores from the testing will be associated with higher quality of life and reduced direct and indirect costs, and participants receiving tenecteplase will demonstrate better cognitive performance than those receiving alteplase.

Conclusions/Significance: Determining the feasibility of a simple, remote cognitive assessment for use in acute stroke trials could facilitate a platform to assess the impact of various stroke interventions on short- and long-term cognition.

Group B: Neuroscience-Brain Health

ESTABLISHING A FEMALE PRECLINICAL MODEL OF PTSD AND THE EFFECTS OF DEEP BRAIN STIMULATION ON POST-TRAUMATIC STRESS DISORDER-TYPE BEHAVIOUR

STUDENT NAME: DELARA EMTYAZI
SUPERVISOR: DR. CLEMENT HAMANI

Background: Post-traumatic stress disorder (PTSD) is a psychiatric disorder that emerges following exposure to a traumatic event. With a higher prevalence of PTSD in females, it is becoming increasingly vital to study sex differences in preclinical models. One important factor driving this difference, is the influence of the menstrual/estrous cycle. In the clinical setting, while most Individuals can improve symptoms via medications and behavioural psychotherapy, ~20-30% remain resistant. Similar to humans, we have employed behavioural criteria to identify and characterize a subpopulation of rats (20-30%) that exhibit impaired fear extinction and long-term fear and anxiety-type behaviour following fear conditioning (Weak extinction rats, WE). Emerging neuromodualtory treatments are being increasingly used as experimental treatment options for neuropsychiatric disorders, namely deep brain stimulation (DBS). Insofar, DBS, a technique of delivering electrical stimulation to precise brain areas using implanted electrodes, has been approved for the treatment of various neuropsychiatric disorders. In male rodents, DBS was shown to be highly effective for the treatment of fear and anxiety behaviours. Despite the higher prevalence of PTSD in females, preclinical models of PTSD-type behaviour have been predominantly conducted in males. We conducted the following experiments with three goals in mind. Firstly, we sought to establish a preclinical model of PTSD in female rats. Secondly, we aimed to assess sex differences in rodent models of PTSD, focusing on the estrous cycle in female rats. Finally, we aimed to study for the first time, whether DBS has anti-fear and anxiolytic effects in a female model of PTSD-like state. Primarily, we expect female PTSD-type rats to exhibit increased fear and anxiety-type behaviours compared to males, reflecting the similar trend exhibited in humans. Furthermore, we predict that DBS will induce anxiolytic responses in females. **Methods:** Study 1: We exposed female Sprague Dawley rats (n=30) to fear conditioning and extinction trials and separated the animals into weak- (WE) and strong-extinction (SE) groups based on behavioural scores reported during extinction. Animals were further investigated for tone and context recall, followed by a battery of behavioural tests to assess anxiety-like behaviours in the marble burying and novelty suppression of feeding (NSF) tests. What is more, vaginal lavages were collected to characterize the phase of the estrous cycle during the fear extinction paradigm. Study 2: Adult female Sprague Dawley rats (N=48) were assigned to 3 groups: DBS, Sham and Surgical Control. DBS group rats received electrodes implanted within the ventromedial prefrontal cortex(vmPFC) and received stimulation for 2 weeks. Furthermore, all rats underwent fear conditioning/extinction paradigms. Subsequently, animals were tested for behavioural tests as mentioned in Study 1: Vaginal lavages were collected to monitor the phase of the estrous cycle during fear extinction. **Results:** Study 1: We found no differences in females undergoing extinction during high and low estrogen phases of the cycle in any of the performed tests. WE females had increased freezing during tone and context recall. In contrast to males, WE females buried less marbles than their SE mates, suggesting decreased anxiety-type behaviour. Study 2: Our preliminary results suggest an effect of vmPFC stimulation on anxiety-type behaviour, but not fear- and aversive-type behaviour. **Conclusions:** Study 1: Future investigation including a larger number of behavioural tests are needed to validate our findings and to explain the sex differences observed in our behavioural results. Study 2: Further analysis of new batches is required to increase the sample size and to improve the reliability of results.

ASE-CONTROL VIRTUAL HISTOLOGY ELUCIDATES CELL TYPES ASSOCIATED WITH CORTICAL THICKNESS DIFFERENCES IN ALZHEIMER'S DISEASE

STUDENT: ISABEL KERREBIJN
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Background: Many neuropsychiatric disorders are characterised by altered cortical thickness, but the cell types underlying these changes remain largely unknown. Virtual histology (VH) approaches map regional patterns of gene expression with regional patterns of MRI-derived phenotypes, such as cortical thickness, to identify cell types associated with case-control differences in those MRI measures. However, this method does not incorporate valuable information of case-control differences in cell type abundance.

Purpose: To develop new methodology to determine the cellular correlates of MRI phenotypes and to test our methodology on cortical thickness group differences in Alzheimer's diseases (AD).

Hypothesis: 1. Cell types that are proportionally decreased in bulk gene analysis of AD correlate to group differences in cortical thickness across regions. 2. Case-control virtual histology can better identify those cell types that are directly lost in AD than traditional virtual histology methods.

Methods: We developed a novel method, termed case-control virtual histology (CCVH), and applied it to AD. Leveraging a multi-region gene expression dataset of AD cases (n=40) and controls (n=20), we quantified AD case-control differential expression of cell type-specific markers across 13 brain regions. We then correlated these expression effects with MRI-derived AD case-control cortical thickness differences across the same regions. Cell types with spatially concordant AD-related effects were identified through resampling marker correlation coefficients.

Results: Among regions thinner in AD, CCVH identified fewer excitatory and inhibitory neurons, and greater proportions of astrocytes, oligodendrocytes, oligodendrocyte precursor cells, and endothelial cells in AD cases vs. controls. In contrast, traditional VH found that only excitatory but not inhibitory neuron abundance was associated with thinner cortex in AD, despite the fact that both types of neurons are known to be lost in the disorder.

Conclusions: Compared to traditional VH, cell types identified through CCVH are more likely to directly underlie cortical thickness differences in AD. As more multi-region brain expression datasets become available, CCVH will be useful for identifying the cellular correlates of cortical thickness across neuropsychiatric illnesses

. TRUCTURAL MAGNETIC RESONANCE IMAGING CORRELATES OF COGNITIVE IMPAIRMENT IN LATE-LIFE DEPRESSION: A SYSTEMATIC REVIEW

STUDENT: TULIP M. MARAWI
SUPERVISOR: BENOIT H. MULSANT

Tulip M. Marawi, Nicholas Ainsworth, Peter Zhukovsky, Neda Rashidi-Ranjbar, Tarek K. Rajji, Aristotle Voineskos, Benoit H. Mulsant

Background: Late-life depression (LLD) is a risk factor for developing Alzheimer's dementia (AD). One third of patients with LLD meet the criteria for mild cognitive impairment (MCI). However, the mechanisms linking LLD and MCI remain poorly understood. Additionally, the structural alterations underlying impaired cognition in LLD and LLD+MCI have not been confirmed.

Purpose: To conduct a systematic review of the reported structural MRI correlates of cognitive impairment in LLD and/or LLD+MCI.

Hypothesis: Evidence will support structural abnormalities (in particular, reduced gray matter volume and fractional anisotropy - FA) in the frontostriatal circuits corresponding to executive function, and corticolimbic circuits associated with memory.

Methods: We conducted a systematic review according to PRISMA guidelines. We searched MEDLINE, PsycINFO, EMBASE, and Web of Science databases from inception through December 11th, 2021 for studies that assessed cognitive performance in patients with LLD and/or LLD+MCI and acquired: (1) T1-weighted imaging (T1W) measuring gray matter atrophy; or (2) diffusion-weighted imaging (DWI) measuring white matter integrity.

Results: Our search identified 23 articles, 13 T1W and 10 DWI. Of those, only 1 study reported on findings in LLD+MCI. Preliminary analysis reveals that impaired cognition in LLD is associated with atrophy in the frontal and medial temporal regions, and with widespread reduced tract integrity particularly in the cingulum bundles and uncinate fasciculus across most patients.

Conclusion: There is a paucity of studies on patients with comorbid LLD+MCI. Future studies are needed to address this gap, and to clarify whether patients with LLD+MCI present with distinct or more severe structural alterations underlying impaired cognition in comparison to LLD or MCI alone.

COMBINED PHYSIOTHERAPY AND DEEP BRAIN STIMULATION TO IMPROVE INDEPENDENT COMMUNITY MOBILITY IN PARKINSON'S DISEASE

STUDENT: RAJASUMI RAJALINGAM
SUPERVISOR: ALFONSO FASANO

Authors: **Rajasumi Rajalingam**, Gianluca Sorrento, Yuri Felloni, Jan Goldstein Elman, Rebecca Zaidlin, Kristin Musselman, Connie Marras, **Alfonso Fasano**

Background: Deep brain stimulation (DBS) is an established and highly effective treatment for individuals in the advanced phase of Parkinson's disease (PD). Despite overall improvements in motor function, studies have reported that DBS alone may not increase community mobility and may be associated with gait instability and increased rate of falling. Physiotherapy has been shown to effectively improve control of balance and gait, and prevent falls, among individuals with PD who are treated with medication only. However, no study has yet established the efficacy of physiotherapy in patients receiving DBS.

Purpose: The overall objective of this study is to determine if DBS combined with physiotherapy is effective for improving safe, independent mobility in the community in individuals with PD compared to patients receiving DBS only.

Hypothesis: We hypothesize that DBS combined with physiotherapy is more effective in improving safe, independent mobility in the community in individuals with PD compared to patients receiving DBS only.

Methods: This is a single-center, single-blind non-randomized controlled study, whereby individuals receiving DBS will be allocated to receive either physiotherapy or no intervention (control group). All participants will undergo a similar timetable with assessments completed at five points throughout an 8 months study period. The primary outcome is the overall amount of independent mobility in the community measured with the Life Space Assessment (LSA) questionnaire. Secondary outcome measures include control of gait and balance, falls, mood, anxiety, health-related quality of life, and PD symptoms.

Results: Study in progress

Conclusions: We expect that the combined effects of physiotherapy and DBS will improve the control of gait and balance for individuals with PD, leading to increased community mobility and reduced falling, and will further improve quality of life. The outcomes of the study if found effective has the potential to contribute to practice guidelines to include rehabilitation as part of the standard of care for patients after DBS.

CAN WE CHARACTERIZE A-P/IAP BEHAVIOURAL PHENOTYPES IN PEOPLE WITH CHRONIC PAIN?

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SUPERVISOR: DR. KAREN DAVIS

Authors: Vaidhehi V. Sanmugananthan, Joshua Cheng, Kasey Hemington, Anton Rogachov, Natalie Osborne, Rachael L. Bosma, Junseok A. Kim, Robert D. Inman, Karen D. Davis

Background: Two behavioural phenotypes in healthy people have been delineated based on their intrinsic attention to pain (IAP) and whether their reaction times (RT) during a cognitively-demanding task are slower (P-type) or faster (A-type) during experimental pain. These phenotypes have not been studied in chronic pain populations to avoid using experimental pain in chronic pain contexts. Pain rumination (PR) measures negative thinking towards pain and could serve as an additional metric of IAP that does not require the application of experimental noxious stimuli.

Purpose: To delineate A-P and IAP phenotypes in people with chronic pain and determine if PR can be used as an additional metric of IAP.

Hypotheses: People with chronic pain will have slower task RTs and greater RT variances (RTv) than healthy controls (HC) in pain and no-pain trials of an interference task. IAP and PR scores will be positively correlated in both groups. IAP and PR scores will be higher in the chronic pain group than HCs.

Methods: Behavioural data acquired in 43 HCs and 43 age-/sex-matched people with chronic pain associated with ankylosing spondylitis were retrospectively analyzed. A-P phenotypes were based on RT differences between pain and no-pain trials of the interference task. IAP was quantified based on scores representing attention towards or mind-wandering away from an experimental pain task. PR was quantified using the pain catastrophizing scale, PR subscale.

Results: There were more A-types than P-types in both groups. RTv was higher during no-pain task trials in the chronic pain group than HCs but were not significantly different in pain-trials. There were no group differences in task RTs in no-pain and pain trials, IAP scores, or PR scores. IAP and PR scores were marginally significantly positively correlated in the chronic pain group.

Discussion/Conclusions: PR can be used as a supplement metric to IAP that quantifies attention to pain. The inclusion of experimental pain stimuli in the current A-P/IAP protocols likely is a confound for testing in chronic pain populations.

ELUCIDATING THE *IN VIVO* MECHANISMS OF FOCUSED ULTRASOUND MODULATION OF HUMAN MOTOR CORTEX

STUDENT: YAZAN SHAMLI OGHLI
SUPERVISOR: DR. ROBERT CHEN

Background: Focused ultrasound (FUS) is a novel non-invasive brain stimulation technique, capable of stimulating with greater spatial resolution and depth compared to other similar techniques (Fomenko et. al, 2018). It has been shown to induce immediate ("online") inhibition, and prolonged ("offline") excitation in the human motor cortex (Fomenko et. al, 2020; Zeng et. al, 2021). This is done by assessing the amplitude of motor evoked potentials (MEPs). Although FUS neuromodulation shows potential, its mechanisms of action are unclear. One method of determining the mechanism is through administration of approved brain-active drugs with known mechanisms together with the stimulation (Ziemann et. al, 2015). Researchers can then assess the interactions of the drugs with the stimulation effects.

Purpose: Therefore, the purpose of this study is to determine the mechanisms of online and offline FUS stimulation in the human motor cortex.

Methods: Nine healthy adult subjects participated in this within-subjects randomized, double-blind, cross-over experiment. Each subject completed 5 visits, representing the administration of carbamazepine (Na^+ channel blocker), nimodipine (Ca^{2+} channel blocker), lorazepam ($GABA_A$ positive allosteric modulator), dextromethorphan (NMDA receptor antagonist), or placebo. 1.5 hours after taking the drug, participants were stimulated using online FUS and offline FUS, and effects were assessed using MEPs.

Results: Preliminary evidence suggests that offline FUS may induce plasticity through a long-term potentiation (LTP)-like mechanism. Online FUS results were not significant, due to low power or possibly an auditory confound (Park et. al, 2021).

Conclusion: This study is the first to directly assess the *in vivo* mechanisms of FUS stimulation in humans and will provide crucial information for researchers and clinicians as they develop FUS as a technique for use in neurological and psychiatric populations.

IMPAIRED INSIGHT INTO ILLNESS IS ASSOCIATED WITH INCREASED REGIONAL CEREBRAL BLOOD FLOW IN THE PARIETAL REGIONS OF PATIENTS WITH SCHIZOPHRENIA

STUDENT: JIANMENG SONG
SUPERVISOR: PHILIP GERRETSEN

Background: Impaired insight into illness is a common feature of schizophrenia that negatively contributes to clinical outcomes. Previous functional MRI studies suggest that impaired insight may be related to hemispheric imbalance in left and right posterior parietal area (PPA) function. Arterial spin labeling (ASL) is a non-invasive brain imaging technique that provides an absolute measure of regional cerebral blood flow (CBF). Little research has explored the relationship between regional CBF and insight into illness in schizophrenia.

Purpose: We aimed to use pseudo-continuous ASL (pCASL) to examine the association between regional CBF and impaired insight in patients with schizophrenia.

Hypothesis: Based on the results of our previous studies using MRI, we hypothesized that increased CBF will be observed in the PPA in relation to impaired insight in patient with schizophrenia.

Methods: A total of 32 participants with schizophrenia with moderate-to-severe impairment into insight (≥3 Positive and Negative Syndrome Scale (PANSS) item G12) were included. Insight into illness was measured using the VAGUS, Self-report version (VAGUS-SR). Regional CBF was measured using pCASL. Whole-brain analysis was conducted to examine the relationship between the VAGUS-SR and regional CBF.

Results: The mean age was 43.8 (SD=12.7) and 28% were female. The mean VAGUS score was 5.8/10 (SD=1.7) with higher scores representing greater insight impairment. Whole brain analysis ($p<0.001$, FEW corrected) revealed that impaired insight into illness was associated with increased regional CBF in the PPA bilaterally, specifically the right angular gyrus and left superior parietal regions after controlled for age, gender and illness severity.

Conclusion: The results support prior studies that identify abnormal brain function in the PPA in schizophrenia patients with impaired insight. The PPA may represent a neuroimaging biomarker that is related to impaired insight and a potential target for neuromodulation interventions.

DECIPHERING THE GENETIC BASIS OF CRANIOCERVICAL ARTERIAL DISSECTIONS IN CHILDREN.

STUDENT'S NAME: ADRIANA CAROLINA VARGAS NINO.
SUPERVISOR: NOMAZULU DLAMINI, MD.

Background: An arterial ischemic stroke (AIS) occurs when there is a sudden disruption of blood flow to a region of the brain. With an incidence of 1.8 to 13 per 100,000 children/year, pediatric stroke is considered a rare condition of childhood. However, it remains one of the top ten causes of death in children and up to 75% have significant lifelong neurological impairments. Over 60% of children presenting with their first AIS have an anomaly of the arteries of the brain or arteriopathy. Craniocervical arterial dissection (CCAD) is one of the commonest arteriopathies of childhood, occurring in up to 1 in 4 of children and young adults with stroke. Children with CCAD face a high risk of stroke recurrence and poor neurological outcome including death. The risk factors described in patients with either spontaneous or minimal trauma CCAD include male sex, migraine, and a number of monogenetic conditions such as, inherited connective tissue disorders, homocysteinemia, fibromuscular dysplasia, and α1 antitrypsin deficit, among others. However, causative monogenic mutations are not found in the majority of CCAD patients. Therefore, the etiology typically remains unknown.

Objectives: to describe the clinical and imaging characteristics, as well as the genetic variants in a cohort of pediatric patients with CCAD diagnosis by performing whole genome sequencing (WGS) in trios, duos, or singletons.

Methods: This is an observational, prospective study. After obtaining consent from the parent/legal guardian, or participant, peripheral whole blood (2-10 mL) will be collected for DNA isolation. Approximately 1 µg of genomic DNA will be used for sequencing library preparation and WGS. Skin biopsies will be obtained in the forearm of the patients who consent to undergo this procedure; the ultrastructural and histological analysis of the dermal connective tissue and arterioles will be then carried out. The study will use the data collected through The Hospital for Sick Children Paediatric Stroke Registry which includes the participant's name, address (for issuing recruitment letters), MRN, date of birth, and health information about stroke risk factors, clinical presentation, Investigations, radiographic features, treatment, neuropsychological evaluation, outcomes, status at discharge, and follow-up. Demographic and clinical data will be examined using descriptive statistics. Continuous variables will be presented as mean ± SD or median and interquartile ranges, as appropriate. Qualitative variables will be presented using frequency distributions and proportions. We will analyze data from WGS for variants in the nuclear genome: single nucleotide variants (SNVs; alternate single bases), insertion/deletions (indels; small segments of DNA that are missing or replicated), structural variants (SVs; variations involving larger segments), including copy number variants (CNVs; deletions/losses or duplications/gains), as well as other rearrangements (inversions or translocations) by comparison to the Database of Genomic Variants.

Expected results: Our hypothesis is that spontaneous or minimal trauma CCAD represent a complex model of genetic susceptibility. The disturbances in the expression of genes associated with inflammation, the cardiovascular development, and the composition, maintenance, and function of the components of the large and medium vessels, could lead to a vascular phenotype with a higher risk of spontaneous dissection and stroke.

INVESTIGATION OF THE EFFECTS OF OLANZAPINE ON CNS-INSULIN MEDIATED BRAIN GLUCOSE UPTAKE USING 2DG AUTORADIOGRAPHY

STUDENT: SALLY WU
SUPERVISORS: DR. MAHAVIR AGARWAL, DR. MARGARET HAHN

Background: Antipsychotics (APs) are the cornerstone of treatment in schizophrenia but are associated with a greatly increased risk of type 2 diabetes. Previous studies examining the effects of APs on insulin and glucose regulation has primarily focused on peripheral pathways. However, insulin-responsive glucose transporters are widely distributed throughout the brain. Emerging evidence suggests that the central nervous system (CNS) may be involved in AP-induced glucose dysregulation. It has been demonstrated that APs can acutely inhibit the effect of CNS insulin infusion in rats to suppress feeding and hepatic glucose production, supporting that APs induce central insulin resistance, likely via hypothalamic pathways. It is possible that APs-induced functional insulin resistance occurs across multiple regions, and that it may be mediated by disruptions in insulin-stimulated glucose uptake. Thus, in this proof-of- concept study, we will examine the neural correlates of olanzapine induced cerebral insulin resistance as measured by an attenuation in brain glucose uptake in response to CNS insulin administration.

Purpose: 1) To examine if rats treated with intracerebroventricular (ICV) insulin will show an increase glucose uptake in the brain and identify the brain regions associated with central insulin glucose uptake; 2) To examine if olanzapine acutely inhibits or attenuates glucose uptake associated with central insulin action in these brain regions.

Hypothesis: 1) ICV insulin will increase cerebral glucose uptake in our regions of interest (ROIs) in the brain, specifically in the frontal cortex, nucleus accumbens, hypothalamus, amygdala, hippocampus, cerebellum, and dorsal vagal complex. 2) Olanzapine will attenuate CNS insulin-mediated brain glucose uptake in the brain regions associated with central insulin action.

Methods: 2DG autoradiography procedures were used to measure cerebral glucose uptake alongside a central infusion of insulin or vehicle into the third ventricle. Male rats were pretreated with olanzapine (3 mg/kg) or vehicle. Groups included (central-peripheral) vehicle-vehicle (n = 6), insulin-vehicle (n = 6), insulin-olanzapine (n = 5), and vehicle-olanzapine (n = 6). Regional tissue radioactivity was quantified on coded films using the MCID Elite system as an index of glucose uptake.

Results: ICV insulin significantly increased cerebral glucose uptake in all ROIs compared to ICV vehicle. This effect was abolished by olanzapine, such that the level of cerebral glucose uptake in the insulin-olanzapine group was comparable to the control groups.

Conclusions: This study demonstrates that an acute dose of olanzapine can induce central insulin resistance in the brain beyond areas responsible for metabolism and energy homeostasis, but to areas involved in cognition. Our findings provide corroborating evidence that central insulin resistance may be the shared pathological feature observed in schizophrenia and diabetes, allowing direct translation to humans for future treatment interventions targeting these mechanisms.

Group C: Neuroscience-Brain Health

THE IMPACT OF EXPERIMENTAL SLEEP RESTRICTION ON NEUROCOGNITION IN HEALTHY ADOLESCENTS

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Project Background: Sleep restriction (SR) is defined as a sleep duration below the basal need of an individual. Despite clinical recommendations of 8 to 10 hours of sleep per night, up to 70% of adolescents sleep less than 8 hours per night, and 30% of report sleeping less than 7 hours per night. SR impacts key performance indicators of physical and mental health. Previous cross-sectional studies have significantly linked SR to adverse impacts on attention, executive function, memory and learning, and leads to poor academic performance. Further, SR has been associated with greater risk-taking behaviours and increased accidental injuries in adolescents. The purpose of this study was to investigate whether experimental sleep restriction, relative to being well-rested, is associated with a change in healthy adolescents' cognitive and behavioral outcomes. We hypothesize that experimental sleep restriction, compared to well-rested sleep, is associated with poorer cognitive outcomes and adverse measures of behavioral function.

Methods: This was a randomized counter-balanced crossover study. Participants underwent a 2-week at-home sleep manipulation protocol, including 5-nights of well-rested sleep (WR; 9 hours in bed), and 5-nights of sleep restriction (SR; maximum 6 hours in bed). Objective measures of sleep were evaluated using a validated accelerometer to record sleep duration across multiple nights. At the end of each sleep condition, participants were assessed using standard neuropsychological assessments and the NIH-Toolbox, a computerized neuropsychological test, for cognitive and behavioural outcomes.

Results: Thirty-three healthy 15- to 18- year old adolescents from the community without history of neurological, psychiatric or sleep disorder were included in this study (mean age 16.9 years, 33% male). The mean sleep duration was 5.2 hours for the SR condition, and 7.1 hours for the WR condition in this sample. With SR compared to WR, participants had significantly lower scores of overall fluid cognition ($p=0.01$) and global cognition ($p<0.01$). Additionally, participants with SR compared to WR had significantly higher scores for sleepiness ($p<0.05$), fatigue ($p<0.01$), and negative affect ($p<0.01$).

Conclusions: This study provides data that acute sleep restriction is associated with adverse cognitive outcomes, specifically to areas of attention and executive function, and poorer behavioral function after less than one week of SR when compared to 5-nights of well-rested sleep. Knowledge from this study may offer early insights for health professionals, clinicians, parents, and teachers on the impact of acute sleep restriction on cognitive ability, and behavioural function in the adolescent population.

METAPLASTICITY ASSOCIATED WITH TRANSCRANIAL FOCUSED ULTRASOUND INDUCED PLASTICITY IN HUMANS

STUDENT: MANDY YI RONG DING
SUPERVISOR: DR. ROBERT CHEN

Background: Low intensity transcranial focused ultrasound stimulation (TUS) is a novel technique for non-invasive brain stimulation (NIBS) that can deliver more focal and deeper stimulation compared to currently used forms of NIBS, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). Neuroplasticity refers to the ability of the brain to modify its connections in response to internal and external experiences. It is an important process for learning, memory, and recovery from injury. Plasticity is primarily mediated by long-term potentiation (LTP), a persistent strengthening of synapses, and long-term depression (LTD), a persistent weakening of synapses. The brain's capacity for plasticity induction is further modulated by a process defined as metaplasticity, which can be homeostatic or non-homeostatic. Homeostatic metaplasticity is a negative-feedback mechanism critical for the maintenance of synaptic volume within the physiological range. Neuroplasticity is also subject to depotentiation, defined as the reversal of LTP by a subsequent stimulation protocol that has no plasticity-altering effects alone.

Purpose: While homeostatic metaplasticity and depotentiation have been shown to regulate plasticity induction using other NIBS protocols such as those with repetitive TMS (rTMS) and tDCS, their effects on TUS are unknown. This study aimed to uncover the effects of metaplasticity on TUS through investigation of the interactions between theta burst TUS (tbTUS), a newly-developed TUS protocol inducing LTP-like effects in humans after 80s of sonication, and continuous theta burst stimulation with 150 pulses (cTBS150), a rTMS protocol considered to be a sub-threshold LTD-like stimulus.

Hypothesis: Based on results in other NIBS, we expected enhanced plasticity induction when cTBS150 is delivered immediately before tbTUS and reversal of plasticity induction when cTBS150 is delivered immediately after tbTUS.

Methods: There were 4 study visits with four interventions: 1) sham cTBS150 → real tbTUS, 2) real cTBS150 → sham tbTUS, 3) real cTBS150 (priming) → real tbTUS, 4) real tbTUS → real cTBS150 (depotentiation) conducted in randomized order on separate days. Using transcranial magnetic stimulation and surface electromyography, motor-evoked potential (MEP) amplitude and motor cortex intracortical circuits (IC) including short-interval intracortical inhibition, long-interval intracortical inhibition, intracortical facilitation, and short-interval intracortical facilitation were measured before and 5, 30, 60, and 90 minutes after each intervention.

Results: Priming with real but not sham cTBS150 increased the duration of plasticity induction by tbTUS. Changes in IC may underlie the observed increase in cortical excitability. Potentiation and IC changes were abolished when cTBS150 was delivered after tbTUS.

Conclusion: The induction of plasticity by tbTUS was altered in manners consistent with homeostatic metaplasticity and depotentiation, supporting TUS to be modulated by metaplasticity.

ELECTROPHYSIOLOGICAL METHODS TO IMPROVE SPATIAL HEARING IN CHILDREN WHO USE BILATERAL COCHLEAR IMPLANTS

STUDENT: ANGELA FUNG
SUPERVISOR: DR. KAREN GORDON

Objectives: We aim to identify asymmetries in bilateral stimulation levels that might disrupt neural processing of binaural cues thereby limiting spatial hearing in pediatric bilateral cochlear implant (CI) users.

Background: Cochlear implants (CIs) stimulate the auditory nerve with electrical pulses, and are a successful hearing prosthesis for individuals with severe to profound hearing loss. Children who are implanted bilaterally (both left and right) with CIs struggle to detect inter-aural level difference (ILD) and timing difference (ITD) cues, which impacts their ability to locate sound and separate target speech from noise. The detection of ILD cues likely requires balanced input from the left and right sides that converge in ascending auditory pathways ("binaural" processing). Differences in neural survival and development and CI device type or placement between the left and right sides will mean that selected bilateral stimulation levels will need to counter-balance existing asymmetries when reaching the auditory system. However, current programming protocols do not consider bilateral balancing, potentially leaving children with poor access to binaural cues. Measures of bilateral function along the auditory pathways could have potential for use as objective measures to set bilateral levels. We hypothesize that: 1) CI input asymmetries can be predicted through auditory nerve and brainstem responses to CI stimulation, and 2) asymmetrical input through bilateral CIs disrupts behavioural and cortical measures of binaural processing.

Methods: Participants in study 1 were 18 pediatric bilateral CI users (mean(SD) = 9.6(5.5) years old)) which included a retrospective cohort of 9 participants' whose data were gathered from a previous study. Electrically-evoked Auditory brainstem responses (eABRs) were recorded to bilateral CI stimulation with different ILDs (from -20 to +20 current units (CU) in the retrospective cohort, and -12 to +12 CU in the prospective cohort, where positive = weighting towards the right CI). Unilaterally-evoked eABRs (retrospective cohort only) and auditory nerve (eCAP) responses were also recorded at similar current level ranges. Behavioral responses to the ILDs were recorded in 8/18 participants using a lateralization task, where participants indicated if they heard the sound to their left or right side. In study 2, ILD processing will be further explored using a measure of cortical change to assess detection of ILD up to 20 dB. The lateralization task will be used to record behavioral responses to the same ILD conditions.

Results: Asymmetries in left vs. right stimulation levels were consistent between amplitude differences in unilaterally-evoked eCAP and unilaterally-evoked eABR ($R^2 = 0.43$, $p < 0.01$). Degree of asymmetry in eCAPs corresponded to larger bilaterally-evoked eABR amplitudes in the retrospective cohort only ($R^2 = 0.11$, $p < 0.05$). Additionally, lateralization responses were not consistently predicted by eCAP asymmetries. Study 2 is in progress.

Conclusions: Asymmetries between left and right input levels may be predicted by comparing peripheral auditory nerve responses. The bilaterally-evoked brainstem response and behavioural measures may lack sensitivity to small level differences and thus not fully demonstrate the effect of asymmetries on central processing.

TRAIT RESILIENCE HAS A SEX-SPECIFIC ASSOCIATION WITH CORTICAL GRAY MATTER OF THE ANTINOCICEPTIVE PATHWAY IN PEOPLE WITH CHRONIC PAIN

STUDENT: MELINDA HECTOR
SUPERVISOR: KAREN DAVIS

Melinda Hector, Joshua Cheng, Kasey Hemington, Anton Rogachov, Andrew Kim, Natalie Osborne, Rachael Bosma, Camille Fauchon, Lizbeth Ayoub, Robert Inman, Jiwon Oh, Dimitri Anastakis, **Karen Davis**

Background: Resilience is an important personal characteristic that influences health and recovery. Evidence from previous studies of chronic pain suggest that highly resilient people may be better able to modulate their pain. Resilience has also been linked to gray matter in antinociceptive brain regions that have been shown to be abnormal in people with chronic pain.

Purpose: Our study aimed to determine whether there is a relationship between resilience and cortical gray matter in key brain regions of the antinociceptive (pain modulation) pathway in people with chronic pain. Given the prevalence of sex-differences in many chronic pain conditions, a secondary aim of this study was to determine if associations between resilience and gray matter are sex-dependent.

Hypothesis: Resilience will be positively correlated with antinociceptive gray matter in people with chronic pain.

Methods: Gray matter was examined from 3T MRIs acquired from 88 people (50% male/female) who had chronic pain (CP) associated with ankylosing spondylitis, multiple sclerosis or carpal tunnel syndrome, and 86 age- and sex-matched healthy controls (HCs). We used FreeSurfer 7.1.1 to extract cortical thickness (CT) and gray matter volume (GMV) from regions of the antinociceptive system, specifically the rostral and subgenual anterior cingulate cortex (rACC, sgACC), anterior insula (aINS), and dorsolateral prefrontal cortex (dlPFC). Participants also completed the Brief Pain Inventory and Wagnild-Young's Resilience Scale. Statistical significance was set at $p<0.05$ and corrected for multiple comparisons.

Results: Mean resilience scores were significantly lower in the CP group compared to HCs. In those with CP, resilience was negatively correlated with pain ratings and positively correlated with GMV in the bilateral rACC, sgACC, and left dlPFC. We also found a statistical trend in the correlation of resilience with GMV of the left rACC, left dlPFC and right aINS of CP males, and the right sgACC of CP females. There were no significant resilience-GMV correlations in HCs, and no resilience-CT correlations in either group.

Conclusions: Our findings indicate that there is a relationship between resilience and gray matter in the antinociceptive pathway of people with chronic pain but not in healthy individuals. These data could reflect the protective nature of resilience against the effects of chronic pain on the brain and quality of life. A better understanding of the brain-resilience relationship may help to advance evidence-based, and even sex-specific, approaches to pain management strategies.

BIALLELIC LOSS OF FUNCTION MUTATIONS IN PYGM CAUSE PRESUMED NON-SYNDROMIC MACULAR DYSTROPHY

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SUPERVISORS: AJOY VINCENT MBBS, MD; ELISE HÉON MD, FRCSC

BACKGROUND: Hereditary Macular Dystrophies (HMDs) are a genetically and phenotypically heterogenous group of disorders that lead to irreversible vision loss. Loss of function mutations in the PYGM gene, encoding glycogen phosphorylase, cause McArdle disease, with a few cases documented to have HMD as an association.

PURPOSE: To identify the disease-causing variants in a recessive family with HMD.

METHODS: The proband tested negative for ~280 known retinal dystrophy genes. Hence, additional family members were recruited (n = 7; two affected) to a research study. Both affected individuals underwent eye exams and paired-end genome sequencing. Candidate variants likely to cause disease were filtered based on customized pipelines. Variants were prioritized if they were shared between the affected, were rare (population frequency < 0.5%), had at least two variants per gene, and had high pathogenicity scores in two predictive algorithms. Variants of interest were segregated in all family members using PCR. Immunohistochemistry (IHC) of human retinal sections was conducted using an anti-PYGM antibody. Additional McArdle disease patients (n = 15) were recruited for eye exams through collaboration with metabolic specialists across the Greater Toronto Area.

RESULTS: Both affected individuals showed progressive vision loss and patchy macular atrophy. Genome filtering revealed a known pathogenic loss of function single nucleotide variant in PYGM (NM_005609 c.148C>T; p.Arg50*) in a homozygous state in both patients. Segregation analysis revealed the variant was also present in a sibling who had not undergone eye exam. IHC results showed PYGM presence in retinal nuclear layers, outer plexiform layer, ganglion cell layer and nerve fiber layer. Although this variant in PYGM is known to cause McArdle disease, the patients in the study were never diagnosed with any systemic features and hence patient re-phenotyping was performed. Affected patients had symptoms and signs of McArdle disease on re-phenotyping. Eye examinations of an additional cohort of McArdle patients revealed 7 of 15 individuals showing signs of retinal involvement.

CONCLUSIONS: This study has identified the cause underlying HMD in the pedigree to be consequent upon biallelic PYGM variants. Other McArdle patients also showed similar signs of retinal pathology as our proband. IHC demonstrated PYGM presence in many retinal layers. Together, these findings suggest a role for PYGM in retinal glucose metabolism and this mechanism should be further explored.

RELEVANCE MAPS: A WEAKLY-SUPERVISED SEGMENTATION METHOD FOR 3D MEDICAL IMAGES

SAJITH RAJAPAKSA
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With the increased reliance on medical imaging, Deep convolutional neural networks (CNNs) have become an essential tool in the medical imaging-based computer-aided diagnostic pipelines. However, training accurate and reliable classification models often requires large fine-grain annotated datasets. To alleviate this, weakly-supervised methods can be used to obtain local information such as region of interest from global labels. This work proposes a weakly-supervised pipeline to extract relevance maps of medical images from pre-trained 3D classification models using localized perturbations. The extracted relevance map describes a given region's importance to the classification model and produces the segmentation for the region. We propose a novel optimal perturbation generation method that exploits 3D superpixels to find the most relevant area for a given classification using U-net architecture. Furthermore, this model is trained with perturbation loss, which maximizes the difference between unperturbed prediction and perturbed prediction. We validated the effectiveness of our methodology by applying it to the segmentation of glioma brain tumours in MRI scans using only classification labels for glioma type. The proposed method outperforms existing methods in both the Dice similarity coefficient for segmentation and resolution for visualizations. Where our method achieved 0.45 DSC compared to 0.11 DSC with Grad-CAM and 0.06 with LIME. We also showed that our proposed method of superpixels-based perturbation masks generator (relevance map) could also generate visualization maps to improve the interpretability of black-box 3D classification models significantly. For future work, we propose using the produced weak segmentations as an auxiliary input to the training of the classification model to improve the classification itself.

EFFECTS OF EXTENDED CANNABIS ABSTINENCE ON CLINICAL AND COGNITIVE OUTCOMES IN PATIENTS WITH CO-MORBID MAJOR DEPRESSIVE AND CANNABIS USE DISORDERS

STUDENT: MARYAM SORKHOU
SUPERVISOR: DR. TONY P. GEORGE
CO-SUPERVISOR: DR. STEFAN KLOIBER

Background:
Cannabis use disorder is a significant problem among individuals with major depressive disorder (MDD) and may contribute to poorer treatment outcome. Previous research indicates that compared to depressed individuals without CUD, depressed individuals with CUD report greater depressive symptoms, greater cognitive complaints, and are less likely to improve in treatment. Despite these findings, most studies employ a cross-sectional design, therefore causal relationships are unclear.

Objectives:
To longitudinally determine whether a contingency reinforcement (CR) cannabis abstinence intervention will produce improvements in Day 28 cannabis abstinence rates, and in cannabis use, cognition, and clinical symptomology, in comparison to noncontingent reinforcement (NCR).

Methods: Patients with comorbid MDD and CUD were randomized into either a CR group or NCR group. In the CR condition, participants received a financial bonus upon 28-days of cannabis abstinence as indicated though urinalysis results, whereas participants in the NCR condition did not receive a financial bonus upon biochemically-verified abstinence. All patients received weekly behavioural support sessions to promote attendance and abstinence. Primary outcomes were % of individuals obtaining abstinence or reductions in cannabis use. Secondary outcomes were changes in cognition and clinical symptoms.

Results: Expected results consist of significantly greater reductions in cannabis use among the CR group in comparison to the NCR group. Similarly, it is expected that the CR group will demonstrate greater baseline to Day 28 improvements in cognition and clinical symptomology than the NCR group. To date, n=4 participants have completed the trial. Preliminary findings will be presented.

Conclusions: Our findings may suggest that extended cannabis abstinence may improve select cognitive domains and clinical outcomes in patients with MDD and co-morbid C UD, which may have important implications for the prevention and treatment of CUD in MDD patients.

ELUCIDATING FUNCTIONAL CONNECTIVITY DIFFERENCES AMONGST OLDER ADULTS WITH MILD COGNITIVE IMPAIRMENT, SUBJECTIVE COGNITIVE DECLINE OR NORMAL COGNITION

STUDENT'S NAME: ARUNAN SRIKANTHANATHAN
SUPERVISOR'S NAME: DR. LINDA MAH|

Subjective cognitive decline (SCD) is a preclinical stage of Alzheimer's disease (AD) describing individuals with cognitive concerns despite normal performance on neuropsychological tests or other formal testing. Neuroimaging studies show that functional connectivity (FC) of neural networks is altered in AD prior to amyloid deposition, beginning in the posterior default mode network (pDMN). These findings have led to the cascading failure hypothesis of AD which suggests that failure begins in the pDMN which shifts the processing burden to other connectivity hubs found in the anterior-dorsal brain regions. Our lab recently reported reduction of FC between the posterior cingulate cortex (PCC) and left posterior parahippocampal gyrus (PHG) in SCD, compared to older adults who were cognitively unimpaired (CU). However, FC within the anterior brain regions was not assessed. In the current study, we examined the pattern of connectivity changes in anterior brain regions in SCD, CU and MCI. We hypothesized that SCD would show decreased FC between PCC and medial temporal lobe (MTL) structures compared to CU, but greater FC between these regions compared to MCI. We also predicted that SCD would show increased FC between the anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC) regions compared to CU, but decreased FC between these regions compared to MCI.

The sample included 72 participants with SCD (n=29), CU (n=26) or MCI (n=17). A 6-minute resting-state functional magnetic resonance imaging scan in an awake state with their eyes closed was acquired. The CONN Toolbox was utilized to compute connectivity matrices between each region-of-interest pair to determine population level-estimates for SCD, CU and MCI groups. ANOVA was performed using Statistical Program for Social Sciences for Windows (Version 26) to compare amongst groups CONN-generated correlation coefficients which served as measures of PCC-MTL and ACC-mPFC FC.

Based on the within-group FC analysis, the PCC was found to have significant FC with the left and right posterior PHG ($T(25)= 5.7$,p-FDR=0.0003; $T(25)=3.02$, p-FDR=0.0242 respectively), and left and right hippocampus (HC) ($T(25)=4.36$,p-FDR=0.0016; $T(25)=4.07$, p-FDR=0.0029 respectively) in the CU group, but not in either SCD or MCI. In contrast, significant connectivity between ACC and mPFC was found in SCD and MCI groups ($T(28)=2.39$,p-FDR=0.0411; $T(16)=2.89$, p-FDR=0.0241 respectively) but not in the CU group. ANOVA showed that PCC-right HC FC was significantly different amongst groups ($F(2,69)=3.408$,$p=0.039$). Post-hoc analyses using Tukey's post hoc criterion for significance indicated that FC between the PCC and right HC differed between CU and MCI ($p=0.042$). ANOVA also showed no significant group effects on FC between ACC and mPFC ($F(2,69)=0.521$,$p=0.596$) or PCC and bilateral posterior PHG ($F(2,69)=2.805$, $p=0.067$; $F(2,69)=0.228$,$p=0.796$ respectively).

Our findings are broadly consistent with the cascading failure hypothesis suggesting reduction of PCC FC with MTL structures in MCI and SCD, and compensatory increases in FC in the anterior DMN, although direct group comparisons of the latter did not yield statistically significant differences. Limitations of this study includes relatively small sample sizes (i.e., MCI group) which may explain the latter and other negative findings, and the lack of AD biomarkers and longitudinal follow-up to establish SCD as a preclinical AD group. Thus, our findings require replication with longitudinal studies including larger sample sizes. The current findings of reduced connectivity between the pDMN and MTL structures in older adults at risk for AD suggest the possibility of assessment of FC as a means to predict AD risk in SCD, as well as targeting of pDMN FC as a potential intervention for older adults at risk for developing AD.

THE EFFECT OF POLYGENIC RISK FOR DEPRESSION ON WHITE BLOOD CELL COUNT IS MODIFIED BY AGE

STUDENT: EARVIN TIO
SUPERVISOR: DR. DANIEL FELSKY

Earvin Tio (student), Milos Milic (research analyst), Dr. Daniel Felsky (supervisor)

Background: Genetic risk for depression has recently been associated with higher white blood cell (WBC) counts, providing support for the link between depression and inflammation. However, the mechanisms and manifestations of depression differ between mid- and late-life, and existing work has not established whether this association holds over the adult lifespan. We tested the interaction between polygenic risk scores (PRS) for depression and age on WBC count in two large independent cohorts.

Purpose: The purpose of this study is to identify age-related effects of depression risk on WBC count. A deeper understanding of the interactions between the genetic risk for depression and inflammatory markers across the lifespan can lead to more accurate and relevant diagnoses and more tailored treatment options.

Hypothesis: We hypothesized that the relationship between polygenic risk for depression and WBC count may also be moderated by age, specifically into the later stages of life.

Methods: PRS were calculated in European ancestry subsets of the UK Biobank (n=324,098; age range: 39-72, mean=57) and the Canadian Longitudinal Study on Aging (CLSA; n=22,758; age range: 45-86, mean=63). Depression GWAS summary statistics from the Psychiatric Genomics Consortium were used to calculate PRS–Continuous Shrinkage. Linear regression was used to model WBC count in each cohort, including an age by PRS interaction term and co-varying for fine population structure, biological sex, smoking status, and other inflammatory conditions.

Results: In both cohorts, the interaction of PRS x age was significant at $P<0.05$ (UKB interaction term: t=-2.9, p=3.7x10-3; CLSA interaction term: t=-2.6, p=9.0x10-3). Importantly, the interaction was directionally consistent in both samples: the positive relationship between genetic risk for depression and WBC was diminished in older individuals. Interactions in both datasets remained significant after including clinical and lifestyle covariates (UKB: t=-2.5, p=0.012; CLSA: t=-3.4, p=6.3x10-4).

Conclusions: We found that the relationship between genetic risk for depression and WBC count is dependent on age. These findings highlight important considerations in the applicability of PRS across life stages and suggest that the inflammatory manifestation of depression risk in elderly may not be the same as in younger individuals.

Group D: Neuroscience-Brain Health

FORENSIC WOMEN'S SUBSTANCE USE TREATMENT EXPERIENCE – A QUALITATIVE STUDY

GIL ANGELA DELA CRUZ
SUPERVISOR: DR. DAVID CASTLE
CO-SUPERVISOR: DR. TONY GEORGE

Purpose: Women with substance use disorders (SUDs) face distinctive challenges compared to men with SUDs, including unique medical consequences (e.g., breast cancer, infertility), barriers to treatment (e.g., pregnancy and childcare), and stressors related to social expectations. Women face increased rates of physical/emotional abuse, highlighting the need for trauma-informed interventions. There is a higher occurrence of coexisting mental illnesses, which is notable since substance users with mental illnesses have worse outcomes when in SUD-only treatment. These issues are aggravated in women involved in the criminal justice system who experience more complex trauma, post-traumatic stress disorder, and poverty. Forensic women's needs are overlooked and not well evaluated due to male-centered measures of health.

Objectives: This project aims to identify useful components of treatment, utility of women's-only group therapies, and barriers forensic women with SUDs face through a qualitative investigation.

Methods: This topic will be addressed in two stages; (1) a qualitative project consisting of (1a) semi-structured interviews of women in the forensic mental health system at CAMH to gather qualitative data on topics like SUD treatment experiences. These data will be combined with interview data from (1b) service providers at CAMH which will inform a future (2) pilot intervention directed at women who have been involved with the criminal justice system.

Predicted Outcome: Based on data collected from the service provider interviews, outcomes will likely be related to challenges accessing treatment, unhelpful program content, and less motivation to attend and engage.

EXAMINING AMYGDALA RESTING-STATE FUNCTIONAL CONNECTIVITY PATTERNS IN MODERATE-SEVERE TRAUMATIC BRAIN INJURY PATIENTS ASSOCIATED WITH DEPRESSIVE SYMPTOMS

STUDENT: LAYAN ELFAKI
SUPERVISOR: DR. ROBIN GREEN

Background: Moderate-severe traumatic brain injury (msTBI) is a prevalent form of injury instigating debilitating physical, cognitive and emotional dysfunction (Lysenko-Martin et al., 2020). Of the detected impairments, depression frequently emerges post TBI (Scheibel, 2017) and manifests as altered resting-state functional connectivity (rsFC) of emotion-regulation regions including the amygdala. Han et al. (2015) examined amygdala rsFC in TBI patients and reported hyperFC between the amygdala and brain regions associated with the somatomotor, default mode, affective and salience networks (SMN, DMN, AN, SN) in depression.

Objectives: Identify a depression signal in msTBI by investigating amygdala rsFC to the brain, voxel-wise.

Methods: This project is a secondary analysis (n= 40) on longitudinal data collected from Toronto Rehab TBI recovery study database (Christensen et al., 2008). Functional scans obtained 5, 12 and 24-months post-injury were preprocessed using fMRIB Software Library. rsFC of bilateral amygdala was investigated using seed-based connectivity analyses (SBCA) with age, sex, years of education, Personality Assessment Inventory (PAI) and Beck Depression Inventory (BDI-II) depression scores as covariates.

Results: In line with literature findings, SBCA using BDI-II and PAI depression scores as covariates revealed hyperFC between bilateral amygdala and regions of the SMN, AN, SN and DMN, though there were some discrepancies between time points and left vs. right amygdala.

Conclusions: Results support the potential value of employing amygdala rsFC in identifying a biomarker of depression in msTBI patients, which would guide treatment development and enhance clinical prognosis. Next steps involve the addition of age and sex-matched healthy control rsFC to provide further confirmation of the depression signal identified in msTBI.

INVESTIGATING TAUOPATHY IN MILITARY OCCUPATIONAL BLAST: A [18F] FLORTAUCIPIR POSITRON EMISSION TOMOGRAPHY STUDY IN CANADIAN ARMED FORCE MEMBERS

STUDENT: SHAMANTHA LORA
SUPERVISOR: DR. ISABELLE BOILEAU

Background: Neurological damage, including long-term chronic traumatic encephalopathy (CTE) – a progressive neurodegenerative disorder resulting from repeated brain trauma – is suspected to occur as a result of repetitive subconcussive exposure to low-intensity occupational blast overpressure during military training and operations. The pathobiological changes underlying this phenomenon are not well-understood and can only be diagnosed definitively through a post-mortem examination. Aggregation of tau neurofibrillary tangles (tauopathy) is a key neuropathological feature of CTE and has been linked with memory loss, cognitive impairment and mood changes. Although animal models putatively link low-level blast exposure with persistent neurological effects and accelerated tau aggregation, studies in humans exposed to blast are limited.

Purpose: o investigate tau accumulation in brain using PET imaging of the radiotracer [18F]flortaucipir in military personnel (Canadian Armed Forces) exposed to low intensity level blast.

Hypothesis: Greater regional [18F]flortaucipir uptake will be associated with greater exposure to low-level military blast.

Methods: Fifteen (n=15) CAF members (male; 44.3 ± 6.3 years old) exposed to repetitive low-intensity blast (i.e., breaching and heavy weapons systems) completed a PET scan following the injection of [18F]flortaucipir, a novel radioligand targeting tau. A magnetic resonance image was acquired for brain regions of interest (ROIs) delineation. Standardized Uptake Value ratio (SUVr) were calculated with the cerebellum as reference tissue. Participants performed a test of executive function (Stroop) and completed mood and clinical questionnaires.

Results: [18F]flortaucipir uptake ranged from 0.7 to 1.1 SUVr in temporal cortex and from 0.6 to 1.12 in frontal cortex. [18F]flortaucipir SUVr values (in temporal and frontal cortices) were positively correlated to years of breaching ($r = 0.9$; p-uncorrected<0.05). Greater [18F]flortaucipir SUVr values in prefrontal regions were related to poorer performance on the Stroop test of executive function ($r = 0.7$; $p<0.05$). [18F]flortaucipir SUVr did not correlate with mood or clinical symptoms.

Conclusion: In line with an earlier PET study linking tau deposition with dose exposure to blast (Robinson et al., 2019), our study finds preliminary evidence for a positive correlation between repeated blast exposure and tau deposition, further suggesting that greater cumulative exposure to subconcussive neurotrauma could potentially increase long-term risk of tauopathy. Studies in a larger cohort of at-risk individuals should aim to quantify tau in order to better understand the pathobiological significance and what constitutes safe exposure limits to military occupational blast.

SEMAGLUTIDE TO THE RESCUE!

STUDENT: FEMIN PRASAD
SUPERVISOR: DR SRI MAHAVIR AGARWAL
CO-SUPERVISOR: DR MARGARET K HAHN

Background and purpose: Off-label use of metformin to mitigate antipsychotic induced weight gain is the currently accepted clinical guideline owing to acceptable safety profile, availability, and positive evidence from well-designed randomised control trials. However, in real world clinical settings not all patients benefit from metformin. Glucagon like peptide 1-receptor agonist like semaglutide have shown great promise in augmenting weight loss in patients with morbid obesity in the general population. However, there are very few studies in extant literature exploring the effectiveness of semaglutide in mentally ill population with iatrogenic obesity in a real-world clinical setting.

Methods: A retrospective chart review of patients attending the mental health and metabolism clinic at Centre for Addiction and Mental Health between May 2019 to May 2021 was conducted. Data of patients initiated on semaglutide was collected. The primary outcome measure was change in weight at 3 months ,6 months and 12 months.

Results: Thirteen patients on semaglutide weekly injections were included in the review.50% of the patients were female.41% had major depressive disorder followed by schizophrenia spectrum disorder (33%). The average age of patients was 38.5(15.37) years, BMI was 37.6(11.3), with a mean waist circumference of 116.1(18.4) cms and HbA1c of 0.05(0.01). A statistically significant weight loss of 4.5±3.01 kg ,5.5 ±6 kg, and 9.1±8.4 kg was seen at 3 ,6 and 12 months respectively.

Conclusion: This review highlights the real-world effectiveness of semaglutide in antipsychotic induced weight gain. Given the preliminary nature of the review, it further reinforces the necessity of trials with larger sample size, diverse population, and longer duration to translate these promising results to applicable clinical guidelines.

INVESTIGATING THE ENDOCANNABINOID SYSTEM IN MAJOR DEPRESSIVE EPISODES: IMAGING WITH THE RADIOTRACER [11C] CURB

STUDENT: DORSA RAFIEI
SUPERVISOR: NATHAN J. KOLLA

Background: The lifetime prevalence of depression is 10-20% among Canadians, and yet over 40% of patients do not respond to traditional, first-line therapeutics such as selective serotonin reuptake inhibitors. Other pathophysiologies besides neurotransmitter system dysfunction have been proposed for depression. The endocannabinoid system (ECS) is a potent modulator of mood, anxiety and stress circuits and its dysregulation has been implicated in both animal and human models of major depressive disorder, an illness composed of recurrent major depressive episodes (MDEs) affecting 4% of Canadians at any one time. Fatty acid amide hydrolase (FAAH), an enzyme in the ECS, plays a vital role in the metabolism of endogenous cannabinoids, such as anandamide, which target cannabinoid 1 (CB1) receptors. CB1 receptors are widely distributed across the brain, particularly within the cerebral cortex and hippocampus, and have functional roles in mood, memory and appetite control. In response to stressors, the breakdown of anandamide by FAAH is initiated and anandamide-CB1 signaling is reduced. A polymorphism (C385A) in the FAAH gene leads to lower levels of FAAH. Rat models of depression have shown higher FAAH and lower anandamide levels in the brain and that inhibition of FAAH elicits antidepressant-like effects (e.g. decreased immobility time in the forced swim test). In depressed human patients, serum anandamide has been negatively correlated with Hamilton Depression Rating Scale (HDRS) subscales in females; lower cerebrospinal fluid levels of ethanolamine, the metabolite of anandamide, have been negatively correlated with total HDRS score; and elevated CB1 receptor expression in post-mortem brains of suicide victims have been found. The ECS, however, has never been characterized in humans with MDE using in vivo imaging techniques.
Purpose: To determine whether brain FAAH is elevated in vivo in patients with MDE and if it is related to depressive severity.
Hypotheses: Since FAAH has been shown to be elevated in animal models of depression, we hypothesize that: (1) brain FAAH will also be elevated in the prefrontal cortex, anterior cingulate cortex, and hippocampus in MDE patients, and (2) brain FAAH will be positively correlated with HDRS scores.
Methods: Eligible psychiatric participants, aged 18 to 65 years, met criteria for a current MDE. Exclusion criteria included a history of manic episodes, psychosis, or a current substance use disorder. All participants were administered the Structured Clinical Interview for DSM-5; control participants were excluded if they had any history of a psychiatric disorder. The HDRS was administered to all participants. FAAH levels were measured using [11C] CURB positron emission tomography (PET) imaging, which were then co-registered with T1-weighted magnetic resonance (MR) images. Additionally, participants underwent genotype sampling to determine the FAAH gene polymorphism. A mixed methods model will be employed to determine the differences in FAAH levels between the two groups, with genotype as a fixed factor.
Results: Nine medication-free participants with MDE (mean age: 26.2 years; 8 females) and 10 age- and sex-matched healthy controls (mean age: 26.1 years; 8 females) completed the study protocol. Six MDE and seven control participants expressed the FAAH genotype associated with higher FAAH protein expression. Statistical analysis showed no significant or trend level differences in FAAH levels between MDE and healthy control groups.
Conclusion: Our study is the first to investigate ECS dysregulation, specifically FAAH elevation, in MDE in vivo. Considering the lack of response to current therapeutics in MDE patients, further research is necessary to explore the ECS in MDE for novel therapeutic targets.

EFFECTS OF CEREBELLAR THETA BURST STIMULATION (TBS) ON WORKING MEMORY

STUDENT: NASEM RAIES
SUPERVISOR: DR. ROBERT CHEN

Background: The cerebellum is well-known for its motor roles, but recent evidence suggests that the cerebellum is also involved in cognitive functions. Lesions of the cerebellum can lead to changes in executive functions, mood, personality, and working memory, which involves a system that temporarily holds and manipulates information. The contribution of the cerebellum to working memory is achieved through its connections with the prefrontal cortex (PFC). Previous studies showed that theta burst stimulation (TBS), a form of repetitive transcranial magnetic stimulation (TMS), on the cerebellum changes its functional connectivity with the PFC. Specifically, excitatory intermittent TBS (iTBS) increases, whereas inhibitory continuous TBS (cTBS) decreases this functional connectivity.

Purpose: This study aims at further exploring the cerebellar contribution to working memory through investigating the effects of cerebellar iTBS and cTBS on working memory performance.

Hypothesis: Based on the reviewed literature, we hypothesize that iTBS on the cerebellum would improve working memory, whereas cTBS would disrupt it.

Methods: Nine subjects (6 women and 3 men; age range: 42 – 79) participated in this ongoing study. Bilateral cerebellar stimulation was applied with a figure-of-eight coil at 3 cm lateral and 1 cm below the inion. The subjects received iTBS, cTBS, or sham iTBS in three separate sessions in random order. Within 30 min after TBS, the participants performed three types of working memory tasks: letter 2-back, digit span forward (DSF), and digit span backward (DSB). In the 2-back task, the outcomes measured were hits (percent correct) and false alarms (responses to non-target trials). The score ranges were 2-9 digits for DSF and 2-8 digits for DSB.

Results: The preliminary results showed marginal significance in the DSB task: scores were lower in the cTBS condition compared to the sham condition ($t=2.286$; $p=0.052$; Cohen's $d=0.762$). There was a trend for better performance in the sham condition in the 2-back task with higher hit rates and fewer false alarm responses compared to the iTBS and cTBS conditions, but the results did not reach significance, likely due to the small sample size. Scores on the DSF task were comparable.

Conclusions: The findings support the hypothesis that the cerebellum is involved in working memory, and this contribution may be disrupted by cTBS. The study is ongoing and more subjects are being recruited.

COMPLEMENT SYSTEM IRREGULARITIES ASSOCIATED WITH SCHIZOPHRENIA

STUDENT: DANIEL UJIC
SUPERVISOR: DR. FANG LIU

Background: Schizophrenia is a mental health disorder characterized by the presence of various symptoms which fall into three categories: negative, positive, and cognitive. Negative symptoms include a loss of normal behaviors and thoughts, positive symptoms are associated with new behaviors and thoughts that are not present before the onset of the disorder and cognitive symptoms include any type of cognitive dysfunction. Epidemiological studies have suggested that environmental insults play a major role in the development of Schizophrenia and one example is maternal infections. The Maternal Immune Activated (MIA) theory has become popular, and the role of the mothers activated immune system on the fetus' brain development and likelihood of developing a Schizophrenic phenotype has been vastly studied. The complement system is a complex network of proteins involved in the innate immune system. One of the roles that several of these complement proteins participate in is in the elimination and pruning of synapses during development. It has been suggested that several neurodevelopmental disorders, such as Schizophrenia, are caused by inappropriate synaptic pruning and that a dysfunctional complement system could promote the synaptic pruning abnormalities seen in these disorders.
Purpose: To determine if there are differences in complement protein expression in animal models of Schizophrenia and to examine which proteins they may overlap with in the brain.
Hypothesis: We hypothesize that the complement proteins are key molecules involved in the development of Schizophrenia. We expect that offspring from polyinosinic-polycytidylic acid (POLY IC) treated mothers compared to offspring from saline treated mothers will result in: Increased expression of complement proteins in the mouse model and that these proteins will have a greater colocalization with proteins associated with synaptic and neuronal integrity. We expect that offspring from POLY IC treated mothers compared to offspring from saline treated mothers will result in previously published findings using the same animal model.
Methods: To conduct biochemical assays and behavioural studies, an animal model of Schizophrenia will be required. An accepted and well cited mouse model of Schizophrenia are mice birthed from mothers who have experienced MIA19. One specific model involves the injection of POLY IC into pregnant mice19. We shall breed mice and shall conduct behavioural tests and biochemical experiments on the offspring at the following ages: Week 1, 3, 6, 8 and 12. We have selected spaced out time points so that we can attempt to measure a quantifiable difference in the expression of the proteins of interest throughout neurodevelopmental stages.
Results: Current results have shown differences in the expression of complement proteins and other proteins associated with synaptic and neuronal integrity at all of the completed time points to date, with some patterns of expression being consistent and others inconsistent throughout the various ages tested. Colocalization of proteins associated with microglia activation, synaptic and neuronal integrity and complement proteins have been found as well throughout the different time points. Animal behavior results have been consistent with literature in adults, but in adolescent mice, we have discovered the opposite finding.
Conclusions: Our findings suggest that the immune system (specifically the complement system) is dysregulated in the MIA mouse model of Schizophrenia. Our findings also support the hypothesis that the complement system plays a role in abnormal synaptic pruning in Schizophrenic phenotype models. Further research is required to determine the exact underlying protein interactions which can be used as a potential drug target.

VASCULAR ENDOTHELIAL DYSFUNCTION, COGNITION AND STROKE IN EARLY LIFE STUDY (VECSELS)

STUDENT: MARIA VU
SUPERVISOR: NOMAZULU DLAMINI

Background: Children with cardiac disease are at risk for arterial ischemic stroke (AIS) and account for up to 30% of all childhood strokes. 50-70% of children have adverse neurological outcomes which include cognitive impairments. An important observation is that cognitive impairments are prevalent in children with congenital heart disease (CHD) in the absence of stroke and up to 70% of impairments seen in children with stroke are not explained by lesion characteristics. We propose a global mechanism of injury, affecting perfusion, present prior to stroke occurrence.

Purpose: 1) To demonstrate that cerebral vascular endothelial dysfunction (cVED) is associated with specific cognitive impairments. 2) To demonstrate a gradient of ischemic injury associated with endothelial function.

Hypothesis: This study hypothesizes that cVED is associated with ischemic injury in children with CHD. It also hypothesizes a gradient of vascular endothelial function associated with cognitive function, among children with CHD with and without AIS.

Methods: 30 participants (n=10 patients with CHD+AIS; n=10 patients with CHD only; n=10 healthy controls) will be enrolled into the study. All participants will undergo a neuropsychological evaluation, peripheral vessel ultrasound scanning, and an MRI scan. The MRI scan assesses cVED using blood oxygen level dependent cerebrovascular reactivity (BOLD-CVR). A vasoactive stimulus (carbon dioxide) will be delivered exogenously from a specialized medical device called the RespirAct, as images are being captured in the MRI.

Results: Recruitment is ongoing. The healthy control cohort has enrolled 10 participants, the CHD+AIS cohort has enrolled 2 participants, and the CHD only cohort has 1 participant.

Expected Outcomes: Children with CHD+AIS will have the most severe VED shown through cerebral and peripheral imaging measures. Less severe VED will be seen in the CHD only cohort and the control cohort should have no VED. The severity of VED is expected to be associated with a gradient of cognitive functioning.

Group E: Neuroscience-Brain Health

A MATHEMATICAL COMPARISON OF INTRACORTICAL AND CORTICOTHALAMIC MODELS OF ALPHA RHYTHMOGENESIS

STUDENT: SORENZA BASTIAENS
SUPERVISOR: DR. JOHN GRIFFITHS

Neural population models (NPM) have been fundamental in advancing our understanding of brain rhythms by describing the coarse-grained activity of neural tissue to replicate macroscale phenomena such as alpha oscillations (8-12Hz) which dominate EEG signals. However, the physiological mechanism behind their generation as well as their functional significance is still unclear, due to the existence of multiple sources interacting and influencing each other. Current theories on the generation of alpha (8-12 Hz) rhythmic activity, which dominates EEG signals, emphasize the importance of communication between various cortical and thalamic neural populations. This is represented by two prevailing types of NPM that simulate alpha oscillations as: 1) Recurrent activity and excitatory-inhibitory interactions within cortical column microcircuits; 2) Delayed inhibitory feedback within cortico-thalamocortical loops. Prominent examples of these two cortical and corticothalamic theories are the NPM models of Jansen & Rit (JR) and Robinson et al., respectively. The aim of this work is to contribute to the mechanistic understanding of candidate theories of alpha rhythmogenesis by simulating, evaluating and synthesizing candidate theories using mathematical modelling. In this study we developed an approach for comparing the dynamical repertoires and parameter space geometries of the JR and Robinson models. The rationale here is that even though these models nominally describe differing neural populations and circuit motifs (e.g. intracortical, corticothalamic), their basic mathematical components, wiring structure, and excitatory/inhibitory sub-motifs can be meaningfully compared. Using this approach, we study their connectivity parameter space, and assess the influence of each loop within the NPM circuit on the stability and frequency of oscillations. We formulate a novel three-dimensional reduction of the five-dimensional coupling strength parameter space of a JR-based model, which allows us to compactly summarize and visualize how the system dynamics change as a function of loop gains. This work contributes to improving our mechanistic and theoretical understanding on candidate theories of alpha rhythmogenesis.

MULTIMODAL LARGE-SCALE DATA-DRIVEN APPROACHES TO RECLASSIFY TRAUMATIC BRAIN INJURY: THE TBI-CLASS STUDY

STUDENT: DR. ABDELHAKIM KHELLAF
SUPERVISOR: DR. MICHAEL D. CUSIMANO

Background: Traumatic brain injury (TBI) is a leading cause of death and disability in people under 40 years of age in Canada and other developed countries. No current pharmacotherapy has clear long-term functional benefits in patients with TBI. This is in part due to a failure to translate the great heterogeneity in the affected host, injury characteristics and management into meaningful clinical targets or improved outcome-based classification systems. Machine learning (ML) works by learning from pre-existing data to predict unseen data. Using patient 'big data', ML and other data-driven approaches can be leveraged to predict individual course of disease and uncover previously unknown associations while removing human bias. No previously published study has examined the full extent of medical and psychosocial variables in large-scale clinical databases for TBI reclassification and prognosis through such approaches.
Purpose: To evaluate the feasibility of latent phenotype identification in TBI patient data using unsupervised ML within large-scale multimodal datasets, and novel phenotype association with patient mortality and morbidity measures. To characterize a reduced, core set of common data elements across TBI datasets that are most relevant in defining injury phenotypes and identify modifiable factors within this set.
Hypothesis: We hypothesize that TBI patient phenotype identification is feasible within both single and aggregate large-scale multimodal databases using unsupervised ML approaches and that the characterized patient phenotypes show association with clinically relevant outcome measures.
Methods: We established international (USA/Europe) collaboration to obtain 6 large-scale prospectively-collected TBI study datasets, comprised of sociodemographics, medical history, imaging (CT/MRI), injury characteristics, laboratory tests, therapies, monitoring data, mortality/morbidity outcome measures. We are performing feature selection for data reduction to identify common data elements, and variable relationships and granularity. As preliminary step prior to aggregate analysis, we first performed clustering using the TBI Model Systems database alone. We employed a partitioning around medoids clustering algorithm to characterize an optimal number of patient clusters chosen from a set of 3 to 10 clusters based on Silhouette technique.
Results: We descriptively characterized and pre-processed 6 large-scale clinical databases (aggregate N>20,000 TBI patients). Moreover, from a development set of 2,863 patients from the TBI Model Systems dataset with 1-year available follow-up data, we identified 2,413 complete patient cases with 97 independent variables post-processing used for clustering. We identified three distinct patient phenotypes that we descriptively characterized and showed association with 1-year morbidity measures (Glasgow Outcome Scale-Extended/Disability Rating Scale). Further results will be presented during poster presentation.
Conclusions: As key landmark, we preliminarily demonstrated feasibility of unsupervised clustering approaches on a single large, multimodal, TBI dataset and identified novel patient phenotypes. Next steps include analysis extension to our aggregate dataset following data dimensionality reduction and processing, using clustering approaches and external data validation. Our research suggest that unsupervised ML approaches show promise in TBI subpopulation characterization and might inform patient stratification for future clinical trials in TBI.

DEVELOPMENT AND TESTING OF A PHOTOSENSITIVE LIQUID POLYMER EMBOLIZATION SYSTEM FOR NEUROVASCULAR DISEASES

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Background: Embolization represents a minimally invasive treatment modality for arteriovenous malformations (AVMs), tumors, hemorrhagic blood vessels, and other neurovascular disease. However, this can be limited by currently available embolic agents, in terms of safety and efficacy. Discovery of new and improved agents could lead to better treatment outcomes.

Purpose: The goal of this project was to develop and test a novel embolization agent for the minimally invasive treatment of AVMs, tumours, and blood vessels.

Hypothesis: We predict that the use of photosensitive liquid polymers can be a safe and effective embolization agent for the treatment of animal models of these neurovascular diseases.

Methods: We formulated low-viscosity, injectable liquid polymers which were mixed with a photo-initiator agent and non-ionic contrast medium. We utilize photo-crosslinking at the tip of the microcatheter with an integrated optical fibre to induce rapid solidification of the embolic agent. The UV intensity can also be adjusted, in real-time, to dynamically modulate the degree of crosslinking and thus the viscosity of the polymer. This allows for improved operational control, which can make embolization treatments safer and more effective. To test this, we utilized the swine rete mirabile as an animal model for AVMs, renal arterial tree (inferior segmental artery) as a model for tumours, and blood vessels (branches of the subclavian artery). 5 animals were utilized without prior preparation. Embolization was graded based on degree of complete obliteration of the vascular target. Any non-target embolization or other complications were recorded. Follow-up angiography was performed at the 4-week interval.

Results: With a combination of shear-thinning properties and dynamic modulation of photo crosslinking, we show that we can deliver an embolic agent with a viscosity range of up to 104 Pa*s from a single low viscosity precursor. Using this methodology, photosensitive liquid polymer embolization was technically successful in all animal trials. Following embolization, 4/5 rete mirabile, 5/5 inferior renal arterial trees, and 5/5 blood vessels were graded as completely obliterated, and there were no instances of clinical or angiographic complications.

Conclusions: We demonstrated a novel method of dynamic photomodulation and delivery of bioengineered liquid polymers to address current limitations of endovascular embolization therapies. This was successful in the treatment of animal models of AVMs, tumours, and blood vessels. This promising technology will be investigated further with longer-term comparative animal trials.

MTSAT IS MORE SENSITIVE TO CEREBELLAR MYELIN ABNORMALITY IN MULTIPLE SCLEROSIS COMPARED TO MTR

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SUPERVISOR: JIWON OH
CO-SUPERVISOR: TOM SCHWEIZER

Background: Damage to the cerebellum in multiple sclerosis (MS) can contribute to a more severe disease course with greater motor and cognitive disability. Quantitative magnetization transfer saturation (MTsat) can provide insight into myelin pathology and is suggested to be superior in quantifying myelin in brain tissue as it is less sensitive to T1 dependent effects and B1 inhomogeneity compared to conventional magnetization transfer ratio (MTR).

Purpose: (1) To compare the ability of MTsat and MTR to detect differences in myelin content in the cerebellum and cerebellar peduncles between people with radiologically isolated syndrome (RIS), relapsing-remitting MS (RRMS), primary progressive MS (PPMS), and healthy control (CON). (2) To evaluate the relationship between cerebellar MTsat or MTR and clinical disability as measured by Expanded Disability Status Scale (EDSS) in people with MS.

Hypothesis: We hypothesized that MTsat will be able to detect greater group differences in cerebellar myelin content and better correlate to clinical disability in MS compared to MTR.

Methods: MT imaging data using a 3D gradient echo sequence with and without MT saturation pulses were acquired on 3T Siemens scanners in 20 RIS (mean age 42y (range 20-59y), median EDSS 0.5 (0.0-3.0)), 82 RRMS (mean age 38y (19-59y), median EDSS 1.5 (0.0-6.0)), 17 PPMS (mean age 49y (26-61y), median EDSS 4.0 (1.5-6.5)) and 16 CON (mean age 33y (19-51y)). 3D T1-weighted image was acquired to account for T1 effect when calculating MTsat. MTsat and MTR were calculated using Spinal Cord Toolbox. Registration and tissue segmentation were performed using FMRIB Software Library (FSL) tools. One-way analysis of variance (ANOVA) with Tukey post hoc test for multiple comparisons was performed to compare MTsat or MTR in the regions of interest (ROI), including whole cerebellum, Crus I, Crus II, and superior/middle/inferior cerebellar peduncles (S/M/ICP), between groups. Spearman's rho was used to evaluate correlations between MTsat or MTR and EDSS scores.

Results: Mean MTsat consistently showed group differences between PPMS and CON (4/6 ROI, $p \leq 0.006$), RIS (4/6 ROI, $p \leq 0.04$), and RRMS (3/6 ROI, $p \leq 0.0001$). Mean MTR showed significant differences only between PPMS and CON (4/6 ROI, $p \leq 0.03$) and RRMS (3/6 ROI, $p \leq 0.007$). The percentage difference of the means between the groups were greater using MTsat (-5.85% to -12.05%, $p \leq 0.04$) compared to MTR (-3.12% to -6.54%, $p \leq 0.03$) in cerebellum, Crus I, Crus II, and ICP. Mean MTsat demonstrated stronger correlations with EDSS in more ROI (5/6 ROI, $r=-0.24$ to -0.33, $p \leq 0.03$) than mean MTR (2/6 ROI, $r=-0.23$ to -0.24, $p \leq 0.04$) in RRMS.

Conclusions: Mean MTsat was able to detect group differences more frequently and demonstrate greater percentage differences of the means between the groups in the cerebellum and cerebellar peduncles compared to mean MTR. The inverse relationship between MTsat (myelin content) and EDSS (disability) was more evident than MTR and EDSS in RRMS. Together, these findings suggest that MTsat may have higher sensitivity and specificity to cerebellar myelin across distinct MS subtypes than MTR. These findings and prospective follow-up of this cohort using MTsat in the cerebellum may allow us to gain deeper insight into early MS disease processes relevant to clinical disability onset and progression in MS.

DETERMINING THE ROLE OF STAT3-MEDIATED ASTROGLIOSIS ON DCM PATHOGENESIS AND SURGICAL DECOMPRESSION AFTER DCM

STUDENT: SARAH SADAT
SUPERVISOR: MICHAEL G. FEHLINGS

Degenerative cervical myelopathy (DCM), is the most common cause of spinal cord dysfunction amongst adults over the age of 55, involving chronic and progressive degeneration of the cervical spinal cord. DCM is thought to occur due to osteoarthritic changes, involving the breakdown of cartilage in the spine, resulting in the collapse of intervertebral discs, and painful pressure on the spinal cord. Depending on the severity of the injury, the symptoms can vary, ranging from mild symptoms, such as numbness or dexterity issues, to severe quadriparesis and urinary incontinence.

The current gold standard treatment for DCM is cervical decompression surgery (DEC), which is shown to effectively delay progression of the disease and improve functioning in most patients. Unfortunately, not all patients are operative, and a significant number of patients experience postoperative complications, most commonly, C5 palsy, within 30 days after surgery. Previously, it has been determined that these post-operative complications can be explained in part, by secondary ischemia-reperfusion injury (IRI). As the ischemic tissue is reperfused with oxygenated blood upon decompression of the cervical spine, aerobic respiration is significantly increased, resulting in the generation of reactive oxygen species (ROS) and ultimately, neuronal apoptosis. Importantly, astrocytes, the most abundant glial cells in the central nervous system, have been shown to play a critical neuroprotective role in many ischemic settings, such as stroke. Furthermore, recent studies have demonstrated that astrocyte activation, mediated by the STAT3-signalling pathway, is critical for the repair of the blood-spinal cord barrier and the restriction of leukocyte infiltration following SCI. However, the role of astrocytes, whether protective or deleterious, in DCM and IRI has yet to be determined.

Thus, this study aims to determine the specific role of STAT3-mediated astrogliosis in the pathogenesis of DCM and in IRI induced by DEC surgery. We hypothesize that if STAT3 signaling in astrocytes is critical for neuroprotection against IRI, then the conditional knockout of STAT3 in astrocytes in a mouse model of DCM (C5-6 injury), followed by decompression surgery, should 1) impede neural repair and 2) worsen functional recovery. This study involves 2 groups of mice; 1) Cre-positive, Aldh1l1-CreERT-Stat3-loxP mice, where STAT3 expression will be inactivated in Aldh1l1-expressing cells (astrocytes) using tamoxifen (i.p. 75mg/kg for 5-days); and 2) Cre-negative, Aldh1l1-CreERT-Stat3-loxP mice treated with tamoxifen, used as controls. Behavioural tests will be performed weekly from 4-12 weeks post-DCM. At 12 weeks post-DCM, all animals will undergo DEC surgery at 24h after DEC, animals will be sacrificed and 1.5cm of tissue centered on the injury epicentre will be extracted for immunohistochemistry or RNA-sequencing. Preliminary behavioural data collected up to 5 weeks post-DEC indicate that STAT3 knockout mice demonstrate worsened motor recovery, suggesting that astrogliosis is critical for neuroprotection in IRI. Ultimately, the outcome of this study will further our current understanding of the cellular mechanisms underlying the pathophysiology of DCM and help determine potential therapeutic targets to improve neural recovery and reduce post-operative IRI-related complications following surgical decompression.

CROSS-SITE VARIABILITY IN EXPOSURE TO ANALGESIA IN VERY PRETERM NEONATES IN RELATION TO 18-MONTH NEURODEVELOPMENT

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Background: In very preterm neonates, repetitive exposure to painful stimuli procedures, while life-saving, is associated with adverse neurodevelopmental outcomes and alterations in brain maturation. Treating neonatal pain is now an accepted component of contemporary intensive care. Yet some analgesic medications are associated with adverse neurodevelopmental outcomes and altered brain maturation, and there are no clear guidelines for analgesic medication use.

Methods: In this prospective study of 276 very preterm neonates, we assessed variability of analgesic medication use across three tertiary NICUs, accounting for early-life exposure to pain, quantified as number of invasive procedures. We then examined whether associations between early-life pain and adverse neurodevelopmental outcomes differ by duration of exposure to analgesic medications using multivariable linear regression models. 18-month neurodevelopmental assessments were completed with Bayley Scales for Infant and Toddler Development-Third ed.

Results: Morphine: Multivariable linear regressions revealed significant differences in the use of morphine across sites, for a given exposure to early-life pain (interaction $p<0.001$), even after adjusting for birth GA, infection, mechanical ventilation, and major surgery. The association between early-life pain and motor scores at 18 months differed by duration of morphine exposure (interaction $p=0.01$); in infants with no or long (>7 days) morphine exposure, greater exposure to early-life pain was associated with lower motor scores, although this association was not observed in infants exposed to short durations of morphine (£ 7 days).
Sucrose: Restricting analyses to the two sites administering sucrose for neonatal pain, greater sucrose use was associated with less morphine use ($b=-0.25$, 95%CI [-0.39, -0.11], $p<0.001$). The association between early-life pain and cognitive (interaction $p=0.08$) and language scores (interaction $p=0.04$) differed by duration of sucrose exposure; in infants exposed to sucrose, greater exposure to early-life pain was associated with lower cognitive and language scores, although this was not observed in infants without sucrose exposure.

Conclusions: Significant cross-site differences in morphine use, for a given exposure to early-life pain, highlights an urgent need for standardized neonatal pain management in preterm infants. Associations between greater early-life pain and adverse motor outcomes differed by duration of morphine exposure in our study and further clinical trials of optimal treatment approaches with morphine in preterm infants are warranted. Sucrose use was associated with less morphine and fentanyl use. However, greater early-life pain was associated with lower cognitive and language scores in infants exposed to sucrose, emphasizing a need for further studies of long-term neurodevelopmental outcomes in very preterm infants exposed to sucrose.

CHARACTERIZATION OF HMGB1 MEDIATED NEUROINFLAMMATION AFTER MODELED TBI

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Background: Traumatic brain injury (TBI) is a leading cause of death and disability world-wide. Secondary injury due to aberrant immune response can contribute to poor outcome. Purpose: Neuroinflammation is a major contributor to secondary injury mechanisms and is a potential target for therapeutic intervention.

Hypothesis: We propose that HMGB1 and its receptor RAGE contribute negatively to the neuroinflammatory response after TBI. HMGB1, a chromatin-associated protein located in the nucleus, acts as a cytokine, and binds to the RAGE receptor resulting in activation of NF-kB mediated pro-inflammatory pathways.

Methods: We have characterized the expression of HMGB1 and RAGE expression in control and injured rats. We used immunohistochemistry and western blot analysis to examine expression at 24 hours, 7 days, and 2 weeks post-injury. To further elucidate the signalling pathways associated with HMGB1 and RAGE expression after injury we are using microglia and primary neuronal cell cultures to examine changes in expression following in vitro stretch trauma. We propose to use inhibitors of RAGE and HMGB1 to parse out the signalling mechanisms involved in expression changes after injury. Furthermore, we aim to determine whether conditioned media from injured neurons and microglia is sufficient to induce a feedforward mechanism of HMGB1 release in non-injured cells.

Results: Our preliminary data suggest a role for HMGB1 and its receptor, RAGE, in the inflammatory response following TBI. We observed expression and translocation of RAGE following injury in neurons and microglia.

Conclusion: These molecules may be potential targets for therapeutic intervention.

TRANSLOCATOR PROTEIN IN OCCUPATIONAL POST-TRAUMATIC STRESS DISORDER: PRELIMINARY FINDINGS USING THE [18F]FEPPA PET RADIOLIGAND

STUDENT: SARAH WATLING
SUPERVISOR: DR. ISABELLE BOILEAU

Introduction: Posttraumatic stress disorder (PTSD), a psychiatric illness developed in response to experiencing or witnessing a traumatic event, is more prevalent in high-risk cohorts (e.g., military and first responders) compared to the general population. Researchers are trying to understand the underlying biology to inform effective drug development. Inflammation, and more specifically, the 18 kDa mitochondrial translocator protein (TSPO, a marker of gliosis in neuropsychiatric disease), has been implicated in the pathophysiology of PTSD. To date, two positron emission tomography (PET) studies have investigated brain TSPO. One study reported decreased TSPO in patients diagnosed with PTSD while another study reported increased TSPO in 9/11 first responders reporting PTSD related symptoms. Therefore, more research is required to fully comprehend potential immune-dysregulation in PTSD. Accordingly, the purpose of this study was to utilize PET of the 2nd generation TSPO probe [18F]FEPPA to investigate TSPO binding in humans with occupational related PTSD. In line with preclinical research and Bhatt and colleagues (2020), we hypothesized that [18F]FEPPA binding would be lower in PTSD compared to Healthy Controls.

Materials and Methods: TSPO binding was measured with PET and arterial sampling in 15 participants who fulfilled DSM-IV/5 criteria for PTSD and 17 healthy controls (HC). A magnetic resonance image was acquired for delineation of regions of interest (ROIs). A repeated-measures analysis of covariance (ANCOVA) was employed to evaluate group differences within 6 ROIs in the limbic-striatum (prefrontal cortex [PFC], anterior cingulate cortex [ACC], insula, striatum, hippocampus, amygdala), controlling for the TSPO polymorphism (rs6971).

Results: The PTSD (mean age: 45) and HC (mean age: 31) groups did not differ in sex, TSPO polymorphism, ethnicity, body mass index, or years of education ($p > 0.05$); however the PTSD group was significantly older than the HC group ($p=0.005$). The PTSD group scored significantly higher on questionnaires assessing depression and anxiety. Fourteen of fifteen participants with PTSD were on medication at the time of the study. A repeated-measures ANCOVA with TSPO polymorphism as a covariate revealed no significant difference in TSPO binding between PTSD and HC ($F(1,26)=0.220$, $p=0.643$) and there was no evidence for a group by ROI interaction ($F(2.543,66.109)=1.203$, $p=0.313$). A second ANCOVA controlling for TSPO polymorphism and age also demonstrated no main group effect ($F(1,25)=0.672$, $p=0.420$) and no interaction ($F(2.576,64.4)=0.845$, $p=0.460$). Interestingly, there was a marginally significant group by genotype interaction ($F(1,23)=2.87$, $p=0.101$), whereby TSPO binding was 34% lower in PTSD middle affinity binders (MABs) compared to HC MABs, and 46% higher in PTSD high affinity binders (HABs) compared to HC HABs. TSPO binding in the amygdala was significantly correlated with Beck Depression Inventory Scores ($p=0.023$, $R=0.7$).

Conclusion: The current study investigating TSPO binding, an index of microglia status, in a cohort of patients with occupational related PTSD did not replicate the recent finding of decreased TSPO in this trauma and stressor related disorder. This finding highlights the importance of characterising the type of trauma in study samples. Furthermore, lower TSPO binding in PTSD MABs and higher TSPO binding in PTSD HABs was an interesting, unexpected finding. This group by genotype interaction merits further investigation to better understand the potential role this genotype may or may not have in PTSD.

Group F: Neuroscience-Brain Health

ADAPTATION & EVALUATION OF AN EXISTENTIALLY ORIENTED GROUP PSYCHOTHERAPY INTERVENTION FOR WOMEN UNDERGOING FERTILITY TREATMENT: A MIXED METHODS FEASIBILITY STUDY

STUDENT: ANDRIA AIELLO
SUPERVISOR: DR. ROBERT MAUNDER
CO-SUPERVISOR: DR. MARY JANE ESPLEN

Background: For many women, the experience of undergoing fertility treatment evokes profound existential concerns related to their sense of self, their identity, and their sense of meaning and purpose. While multiple existentially oriented group psychotherapies have been developed for women with breast cancer and patients with advanced cancer, and subsequently adapted for other medical populations, there are no existing studies evaluating the effectiveness of interventions rooted in existential philosophy for women undergoing fertility treatment.

Purpose: To test the feasibility of an existentially oriented group psychotherapy intervention (EOGPI), which will be adapted and manualized for women undergoing fertility treatment. Research Questions: (1) What are the existential concerns of women undergoing fertility treatment, and (2) what is the feasibility of an EOGPI adapted for this population?

Hypothesis (for quantitative phase): An EOGPI adapted for women undergoing fertility treatment is feasible. Exploratory Hypothesis (for quantitative phase): Measures of psychological distress, self-concept, self-esteem, and quality of life will differ pre- and post-intervention.

Methods: A mixed methods exploratory sequential design is being used to first explore qualitatively to develop a context specific EOGPI that will be quantitatively tested. The first phase of the study involved the development of an Existential Theory of Women in Fertility Treatment based upon a comprehensive review of the literature. The current phase of the study involves a qualitative exploration of the existential concerns of women undergoing fertility treatment in which qualitative interview data is being collected from a purposeful sample of women at Mount Sinai Fertility in Toronto. From this initial exploration, the qualitative findings will be used to further develop and refine our Existential Theory. Our refined theory will then be used to adapt an EOGPI for women undergoing fertility treatment that can be administered to a larger sample. In the tentatively planned quantitative phase, data will be collected from a convenience sample of women at Mount Sinai Fertility to test the feasibility of the EOGPI.

Results (to date): Based upon a comprehensive review of the literature, we have developed an Existential Theory of Women in Fertility Treatment, which captures the experiences of women undergoing fertility treatment through the lens of existentialism and the four ‘ultimate concerns’ of human existence, namely death, freedom, isolation, and meaninglessness. Our Existential Theory incorporates the dialectical dimensions of challenge and opportunity associated with each concern as they pertain to the experience of fertility treatment. Preliminary themes from the interviews conducted thus far include: (1) loss and grief, (2) uncertainty, liminality, and lack of control, (3) alienation from the fertile world, and (4) reflecting on meaning and purpose.

Conclusions (to date): The preliminary qualitative themes validate our current Existential Theory of Women in Fertility Treatment, suggesting that the theory is appropriate to inform the EOGPI.

INVESTIGATING THE IMPACT OF NEUROGENESIS-MEDIATED SYNAPTIC REMODELING ON REPRESENTATIONAL DRIFT IN THE HIPPOCAMPUS

STUDENT: MITCH DE SNOO
SUPERVISOR: DR. PAUL FRANKLIN

Background: Stable neural ensembles are often thought to underly stable learned behaviours and memory. However, recent longitudinal imaging experiments in animals stably exposed to the same stimuli, or repeatedly performing the same task, have demonstrated that there is instability in the neural representations produced in many brain areas including the sensory cortex, the hippocampus, and motor cortex. This phenomenon is referred to as representational drift, and while there are potential neurobiological mechanisms, none have been tested directly. For example, it is possible that the ongoing turnover of synaptic connections between neurons drives representational drift in neural population codes. I aim to test this possibility by experimentally modulating levels of hippocampal neurogenesis, a unique plasticity process that is known to actively remodel hippocampal synaptic connectivity, while observing changes in the neural representation of a familiar spatial environment.

Hypothesis: Increasing and decreasing the rate of hippocampal neurogenesis will alter the degree of synaptic remodeling within the hippocampal circuitry and result in accelerated and attenuated representational drift, respectively.

Methods: Transgenic mice expressing the genetically encoded calcium indicator, GCamP6f, were implanted with miniaturized endoscopes above the CA1 region of their hippocampus to image their neural activity. Implanted mice were divided into control (CTR) and experimental groups that had their levels of adult neurogenesis either reduced by whole brain γ-irradiation (IRR) or enhanced through home cage access to a voluntary running wheel (RUN). The mice were then trained to navigate a linear track while their neural activity was imaged over two weeks. Comparisons in hippocampal spatial representation were made between CTR, IRR, and RUN mice.

Results: Irradiation and voluntary exercise were confirmed to decrease and increase the rate of adult neurogenesis, respectively. CTR, IRR, and RUN mice all form comparable spatial representations of the linear track with similar numbers and distributions of place cell fields. Preliminary results indicate IRR animals show a trend towards greater stability in their spatial representation of the linear track and RUN animals show a trend towards greater instability.

CHARACTERIZING ANHEDONIA AS A CLINICAL AND NEUROBIOLOGIAL MARKER OF SUICIDALITY

STUDENT: MOLLY HYDE
SUPERVISOR: SAKINA RIZVI
CO-SUPERVISOR: SIDNEY KENNEDY

Background: Anhedonia (a loss of interest and pleasure) is a potential trait marker of suicidality, which may be indexed by a deficient reward syndrome. Reduced response to rewarding stimuli and difficulty adapting behavior to acquire it have been reported in suicide attempters (SA). While aberrations in brain reward networks that subserve anhedonia are frequently reported in major depressive disorder (MDD), the functioning of reward neurocircuitry in suicide risk has not been extensively studied. Given that anhedonia may be predictive of suicide risk, it may also predict whether at-risk individuals respond to suicide intervention. The Brief-"Skills for Safer Living" (SfSL), is a novel single session individual psychotherapy delivered via online videoconference to mitigate risk in suicidal patients. B-SfSL emphasizes safety planning, skills training, and engaging in opportunities for positive reinforcement.

Objectives: To determine the extent of reward deficits and their underlying neural correlates in depressed patients with suicide risk (Study 1) and evaluate the stability over one year (Study 2); and determine the ability of anhedonia to predict reduction in suicide risk 3 months following B-SfSL (Study 3).

Methods: In Study 1 and 2, I will collect and analyze functional magnetic resonance imaging (fMRI) and clinical data from 90 MDD (60 SA; 30 non-attempters) patients and 30 healthy controls at baseline and after 1 year. An effort-based decision task will be used to identify abnormal neural activation reflecting impaired encoding of effort and reward integration in SA. In Study 3, I will recruit 50 depressed individuals with suicide risk across Canada to receive open-label B-SfSL. Patients will complete baseline self-report measures of suicide severity and anhedonia, as well as computerized cognitive and reward tasks. A single session of B-SfSL will be delivered and follow-up clinical assessments will be performed at 1 week, 1 month, and 3 months.

Results: To date, 22 patients and 21 HCs have completed Study 1 and Study 2. In Study 3, 27 participants have received B-SfSL and are in follow-up.

Conclusion: By revealing correlates of reward function within a longitudinal design, I will determine whether some patients will benefit from intervention compared to others and whether anhedonia is reflective of a state or trait in SA. In addition to direct patient benefit, my research will assist with transforming mental health systems for those at risk for suicide.

AN ARTIFICIAL INTELLIGENCE-DRIVEN TRIGEMINAL NEURALGIA GRADING SYSTEM

STUDENT: TIMUR H. LATYPOV
SUPERVISOR: MOJGAN HODAIE

Background: Trigeminal neuralgia (TN) is a severe chronic neuropathic facial pain condition. TN patients face intense shock-like paroxysms of pain. They can also experience longer bouts of pain, or a transformation of their pain into one that is constant in nature, with burning or dull features. Given the patterns of pain expression over time, we hypothesize that TN is a singular pain syndrome that evolves and exhibits a spectrum of grades associated with different brain imaging correlates and pain characteristics, rather than discrete pain subtypes. These correlates can be assessed using artificial intelligence (AI) techniques.**Purpose:** In this two-armed study, we apply AI on brain imaging and clinical data to propose a novel grading system for TN pain based on patients' duration of surgical response. This framework facilitates a combined assessment of TN progression in accordance with surgical response duration using (1) brain imaging data through a convolutional neural network classification task, and (2) clinical characteristics through a dimensionality reduction task. Our approach offers objective insights derived from imaging data, while leveraging the individual characteristics of pain using clinical data. **Methods:** We included 95 classical TN patients with 3T T1-imaging data who were treated with either Gamma Knife radiosurgery (GKRS) or microvascular decompression (MVD). Surgical response was defined as a ≥ 50% reduction in pain intensity, and a score of I-III on the Barrow Neurological Institute (BNI) scale. Duration of response was defined as the time between surgery and pain recurrence, measured by a reversion to a score of IV-V on the BNI scale within a five-year follow-up period. The first arm of this study combined structural imaging data with a convolutional neural network classifier (implemented in PyTorch) to predict surgical response (non-responder vs. responder) and surgical response duration (< 5 years pain relief vs. ≥ 5 years pain relief), after surgical intervention. Classifiers were trained using voxel-wise intensity measures obtained from skull-stripped T1-weighted images. Model performance assessment and hyperparameter tuning was performed using a 10-fold cross validation algorithm. The second arm combined retrospective clinical data and dimensionality reduction machine learning algorithms to produce a prediction framework of pain relief duration after surgery. Clinical data was one-hot-encoded for principal component analysis (PCA). PCA was applied to compute novel 'pain grades'. Correlation of principal components (PCs) and duration of surgical response was assessed using the Spearman correlation test, and significance was evaluated using Bonferroni multiple-comparison correction. **Results:** In the first arm, the surgical response and response duration classification tasks performed with accuracies of 86% in distinguishing responders from non-responders and 78% in distinguishing < 5 year-responders from ≥ 5 year-responders, respectively. Therefore, this classification framework identifies responders from non-responders, while also estimating responders' duration of post-surgical pain relief. In the second arm, the PCA of raw and feature selected data yielded 19 and 15 PCs, respectively. In both trials, PC1, largely representing TN pain-related variables, showed a significant negative correlation with duration of surgical response ($r = -0.48$ ($p = 0.0007$) and $r = -0.51$ ($p = 0.0002$), respectively). These findings indicate that PC1 may be defined as a novel measure for disease severity in TN patients. **Conclusion:** In this study, we demonstrate a data-driven approach to derive a novel grading system for TN based on brain imaging and clinical characteristics that predict surgical response duration. This framework may provide a foundation for the future development of AI-driven, clinical tools for TN assessment and surgical outcome prognostication.

FUNCTIONAL ANALYSIS OF CHD2 AND CHD8 DE NOVO MUTATIONS AND RELATED MOLECULAR EVENTS INVOLVED IN NEURODEVELOPMENTAL DISORDERS

STUDENT: TAHIR MUHAMMAD
SUPERVISOR: DR. JOHN B. VINCENT

Intellectual disability (ID) is associated with impaired intellectual and adaptive functioning while autism spectrum disorder (ASD) is characterized by deficits in social communication accompanied by the presence of limited interests and repetitive behaviors. Together, ID and ASD affect between 3-5% of the global population and pose a huge challenge to clinicians in diagnosis. Chromodomain helicase DNA-binding (CHD) proteins are important factors for remodeling chromatin and for gene regulation. To date, nine CHD proteins have been identified in humans and de novo variants in CHD2 and CHD8 have been reported in numerous ASD genetic studies and appear to be among the most common and consistent mutations associated with NDDs. CHD2 and CHD8 pathogenic variants have extensively been reported to cause a brain-related phenotype, demonstrating the specific roles of these two in NDDs. The initial goals of this study are to evaluate the effects of CHD2 & CHD8 pathogenic missense mutations on DNA/chromatin binding dynamics, on protein stability and gene expression, and to assess the effects of mutations in these genes on neuronal morphology and differentiation. Preliminary findings revealed that these mutations affect the expression of key methyltransferases that are required for numerous gene expressions and regulation. Similarly, ChIP data shows that these mutations also affect the binding dynamics of the CHD2 and CHD8 proteins. Currently, in utilizing the CRISPR/Cas9 system to knockout CHD2 and CHD8, and to introduce de novo missense mutations in HEk293T, mouse embryonic stem cells (P19) and iPSCs primary cells. Based on in vitro findings, we will generate mouse models with the most pathogenic mutations and examine the effects of those mutations on social behavior and/or biochemical analysis of these mutations on neuronal development and differentiation.

A LONGITUDINAL ANALYSIS OF CEREBRAL BLOOD FLOW CHANGES IN GENETIC FRONTOTEMPORAL DEMENTIA

STUDENT: MAURICE PASTERNAK
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Background: Mutations in the C9orf72, GRN, or MAPT genes are the most prevalent genetic causes of familial frontotemporal dementia (FTD). In a cross-sectional study of genetic FTD, lower cerebral blood flow (CBF) was observed in presymptomatic FTD mutation carriers vs. controls from the same families in 6 regions of interest: the bilateral anterior cingulate cortex, the left medial temporal gyrus, the left and right insulae, and the left and right supramarginal gyri.1

Purpose: There have been no studies to date that identify longitudinal changes in CBF between FTD genetic subgroups (both presymptomatic and symptomatic) compared to controls. As such, this study serves to re-evaluate the previous findings under a longitudinal context.

Hypotheses: We hypothesize that CBF signatures previously identified in the cross-sectional study will generally be replicated, with FTD mutation carriers featuring lower CBF over time. Furthermore, these signatures will differ by FTD genetic subgrouping.

Methods: We compared longitudinal changes in CBF, as measured by ASL-MRI, in 317 FTD mutation carriers (118 C9orf72, 141 GRN, and 58 MAPT) vs. 261 non-carrier controls from the Genetic FTD Initiative (GENFI). Linear mixed effects models tested the main effect of carrier status, and its interaction with age, along with covariates of age, sex, site-of-scan against each subject's mean regional cerebral blood flow within the regions of interest from previous studies.1 Family membership was a random intercept in the model to control for similar genetic and environmental backgrounds.

Results: Differences between genetic subsets of FTD were apparent, with GRN carriers preferentially experiencing decreases within the salience network regions while MAPT carriers expressed differences within the left medial temporal gyrus and bilateral anterior cingulate gyrus. C9orf72 carriers only saw a decrease in CBF within the left insula. Significant interactions between carrier status and age were exclusively evident in MAPT carriers across all regions of interest.

Conclusions: Differentiating CBF signatures were found to exist between the genetic FTD subgroups, with the most prominent changes being seen in GRN and MAPT subsets. Evidence continues to mount that CBF may be a viable biomarker in the earlier detection and delineation of genetic FTD variants.

DOWNSTREAM OPEN READING FRAMES IN THE PTCHD1-C TRANCRIPT MAY MITIGATE SOCIAL DEFICIENCIES IN PTCHD1D2 MICE

STUDENT: STEPHEN F. PASTORE
SUPERVISOR: DR. JOHN B. VINCENT

Background: Patched domain-containing 1 (PTCHD1) is a risk factor gene for autism, and is predicted to produce a multi-pass protein encoded by three exons. Two isoforms have been identified in the brain: PTCHD1-a (exons 1-3; high expression), and PTCHD1-c (exons 1 and 3; low expression). To investigate the role of Ptchd1 on behaviour and cognition, researchers have deleted exon 2 (Ptchd1D2). This relegates expression to only the Ptchd1-c isoform; absence of the 661-bp exon 2 creates a frameshift that is predicted to truncate Ptchd1. Ptchd1D2 mice recapitulate many clinical symptoms of ASD, but unexpectedly do not exhibit social deficits. Our collaboration has generated a mouse model with a mutation disrupting a downstream ORF that exists in the final exon of the Ptchd1-c transcript (Ptchd1G387Vfs*2), and these mice display pronounced social deficits.
Purpose: This study seeks to determine a molecular mechanism for the social phenotypic disparity between the Ptchd1D2 and Ptchd1G387Vfs*2 mice.
Hypothesis: We hypothesize that the downstream ORF in the final exon of the Ptchd1-c transcript may be used to translate one or more C-terminal Ptchd1 proteins that retain some degree of wildtype function, collectively mitigating social deficiencies in the Ptchd1D2 mice.
Methods: To investigate translation from exogenous Ptchd1 transcripts, the Ptchd1-a isoform was first amplified by RT-PCR, cloned into an expression vector and fused with a C-terminal 3xFlag epitope tag. Site-directed mutagenesis was then used to generate Ptchd1D2 and Ptchd1G387Vfs*2 constructs. These constructs were transiently transfected into HEK293T cells for 48 hours, at which point total protein was isolated and quantified using the Bradford assay, followed by western blotting of the protein lysates. To examine endogenous Ptchd1, CRISPR-Cas9 was first used to insert a 3xFlag epitope tag at the C-terminus of Ptchd1 (Ptchd1-3xFlag) in the male mouse embryonal carcinoma cell line P19. CRISPR-Cas9 was next used to generate subclones analogous to the two mouse models, with either a deletion of exon 2 (Ptchd1D2-3xFlag) or a disruption of the downstream ORF (Ptchd1G387Sfs*44-3xFlag). Transgenic P19 lines were differentiated into neurons using retinoic acid and fractionated by differential centrifugation to enrich for synaptosomal proteins. Lastly, protein fractions were analyzed by western blotting.
Results: In HEK293T cells, western blotting detected Ptchd1 protein from the Ptchd1-a transcript, but N-truncated Ptchd1 was not generated from the Ptchd1D2 or Ptchd1G387Vfs*2 constructs. Conversely, western blotting revealed that Ptchd1D2-3xFlag neurons endogenously express several putative N-truncated Ptchd1 proteins. These products approximately correlate with computationally-predicted alternative start codons, and are all absent in Ptchd1G387Sfs*44-3xFlag neurons.
Conclusion: The downstream ORF in Ptchd1-c appears to produce putative N-truncated Ptchd1 proteins which may prevent social deficits in Ptchd1D2 mice.

SENSORY ARTIFACTS IN SUPRATHRESHOLD TMS-EEG OF DLPFC

STUDENT: MOHSEN POORGANJI
SUPERVISOR: DR. DANIEL BLUMBERGER

Objective: Combined transcranial magnetic stimulation and electroencephalography (TMS-EEG) is an effective way to evaluate neurophysiological processes at the level of the cortex. To further characterize the TMS-evoked potential (TEP) generated with TMS-EEG, beyond the motor cortex, we aimed to distinguish between cortical reactivity to TMS versus non-specific somatosensory and auditory artifacts using both single-pulse and paired-pulse protocols at suprathreshold stimulation intensities over the left dorsolateral prefrontal cortex (DLPFC).

Method: Fifteen right-handed healthy participants received six blocks of stimulation including single and paired TMS delivered as active-masked (i.e., TMS-EEG with auditory masking and foam spacing), active-unmasked (TMS-EEG without auditory masking and foam spacing) and sham (sham TMS coil). We evaluated cortical excitability following single-pulse TMS, and cortical inhibition following a paired-pulse paradigm (long-interval cortical inhibition (LICI)).

Hypothesis: We hypothesized that single-pulse TMS and paired-pulse protocol would result in larger response amplitudes in the active-unmasked condition compared to the active-masked (without the same degree of sensory co-activation) and sham (sensory elicitation only - without direct TMS brain responses) conditions. Moreover, we hypothesized that the scalp topographic distribution of cortical evoked activity would be different between each of the three conditions. Finally, we hypothesized that LICI (signal inhibition) would be significant in both masked and unmasked conditions but not in the sham condition.

Results: Repeated measure ANOVAs revealed significant differences in mean cortical evoked activity (CEA) of active-masked, active-unmasked, and sham conditions for both the single-pulse ($F(1.76, 24.63)=21.88$, $p<0.001$, $\eta2=0.61$) and LICI ($F(1.68, 23.49)=10.09$, $p<0.001$, $\eta2=0.42$) protocols. Furthermore, global mean field amplitude (GMFA) differed significantly across the three conditions for both single-pulse ($F(1.85, 25.89)=24.68$, $p<0.001$, $\eta2=0.64$) and LICI ($F(1.8, 25.16)=14.29$, $p<0.001$, $\eta2=0.5$). Finally, only active LICI protocols but not sham stimulation ([active-masked (0.78 ± 0.16, $P<0.0001$)], [active-unmasked (0.83 ± 0.25, $P<0.01$)]) resulted in significant signal inhibition.

Conclusion: While previous findings of a significant somatosensory and auditory contribution to the evoked EEG signal is replicated by our study, an artifact attenuated cortical reactivity can reliably be measured in TMS-EEG signal with suprathreshold stimulation of DLPFC. Artifact attenuation can be accomplished using standard procedures and even when masked, the level of cortical reactivity is still far above what is produced by sham stimulation. Furthermore, our study illustrates that TMS-EEG of DLPFC remains a valid investigational tool.

EVALUATION OF NEUROINFLAMMATORY RADIOTRACERS IN CHRONIC TRAUMATIC ENCEPHALOPATHY

STUDENT: CASSIS VARLOW
SUPERVISOR: DR. NEIL VASDEV

Background: Chronic traumatic encephalopathy (CTE) is a neurological disorder associated with head injuries and is diagnosed upon autopsy. Positron emission tomography (PET) imaging of neuroinflammatory processes following injury could enable ante-mortem diagnosis of CTE for the first time and insights into monitoring disease progression and therapeutic interventions.
Purpose: The present study evaluates four tritium-labeled PET tracers for neuroinflammatory targets: [3H]PBR-28 for the 18 kDa translocator protein (TSPO), [3H]L-deprenyl for monoamine oxidase-B (MAO-B), [3H]CPPC for colony stimulating factor-1 receptor (CSF-1R) and [3H]SMW-139 for the P2X7 purinergic receptor, using in vitro radioligand binding assays in pathologically diagnosed cases of human CTE brains.
Hypothesis: PET imaging of neuroinflammatory biomarkers will detect changes in immune response between CTE and HC.
Methods: Thin-section autoradiography was employed to assess specific binding and distribution of [3H]PBR-28, [3H]L-deprenyl, [3H]CPPC and [3H]SMW-139 in fresh-frozen human post-mortem CTE frontal cortex. [3H]PBR-28 activity was quantified by autoradiography and correlated with TSPO expressing cells by immunohistochemistry. [3H]L-deprenyl signal was detected by autoradiography and correlated with GFAP positive cells by immunofluorescence. TSPO cell-type expression was shown by immunofluorescent co-localization to Iba1, GFAP and CD68 positive cells. Target density (Bmax) of TSPO and MAO-B in both CTE and HC tissue was quantified by saturation analysis.
Results: [3H]CPPC demonstrated low specific binding (15.34 ± 8.12%; n = 4) and [3H]SMW-139 had negligible radiotracer signal in CTE tissues and were not further evaluated. [3H]PBR-28 revealed high specific binding in both CTE (95.40 ± 1.87%; n = 11) and healthy controls (HC; 89.89 ± 8.52% , n = 3) and co-localized to TSPO immunostaining. TSPO expression was localized to Iba1, GFAP and CD68 positive cells, indicating microglial, astrocytic and macrophage expression in CTE. [3H]L-deprenyl displayed high specific binding in CTE (96.95 ± 1.43%; n = 12) and HC (93.24 ± 0.43%; n = 2), with distribution co-localized to GFAP positive cells. Using [3H]PBR-28 the Bmax of TSPO in HC was 177.91 ± 56.96 nM (n = 7; mean ± SD) however, a highly variable Bmax (345.84 ± 372.42 nM; n = 11; mean ± SD) was measured in CTE. [3H]L-deprenyl quantified a MAO-B Bmax of 304.23 ± 115.93 nM (n = 8; mean ± SD) in HC tissue and is similar to the Bmax in CTE tissues (365.80 ± 128.55 nM; n = 12; mean ± SD). A two-sample t-test determined no significance in TSPO or MAO-B Bmax values between HC and CTE ($P > 0.05$), albeit a trend of increased TSPO and MAO-B expression was observed in CTE compared to HC.
Conclusions: To our knowledge, this work represents the first in vitro evaluation of neuroinflammatory biomarkers for PET imaging in CTE tissue and reveals the variability in neuroinflammatory pathology of head injuries. PET imaging of TSPO and MAO-B in patients after head injuries could be used to quantify neuroinflammation and shows potential toward the ultimate goal of imaging CTE in the living human brain.

Group G: Cancer

MULTIFOCAL EXTRAMURAL VENOUS INVASION DETECTED WITH AN ELASTIN STAIN IS A POWERFUL PREDICTOR OF CANCER-SPECIFIC OUTCOMES IN STAGE I-III RESECTED COLORECTAL CANCER

STUDENT: DAVID P CYR
SUPERVISOR: CAROL J SWALLOW

Background. ExtraMural Venous Invasion (EMVI) is considered an indicator of poor prognosis in patients who have undergone resection of primary colorectal cancer (CRC), but its use has not been widely adopted in staging systems or nomograms. Staining for elastin may facilitate the accurate detection of EMVI and minimize interobserver variability, as well as enable the assessment of specific features of EMVI including focality and size.

Purpose. Examine the prognostic potential of EMVI detected by elastin staining at a tertiary center that performs a high volume of CRC resections.

Methods. This is a single-institution, observational study of consecutive patients who underwent resection of primary CRC between 01/2011 and 12/2016 (n=585). All pathology specimens were re-assessed by expert reviewers who were blinded to patient outcomes. Venous invasion was detected using an elastin trichrome stain and classified as IntraMural or ExtraMural. The number of VI foci, as well as the maximum foci width and length, were also determined. Disease-specific and recurrence-free survival (DSS, RFS) were estimated using the Kaplan-Meier method and group differences were assessed using the log-rank test. Cox proportional-hazard models were used to calculate hazard ratios (HR) and 95% CI. For the present analysis, patients with stage IV (n=87) CRC, grossly positive resection margin (n=2) and post-operative mortalities (n=15) were excluded.

Results. The study cohort included 481 patients (269M, 212F; AJCC TNM 8th edition Stage I/II n=301; Stage III n=180) with a median follow-up time of 70 months (0.1-131). EMVI was detected in 34% of all cases (25% in Stage I/II vs. 56% in Stage III; p<0.0001). For the entire cohort, DSS and RFS at 5 years were 88% and 76%, respectively. The presence of EMVI was associated with significantly worse DSS and RFS at 5-years (77% and 56%) compared to patients with no VI (94% and 86%) or IMVI alone (93% and 89%; p<0.0001). The majority of EMVI was multifocal (69%) vs. unifocal (31%). Interestingly, multifocal EMVI was prognostic for worse 5-year DSS (69%), whereas unifocal EMVI was similar to the absence of EMVI (94% and 93%, respectively; p<0.0001). A Cox-proportional hazards model including T and N stage showed worsening DSS with increasing number of detected EMVI foci (1 focus: HR 0.6, 2-4 foci: HR 2.1, >4 foci: HR 5.9; p<0.0001). A similar trend was observed for RFS. Neither the maximum width or length of EMVI foci were prognostic of DSS or RFS.

Conclusions. To our knowledge, the prognostic role of EMVI focality in CRC has not been previously explored. In this cohort of Stage I - III CRC patients, multifocal EMVI as assessed by elastin staining was a powerful predictor of cancer-specific death and recurrence-free survival. Elastin staining, which improves the accuracy and objectivity of EMVI detection, may allow validation of EMVI as an independent prognostic variable that should be incorporated into staging systems and nomograms.

TRAJECTORIES OF BLOOD COUNTS DURING CYTOTOXIC CHEMOTHERAPY

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SUPERVISOR: STEVEN GALLINGER

Background
Cytotoxic chemotherapy for cancer causes reductions in blood counts including red blood cells, platelets, and neutrophils, which is called cytopenias. Cytopenias place patients at risk of anemia, bleeding, and infection. Scarce data exists on population-level blood count trajectories after cytotoxic chemotherapy and their relationship with patient-level characteristics.

Purpose
We aimed to define population-level trajectories of blood counts during cytotoxic chemotherapy and to develop machine-learning systems that predict blood counts after chemotherapy.

Hypothesis
We hypothesize that blood count trajectories can be inferred from administrative data and cytopenias after chemotherapy can be predicted from features available in administrative data.

Methods
We identified individuals treated with cytotoxic chemotherapy in Ontario between July 1, 2014 and June 30, 2020 within the Activity Level Reporting database, a population-level dataset Cancer Care Ontario/Ontario Health held at ICES. For each day after chemotherapy, we estimated the proportion of patients with low hemoglobin, platelets, and neutrophils as defined by the Common Terminology Criteria for Adverse Events v5.0. We developed and evaluated machine-learning models to predict cytopenias when patients are due for their next chemotherapy treatment. We evaluated models using the final year of data.

Results
We analyzed over 400,000 blood count measurements from over 40,000 patients. We defined empirical blood count trajectories after each chemotherapy regimen; for example, after treatment with carboplatin and paclitaxel every three weeks, 21% of patients face grade 2 neutropenia on day 14. Grade 2 anemia, thrombocytopenia, and neutropenia were present when patients were due for their next chemotherapy after 17.8%, 2.6%, and 11.0% of chemotherapy treatments, respectively. In the held-out evaluation year starting July 1, 2019, machine learning models accurately predicted anemia (area under the receiving operating characteristic curve 0.96), thrombocytopenia (0.95), and neutropenia (0.88).

Conclusions
We present the first empirical population-level trajectories of blood count during cytotoxic chemotherapy treatments and demonstrate that patient-level cytopenias can be accurately predicted after chemotherapy. Future work should focus on deployment of the predictive models in clinical practice, accounting for potential differences across health systems.

ANALYSIS OF PROGNOSTIC GERMLINE POLYMORPHISMS IN PATIENTS WITH ADVANCED HEPATOCELLULAR CARCINOMA

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SUPERVISOR: DR. GEOFFREY LIU

Introduction: Hepatocellular carcinoma (HCC) is a leading cause of death globally and sorafenib, an oral tyrosine kinase inhibitor targeting multiple cellular receptors including VEGFR, is a mainstay of treatment.

Purpose: Accurate estimation of prognosis of advanced HCC is critical for clinical decision making. Existing prognostic frameworks are still limited in determining patient prognosis in advanced HCC, and do not incorporate any information about single nucleotide polymorphisms (SNPs). Validation of existing SNPs, as well as analysis of novel exploratory SNPs has the potential to improve estimates of prognosis.

Hypothesis: SNP's influential in the prognosis of patients with incurable HCC treated with sorafenib and can be externally validated.

Methods: Our aim was to validate SNPs prognostic of outcome in advanced HCC from the literature, and to analyze exploratory SNPs chosen from evaluation of the HCC tumor immune microenvironment. Using a database of patients with HCC treated with sorafenib, blood samples were genotyped, clinical variables were retrospectively collected, and SNPs were analyzed for association with PFS and OS.

Results: Literature review identified 7 SNPs in VEGF, eNOS, ANGPT2 and VEGFR2, however none were externally validated in our dataset. Of the 35 exploratory SNPs, the following were associated with PFS or OS: rs1024611, rs1800896, rs231775, and rs28362491 with hazard ratios between 1.14-1.5.

Conclusion: SNPs identified by literature review to be prognostic in sorafenib treated patients with advanced HCC were not validated in our dataset. Our findings suggest potentially important prognostic implications of SNPs in VEGFR2, CCL2, IL-10, CTLA-4 and NFKB1 that deserve further study.

NON-INVASIVE BRAF STATUS DIFFERENTIATION OF PEDIATRIC LOW-GRADE GLIOMAS

STUDENT: KAREEM KUDUS
SUPERVISOR: DR. FARZAD KHALVATI

Background: To create a treatment plan for pediatric low-grade glioma (pLGG) patients it is currently necessary to determine the molecular landscape of pLGG (BRAF status) through biopsy. There are, however, multiple drawbacks to biopsy, such as infection and hemorrhage. Previously, our group showed that it is possible to predict the BRAF status of pLGG patients using machine learning models trained on quantitative radiomic features extracted from FLAIR MR images, a non-invasive approach that alleviates the risks and concerns associated with biopsy.

Purpose: Design a novel thresholding approach to improving model performance. Build a nomogram, a graphical representation of our model, to drive clinical adoption. Replace the radiomics model with a neural network.

Hypothesis: New thresholding approach should increase model accuracy, at the expense of the number of patients the model will work for. Neural networks should produce better accuracy than the radiomics model, since they are a more powerful class of models that automatically detect important features in the MR image, unlike the radiomics approach, which relies on handcrafted algorithms to extract features from the image.

Methods: All patients were identified from the electronic health record database at the Hospital for Sick Children (internal dataset) and Lucile Packard Children's Hospital (external dataset) from 1999 to 2018. A total of 253 patients were included. All patients had either a 2D axial or coronal FLAIR sequence. Tumor segmentation was performed by a fellowship-trained pediatric neuroradiologist using 3D Slicer. Models were implemented using the Python programming language. Experiments were run under a nested cross-validation framework.

Results: New threshold approach increased the mean accuracy of the model from 84.5% to 92.2%, but the model made a prediction for 80.7% of patients, on average, rather than for all of them. Radiomics and neural network performed similarly (mean AUC of 0.880, and 0.885 respectively), but a combined model, which used a simple method of fusing the radiomics and neural-network predictions, resulted in a higher mean AUC of 0.902.

Conclusions: It is possible to increase the accuracy of the model using our new thresholding method. There is potential to build a more accurate model by combining the radiomics and neural-network approaches.

DEVELOPMENT OF AN IMPLEMENTATION STRATEGY FOR AN ELECTRONIC PROSPECTIVE SURVEILLANCE MODEL FOR CANCER REHABILITATION

STUDENT: CHRISTIAN LOPEZ
SUPERVISOR: DR. JENNIFER JONES

Background: Electronic prospective surveillance models (ePSMs) are online systems that regularly screen people diagnosed with cancer for their wellbeing and provide rehabilitation resources based on their responses. Clinical trials have demonstrated that ePSMs are effective at identifying and managing cancer-related impairments; however, less is known about the implementation of this intervention into routine clinical care. Implementation of evidence-based practices is highly dependent on local context, and tailoring implementation strategies to a given setting has been shown to increase implementation success.

Purpose: The purpose of this study was to develop a tailored implementation strategy for an ePSM called REACH by understanding potential barriers and facilitators to implementation.

Methods: This is a multi-centre, descriptive qualitative study. We recruited clinical staff from each site and tumour group under study, as well as members of the REACH Patient and Family Advisory Committee to participate in virtual qualitative interviews and focus groups. Interview questions and analyses were guided by the Consolidated Framework for Implementation Research (CFIR) and attempted to understand potential barriers and facilitators to implementing REACH. The CFIR identifies five implementation domains including the following: 1) characteristics of the intervention; 2) characteristics of the inner setting; 3) characteristics of the outer setting; 4) characteristics of the individual; and 5) the process of implementation.

Results: Fourteen interviews were conducted with a total of 41 clinic staff and 14 patients. At the intervention level, stakeholders highlighted the need to ensure REACH is perceived as easy to use and beneficial for patients. At the inner setting level, staff suggested that tasks to implement REACH should be compatible with existing clinic processes and that REACH should be integrated with other electronic systems already used by the setting. At the outer setting level, staff underscored the need to ensure there are adequate resources to meet the needs of patients identified by the REACH system. At the individual level, stakeholders indicated the need to have sufficient knowledge about REACH. Lastly, at the process level, staff suggested several methods to enhance patient awareness and uptake.

Conclusions: The findings from this study provide insight into determinants to implementation that may be targeted by a tailored implementation strategy for REACH. This strategy will be used to pilot the implementation of REACH through a one-year process evaluation to understand how and why implementation was or was not successful and identify changes that need to be made to the implementation plan.

IMPACT OF ISG15 OR ISGYLATION ON IRF3 ACTIVATION BY DAMPS IN IMMORTALIZED FALLOPIAN TUBE EPITHELIAL CELLS

STUDENT: VIDUSHI MADAAN
SUPERVISOR: THEODORE BROWN

Background: High-grade serous epithelial ovarian cancer (HGSOC) is a predominate lethal form of ovarian cancer thought to arise from fallopian tube epithelial (FTE) cells. Pathogenic germline mutations in *BRCA1* (DNA repair gene) predisposes to this cancer with other identified risk factors suggesting a role for recurrent exposure to follicular fluid. We previously reported that BRCA1 deficiency in FTE cells increased pro-inflammatory and cell proliferation signaling i.e. NF-κB and EGFR signaling with periovulatory follicular fluid exposure further increasing interferon-stimulated genes (ISGs), such as ISG15 and ISGylation pathway members. ISG15 can act as a free secreted or intracellular protein or can covalently conjugate to target proteins (ISGylation) to modulate their function. ISG15 and components of ISGylation pathway can be upregulated by the stimulation of cGAS/STING or MDA5/RIG-1 DNA or RNA Pattern Recognition Receptors (PPRs) that recognize aberrant nucleic acid accumulation (DAMPS) in cells which could accumulate due to BRCA1 insufficiency. Stimulation of PRRs results in the activation of IRF3, a major driver of the interferon pathway and NF-κB signaling. Synergistic activation of IRF3 and NF-κB induces pro-inflammatory cytokines, interferons, ISG15, and ISGylation components. ISGylation might in turn prolong pro-inflammatory interferon and NF-κB signaling in FTE cells by targeting MDA5, RIG-1, STING and key downstream proteins, in particular IRF3. Though, intracellular ISG15 or ISGylation can promote tumorigenesis in many cancer types and is upregulated in HGSOC, the mechanistic role of ISG15 or ISGylation in HGSOC development is unknown. **Purpose:** This study will elucidate whether ISGylation impacts MDA5-RIG-1 or cGAS-STING dependent activation of IRF3 and increase pro-inflammatory signaling that could contribute to HGSOC development. **Hypothesis:** ISGylation promotes pro-inflammatory signaling in FTE cells by increasing the activation of IRF3 via either the MDA-RIG1 or cGAS-STING pathway. **Methods:** Immortalized non-malignant FTE cells were characterized to expression of ISG15 and a functional ISGylation pathway using quantitative real-time PCR and western blot. These cells were subjected to prime editing and conventional CRISPR to disrupt *ISG15* and *UBE1L* expression. UBE1L is considered the sole E1 enzyme of ISGylation pathway. *ISG15* and *UBE1L* knock-out clones were screened and selected using restriction digestion and western blot analysis. These clones are currently being treated with an inducer of MDA5-RIG1 or an inducer of cGAS-STING to assess the impact of loss of ISG15 or ISGylation on IRF3 activation and downstream cytokine expression. ISGylation of key pathway components will be determined by immunoprecipitation and western blotting as appropriate. **Results:** Immortalized non-malignant FTE cells express ISG15, components of ISGylation pathway and exhibit functional ISGylation. Conventional CRISPR and Prime Editing effectively disrupted ISG15 and UBE1L expression in these cell lines. Selected clones were confirmed to express low to undetectable levels of ISG15, UBE1L and functional ISGylation. Experiments studying the effect of ISG15/ISGylation on IRF3 activation and PRR activation by DAMPS are on their way. **Conclusion:** This study will provide evidence as to whether an increase in ISG15 and members of the ISGylation pathway, such as that triggered by follicular fluid exposure, can lead to increased pro-inflammatory signaling in FTE cells. This work could identify a potential preventative strategies to mitigate risk of HGSOC, particularly in BRCA1 mutation carriers.

SELECTIVELY TARGETING THE EPIGENOME IN RHABDOMYOSARCOMA

STUDENT: ANNA MANDEL SUPERVISOR: REBECCA GLADDY

Background: Rhabdomyosarcoma (RMS) is a tumor originating from the skeletal muscle lineage and is the most common type of soft tissue sarcoma in children and adolescents. Current treatment includes chemotherapy, radiation therapy, and/or surgery. Vincristine, the primary standard of care drug, has high systemic toxicity and >70% of high-risk RMS patients develop resistance to this therapy over time. The most common RMS histological subtypes are embryonal (ERMS), alveolar (ARMS), pleomorphic (PRMS) and spindle cell/sclerosing (SRMS). Childhood RMS (mainly ERMS and ARMS) contains relatively few genetic mutations compared to adult cancers, and research has failed to uncover obvious and targetable molecular factors. Therefore, the investigation of underlying molecular pathways and development of more effective therapeutic approaches is strongly warranted. **Purpose:** Previous studies identified epigenetic modulation, or phenotype changes which do not involve alterations in the DNA sequence, as a crucial mechanism controlling developmental gene expression and potentially contributing to malignant transformation in childhood tumors. Some of the main targetable epigenetic factors include bromodomain and extra-terminal domain (BET) and histone deacetylase (HDAC) family proteins. My research aim is to explore the molecular mechanisms behind promising epigenetic inhibitors in a subtype-specific manner in RMS, and to test these drugs *in vivo* in combination with vincristine.**Hypothesis:** Aberrant epigenetic signaling is prevalent in RMS and epigenetic enzymes can be exploited for clinical drug development.**Methods:** Hit compound validation with EC_{50} curves confirmed that a panel of patient-derived ARMS, ERMS, PRMS and SRMS cell lines, and murine RMS tumor-derived cell lines are sensitive to a wide range of epigenetic inhibitors obtained from the Ontario Institute of Cancer Research (OICR) and Structural Genomics Consortium (SGC) that mainly target BET or HDAC proteins. The effect of the compounds on apoptosis was assayed using immunoblot and flow cytometry. To investigate endogenous target expression and the mechanism of action of the inhibitors we performed immunoblots and qPCR. To analyse cell viability after knocking down the target proteins with siRNA and to investigate the kinetics of the compounds, we used IncuCyte live cell imaging. In the future, we plan to investigate the transcriptomic profile of RMS after treatment with the inhibitors using mRNA sequencing with differential expression analysis, and conduct *in vivo* drug combination studies in ERMS and ARMS mosaic mouse models and patient-derived xenografts (PDXs).**Results:** Our experiments confirmed that the selected compounds decreased cell proliferation in ERMS, ARMS, PRMS and SRMS *in vitro*, and that the compounds induced apoptosis in ERMS and ARMS as indicated by PARP cleavage and caspase-3 activation assays. The BETi compounds induced the highest level of apoptosis, especially in ARMS ($p<0.01$). The primary target of the BETi compounds is BRD4 and the endogenous expression assay showed that this protein is overexpressed in ERMS and ARMS when compared to control myoblasts. Furthermore, the kinetics study suggested that the BETi compounds inhibited cell proliferation early on within 24-48 hours, which will be the time-points used in future cellular response experiments. MYC is a common downstream target of BETi inhibitors, however our assays so far show that MYC is not reduced on the protein or transcriptional level following treatment *in vitro* in ERMS and ARMS. The data might indicate that BETi compounds inhibit proliferation in RMS via alternate downstream mechanisms. Other targets of the BETi compounds include BRD2 and BRD3. Ongoing on-target effect experiments suggest that knocking down BRD4 or BRD2 (but not BRD3) in ARMS reduces cell viability, however knocking down any of the three BET proteins individually does not reduce viability in ERMS.
Conclusions: Based on our data, we propose that RMS has underlying epigenetic mechanisms which can be exploited for therapeutic targeting. The overall aims of this project are to introduce epigenetic targeting compounds into clinical use against RMS and combine them with standard of care treatment to design more effective and less toxic treatment options for this devastating disease.

EXPLORING THE ROLE OF NOVEL CANDIDATE GENES IN SUSCEPTIBILITY TO BREAST AND OVARIAN CANCERS

STUDENT: NEDA ZAMANI
SUPERVISOR: DR. MOHAMMAD R. AKBARI

Breast cancer is the most common malignancy and the first leading cause of cancer-related mortalities in women. About 10% of all breast cancer cases are hereditary, 15%-20% of which are due to deleterious mutations in BRCA1/2 genes. Other genes associated with breast cancer predisposition include ATM, BARD1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD50, STK11, and TP53. Together, mutations in known breast cancer susceptibility genes account for almost half of hereditary cases and research is underway to identify other genes that may predispose to the other half of inherited breast cancer cases.

Recently, our research team has identified four novel candidate breast cancer susceptibility genes among the Polish and Bahamian populations. In the next step and to achieve a deeper understanding of the hereditary landscape of breast cancer among other populations, we need to evaluate the role of these four novel candidate genes in susceptibility to breast cancer among other populations. Therefore, this study aims to apply targeted full-gene sequencing of the four novel candidate genes identified among the Polish and Bahamian populations on cohorts of breast cancer patients and healthy controls from other populations. As an exploratory aim, we want to study if mutations in the four newly identified candidate genes may increase the risk of ovarian cancer.

We will perform targeted sequencing of the four candidate genes among 1,000 familial breast cancer patients in Ontario, 1,400 familial French-Canadian breast cancer patients in Quebec, 560 familial breast cancer patients from Iran, 1,422 ovarian cancer patients from Ontario, and 930 healthy women in Ontario used as controls for association analysis with the Ontario breast/ovarian cancer patients. We will use the Iranome database of 800 healthy individuals as control group for comparison with the Iranian breast cancer patients. Also, we will use the sequence data of 450 healthy individuals from the Gen3G study recruited from Sherbrooke in Quebec as the control group for the French-Canadian breast cancer patients. Within the UK Biobank study, we will include all women with invasive breast cancer (about 16,000 incident and prevalent cases) for whom WES data is available. All the remaining healthy women with available WES data will be considered as the control group for the association analysis among the UK Biobank participants. We will do gene-based association analyses to study the association of the candidate genes with breast cancer risk among studied populations. Also, we will perform loss of heterozygosity analysis in tumours of mutation carriers to investigate further the role of the novel candidate genes in susceptibility to breast cancer; FFPE sections will be macro-dissected and the candidate genes will be sequenced in tumour DNA of mutation carriers using NGS technology. We will also analyze tumour mutational signatures in cancer genome of the germline mutation carriers; we will perform whole genome sequencing on matched tumour-normal DNA of breast cancer patients harboring germline mutations in the four candidate genes. Using web-interfaces and R packages, we will extract signatures of base-substitutions, indels, and rearrangements from each tumour and compare them with the already known signatures listed on the COSMIC database using cosine similarity.

Group H: Infection-Immunology

ANALYSIS OF THE T CELL COMPOSITION OF CLINICALLY STABLE ISHLT GRADE A1 REJECTION LESIONS

STUDENT: SAMUEL BEBER
SUPERVISOR: STEPHEN JUVET

Purpose: CD4+FOXP3+ Regulatory T cells (Tregs) may modulate acute cellular rejection (ACR)-associated immune responses and the progression of ACR to Chronic Lung Allograft Dysfunction (CLAD) following lung transplantation (LTx). Treg stability is enhanced by demethylation of the Treg-specific demethylated region (TSDR) in the Foxp3 locus. Grade A1 ACR is not treated in clinically stable patients in the Toronto Lung Transplant Program, affording the opportunity to study its natural history in relation to its cellular composition. Our hypothesis was that a greater Treg content in first, clinically stable, grade A1 biopsies is associated with a lower risk of CLAD.

Methods: In this retrospective study, we selected clinically stable patients who received a bilateral LTx between 1 Jan 2010 and 18 Dec 2016 with a first episode of A1B0 ACR and who remained CLAD free for at least 5 years (n=16) or who developed CLAD within 2 years (n=9) following LTx. Immunofluorescence was used to quantify CD4+, CD8+ and FOXP3+ cells in transbronchial biopsies (Figure 1A). Separately, CD3+ cells were fluorescently labelled, micro-dissected (Figure 1B) and, using bisulfite conversion and pyrosequencing, the degree of TSDR methylation was determined.

Results: TSDR methylation was slightly lower in the CLAD group as compared to the CLAD-free group (Figure 1C), indicating increased Treg infiltration. Accordingly, the ratio of CD4+FOXP3+ cells to total CD4+ cells was slightly higher in the CLAD group (Figure 1D). These measures were weakly negatively correlated (r^2 = 0.250, p=0.0152) (Figure 1E). The ratio of CD4+:CD8+ cells was significantly lower in the CLAD group (p=0.0065) (Figure 1F).

Conclusion: Patients who developed CLAD within 2 years of LTx showed no significant difference in Treg, though greater CD8+ T cell, infiltration compared to stable patients. These results suggest that in asymptomatic patients with a first episode of A1 rejection, greater CD8+ T cell content may be indicative of a poorer long-term outlook. Validation in additional patients is now underway.

THE ROLE OF COMPLEMENT IN PRIMARY MEMBRANOUS NEPHROPATHY

STUDENT: VALENTINA BRUNO
SUPERVISOR: CHRISTOPH LICHT

Background:
Primary membranous nephropathy (PMN) is the leading cause of nephrotic syndrome in adults and a common cause of end-stage kidney disease (ESKD). The Heymann's nephritis mouse model of PMN shows that proteinuria is complement-mediated. However, the pathogenetic role of complement in human PMN remains unclear.
Our preliminary data showed that complement deposition (C3b and C5b9) can be detected on podocytes exposed to serum of PMN patients (recruited from the Toronto GN Registry), leading to disruption of actin cytoskeleton.

Hypothesis and aim:
We hypothesize that complement system plays a pivotal role in the pathogenesis of PMN, leading to alterations in both the structure and function of podocytes.

Material and Methods:
An in-vitro model of immortalized human podocytes (from Moin Saleem, Bristol, UK) was used for all the experiments. Cells pre-sensitized with anti-CD59 were exposed to 50% normal human serum (NHS) to obtain complement deposition on the podocytes surface. Subsequently, changes in intracellular calcium levels were monitored using a fluorescent dye (Fluo8-AM), acquiring images every 20 seconds (up to 10 minutes) by confocal microscopy. Calcium effects on mitochondrial membrane potential were measured by flow cytometry using tetramethylrhodamine, methyl ester (TMRM) dye. Wound healing assays were performed to study functional effects on podocyte migration.

Results:
Complement activation led to a significant rise in the intracellular calcium levels. Loss of mitochondrial membrane potential was also observed, together with disruption of the actin cytoskeleton and impaired cell migration.

Conclusions:
Complement is active in PMN, leading to both structural and functional effects on podocytes. Further studies are needed to better understand the consequences on the podocyte energy machinery and the possibility of its reversibility by using complement inhibitors. Our research of such alternate therapy could lead to improvement in outcome in PMN where, despite current therapies, up to one third of patients develops ESKD.

MIR-187 MIMIC TARGETS PRO-INFLAMMATORY CYTOKINES IN CARDIOMYOCYTES TREATED WITH BACTERIAL ENDOTOXIN

AMIN MOTAMED EKTESABI
SUPERVISOR: DR. CLAUDIA DOS SANTOS

Introduction:1 in 18 Canadians dies from sepsis, a life-threatening dysfunction caused by a dysregulated host response to infection which can lead to sepsis induced Cardiac dysfunction. In the past we have shown that administration of mesenchymal stem cells (MSCs) in cecal ligation and puncture (CLP) model of polybacterial sepsis leads to the mitigation of sepsis-induced cardiac dysfunction by modulating microRNAs in septic heart. Differential expression of miRNAs alters mRNA expression profile leading to a change in cellular response. In our CLP model, we identified miR-187 as a host-derived MSC-regulated miRNA. Here we investigate the broader immunoregulatory of miR-187. Therefore, we tested the hypothesis that miRNA-187 inversely regulates sepsis-induced cardiac dysfunction by attenuating pro-inflammatory gene expression.

Methods: Primary cardiomyocytes were isolated from 1-2 days old CD1 neonates. Cultured cells were transfected with miR-187 mimic (25 nM/mL) for 24 hours. Transfected cells were then subsequentially exposed to lipopolysaccharide (LPS, 1 μg/mL) for an additional 24 hours post-transfection. We chose to measure the expression of pro and anti-inflammatory cytokines and some cardiac injury markers via Real-Time Polymerase Chain Reaction (RT-qPCR).

Results: Cardiomyocytes treated with bacterial LPS showed a significant decline in expression of miR-187 ($n=4$, $p<0.05$) and a significant surge ($n=4$, $p<0.05$) in the expression of pro-inflammatory cytokines such as IL-1β, IL-6, TNFα and IL-12 in comparison to untreated cells. Conversely, LPS-stimulated cardiomyocytes transfected with exogenous miR-187 mimic versus non-transfected LPS-stimulated cardiomyocytes demonstrated reduced expression of IL-1β, IL-6, and IL-12 ($n=3$, $p<0.05$) and FasI, S100A4 and S100A9 ($n=3$, $p<0.05$).

Conclusions: Transfecting neonatal cardiomyocytes with mimic miR-187 in the presence of LPS decreases the production of key inflammatory genes involved in sepsis-induced cardiac dysfunction. Our findings show miR-187 to be a potential therapeutic target to improve cardiac function in a pre-clinical model of sepsis by reducing pro-inflammatory cytokine translation.

MIF IS AN IMPORTANT MEDIATOR IN SPA ASSOCIATED GUT INFLAMMATION IN SKG MOUSE

STUDENT: SHAGHAYEH FOROOZAN BOROOJENI
SUPERVISOR: DR. NIGIL HAROON

Shaghayeh Foroozan Boroojeni , MSc. PhD student & Nigil Haroon MD, PhD, DM, FRCPC

Background: Axial Spondyloarthritis (AxSpA) is a chronic inflammatory disease with multifactorial origins, primarily affecting the musculoskeletal system. AxSpA often is accompanied by extra-articular manifestations, such as Inflammatory Bowel Disease (IBD). Over the years many pathways and cell populations have been linked to the pathogenesis of gut inflammation in AxSpA.
Macrophage migration inhibitory factor (MIF) plays a critical role in the pathogenesis of AxSpA. Over-expression of MIF in a mouse model of SpA (SKG mice) causes major clinical features of AxSpA. On the other hand, blocking or depletion of MIF significantly suppresses these symptoms. However, it is mostly unknown what the role of MIF is in gut inflammation in AxSpA. We hypothesized that MIF is a key player driving gut inflammation in AxSPA.
Hypothesis: MIF as an immune driver plays an essential role in gut hemostasis in the SKG mouse model of SpA.
Methods: 6 SKG-MIFKO and 6 SKG control 16 weeks old mice were used for this study. H&E slides were prepared for histopathology assessment considering global architecture, Epithelium, and Lamina propria (2 individual persons scored the samples blindly) on formalin-fixed paraffin-embedded (FFPE) blocks of ileum tissue. Immunohistochemistry (IHC) was performed on slides for occludin. qPCR was performed to measure the expression level of IL-17, Muc2, Mmp7, and Lyz1. Mann Whitney test was used to analyze the difference between the two groups.
Results: We observed significantly increased levels of inflammation in the ileum of MIFKO SKG mice compared to SKG control ($p = 0.01$). We also observed disruption of ileum epithelium in MIFKO SKG mice compared to the SKG control group which was confirmed with decreased expression of occludin suggesting disruption of tight junctions. IL-17 and Muc2 levels were decreased in MIFKO-SKG ileum. Expression of Mmp7 and Lyz1 were increased in the ileum of MIFKO-SKG mice.
Conclusion: Knocking out MIF in SKG mice has shown improvement in AxSpA symptoms. However, based on our findings, MIF seems to have a protective role in the gut of SKG mouse unlike what we see in joints. MIF can stimulate IL-17 (which also has a protective role in the gut). Thus, decreased levels of IL-17 in the MIFKO group can contribute to gut inflammation as well. The absence of MIF leads to decreased production of mucin and increased production of Mmp7 and Lyz1 from goblet and paneth cells, respectively. These findings together suggest that MIF is essential for the integrity of the gut epithelial barrier. The impact of excess MIF and if suppressing MIF to an optimal level will help control AxSpA inflammation both in joints and intestine is being studied.
Declaration of interest: Authors have no conflict of interest to declare

WHOLE BLOOD HIGH DIMENSIONAL FUNCTIONAL SINGLE CELL PROFILING OF BASOPHILS IN PEANUT ALLERGY

STUDENT: CARMEN LI
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Background: Food allergies are a common cause of anaphylaxis in childhood. Controlled exposure via oral immunotherapy (OIT) is emerging as a food allergy treatment. However, the molecular and cellular mechanisms that define the difference in basophil activation between peanut allergic and non-allergic individuals and during OIT are not fully understood.
Purpose: To establish a model to evaluate differences in allergen-specific signaling pathways upon allergen stimulation in basophils in the context of allergy and during OIT.
Hypothesis: Our model will define basophil subsets based on differences in activation and expression of cellular markers.
Methods: Blood samples from clinically confirmed peanut-allergic (n=6) and non-allergic (n=3) children were collected at The Hospital for Sick Children. Mass cytometry (CyTOF) was used to observe differences in downstream signaling pathways in basophils following stimulation with 0.01-100 ng/mL of peanut extract for 3 or 15 minutes. Samples were barcoded, pooled, and stained with a panel of surface markers and anti-phosphorylation antibodies for p38, Erk, mTOR and AKT markers, followed by acquisition on a CyTOF Helios2 and basophil activation patterns were analyzed.
Results: Relative basophil abundance did not differ between the allergic and non-allergic individuals. No significant differences were seen in the expression of IgG receptors, IgE receptors or other granulocyte cell surface markers. Results from peanut-allergic individuals showed allergen-induced activation and subsequent phosphorylation of Erk and p38, which occurred in a dose-dependent manner at 3 mins post-stimulation, and phosphorylation of AKT and mTOR at 15 mins. This activation signature was significantly lower to absent in non-allergic individuals. Pooled basophils from peanut-allergic individuals were clustered using the Leiden algorithm and then analyzed by duration and type of stimulation, including PMA/Iono, FcεRI-crosslinker, and 10 ng/mL peanut extract. During basophil stimulation, shifts in the proportion of stimulated basophils in subsets and surface marker expression were found.
Conclusions: High dimensional mass cytometry data provides single-cell measurements that aid in elucidating differences in cellular activation in allergic and non-allergic individuals. This model can be further applied to understand the mechanisms differentiating allergic and non-allergic patients during OIT studies.

SOUBLE E-CADHERIN: A MARKER OF EPITHELIAL DISRUPTION

STUDENT: RACHEL LIU
SUPERVISORS: DR. RUPERT KAUL & DR. BRYAN COBURN

Background: The genital epithelial barrier is one of the first line of defense against urogenital pathogens, including HIV. Epithelial disruption and dysfunction enhance HIV susceptibility, as virions permeate through the barrier and gain access to HIV susceptible immune cells.

Purpose: Currently there is no standardized biomarker for evaluating the epithelial barrier integrity *in vitro.* We propose that soluble E-cadherin (sE-cad), a soluble monomer of the E-cadherin protein, is a marker of epithelial disruption in the genital tract.

Methods: Immortalized endocervical epithelial cells were physically disrupted and assessed for levels of interleukin (IL)-6, IL-1a, IL-1b and sE-cad. We analyzed clinical cohorts to identify changes in sE-cad following minor physical disruptions, and the relationship between sE-cad and membrane bound E-cad.

Results: Physical disruption immediately elevated sE-cad, IL-1B, and IL-1a levels and a delayed increase in IL-6. Similarly, sE-cad was significantly elevated 6 hours after endocervical cytobrush sampling *in vivo* compared with baseline levels. Furthermore, we observe a negative association between soluble E-cadherin and membrane bound E-cadherin, supporting a model whereby a loss of intact membrane-bound E-cadherin in the genital epithelium results in elevated soluble E-cadherin levels.

Conclusion: Our results validate the usage of soluble E-cadherin as a marker of epithelial disruption independent of inflammation and demonstrate that physical disruption in the genital tract is strongly intertwined with inflammation. Further work is needed to evaluate the association between sE-cad levels, inflammation, and HIV risk.

PREDICTING CLINICAL AND IMMUNE SECUKINUMAB EFFECTS IN AXIAL SPONDYLOARTHRITIS (PreCISE.AS)

STUDENT: ADDISON PACHECO
SUPERVISOR: ROBERT D. INMAN

Authors: **Addison CA. Pacheco, Robert D. Inman**

Background:
Axial spondylarthritis (axSpA), which encompasses Ankylosing Spondylitis (AS) and non-radiographic axial spondyloarthritis (nr-axSpA), is an inflammatory disease that predominantly affects the spine and the sacroiliac joint. Although the pathobiology of axSpA is not completely known, biologics that target the cytokine IL17a, such as secukinumab, have demonstrated improved clinical outcome for majority of patients.

Objectives:
The PreCISE study aims to address some biomarkers that may predispose treatment failure.

Hypothesis:
We hypothesize a decrease in IL-17 producing cell subsets in secukinumab responders and an increase in anti-inflammatory cell subsets. We expect this difference to be absent in non-responders.

Methods:
Multi-parametric flow cytometry analysis of the different IL-17 producing cell subsets was used to assess differences between responders and nonresponders before and after secukinumab treatment.

Results:
Patients demonstrate no change in the percentage of regulatory T-cells (Tregs) relative to CD4+ cells after secukinumab treatment. AS patients show less RORyt+ Tregs in the periphery compared to healthy controls and this remains throughout drug treatment. However, upon stimulation with PMA and ionomycin for 5 hours, AS patient Tregs demonstrate healthy control RORyt+ Treg percentages after secukinumab treatment, a phenomenon not observed before the treatment is provided.

Conclusions:
An increase in RORyt+ Tregs upon stimulation may demonstrate an important role in type 3 immunity suppression through Tregs that has not been demonstrated through secukinumab treatment previously. Further assessment into the epigenetics of this Treg subset may address reasons to treatment failure.

ROLE OF THE COMPLEMENT SYSTEM AND VASCULAR ENDOTHELIUM IN COVID-19 PATHOGENESIS

NIYOUSHA ROSTAM SHIRAZI INFECTION-IMMUNOLOGY
SUPERVISOR: CHRISTOPH LICHT

Coronavirus Disease 2019 (COVID-19) caused by SARS-CoV-2 infection has become a global pandemic with more than hundred million cases worldwide, presenting in varying degrees of severity ranging from acute respiratory distress syndrome (ARDS) to multi-organ failure. While the underlying mechanisms contributing to pathogenesis and lethality of COVID-19 remain poorly understood, there have been evidence linking the activation of the complement system and vascular endothelium injury to the cardiovascular complications that increase the risk of mortality in severe COVID-19 cases. Using the evidence from previous coronavirus epidemics such as SARS (2003) and MERS (2012), we hypothesize that vascular endothelial injury and energy homeostasis dysregulation as a result of complement activation contributes to COVID-19-associated tissue injury seen in severe COVID-19 cases. Thus, the aim of this research project is to identify complement activation in severe COVID-19 patients and to investigate consequent endothelial cell (EC) injury, and energy homeostasis disruption. Clinical information, and sera from SARS-CoV-2 positive patients with mild and severe COVID-19 disease were obtained at immediate and acute phases through collaborations with The Canadian COVID-19 Prospective Cohort Study (CANCOV) at the University Health Network. Complement activation on ECs is evaluated via immunofluorescence assay, measuring the deposition of complement products C3b and C5b-9 on Human Umbilical Vein Endothelial Cells exposed to control or patient sera. A permeability assay using a transwell model is used to measure the integrity of the endothelial monolayer exposed to patient sera and the mitochondrial potential will be assessed using a TMRM assay. This study will provide insights regarding the role of complement system in COVID-19 and will provide scientific rationale for the use of complement blockers to reduce the adverse outcomes associated with overactivation/dysregulation of complement system in COVID-19.

PROTON PUMP INHIBITORS SUPPRESS IL-1 MEDIATED CARDITIS IN A MURINE MODEL OF KAWASAKI DISEASE

STUDENT: PAUL TSOUKAS MD
SUPERVISOR: RAE YEUNG MD PHD

Background: Kawasaki disease (KD), is the leading cause of acquired heart disease in childhood, with a portion of children developing coronary artery lesions (CAL) despite standard of care treatment, intravenous immunoglobulin (IVIg). Murine and patient data indicate Interleukin-1 (IL-1) contributes to CALs. Although the second line agent, anakinra (recombinant IL-1 receptor antagonist), trial results are promising, the medication is unavailable to half the population. Calcium mobilization plays a role in inflammasome activation and is key to the immunobiology of KD. Proton pump inhibitors (PPI), a class of medications used to limit gastric acid secretion, have also been shown to have anti-inflammatory properties.

Purpose: To determine if PPIs reduce CALs in the Lactobacillus casei cell wall extract (LCWE) induced coronary arteritis murine model of KD.

Hypothesis: PPIs inhibit inflammasome assembly limiting IL-1β production resulting in reduced carditis

Methods: Human monocyte cell line (THP1) derived macrophages and bone marrow derived macrophages (BMDMs) were stimulated with a TLR1/2 agonist Pam3Cys-Ser-(Lys)4 (Pam3Cys) and LCWE, in the presence or absence of PPIs. To exclude toxic effects, viability was tested via flow cytometry and trypan blue exclusion. Western blot analysis was performed on BMDM cell lysates. Calcium flux was measured via fluorescent imaging plate reader on THP-1 macrophages. In vivo, KD was induced by intraperitoneal LCWE injection. Mice were injected with LCWE, anakinra (recombinant IL-1 receptor antagonist), saline or PPI alone as well as LCWE treated with PPI or Anakinra. Coronary artery inflammation was blindly scored by a pathologist.

Results: Following stimulation with either Pam3Cys or LCWE, PPIs inhibited BMDM IL-1β production in a dose-dependent manner with an IC50 similar to that seen in humans. Inflammasome activation is prevented by PPI inhibition of signal two, seen via cytokine levels as well as immunoblot results. Stimulated macrophages treated with a PPI, in vitro, had a less calcium flux than untreated stimulated macrophages. In vivo, compared to untreated KD diseased mice, those treated with PPI were shown to have significantly reduced coronary artery inflammation based on overall cardiac severity score ($p<0.01$), area of inflammation ($p<0.05$) and elastin breakdown ($p<0.01$). Additionally, efficacy of PPI was shown to be non-inferior to anakinra.

Conclusion: Our data indicate that PPIs have anti-inflammatory properties: decreasing macrophage IL-1 production in vitro and in an in vivo KD murine model preventing IL-1 induced coronary artery inflammation. The data suggest two novel findings. Firstly, PPIs may inhibit inflammasome activation by preventing intracellular calcium accumulation. Secondly, PPIs have the potential to be a novel inexpensive, oral, and safe adjuvant anti-IL-1 medication to treat KD.

Group I: Cardiovascular-Respiratory-Musculoskeletal

EFFECT OF TRANSIENT RECEPTOR POTENTIAL MELASTATIN 4 (TRPM4) INHIBITION BY 9-PHENANTHROL ON VENTRICULAR ARRHYTHMIA VULNERABILITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT.

STUDENT: PRALOY CHAKRABORTY
SUPERVISOR: DR. KUMARASWAMY NANTHAKUMAR

Background: Treatment of ventricular arrhythmia (VA), the commonest cause of sudden death, in patients with heart disease is difficult due to hemodynamic and proarrhythmic side effects of available drugs. Cytosolic Ca^{2+} overload-induced activation of transient receptor potential melastatin 4 (TRPM4) channels in the diseased heart is associated with perturbation of membrane action potential (AP) the substrate for arrhythmias. The role of 9-phenanthrol, an inhibitor of TRPM4, on VA in cardiomyopathy (CMP) has not been explored.

Purpose: To evaluate the role of TRPM4 inhibition by 9-phenanthrol on ventricular arrhythmia inducibility, Ca^{2+} dynamics, membrane AP dynamics, and Ca^{2+} handling proteins in the spontaneously hypertensive rat (SHR) ventricle.

Hypothesis: In the SHR ventricle, TRPM4 inhibition by 9 phenanthrol will reduce ventricular arrhythmia by reducing action potential duration (APD) and APD alternans despite negligible effects on cytosolic Ca^{2+} dynamics and sarcoplasmic Ca^{2+} handling proteins

Methods: Langendorff-perfused hearts of young (10 to 14 weeks), old (10 to 14 months) SHRs and WKY (10-14 months) rats will be treated with 9 -phenanthrol (20μM) or DMSO (0.1%). Simultaneously, LV epicardial AP and cytosolic Ca^{2+} transients (CaT) will be optically mapped following the “pace & pause” protocol. VA vulnerability will be assessed by electrically inducing ventricular fibrillations (VFs) in each heart. Western blot analysis for TRPM4 and Ca^{2+} handling proteins (RyR2, CaMKII, PLB, SERCA2a) will be performed on LV tissues.

Results: To date, the experiment numbers are not adequate to get a meaningful statistical result. However, APD reduction in SHR ventricles is a consistent finding. We expect to complete the study analysis by end of April. From our hypothesis we are expecting the following results: in SHR heart, 9-phenanthrol treatment will be associated with reduction of action potential duration (APD), APD alternans, and mitigation of VF inducibility without any change in calcium transient duration and alternans. TRPM4 expression will be higher in the SHR ventricle compared to WKY rats. 9-phenanthrol treatment will not be associated with any change in expression or phosphorylation status of calcium handling proteins.

Conclusions: Results from the study are expected to provide a unique antiarrhythmic effect of TRPM4 inhibition in patients with cardiomyopathy without significant proarrhythmia.

IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF FIBRO-ADIPOGENIC PROGENITORS AND MYOGENIC PROGENITORS IN SKELETAL MUSCLE IN THE RAT

STUDENT: CHRISTINA DOHERTY
SUPERVISOR: DR. JANE BATT

Background/Purpose: Duration of muscle denervation determines whether re-innervation can reverse muscle atrophy and fibrofatty degradation. With acute injury, fibro-adipogenic progenitor cells (FAPs), a progenitor population known to carry both fibrogenic and adipogenic potential, has been shown to mediate a favourable response to muscle injury by enabling myogenic progenitor (MP) mediated muscle repair. In contrast, in states of chronic injury, FAPs are known to mediate fibrosis and fat deposition, switching from a pro-regenerative phenotype to a pathogenic one as time post injury ensues. Currently, the molecular mechanisms regulating the phenotypic change in FAPs from pro-regenerative to pathogenic are not completely understood. To date, the study of FAPs via flow cytometric and fluorescence activated cell sorting (FACS) has been conducted solely in mice, which is limiting in studies of long-term denervation injury due to inadequate tissue for downstream assays as a result of severe atrophy. The larger inherent size of the rat allows for a more comprehensive analysis of FAPs in skeletal muscle injury models, especially in severely atrophic muscle or when investigators require substantial tissue mass to conduct multiple downstream assays. **Objective:** To develop a method for isolation of FAPs from the rat to enable the study of molecular regulation of FAPs in long-term denervated muscle **Methods:** We developed a novel flow cytometry/FACs antibody panel to identify and isolate both FAPs and MPs populations in rat muscle, using a unique panel of antibodies comprised of CD31/CD45, VCAM and SCA-1. FAPs are identified by CD31-/CD45-/Sca-1+/VCAM-1- events, whereas MPs by CD31-/CD45-/Sca-1-/VCAM-1+ events. Purported pure FAPs and MPs populations were verified by i) immunostaining of FACS freshly-sorted cells with alternative markers and ii) by *in vitro* culture and differentiation of sorted cells into myotubes, adipocytes and fibroblasts. The validated flow cytometry/FACS antibody panel was used to evaluate the dynamics and phenotype of the FAPs population in short-term and long-term denervated gastrocnemius muscle using the tibial nerve transection model. **Results:** Application of the novel flow cytometry antibody staining panel and gating strategy effectively isolated purported FAPs (CD31-/CD45-/Sca-1+/VCAM-1-) and MPs (CD31-/CD45-/Sca-1-/VCAM-1+) populations. Validation of the specificity and purity of the population sorts was confirmed by demonstrating i) sole immunostaining of freshly sorted FAPs with secondary marker PDGFRα, and MPs sole staining with secondary marker Pax7, ii) *in vitro* differentiation of FAPs into fibroblasts or adipocytes with an absence of myotubes and iii) *in vitro* differentiation of MPs into mature myotubes in the absence of fibroblasts and adipocytes. We next serially assessed the dynamics of FAPs and MPs in the gastrocnemius muscle over a 14-week time course post tibial nerve transection. We demonstrated a changing FAPs phenotype with long term denervation that correlated with the onset of irreversible fibrosis. Initially (2 weeks post denervation), low Sca-1 expressing FAPs dominate during the period of reversible denervation injury. Long term denervation (14 weeks) showed a significant shift to a population of high Sca-1 expression FAPs (from 5% at 2 weeks, to 51% at 14 weeks), suggesting that Sca-1 can delineate a population of FAPs that induce fibrogenesis. **Conclusions:** After successfully developing and validating a novel flow cytometric method for FAPs identification and isolation in the rat, we observed FAPs dynamics over a 14-week course of muscle denervation. We identified low and high Sca-1 expressing populations over this time course directly correlated with the transition of denervated muscle to irreversible fibro-fatty degradation. This novel finding paves the way for future studies delineating the biologic relevance of these two FAPs populations, in addition to the upstream mediators of this changing phenotype.

ACCURACY OF THE FRAIL SCALE FOR PREDICTING POST-OPERATIVE COMPLICAITONS IN OLDER SURGICAL PATIENTS, A SYSMTEMATIC REVIEW AND META-ANALYSIS

STUDENT: SELENA GONG
SUPERVISOR: DR. JEAN WONG

BACKGROUND: Frailty is associated with increased risk for postoperative complications and mortality. During the COVID-19 pandemic, preoperative assessments have shifted to virtual assessments, precluding the use of frailty tools that require in-person assessment. The FRAIL scale is a brief assessment that can be conducted virtually, but its ability to predict postoperative outcomes in older surgical patients is unknown. Despite recommendations from medical societies that frailty be assessed routinely before surgery, frailty assessment may not be performed due to barriers including a lack of time in busy preoperative clinics, length of time it takes to administer assessments, and reliance on an administrator for assessments. **PURPOSE:** to determine whether the FRAIL scale predicts mortality and postoperative outcomes in older surgical patients, and whether it is comparable with validated frailty assessments. HYPOTHESIS: We hypothesize the FRAIL scale will be associated with postoperative mortality and outcomes in older surgical patients. **METHODS:** Medline, Medline ePubs/In-process citations, Embase, APA PsycInfo, Ovid Emcare Nursing, (all via the Ovid platform); Cumulative Index to Nursing & Allied Health Literature (CINAHL) EbscoHost; the Web of Science (Clarivate Analytics), and Scopus (Elsevier) were searched from 2008 to Dec 17th, 2021 to identify English language studies using the FRAIL scale in surgical patients and reporting postoperative outcomes, mortality and postoperative complications. No restrictions were placed on the use of a comparator, if any. We included randomized controlled trials, quasi-experimental studies (non-randomized controlled trials), and observational studies (prospective and retrospective). The risk of bias was assessed using the quality in prognosis studies tool. All citations were de-duplicated, screened, extracted, and assessed for quality in duplicate using Covidence. **RESULTS:** A total of 18 studies with 4,479 participants were included after screening 7,800 citations and 85 full text articles. Mortality was assessed in seven studies, with five studies reporting 30-day mortality OR: 6.28 [95% CI: 2.15. 18.30, p<0.01, I^2=59.3%], three studies reporting mortality at 6 months OR 2.97 [95% CI: 1.54, 5.72, p<0.01, I^2=20%], and three studies reporting 1-year mortality OR:1.54 [95% CI: 0.91, 2.58, p=0.11, I^2=0%]. Postoperative complications were reported in five studies, with all studies indicating greater risk of postoperative major complications for frail patients OR:2.99 [95% CI: 1.99, 4.49, p<0.01, I^2 =28.7%]. Four studies showed frail patients had a greater likelihood to develop postoperative delirium OR: 2.65 [95% CI: 1.85, 3.80, p<0.01, I^2=0%] and 1 study found cognitive recovery to be inversely correlated with frailty. The FRAIL scale and clinical frailty scale (CFS) were comparable OR: 1.04 [95% CI: 0.939, 1.16, p=0.42] for classifying frail patients. The risk of bias was low in 16 studies, and moderate in two. **CONCLUSION:** Our SRMA shows that frailty assessed with the FRAIL scale was associated with increased odds of mortality at 30-day, 6-months, postoperative complications, and postoperative delirium. The FRAIL scale may be an acceptable alternative to in-person frailty assessments.

CROSS-SECTIONAL ANALYSIS OF PULMONARY FUNCTION 6 MONTHS FOLLOWING COVID-19 INFECTION USING CONVENTIONAL AND NOVEL TECHNIQUES

STUDENT: ANNIE JIANG
SUPERVISOR: DR. CHUNG-WAI CHOW

Background: Early follow-up studies of lung function after COVID-19 identify a primary restrictive defect with reduced diffusing capacity. The long-term pulmonary consequences of COVID-19 are unknown. Oscillometry is a pulmonary function test (PFT) modality that is highly sensitive to lung mechanics and has been shown to identify lung disease earlier than conventional PFT.

Purpose: Our primary objective is to determine the prevalence of abnormal lung function in COVID-19 survivors 6 months post-infection. A secondary objective is to compare oscillometry with conventional PFT in detecting respiratory dysfunction in this patient population.
Hypothesis: Oscillometry detects pulmonary physiological abnormalities with greater sensitivity than conventional PFT to provide an early biomarker of sustained lung function impairment following COVID-19.

Methods: In this cross-sectional observational study, all patients enrolled in the CANCOV study and followed at the University Health Network are eligible for enrollment for oscillometry prior to spirometry with/without plethysmography, followed by a 6-Minute Walk Test and survey using the Borg CR10 Scale. Demographic and clinical data are collected from electronic patient records and/or questionnaires.

Results: This interim analysis included 216 participants (91M/125F, mean age=46.0±13.4 years) with follow-up 6.2±0.42 months post-infection. Spirometry showed restrictive defects in all patient groups (outpatient=6.7%, ward=33.3%, ICU=42.4%, $p<0.001$), with ward and ICU patients experiencing significantly higher prevalence of restriction than outpatients. Abnormal oscillometry was defined as R5-19 (difference in respiratory resistance between 5 and 19 Hz) > 0.80 cmH2O/L/s and/or AX (area of reactance) > 10 cmH2O/L. Oscillometry (n=178) was similarly abnormal in all groups (outpatient=17.6%, ward=35.0%, ICU=57.6%, $p<0.001$). Abnormal AX only, and abnormal R5-19 and/or AX, were significantly higher in ward and ICU patients than in outpatients, whereas that of abnormal R5-19 only was just higher in ICU patients compared to outpatients. Finally, 35.1% (53/151) of patients with normal spirometry had abnormal R5-19 and/or AX, but only 7.55% (8/106) of patients with normal oscillometry had abnormal spirometry ($p<0.01$).

Conclusions: In a cross-section of COVID-19 survivors 6-months post-infection, spirometry revealed restrictive defects in all groups with oscillometry findings of peripheral airway obstruction and ventilatory inhomogeneity as measured by R5-19 and AX. Both PFT modalities found higher prevalence of abnormalities in hospitalized patients than those managed as outpatients. The higher prevalence of abnormalities detectable by oscillometry suggests that it may provide an early biomarker of sustained lung function impairment that will need to be evaluated with longer follow-up.

THE ASSOCIATION OF PERIOPERATIVE HEMOGLOBIN WITH ADVERSE OUTCOMES IN PATIENTS UNDERGOING CARDIAC SURGERY: A RETROSPECTIVE STUDY

STUDENT: MICHELLE MUZHI LI
SUPERVISOR: KEYVAN KARKOUTI

Background: Anemia is a common blood disorder that occurs due to a lack of healthy red blood cells (RBCs), subsequently decreasing the amount of hemoglobin—an iron-rich protein found in RBCs—available to transport oxygen throughout the body. Approximately one-third of patients undergoing major surgical procedures are diagnosed with anemia and those undergoing cardiac surgeries are at an increased risk, with just over one-half of patients presenting with anemia. Many studies have demonstrated a relationship between low preoperative hemoglobin levels and poor postoperative clinical outcomes such as longer hospital stays and increased rates of infection and mortality. However, few have explored the association between postoperative hemoglobin level and adverse outcomes, and the influence of preoperative anemia on this relationship. Given the excessive blood loss that occurs during cardiac surgical procedures, anemia becomes even more common after surgery, affecting ~90% of patients. Additionally, patients with low hemoglobin preoperatively may be at a greater risk of being harmed due to reduced physiological resilience.

Purpose: The purpose of this study is to examine the association between postoperative hemoglobin level and adverse outcomes in patients undergoing elective cardiac surgery as well as investigate whether the association is influenced by the patient's preoperative hemoglobin level.

Hypothesis: We hypothesize that lower levels of postoperative hemoglobin are independently associated with decreased days alive and out of hospital at 30 days (DAOH-30) and increased rates of adverse clinical outcomes, and this relationship is influenced by patients' preoperative hemoglobin level, placing those with chronic anemia at increased risk.

Methods: We will conduct a retrospective single-centre cohort study including ~10,000 patients who underwent elective cardiac surgery at Toronto General Hospital (TGH) between January 1, 2012, and December 31, 2021. We will link existing clinical databases at TGH, including cardiovascular surgery, anesthesia, and blood bank, with ICES databases to obtain the required data after patients are discharged from the hospital. The primary outcome is DAOH-30—an important patient-centred outcome that reflects the cumulative impact of survival, hospital length of stay, readmission, and discharge to alternative facilities. Secondary outcomes of interest include adverse clinical events such as lengths of hospitalization and ICU stay and rates of acute kidney injury, myocardial infarction, sternal wound infection, stroke, and mortality. Our primary analysis will examine the association of lowest postoperative day 1 (POD 1) hemoglobin level with DAOH-30, but we will also analyze other postoperative hemoglobin measures. Unadjusted and adjusted analyses will be conducted utilizing multilevel generalized estimating equation methods.

Expected Results: Our study will determine if low postoperative hemoglobin is associated with poor clinical outcomes and whether preoperative hemoglobin plays a modifying role.

Conclusions: Clarifying the relationship between perioperative hemoglobin and clinical outcomes following surgery may identify a large subgroup of patients in whom therapies to correct postoperative anemia, such as intravenous iron, may lead to improved outcomes.

DEEP GENERATIVE MACHINE LEARNING TO OBTAIN SYNTHETIC ULTRASOUND DATA FOR HEMOPHILIA RESEARCH

STUDENT: MAURO MENDEZ MORA
SUPERVISOR: DR. PASCAL TYRRELL

Background: Modern medicine is transitioning to the involvement of artificial intelligence (AI) to assist healthcare professionals in their assessment of patients. Imaging modalities such as computed tomography (CT), magnetic resolution (MRI), and ultrasound (US) are used for pathology detection, segmentation, and monitoring, evaluating a group of examinations in a matter of seconds. Recent advances in AI require a large amount of data to train; however, in healthcare, privacy concerns, exam costs, or the rarity of diseases limit the acquisition of patient exams. Hemophilia is a rare blood disorder that obstructs the ability to clot. To diagnose and treat hemophilia, US examinations of a joint cavity are performed to detect the presence of blood accumulation (i.e., hemarthrosis). US is commonly used because of its relatively low cost; however, its operator dependence and limited hemophilia sample acquisition, pose a challenge for the development of artificial intelligence (AI) solutions related to the disease. New research has explored artificial sample generation (synthetic data) to increase the number of samples to train on, allowing research and development to move forward without ignoring the need for prospective validation.
Purpose: This study aims to simulate synthetic images of hemophilia US using domain knowledge and a similar condition as a basis (e.g., synovial fluid recess strain). In this way, missing data are created to augment the information present in the dataset and improve the training of a model compared to regular data augmentation techniques.
Hypothesis: Using expert domain knowledge and a common proxy disease of similar appearance, it is possible to simulate synthetic data that are effective for training AI detection models for the target disease.
Methods: The data used in this study comprised 48317 images from 10958 patients collected retrospectively in community clinics using multiple US devices. Knee examinations were performed on adult subjects for suspected recess distension. The examinations were annotated by two experts and filtered by suprapatellar view. Two main models were trained to generate synthetic US images: StyleGAN 2 ADA and FUNIT. A classification model was trained to detect the presence of blood in the US images of the knee joint, as a validation of the applicability of the synthetic data.
Results: A filtered dataset of 10074 images was used to train the models. The StyleGAN 2 ADA was trained for knee US generation under multiple conditions (e.g., contrast, orientation, distension size). The model was able to create new and realistic images showing recess distension sharing visual similarities with real data. The FUNIT model was trained to transform an image to another domain (e.g., from recess distension to hemarthrosis). This model was able to simulate the presence of blood, increase or decrease in the size of the distension, and contrast enhancement in real data. Our approach shows an increase of 15% when training an AI classification model with our synthetic data versus using regular data augmentation techniques, with the added feature of customization control that clinical experts can use to explore rare conditions.
Conclusion: While our results show promise for introducing synthetic data into current AI solutions, the need for real data remains for validation and deployment. However, our approach advances hemophilia research by creating missing data and enabling expert exploration by replicating rare conditions.

LINKS BETWEEN CORONARY MICROVASCULAR DYSFUNCTION AND EVIDENCE OF HFPEF

STUDENT: SUMIN (JOY) PARK
SUPERVISOR: DR. SUSANNA MAK

Background: It has been hypothesized that coronary microvascular dysfunction (CMD) may be associated with the development of myocardial abnormalities associated with heart failure with preserved ejective fraction (HFpEF). Making a diagnosis for HFpEF is aided by exercise right heart catheterization (RHC) based on an abnormal pulmonary artery wedge pressure (PAWP) response during exercise. An exercise-associated increase in PAWP adjusted for the change in cardiac output (ΔPAWP/ΔCO) greater than 2 mmHg/L/min is a clinical predictor for HF outcomes and predicts exercise capacity. Although there is speculation that CMD may play a role in the development of early HFpEF, there is limited evidence that directly links CMD as assessed by an invasive coronary physiology study (ICPS) with HFpEF as measured by an exercise RHC.

Purpose: We will analyse the relationship between ΔPAWP/ΔCO and measures of coronary microvascular physiology in a cohort of patients that have had both an ICPS and an exercise RHC to investigate unexplained cardiovascular symptoms (UCS).

Hypothesis: In patients with an abnormal coronary microvascular function, we will identify a high proportion of patients who demonstrate ΔPAWP/ΔCO greater than 2.

Methods: Our study design is an exploratory, retrospective cohort analysis. The study population consists of patients experiencing UCS who were referred to Mount Sinai Hospital (MSH) for a RHC with exercise from our clinical partner at Southlake. Our inclusion criteria are patients with UCS who have undergone an ICPS to assess coronary microvasculature function and have then been referred to MSH for an exercise hemodynamic study.

Results: To date, we identified a cohort of 20 patients who met the study's inclusion criteria. Of these, 19 completed a RHC exercise study from which 9/19 (47%) had a ΔPAWP/ΔCO > 2. A mean pulmonary arterial pressure (mPAP) adjusted for CO greater than 3 defines pulmonary hypertension at exercise, of which we found 8/19 (42%) meeting criteria. The mean index of microvascular resistance (IMR) for patients with abnormal exercise hemodynamics was 32.6 versus 20.9 (p=0.08).

Conclusions: Among patients undergoing an ICPS, a relatively high proportion of these patients exhibit a ΔPAWP/ΔCO > 2 mmHg/L/min. We have also observed that in patients with an abnormal PAWP response, the mean IMR values were higher compared with patients with a normal PAWP response. Our observations support the indication that CMD may play a role in the development of early HFpEF. More patients are needed to further characterize this relationship.

VIDEO PLETHYSMOGRAPHY FOR CONTACTLESS VITAL SIGNS MEASUREMENT: A PILOT STUDY

STUDENT: CHI PHAM
SUPERVISOR: DR. FRANCES CHUNG

The following abstract presents preliminary data while data collection is ongoing and does not represent the final results. Results and conclusions in this abstract might change when additional data is added.

Introduction: Transdermal Optical Imaging (TOI) is a contactless vital sign monitor that can provide physiological measures such as blood pressure (BP), heart rate (HR) and respiratory rate (RR) using a facial video. It utilizes the light information captured by the camera optical sensors, reflected from a subject's face, then analyzes it using advanced machine learning methods to measure changes in facial blood flow. TOI has been demonstrated to measure BP with accuracy to international standards (5 ± 8 mmHg) in normotensive subjects under controlled conditions and detects heart rate with accuracy equal to an electrocardiogram.

Purpose: This proof-of-concept study aims to determine the feasibility and effectiveness of implementing TOI technology for contactless measurements of BP, HR and RR from surgical patients in preoperative medicine. Our study will be the first to validate the use of contactless monitoring technology in a clinical setting while including a diverse group of participants with various cardiovascular and health conditions.

Hypothesis: We hypothesized that TOI predicted vital signs will have a high agreement compared to standard measurement for HR, BP and RR since TOI has been validated in measuring BP and HR in a lab setting.

Methods: To date, we enrolled 61 out of 216 participants from Toronto Western Hospital. All patients undergoing elective surgery, are 18 years or older and able to comprehend study instructions in English were eligible to participate in the study. An iPad Pro was used to record a 1.5-minute facial video of the participant's face and the video was processed through TOI to extract vital signs measurements. For comparison of accuracy, the hospital's standard device was used measure the participant's blood pressure and heart rate. TOI derived respiratory rate was compared against counting breaths per minute.

Results: We found that TOI predicted vital signs with a measurement bias ± SD of 1.15 ± 6.06 beats/min for HR, -9.61 ± 16.56 mm Hg for systolic BP, −7.65 ± 8.42 mmHg for diastolic BP, and 1.20 ± 2.35 breaths/min for RR, respectively.

Conclusions: TOI predicted vital signs with high agreement compared to standard measurement for HR, BP and RR.

SEX-RELATED DIFFERENCES IN HEALTHCARE ACCESS AND UTILIZATION IN ADULT PATIENTS WITH INFLAMMATORY ARTHRITIS IN ONTARIO: A POPULATION-BASED STUDY

STUDENT: SANJANA TARANNUM, MSC CANDIDATE
SUPERVISOR: DR. LIHI EDER

Background: Timely access and appropriate utilization of healthcare resources are critical for management of patients with inflammatory arthritis (IA) including rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA). Sex (biological) and gender (sociocultural) are important health determinants that influence different aspects of care including referral to specialists, performance of diagnostic tests, continuity of care and prescription patterns which ultimately affect disease outcomes.
Purpose: To compare musculoskeletal (MSK)-related healthcare utilization between male and female patients with RA, AS and PsA in Ontario.
Hypothesis: I hypothesize that female patients with IA have higher healthcare utilization compared to male patients both before and after diagnosis.

Methods: Three inception cohorts of adult patients with RA, AS and PsA were assembled using Ontario health administrative data. Healthcare utilization was assessed yearly for 3 years before and after diagnosis (2010-2017), and compared between male and female patients. Dispensation of drugs was ascertained yearly for 3 years after diagnosis in patients ≥66 years at the time of diagnosis. Regression models compared healthcare utilization indicators between male and female patients after adjusting for demographics and comorbidities. Results were expressed as female to male adjusted odds ratio (aOR) with 95% confidence intervals and, a standardized difference > 0.1 and a p-value < 0.05 were considered statistically significant.
Results: A total of 41,277 patients with RA (69% females), 8,150 patients with AS (51% females) and 6,446 patients with PsA (54% female) were analyzed. Male patients were significantly older than female patients only in the RA cohort (mean age M 60.4 y, F 57.1 y). Multimorbidity, depression and osteoporosis were more common in female patients and cardiovascular disease in male patients across the cohorts.
Before diagnosis, female patients with IA were more likely to visit family physicians (aOR 1.10–1.15) and rheumatologists (aOR 1.2–2.3), receive simple imaging (aOR 1.15-1.2 for X-rays; 1.07-1.44 for ultrasounds) and laboratory tests (aOR 1.10-2.17) across the three cohorts. These sex-differences were most notable among patients with RA and AS, and in earlier pre-diagnosis periods. Male patients were more likely to visit the emergency (aOR 0.76–0.87) immediately before diagnosis. After diagnosis, female patients were more likely to remain under rheumatology care for up to 3 years compared to male patients (aOR 1.12-1.24). Prescription dispensations were higher for NSAIDs (aOR 1.14–1.16) and opioids (aOR 1.39–1.51) in female RA patients and for conventional disease modifying anti rheumatic drugs (DMARDs) in female AS patients (aOR 1.51-1.82).
Conclusion: Female patients with IA have higher MSK-related healthcare utilization than male patients which may indicate sex-related biological differences in disease course or gender-related sociocultural differences in healthcare seeking behavior.

Group J: Population Health-Education

DEATHS AMONG PREGNANT WOMEN: A RETROSPECTIVE REVIEW WITH THE ONTARIO CORONER

STUDENT: KAYVAN AFLAKI, MSC. CANDIDATE
SUPERVISOR: DR. JOEL G. RAY, MD MSC.

BACKGROUND

Maternal mortality is an often-preventable event that can have devastating consequences for families and care providers. In 2010, the WHO reported an increase in maternal mortality in Canada. As Canada's most populous province, there is a major opportunity in Ontario to audit patterns, and identify potentially modifiable factors, contributing to peri-pregnancy deaths.

PURPOSE

1. To ascertain all peri-pregnancy deaths reported to the Ontario Coroner arising during a pregnancy or up to 365 days thereafter and collect detailed information including demographic characteristics, the nature and cause of death, as well as antecedent health factors around the time of each death.
2. To classify these maternal deaths into four or five distinct sub-types, with somewhat distinct causes and maternal phenotypes.

HYPOTHESIS

We hypothesize that such deaths can be characterized into distinct sub-types vis-à-vis a latent class analysis (LCA).

METHODS

A keyword search was performed in the Ontario Coroner archives to capture maternal deaths. Granular details surrounding each death were abstracted using medical examiner investigation reports, autopsy, and toxicology filings reported to the Ontario Coroner. LCA was performed to identify sub-groups.

RESULTS

Preliminary findings show 181 peri-pregnancy deaths. LCA typifies these deaths into 4 distinct classes: a group who die predominantly of hypertensive disorders of pregnancy during or shortly after delivery ("high-risk"), a group experiencing mental health crises throughout the perinatal period ("suicide/overdose"), a group with poor antenatal care who die from unmanaged conditions ("unmanaged conditions"), and a group who die due to undiagnosed genetic disorders ("invisible conditions"). Data collection is ongoing.

DEVELOPMENT AND VALIDATION OF A CLINICAL TOOL TO ESTIMATE 1-YEAR MORTALITY IN HOSPITALIZED PATIENTS WITH DEMENTIA

STUDENT: MICHAEL BONARES
CO-SUPERVISORS: KIRSTEN WENTLANDT, PETER TANUSEPUTRO

Background: Patients with dementia are frequently admitted to hospital. Prognostic uncertainty in this patient population may inform clinical care decisions during hospitalization, including whether or not to engage specialist palliative care services. A prognostic tool could inform these decisions and increase access to these services. Unfortunately, existing prognostic models have limitations, including the exclusion of clinically meaningful and readily available variables that decrease their validity and scalability.

Purpose: The study seeks to develop and validate a clinical tool for estimating mortality within 1 year of hospitalization among patients with dementia.

Hypothesis: I hypothesize that the clinical tool will demonstrate acceptable performance among hospitalized patients with dementia.

Methods: This study will use population-level health administrative data from Ontario to study a cohort of hospitalized patients with dementia between March 31st, 2009 and April 1st, 2019. A logistic regression model will be used, whereby the outcome variable is mortality within 1 year of admission, and the predictor variables have been selected a priori based on a literature review of existing prognostic models and on clinical expertise. The cohort will be divided into derivation and validation cohorts. Model development, including data cleaning, model specification, and model estimation, will be performed in the derivation cohort. Model performance, measured by predictive accuracy, discrimination, and calibration, will be assessed in the validation cohort.

Results: The cohort comprised 298,586 patients, of whom 103,543 (34.7%) died within one year of admission. The mean age was 83.63 (SD 7.36) years, and most patients were female (58.9%). Most patients were admitted from living at home without home care (67.2%) followed by a nursing home (29.9%). The most common comorbidities were hypertension (41.9%), diabetes mellitus (27.0%), and arrhythmia (23.0%). A high proportion of patients experienced a urinary tract infection (25.2%), delirium (19.9%), a fall (19.7%), or pneumonia (17.4%) in the 3 years before their index admission. The median length of stay during the index admission was 7.0 (IQR 12.0) days. Most hospitalizations were urgent via ambulance (69.8%) and under the service of general internal medicine (66.4%), followed by orthopedic surgery (7.3%), and gastroenterology (6.3%). Model development will be completed and model performance will be assessed by Spring 2022.

Conclusions: Development of a clinical prediction tool that provides an accurate and personalized risk of 1-year mortality among hospitalized patients with dementia may increase access to specialist palliative care services, assist in the identification of eligible participants for clinical trials, and inform quality reporting for healthcare institutions and health policy.

MOTIVATIONS, PERCEPTIONS, AND EFFECTS OF CANNABIS USE IN INDIVIDUALS WITH MOOD AND ANXIETY DISORDERS.

STUDENT: ANKITA DAS
SUPERVISOR: STEFAN KLOIBER

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Assistant Professor, Clinician Scientist, Centre for Addiction and Mental Health and Department of Psychiatry, University of Toronto

Introduction: Cannabis use is common in individuals with mood and anxiety disorders**,** the most prevalent mental health conditions in Canada and worldwide. In the era of cannabis legalization, medical cannabis program and a variety of available cannabis products in Canada, there is controversy and uncertainty about the potential risks and benefits of cannabis in this population.
Objective: To conduct a systematic review of the current scientific literature and a mixed methods study including an anonymous survey and qualitative interviews on perceptions, motivations, knowledge, and effects of cannabis use in individuals with mood and anxiety disorders.
Methods: The systematic search of PubMed database has conducted. A mixed methods study consisting of an anonymous survey and in-depth interviews in patients with mood and anxiety disorders is currently ongoing.
Results: Results from the systematic review and preliminary analysis of mixed methods study indicate that cannabis use is reported to provide symptom relief for individuals with mood disorders though appears to potentially exaggerate depressive symptoms over time. Most frequent self-reported reasons for cannabis use were coping with negative affect and sleep problems. Individuals with social anxiety reported expectation of greater cognitive and behavioural impairment from cannabis use. Some veterans with PTSD reported reasons for cannabis use related to relief of side effects from pharmacological treatment, facilitation of social competency and for confrontation of the source of trauma. Individuals using medicinal cannabis reported difficulty in stopping cannabis use, failing to meet responsibilities due to use and problems with concentration and memory associated with cannabis use.
Conclusion: Our preliminary results emphasize the importance of understanding cannabis use motives and potential risks in individuals with mood and anxiety disorders. Targeting coping motives could be a potential approach in development of intervention and prevention strategies. We expect that additional results from this work will be relevant for policy, prevention strategies, and clinical practice.

EVALUATING THE USE OF ELECTROCONVULSIVE THERAPY IN LOW-MIDDLE INCOME COUNTRIES: A NARRATIVE REVIEW

STUDENT: DASKALAKIS AA
SUPERVISOR: RAVINDRAN A

Background: Electroconvulsive therapy (ECT) is a well-accepted intervention for treatment-resistant and serious mental illness. Its use, efficacy, and tolerability have been well evaluated in high but not in lower- and middle-income countries (LMICs).

Purpose: This report provides a narrative review of ECT practice in LMICs.

Hypothesis: We anticipated lower rates of ECT use in LMICs due to stigma. We also predicted greater use of ECT in an unmodified form and that its efficacy would be similar compared to developed countries.

Methods: A literature search was conducted using Medline and PubMed. Initial results yielded 81 studies from LMICs. Following screening, a total of 22 papers were included to evaluate the information on the practice and perception of ECT use.

Results: Reports from LMICs on the efficacy, tolerability, and perceptions of ECT were relatively sparse. Clinicians in LMICs report modified ECT to be effective and better tolerated. However, unmodified ECT continues to be frequently used in LMICs due to limited resources, convenience of use, and lack of trained anesthesiologists. ECT in LMICs was used for depression, schizophrenia, and bipolar disorder. The review suggests that better dissemination of information on ECT, education to reduce misconceptions and stigma, and greater emphasis on ethical processes would significantly improve practice in LMICs.

Conclusions: ECT appears effective and is perceived as such in LMICs, but the practice of unmodified ECT remains an issue with less rigorous standards of practice. There is a recognized need for national guidelines, more formal training, and better outcome research in LMICs.

CAREGIVER MENTAL HEALTH AND CAREGIVER-MEDIATED INTERVENTION IN CHILDREN WITH AUTISM SPECTRUM DISORDER (ASD): AN INTEGRATED APPROACH

STUDENT: CECILIA LEE
SUPERVISOR: DR. MELANIE PENNER

Background:
Caregivers of children with autism spectrum disorder (ASD) face high levels of stress and are at risk of developing mental health disorders. In particular, caregivers participating in caregiver-mediated interventions, such as the *Social ABCs* where they provide the intervention to their young child, juggle many responsibilities to their child and family, leaving self-care as the last priority. Caregivers may not seek mental health support, though interventions for caregiver stress, such as acceptance and commitment therapy (ACT), exist.

Purpose:
This study is the first step in addressing the gap in mental health intervention for caregivers who are also participating in caregiver-mediated interventions.

Objectives:

1. To understand the mental health needs of caregivers participating in caregiver-mediated early intervention;
2. To identify caregivers' perspectives on the benefits and disadvantages of ACT, and its potential application to those caregivers whose children are participating in early intervention.

Methods:
Utilizing a constructivist paradigm, this qualitative study involves conducting one-on-one semi-structured interviews with: a) caregivers who have participated in a caregiver-mediated early intervention program (*Social ABCs*) and b) caregivers with children with ASD who have participated in ACT. Interviews will be audio-recorded, transcribed and then coded. Coded data will undergo reflexive thematic analysis, and participants will review finalized themes to ensure they accurately reflect participants' experiences.

Results:
Data collection and analysis are currently underway.

Expected Outcomes:
Knowledge gained from this study will highlight the gaps in mental health support for caregivers with young children with ASD. It will also identify elements of an existing mental health intervention that may contribute to supporting a caregiver's mental health early on in their journey through their child's diagnosis and treatment. Our hope is that ACT may be integrated with *Social ABCs* in a way that will be complementary and synergistic, allowing caregivers to optimize their own well-being which will in turn enhance their effectiveness in applying early intervention strategies.

SCHOOL-BASED HEALTH CARE: A MODEL FOR IMPROVING ACADEMIC OUTCOMES AND EARLY IDENTIFICATION OF DEVELOPMENTAL AND MENTAL HEALTH DISORDERS FOR INNER CITY CHILDREN– A CLUSTER RANDOMIZED CONTROL TRIAL

SUPERVISOR: DR. MICHAEL SGRO
STUDENT: SAISUJANI RASIAH

Background: School-based health centres (SBHCs) aim to reduce barriers to accessing developmental and mental health care for children.

Purpose: The study objectives were to examine if access to SBHCs improves school grades, standardized test scores, school attendance, identification of students needing a physician's assessment and wait time for inner city children.

Hypothesis: Introducing SBHCs is anticipated to alleviate barriers to accessing health care services, thus improving children's academic achievement, school attendance, identification of children with developmental and mental health disorders, and decreasing wait times for developmental assessments.

Methods: This was an open label, cluster randomized control trial involving 16 elementary schools. In the intervention arm (IA), physicians attended School Support Team meetings to triage referrals to SBHCs for assessments. The primary outcome was academic achievement. Secondary outcomes were sociodemographic data and its association to grades, wait time, school attendance, the number of students identified needing a physician assessment, and diagnoses/management.

Results: There was insufficient evidence to support that the change in reading (p= 0.51, Estimate = 0.83%, 95% CI [-1.82, 3.48]), writing (p= 0.28, Estimate = 1.11%, 95% CI [-1.03, 3.25]), math grades (p= 0.98, Estimate = 0.07%, 95% CI [-2.94, 3.08]) or school attendance (p= 0.76, Estimate = 0.20, 95% CI [-1.22, 1.63]) differed between the groups. The IA's average wait time for assessment was 3.4 months. However, the proportion of students identified and referred for a physician's assessment was higher in the IA (48% (n=76/158)) than the control group (18.1% (n=30/166)), p=0.028. Additionally, many children in the IA received a diagnosis and/or further management and had a low annual income.

Conclusion: Additional time may be needed to observe differences in academic achievement. However, the SBHC model identified twice as many students needing a physician's assessment.

AN INTERACTIVE EDUCATIONAL RESOURCE FOR KNEE BIOMECHANICS INSTRUCTION

STUDENT NAME: SHEHRYAR (SHAY) SAHARAN
SUPERVISOR NAMES: MICHAEL CORRIN & TIMOTHY BURKHART

Biomechanics is formally defined as the application of mechanics in biological systems. The proposed project - titled kneeMo - tackles an area of biomechanics that is fundamental for human movement and relates to one of the most important joints in the body – the knee. Although the movement of the knee joint seems rather simple, the underlying biomechanics is highly complex and depends on several structures to ensure proper stability and mobility. Knee biomechanics is a challenging subject area to teach and learn as it is highly integrative, spatially demanding, and lacks proper instructional techniques and resources. Therefore, the primary aim of this project is to develop an interactive educational resource (designed for biomechanics instructors and undergraduate students) that consolidates cross-disciplinary information and facilitates a holistic understanding of this subject area. Following a preliminary literature review and media audit, a parallel mixed methods research design was implemented, which encompassed interviews with biomechanics instructors and a detailed analysis of course syllabi in undergraduate biomechanics courses. The consolidation of this research was used to identify the functional and technical requirements of the final design. Ultimately, it was concluded that a module-based website (with strategically designed 2D/3D interactive displays, animations and illustrations) would be the optimal solution. Once complete, the final resource will guide a student from a fundamental understanding of the knee joint to an advanced knowledge of its multidimensional movement. Ultimately, kneeMo will enable future professionals to gain a better understanding of knee biomechanics and improve injury management, treatment, and prevention options for patients.

THE VALIDATION OF THE MICHIGAN RETINAL DEGENERATION QUESTIONNAIRE AND MICHIGAN VISION-RELATED ANXIETY QUESTIONNAIRE IN ADOLESCENT PATIENTS WITH INHERITED RETINAL DISEASES

STUDENT: KAVIN SELVAN
SUPERVISOR: DR. ELISE HÉON, CO-SUPERVISOR: DR. AJOY VINCENT

Background: Advances in ophthalmology have led to the first Health Canada-approved gene therapy for a gene variant of inherited retinal disease (IRD). IRD is a group of hereditary disorders of the retina that can cause progressive photoreceptor degeneration and subsequent vision loss, sometimes leading to blindness, among other sequelae. Vision structure and function measures have been thoroughly studied and implemented to assess the patient's condition and efficacy of therapies in the IRD population; however, the condition's impact on patients' daily lives has not been sufficiently explored. Patient-reported outcome measures (PROMs) can breach this gap; they are self-report questionnaires that measure health outcomes from the patient's perspective. Recently, PROMs were created for adult patients with IRD, the Michigan Retinal Degeneration Questionnaire (MRDQ) and Michigan Vision-related Anxiety Questionnaire (MVAQ). However, there are no PROMs that have been tailored to adolescent IRD patients. Clinical trials have begun to include adolescent patients as early intervention increases the efficacy of such therapies, so PROMs for adolescent IRD patients are required. The MRDQ and MVAQ measure vision disability and vision-related anxiety, respectively, and have subscales based on retinal physiology also observed in adolescents. This study aims to validate the adult MRDQ and MVAQ in adolescent patients with IRD.

Purpose: Validate the adult Michigan Retinal Degeneration Questionnaire (MRDQ) and Michigan Vision-related Anxiety Questionnaire (MVAQ) in adolescents (13–<18 years).

Methods: Ninety-one adolescent patients diagnosed with IRDs were recruited at the Hospital for Sick Children (University of Toronto) and the Kellogg Eye Center (University of Michigan). The patients were administered the MRDQ, MVAQ, and Patient-Health Questionnaire-4 (PHQ-4). Test-retest variability was assessed in eighteen patients within 14 days of the initial administration. Additionally, adolescent responses were psychometrically analyzed to establish that responses did not show skewed distribution (*i.e.*, floor or ceiling effects), and there were no correlations to extraneous variables at a domain level (*i.e.*, domain and trait associations) and item level (*i.e.*, differential item functioning). As a further validation step, comparisons were made to adult data from the original MRDQ and MVAQ studies.

Results: Psychometric analysis showed that the existing MRDQ and MVAQ content and format were applicable in adolescents with IRD. Test-retest reliability was established ($r = 0.84$), floor/ceiling effects were not identified, and responses, as measured by domain and trait associations and differential item functioning, only correlated to visual acuity and IRD phenotype.

Conclusion: The MRDQ and MVAQ are psychometrically validated questionnaires that have established applicability in evaluating adolescent patients with inherited retinal diseases.

Group K: Population Health-Education

EVALUATION AND ADAPTATION OF THE FACE-Q | CRANIOFACIAL PATIENT-REPORTED OUTCOME MEASURE FOR OPHTHALMOLOGY PATIENTS

STUDENT: FARHEEN KHAN
SUPERVISOR: HELEN DIMARAS

Farheen Khan, Roxanne Noronha, Sara Williams, Dr. Karen Wong-Riff, Dr. Asim Ali, Dr. Helen Dimaras

Background and Purpose: FACE-Q is a patient-reported outcome measure (PROM) that evaluates outcomes of craniofacial surgery from the perspective of individuals aged 8-29. This study aimed to assess the content validity of FACE-Q questions among patients/survivors and parents/legal guardians of patients/survivors treated for corneal anesthesia (CA), retinoblastoma (RB), or strabismus (SB).

Methods: This is a cross-sectional qualitative study performed in two rounds. Eligible participants were identified in the medical record and recruited in-person during clinic visits or by a mailed introduction letter, followed by phone call. Individuals were eligible if they were (i) CA, RB, or SB patients/survivors aged 8 years or older, or (ii) parents/legal guardians of CA, RB, or SB patients/survivors aged under 8 years, developmentally delayed, or hard of hearing. The target sample size was n = 7 per participant type and condition, per round.

Participants completed cognitive debriefing interviews where they reviewed FACE-Q sections measuring eye-related, appearance-based, and psychosocial outcomes. The first round ascertained the relevance of, identified problems with, and solicited modifications to the FACE-Q. Input from round 1 informed the modification of applicable FACE-Q questions, which were evaluated in round 2. The interviews were transcribed, coded, and analyzed by thematic analysis and descriptive summaries to identify confirmatory and non-confirmatory feedback, and new concepts. The number of participants offering confirmatory, non-confirmatory, and new-concept feedback was tallied; a minimum of three instances of non-confirmatory and new-concept feedback was required for modifications to be made to the FACE-Q for round 2.

Results: Participant recruitment, data collection, and analysis are ongoing. Round 1 interviews with 5/7 RB survivors and 5/7 RB parents were completed. One transcript from each participant group was transcribed and coded. Descriptive summaries for all ten interviews have also been devised. Thus far, based on the coding and descriptive summaries, all FACE-Q sections reviewed were deemed to be relevant to the RB participants. Further, new concepts related to prosthetic eyes and mental health were identified in both, survivor and parent interviews. Concepts related to interpersonal relationships were identified in survivor interviews. There was common consensus among 5/5 parents and 4/5 survivors interviewed on modifying the response options for all sections.

Conclusion: The study results will be used to adapt the FACE-Q to ensure that it is comprehensible, comprehensive, and relevant to CA, RB, and SB patients/survivors. The adapted FACE-Q will then undergo field testing for assessment of validity and reliability as part of a future study.

HETEROSEXUAL HIV TRANSMISSION IN ESWATINI: A DESCRIPTIVE MODELLING ANALYSIS

STUDENT: JESSE KNIGHT
SUPERVISOR: SHARMISTHA MISHRA

BACKGROUND. As of 2020, eSwatini had the highest HIV prevalence in the world, estimated at 27%.[1] Even in this generalized epidemic context, female sex workers (FSW) experience disproportionate burden of HIV, with prevalence as high as 70%.[2] Recent scale-up of HIV treatment, which can prevent onward transmission,[3] is anticipated to curb the epidemic in eSwatini. However, many worry that unique needs of FSW may continue to go unmet, thus sustaining networks of transmission and potentially undermining the expected prevention benefits of treatment.[4] To understand the role of these unmet needs, we sought to describe who acquires infection from whom – i.e. networks of transmission – throughout the past, present, and future of the HIV epidemic in eSwatini.

PURPOSE. Describe the past, present, and future networks of HIV transmission in eSwatini.

HYPOTHESES. 1) Early epidemic emergence was driven by sex work partnerships; 2) Unmet needs of FSW continue to play a disproportionate role in transmission persistence.

METHODS. We developed a deterministic compartmental model of heterosexual HIV transmission in eSwatini. The model included 8 risk groups: men and women with 0-1 partners in the past 12 months, men and women with 2+ partners but no engagement in sex work, higher and lower risk female sex workers (FSW), and higher and lower risk clients of FSW; as well as 4 partership types: main/spousal (long, low condom use); casual (short, medium condom use); one-off sex work (one visit, high condom use); and regular sex work (short, medium condom use). The model also included differences in transmission risk by HIV infection stage, STI co-infection, and sexual positioning, as well as increasing condom use, circumcision, and treatment coverage over time. We calibrated uncertain model parameters to reproduce available epidemic data, including HIV prevalence, incidence, and treatment cascade, stratified by risk group where possible.[5] Finally, we quantified the proportions of yearly infections transmitted between all combinations of risk groups and partnership types.

RESULTS. Based on our calibrated transmission model, we estimated the following. Epidemic emergence before 2000 was driven by regular sex work partnerships. By 2000, main/spousal partnerships contributed the majority of infections, while casual and one-off sex work partnerships contributed relatively little to overall transmission. Since 2000, lower risk women consistently acquired the most infections each year, while clients of FSW transmitted the most infections. Beyond 2020, we project a rebounding proportion of transmission via regular sex work partnerships, and two dominant transmission networks: between FSW and clients, and between lower risk women and men, linked mainly by transmission from clients to lower risk women. We also estimate that FSW may soon represent a net sink (vs source) of infections, but that most infections among clients originate still from FSW.

CONCLUSIONS. Unnmet needs of FSW and clients of FSW have likely played a central role in HIV epidemic emergence and persistence in eSwatini. Reaching FSW and their clients with prioritized access to care will likely be critical to realizing the anticipated prevention benefits of HIV treatment scale-up in eSwatini, and in other generalized epidemics.

1 UNAIDS (2022) AIDSinfo.
2 Baral et al. (2014) PLOS ONE, 9 (12) e115465.
3 Eisinger et al. (2019) JAMA, 321 (5) 451-452.
4 Baral (2019) Lancet HIV, 6 e632-638.
5 SDHS (2006/07) MOH; SHIMS (2011) MOH/ICAP; SHIMS2 (2016) MOH/ICAP.

ASSESSMENT OF KNOWLEDGE, ATTITUDE, PRACTICE AND BARRIERS OF SMOKELESS TOBACCO USE AND CESSATION DURING COVID 19 PANDEMIC IN MANIPUR, INDIA

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SUPERVISOR NAME: DR. MICHAEL CHAITON, DR. JAGDISH KAUR

INTRODUCTION: The novel Coronavirus (COVID-19) was first reported in December 2019, as a cluster of acute respiratory illnesses in China, from where it spread rapidly to over 198 countries. It was declared as a global pandemic by World Health Organization (WHO). (1) **BACKGROUND:** Tobacco users showed a higher risk of being infected with the virus. (2) **PURPOSE:** This study was planned to assess the knowledge, attitude, practice and barriers of smokeless tobacco use and cessation during the COVID 19 pandemic. **METHODS:** A pretested, structured questionnaire was self-administered among 810 participants between 15-65 years, who reported to the Out-Patient Department of Regional Institute of Medical Sciences, Imphal, Manipur. **RESULTS:** 88.6% participants were aware about COVID. 23.2% thought that there was a relationship between chewing tobacco and COVID. 46.6% were willing to quit tobacco after the pandemic. Consciousness about health was a major to quit with 35.8% thinking positively about health, 26.2% getting time to think about health hazards, followed by 12.7% having a fear of contacting with COVID 19. Not feeling the need (31.3%) and addiction (22.1%) were the major barriers to quitting tobacco. **CONCLUSION:** Pandemic has more impact on occasional users than daily users of SLT, who are more aware of the relationship between chewing tobacco and COVID, are more cautious and willing to quit tobacco.

Source of Funding: This project was funded by the World Health Organization, South East Asia Regional Office

DEVELOPING, IMPLEMENTING, AND EVALUATING A MULTI-CRITERIA DECISION ANALYSIS (MCDA) MODEL TO AUGMENT DELIBERATIONS WITHIN HOSPITAL PERIOPERATIVE VALUE ANALYSIS TEAMS

STUDENT: AARON MILLER
SUPERVISOR: TEODOR GRANTCHAROV

BACKGROUND

Healthcare decision making is complex and often necessitates difficult trade-offs. A structured approach to improving value analysis decisions involving sets of relevant criteria can inform the development of an evaluation methodology for use with value analysis teams (VATs) and improve overall decision making. Multi-criteria decision analysis (MCDA) models have seen increasing use in healthcare in recent years and hold the potential to establish, reconcile, and weight the various criteria which underlie a given decision problem.

PURPOSE

Perioperative VATs conduct basic reviews of new equipment, tools, and products, including staplers and energy devices among others before they are adopted for use by surgical teams. The VAT is primarily deliberative and currently lacks a robust evaluative framework to support the decision-making process. This project proposes to leverage the iterative, incremental approach of plan-do-study-act (PDSA) cycles to engage VAT stakeholders in the development, implementation, and evaluation of MCDA models to support decision-making.

HYPOTHESIS

MCDA models are feasible and acceptable in their ability to augment value analysis deliberations and the utilization of evidence for perioperative products under trial.

METHODS

This prospective, multi-institutional, mixed methods, within groups PDSA Model for Improvement for leverage MCDA best practice guidelines, together with surveys and interviews, to iteratively develop, implement and evaluate MCDA models to support deliberations.

RESULTS

This project aims to understand which criteria are effective to support VAT decision making, understanding the change and implementation management strategies necessary to embed MCDA into practice in this context, understanding mitigating factors which influence VAT decision making, and what are end user and VAT stakeholder perceptions around the use of MCDA models in this context.

CONCLUSION

It is expected that this project will make several novel contributions to the growing body of knowledge around the use of MCDA models to support deliberative processes within perioperative VATs and the findings will generate strategies for the implementation and evaluation of MCDA models.

EQUITY, DIVERSITY, AND INCLUSION IN THE TREATMENT OF TRAUMATIC SPINAL CORD INJURY: JUSTIFICATION FOR A TRANSPARENT "CODE SPINE" PROTOCOL.

STUDENT: ALI MOGHADDAMJOU
SUPERVISOR: MICHAEL G. FEHLINGS.

Ali Moghaddamjou, Alex Bak and Michael G. Fehlings.

Background Context
There is a culmination of research supporting the role of early surgical decompression in the treatment of acute traumatic spinal cord injury (tSCI). Clinicians must ensure that there is equity in the delivery of early surgical decompression in tSCI patients.
Purpose: To analyze equity in the delivery of early surgical decompression in tSCI.
Study Design/Setting: Multicenter, prospective data from the STASCIS clinical trial and the NACTN database were leveraged for this study.
Patient Sample: All adult patients with tSCI that underwent surgery from the STASCIS and NACTN registries were selected.
Outcome Measures:
Time to surgery was calculated from the time of injury. The 24hr mark was selected as the cut-off for early surgery.
Methods
ASIA Impairment Scale (AIS) was assessed up to 72 hours after injury. Missing information was imputed with multiple imputations at 40 iterations. A multi-level mixed-effects multivariate logistic regression model with hospital center as the random effect and early surgery as the dependent variable was performed with the patient- and injury-specific variables recorded before surgery. Odds ratios were calculated for each inputted variable with 95% confidence intervals and p-values. The level of significance was set as $p<0.05$.
Results
A total of 580 patients (46.3±17.9 years, 20.0% female) met the inclusion criteria, with 223 patients receiving early surgery (38.4%). The majority of patients were educated at or above secondary school (86.4%), married (50.7%), and of White Caucasian race (77.2%). Injuries were mostly cervical (63.0%) followed by thoracic (23.3%), 49.5% of patients presented as motor complete, 82.9% were blunt, and 56.4% were from high energy mechanisms. In the mixed-effects regression model, being White Caucasian (OR: 1.73[1.02–2.92], p=0.041) was associated with receiving early surgery. Being transferred from another hospital for surgery (OR: 0.50[0.32–0.78], p=0.002), lower baseline lower extremity motor score (LEMS, OR: 0.97[0.95–0.98], p=0.001), and older age (OR: 0.98[0.96–0.99], p=0.001) were associated with receiving delayed surgery.
Conclusions
Our results suggest there is discrepancy when it comes to the equitable delivery of early surgical decompression in tSCI. Race, age and geographical remoteness all are associated with delays in surgical intervention which is one of the only proven treatments for tSCI. The results of this study provide justification for the implementation of systemic transparent protocols to ensure all tSCI patients are decompressed within 24-hours of injury.

EXPLORING HEALTH CARE WORKERS' PERSPECTIVES WITH PPE USE IN TRAUMA: A QUALITATIVE STUDY.

STUDENT: ANISA NAZIR
SUPERVISORS: DR. TEODOR GRANTCHAROV

Background

Frontline health care workers (HCWs) and other hospital staff are the highest risk populations of nosocomial transmission of airborne and droplet pathogens.[1] The COVID-19 pandemic has highlighted this problem and has stressed the importance of safety protocols and PPE donning and doffing procedures for frontline staff.[2] One contributory factor may be the breaches and errors in personal protective equipment (PPE) donning and doffing processes leading to transmission of pathogens to other staff and patients. Studies have discussed reasons for these errors, such as PPE types, combinations of donning and doffing procedures, adequate training modalities, and staff behaviours while using PPE in clinical settings.[3,4] There is also little evidence on transmission dynamics in the hospital and the staff's role in initiating or amplifying nosocomial outbreaks.

Purpose

At the beginning of the pandemic, OR BlackBox technology was repurposed to guide healthcare providers on PPE donning and doffing at St. Michael's Hospital. The preliminary simulation study reported significant risks in 70% of cases where a breach in sterility would expose the HCW to the virus. After adequate training and data-driven coaching, results showed 80% improvement in donning and doffing processes and compliance. It reduced infection breaches and transmission to other staff members and patients by more than 50%. However, these results are limited to a simulated environment and in practice, there are still challenges that HCWs face.

Hypothesis

Despite HCW wearing PPE for safety, there is significant self-contamination across PPE types (for example gloves and gowns). In addition, there are environmental challenges that can also result in contamination such as tight spaces, the number of people sharing the space at particular times as well as the design of the ante-rooms. Due to staff turnover and human resource constraints, it is also challenging for HCWs to have help with wearing PPE (i.e., buddy system). This study will help identify challenges HCWs face in the trauma bay while donning and doffing PPE. Additionally, it will provide a framework for understanding environmental and team challenges in donning and doffing PPE, particularly for aerosol-generating procedures.

Methods

This objective will employ a qualitative methodology. We will use a combination of stakeholder and maximum variation sampling strategies for this qualitative interview study of multi-disciplinary ED and trauma staff at St. Michael's Hospital. Stakeholder sampling involves identifying the main participants who are involved in designing, giving, receiving, or administering care in the trauma bay, and who might be affected by the evolving use of PPE in trauma care. Participants will be approached from a variety of occupational groups in order to ensure there is broad representation, including senior leadership, physicians, nurses, health discipline professionals, and non-clinical staff. In addition, we will purposefully sample hospital staff directly involved in the preparation and implementation of PPE training and protocols since the start of the COVID pandemic. The primary method for collecting data will be semi-structured qualitative interviews with ED and trauma staff using a draft interview guide informed by the study objectives and input of study investigators and collaborators. Participants' perceptions of trauma care, PPE use and individual and institutional readiness for PPE training during a pandemic will be sought.

ROLE OF SOCIAL INEQUITY IN HEART FAILURE PATIENT USAGE AND OUTCOMES OF DIGITAL COUNSELLING OR SELF-CARE

STUDENT: CHELSIA WATSON
SUPERVISOR: DR. ROB NOLAN

Background. Digital health is recognized by professional associations and international task forces as a critical element in outpatient care and accessibility. Individuals from marginalized populations (e.g., low income, non-White descent, non-male gender) experience improved outcomes in health and quality of life following conventional in-person counseling programs for self-care behavior, quality of life, and health. It is unknown whether marginalized groups with chronic medical conditions demonstrate therapeutic benefit from automated digital counseling programs for health promotion. **Purpose.** To assess the association between improvement in a validated index of mental health and program engagement (logon hours) with the ODYSSEE-vCHAT program among marginalized vs. nonmarginalized patients with chronic kidney disease (CKD) or chronic heart failure (CHF). **Hypotheses**. **Primary:** Marginalized participants (i.e., annual household income ≤ $70K/year, or non-white descent, or non-male gender) will demonstrate a dose-response association between ODYSSEE-vCHAT total logon hours (tertiles of low, moderate, and high) and improvement in the Mental Component Summary (MCS) score of the Short-Form survey at 6 months. **Secondary:** Marginalized versus non-marginalized participants will exhibit (i) lower baseline scores for the MCS and health related quality of life (HRQL), defined by the Kansas City Cardiomyopathy Questionnaire-Overall Summary score (KCCQ-OS) and the Kidney Disease Quality of Life scale (KDQOL), and (ii) decreased total logon hours on vCHAT at the 6 month assessment. We will also observe an interaction between marginalized versus non-marginalized participants and tertile of program usage on 6-month MCS and HRQL measures. **Methods**. **Sample**: CKD and CHF patients. **Assessments:** Background characteristics, MCS, KCCQ-OS, and KDQOL at baseline; MCS, KCCQ-OS, and KDQOL at 6 months; sum login hours, sum logins at baseline and 6 months. **Analyses:** Primary endpoint (6 months following enrollment). We will assess: (a) patient engagement with CHF self-care resources (sum logon hours, sum logons, and number of login days prior to a logon lapse ≥ 1 month) and (b) Patient-Reported Outcomes (health-related quality of life, self-care behaviours, quality of life activities, overall mental health, depression) at baseline and the 6-month interval. **Secondary endpoint:** Assessed at 6-months after enrollment. (a) Patient engagement with CHF self-care resources: We will assess (i) sum logon minutes, (ii) sum logons, and (iii) # logon days prior to a logon lapse ≥ 1 month. **Analysis-Primary Hypothesis:** A multivariate linear regression model with covariates that include the baseline MCS will determine if there is a significant interaction between program engagement (tertiles of total logon hours) and marginalized vs. nonmarginalized status in associated with the 6-month MCS score, which indicates increased improvements of marginalized participants in the top tertile of program engagement. For the secondary hypotheses, t-tests will compare total logon hours for marginalized and non-marginalized groups at baseline. Separate multivariable linear regression analyses will evaluate the association between tertile total logon hours and the MCS score, as well as the HRQL measures. **Results.** The outcomes are expected to support the above hypotheses. There are no results available at this time. **Conclusions.** The present findings will be applied to the ongoing development of equitable digital health services with the ODYSSEE-vCHAT program. It will help specify treatment priorites for marginalized patients with CHF or CKD to improve mentall health and HRQL.

THE VISUAL SCIENCE COMMUNICATION TOOLKIT: BUILDING UNDERGRADUATE LIFE SCIENCE STUDENTS' CONFIDENCE AND COMPETENCE IN VISUAL SCIENCE COMMUNICATION

STUDENT NAME: KE ER ZHANG
SUPERVISOR NAMES: JODIE JENKINSON, NICHOLAS WOOLRIDGE, MARC DRYER

Objective: Many undergraduate life science students demonstrate poor visual literacy and struggle to communicate scientific concepts through visuals. Yet, visualizations play an increasing role in the communication of scientific findings to the general public in the digital age, particularly in the realm of science, medicine, and health. Undergraduate students are increasingly assessed on their application of visual science communication principles, but instruction to improve their understanding of these principles is not well-integrated into their curriculum. This study aims to identify gaps in current visual science communication education through a needs assessment of undergraduate biology students and instructors at the University of Toronto St. George and Mississauga. This needs assessment informs the design and development of the “Visual Science Communication Toolkit”—a web-based system of guidelines and resources that educate users on the best practices of visual science communication.

Methods: We used a mixed methods research design; this encompassed a preliminary literature review and media audit, followed by a two-stage formative needs assessment consisting of questionnaires (n=74), a focus group interview (n=8), and one-on-one semi-structured interviews with undergraduate students, instructors, and teaching assistants (n=8). Data was analyzed descriptively and thematically (open coding).

Results: Students turn to self-directed learning due to a lack of support and guidance from instructors regarding the creation of visual science communication materials. Ambiguous standards and expectations from instructors, and insufficient feedback and critique, are obstacles to students who want to elevate their skills and effectively communicate their ideas. The ideal solution is an accessible, concise, and self-paced reference material that incorporates activities that reinforce communication skills and can be integrated into undergraduate courses.

Conclusions: Our findings support the design and development of the “Visual Science Communication Toolkit”—there is a need for guidelines and resources that improve student understanding of visual science communication principles and establish guidelines for instructors evaluating visual communication materials.

Group L: Regenerative Medicine/Development

EX VIVO PERFUSION DECELLULARIZATION OF RAT HINDLIMBS FOR VASCULAR COMPOSITE ALLOTRANSPLANTATION

STUDENT: AISHA ADIL
SUPERVISOR: SIBA HAYKAL

Aisha Adil, HBSc, Golnaz Karoubi, PhD, Siba Haykal, MD, PhD, FRCSC, FACS

BACKGROUND: Traumatic injuries and tumor resections result in volumetric tissue loss that can be difficult to reconstruct. Vascular composite allotransplantation (VCA) is a promising reconstructive surgical avenue for treating severe musculoskeletal and tissue loss. VCA allows the transfer of multiple tissue types such as the bone, muscle, nerves, skin, and vessels, as a composite subunit from donors to recipients. However, VCA's clinical implications are limited due to high allograft immunogenicity and the need for long-term immunosuppression which poses increased risk of infections, malignancies, and end-organ toxicity. Tissue engineering acellular composite scaffolds can help circumvent the need for immunosuppression and significantly advance VCA strategies. Tissue decellularization involves the removal of cellular and nuclear content while retaining the extracellular matrix structure. This decellularized scaffold can then be repopulated with patient-specific cells. Decellularization of composite tissues is of challenge due to the presence of multiple tissue types with varying tissue densities, architectures, and anatomic locations within a scaffold. **HYPOTHESIS:** Sodium dodecyl sulfate (SDS)-based decellularization will remove cellular content while preserving extracellular matrix structure in composite rat hindlimb grafts. **METHODS:** Rat hindlimbs were procured from cadaveric male Lewis rats whereby the common femoral artery was cannulated. A customized *ex vivo* machine perfusion-based, single-pass, closed-system bioreactor was designed to apply detergent perfusion at 0.1 mL/minute via the cannulated artery using 0.25% SDS concentration. Endpoint of decellularization was determined by visual examination for systemic white, translucent appearance and by histological analyses. All tissue compartments including the skin, femoral vessels, nerves, muscle, and femur were histologically assessed for preservation of tissue architecture and absence of cellular content. For recellularization, 20 x 10^6 human umbilical vein endothelial cells (HUVECs) were seeded by arterial perfusion and 20 x 10^6 L6 rat myoblasts were seeded by injection. Using the same bioreactor design, scaffolds were cultured and monitored for cell engraftment for 24 hours. **RESULTS:** Gross morphology showed systemic white, translucent appearance of decellularized hindlimbs in 0.25% SDS condition relative to native. Histologically, 0.25% SDS concentration preserved tissue architecture across all tissue compartments. Notably, it retained up to the innermost nerve tissue, the endoneurium, as well. Cellular content was absent across all decellularized tissues. Additionally, the construction of a perfusable, single-pass, and closed-system bioreactor circuit was suitable for decellularization. For recellularization, cells could be detected histologically after 24 hours of seeding. **CONCLUSIONS**: Perfusion decellularization was successfully implemented and 0.25% SDS perfusion retained all respective tissue compartments of the rat hindlimb. This is a lower concentration of SDS than what is commonly used, suggesting a less toxic approach. The successful bioreactor design can serve as a foundational model for sterilization and recellularization. Initial recellularization work shows cell engraftment within 24 hours. Further work with recellularization will involve examining long-term culturing of cells to test cell proliferation and survival within the acellular scaffolds. Next steps for decellularization include further characterization via. biochemical analyses for DNA and extracellular matrix content. The present study offers a proof-of-concept model for applying this tissue engineering technique for composite tissues and acts as a first step towards regeneration of a bioartificial hindlimb using perfusion de- and recellularization.

CIRCULATING PLACENTAL EXOSOMES IN GESTATIONAL DIABETES MELLITUS ARE ENRICHED WITH SPHINGOLIPIDS

STUDENT: VANESSA DI CECCO
SUPERVISOR: DR. ISABELLA CANIGGIA

Background: Gestational diabetes mellitus (GDM) affects up to 20% of all pregnancies, and of these, ~4-12% will be poised to also develop pre-eclampsia (PE), a serious and unpredictable hypertensive syndrome. GDM leads to immediate and/or future complications in the mother (e.g. increased risk of cardiovascular disease) and fetus (e.g. macrosomia). The placenta, an organ positioned at the fetomaternal interface, plays a central role in the pathophysiology of these disorders. We reported differential changes in sphingolipid metabolism within GDM and PE placentae, suggesting their contribution to the pathogenesis of these common pregnancy-related disorders.
Purpose: The aim of this study is to identify changes in lipid content of placenta-derived exosomes (pEXOs) circulating in GDM and GDM+PE maternal blood compared to normoglycemic and normotensive controls.
Hypothesis: Lipid cargo in circulating pEXOs reflect the altered sphingolipid metabolism found in GDM and PE placentae.
Methods: We used plasma samples obtained from a prospective heterogeneous cohort of 253 pregnant patients (n=220 normoglycemic and normotensive controls, n=24 GDM, n=9 GDM+PE) across four gestational time-points (10-14 weeks, 16-22 weeks, 26-32 weeks, and at delivery). Ultracentrifugation was used to isolate total plasma exosomes, and this was followed by immunoprecipitation targeting PLAP-positive pEXOs. The pEXOs were characterized by western blotting for exosomal markers (CD63 and Alix) and morphologically assessed using transmission electron microscopy (TEM). Nanoparticle tracking analysis (NTA) was used to quantify exosome size (50-150 nm) and concentration . MS/MSALL analysis was used to measure individual lipid species (954 total), comprising several classes. Lipid concentrations of these pEXOs were examined in control, GDM, and GDM+PE patients. Targeted sphingolipidomic analysis using LC-MS/MS was used for more accurate quantification of specific sphingolipids.
Results: NTA revealed that the concentration and size of circulating pEXOs was decreased in GDM compared to controls at 10-14 weeks and delivery ($p<0.01$). Of the 8 lipid classes studied in the unbiased lipidomic analysis (ceramides, sphingomyelins, phosphatidylserine, phosphatidylcholine, phosphatidylinositol, phosphatidic acid, phosphatidylethanolamine), all were collectively higher in GDM ($p<0.05$) than in control, and this increase was even higher in GDM+PE patients ($p<0.01$). Increased pEXO lipid classes was observed at all 4 gestational time-points (excluding phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine between 16-22 weeks). Targeted sphingolipidomics revealed that several ceramide species (i.e Cer 18:0) increased in GDM and, to a greater extent in GDM+PE, across gestation. Additionally, the sphingolipidomic analysis detected quantifiable amounts of sphingosine-1-phosphate and sphinganine-1-phosphate in these pEXOs.
Conclusions: The pEXOs of GDM and GDM+PE patients exhibit higher lipid concentrations compared to controls. Sphingolipidomic analysis confirmed increased sphingolipid species across gestation in GDM pregnancies. Our findings verify that placental lipids are shuttled into the maternal circulation via exosomes and their content reflects the placenta's pathological state. Changes in pEXO sphingolipid content may provide diagnostic biomarkers for early detection of GDM and associated comorbidities like PE.

A HUMAN MISSENSE INTEGRIN-LINKED KINASE VARIANT NEGATIVELY REGULATES MURINE RENAL DEVELOPMENT VIA MTOR SIGNALING IN METANEPHRIC MESENCHYME

STUDENT: XIANGYUE HU
SUPERVISOR: NORMAN D. ROSENBLUM

Nephrogenesis and branching morphogenesis are critical to kidney development and the pathogenesis of Congenital Anomalies of the Kidney and Urinary Tract (CAKUT). Identification of gene variants via genomic sequencing aims to elucidate molecular mechanisms underlying CAKUT. Functional analyses on such variants are required to identify their pathogenic mechanisms. Here, we hypothesize a deleterious role of a CAKUT-associated human missense variant of Integrin-Linked Kinase (ILK), a key regulator of renal branching morphogenesis, and elucidate its pathogenic effects on renal development.

An ILK missense variant, *ILK-T173I*, was identified in a CAKUT patient and mother by targeted gene panel sequencing and verified by Sanger sequencing. To begin to identify the molecular pathways downstream of *ILK-T173I*, lentivirus-mediated overexpression of *ILK-T173I* in mouse inner medullary collecting duct (mIMCD3) cells demonstrated dysregulated expression of AKT/mTOR target mRNAs, identified by RNA microarray and qPCR, and elevated levels of phospho-p70-S6Kinase, a mTOR target (n=3, P=0.03). *ILK-T173I* overexpression in mouse embryonic kidneys showed increased phospho-p70-S6Kinase (n=3, P=0.03) and decreased ureteric tip number by 50% (n=15, P=0.003), both of which were rescued by treatment with Rapamycin, an mTOR inhibitor (n=4, P=0.04). Analysis of the pathogenic effects of *ILK-T173I* in a physiologic genetic context was performed in mice in which the *Ilk-WT* allele was replaced with *Ilk-T173I* using CRISPR/Cas9. *Ilk-T173I* knock-in mice were characterized by low nephron number (n=6, P=0.04), decreased ureteric branching (n=5, P=0.006), and increased expression of phospho-p70-s6Kinase (n=3, P=0.014). Treatment of *Ilk-T173I*-knock-in embryonic kidney explants with Rapamycin rescued ureteric branching to levels observed in *Ilk-WT* mice. Unbiased analysis of gene expression by RNA microarray in FAC-sorted ureteric and mesenchymal cell populations isolated from *Ilk-T173I*-knock-in embryonic kidneys indicated elevated mTOR signaling is limited to the mesenchymal cell population.

Together, our data indicate that human Ilk-T173I variant impairs renal development in a mTOR-dependent manner, specifically acting within the mesenchymal cell population.

A COMBINED CELL AND GENE THERAPY FOR THE TREATMENT OF RETINITIS PIGMENTOSA

STUDENT: ERIC JONG
SUPERVISOR: ANDRAS NAGY

Background and Purpose: Retinitis pigmentosa (RP) is one of the most common inherited retinal diseases that causes progressive photoreceptor degeneration, resulting in severe vision loss or blindness. Gene replacement therapy has demonstrated great promise in the clinic, however, developing a treatment for every genetic cause is infeasible as mutations in over 70 different genes that affect the photoreceptors or retinal pigment epithelium (RPE) contribute to this disease. Photoreceptor transplantation to restore vision has potential but this strategy is limited by poor cell survival and integration with the host retina. Here, we propose a therapeutic approach that could be used to treat most, if not all, cases of RP by delivering relatively inert, long-lived cells that can produce a biologic *in situ*.

Hypothesis: Transgenic cells delay photoreceptor degeneration in a mouse model of RP by inhibiting microglia activation, a non-cell autonomous mechanism of photoreceptor death.

Methods: Human embryonic stem cells (hESC) equipped with our FailSafe™ system were genetically engineered to overexpress soluble CX3CL1 (sCX3CL1) using the *piggyBac* transposon – it has been demonstrated that exogenous sCX3CL1 delays photoreceptor degeneration in mouse models of RP, possibly via microglia inhibition, a non-cell autonomous mechanism of photoreceptor death. sCX3CL1-hESC were differentiated into RPE cells due to their longevity and quiescent state – cells were then characterized by RT-qPCR, flow cytometry, and immunocytochemistry. Western blotting and ELISA of conditioned media confirmed secretion of sCX3CL1. sCX3CL1-RPE were delivered into the subretinal space of immunosuppressed (Cyclosporine A) Rd10 mice, a model of RP, prior to the onset of photoreceptor degeneration.

Results: We show that sCX3CL1-RPE can survive in the degenerating retina for up to two weeks, retain retinal cell fate and transgene expression; however, donor cells were absent three weeks post-treatment. Subretinal injection of the same RPE cells into immunocompromised mice reveal their capacity for long-term survival and that poor cell survival in the Rd10 mouse could be attributed to insufficient immunosuppression or the degenerating retina. Nonetheless, rhodopsin staining indicates that eyes treated with sCX3CL1-RPE contained more rod photoreceptors in the outer nuclear following bulk rod degeneration compared to control groups, yet this effect was only observed where donor cells were present. Recently, we delivered sCX3CL1-RPE into the vitreous to assess whether the administration route can affect therapeutic outcome. To determine its mechanism of effect, mice will be treated with minocycline, an inhibitor of microglia activation, which has been shown to delay photoreceptor degeneration in the Rd10 mouse.

Conclusions: Overall, our results demonstrate the potential for a combined cell and gene therapy to prevent photoreceptor degeneration in a localized manner. This strategy could be employed to treat the cone-rich macula by using cells that produce a cone-specific trophic factor. The success of this proof-of-concept will provide insight into the treatment of other neurodegenerative diseases and will also support the application of safe, therapeutic cells in the clinic.

THE FIBRINOGEN-LIKE PROTEIN 2 MOLECULE INFLUENCES THE DEVELOPMENT OF THYMIC REGULATORY T- CELLS

STUDENT: CHRISTINA LAM
SUPERVISOR: DR. STEPHEN JUVET

Solid organ transplantation is the primary treatment for patients with end-stage organ failure. Advancement in surgical technique and development of immunosuppressants have significantly improved short-term graft survival. Unfortunately, long-term graft survival has stagnated as most will be rejected by patients over time. Although the current immunosuppressants can blunt the initial immune response, they are inefficient at preventing a process called chronic graft rejection. This is when the recipients' immune system perceives the grafts as foreign and continually attack them. Grafts' function will decline steadily and eventually fail, resulting in patients returning to the transplant list for a second graft. Considering how limited organs are, one of the main interests in the field is finding alternative methods that can induce safe and lasting tolerance, eliminating the need for re-transplant.

An attractive strategy is to give patients regulatory T cells, or Tregs. These are T cells that express the markers CD4, CD25, and FOXP3. Tregs play critical role in maintaining self-tolerance and immune homeostasis. Deficiency in Tregs results in systemic autoimmune diseases and excessive immune response. Much of Tregs' development and commitment stages currently remains unknown. A strong understanding of this process is needed if we were to fully realize Tregs' potential in solid organ transplantation. One of the main modulators of Tregs' functions is a protein called Fibrinogen-like protein 2 (Fgl2). Previous research has demonstrated that Fgl2-deficient Tregs are less effective at immune suppression, so, we tested the hypothesis that Fgl2 is required for normal Treg development.

Since Tregs develop from stem cells in our bone marrow (BM), we first generated reciprocal Fgl2 bone marrow chimeras by irradiating mice to remove their respective BM stem cells and replace them with BM from either $Fgl2^{-/-}$ (KO) or $Fgl2^{+/+}$ (WT) mice, producing WT→KO and KO→WT chimeras and the corresponding KO→KO and WT→WT controls. We waited around 3-4 months for the new cells to engraft in the recipients. Once the reconstitution reached ≥90%, we collected the mice's plasma and their thymi since this is where Tregs finish their maturation. We analyzed the plasma for Fgl2 level with an ELISA and the thymi with flow cytometry.

We found that WT→KO chimeras had a faster rate of reconstitution than WT→WT chimeras. Analysis of thymocyte developmental stages revealed no significant differences between the groups. Thymi from KO→WT chimeras had a significantly lower number of donor-derived Tregs, whereas thymic Tregs were present in higher numbers in KO→KO and WT→KO chimeras. The expression of PD-L1, a marker of Treg's function, differed dramatically among the groups, with KO→WT and WT→KO chimeras exhibiting an elevated percentage and absolute number of PD-$L1^{+}$ cells compared with the other chimeras. There were no significant differences in Fgl2 plasma levels between the different chimeras.

Our results indicate that circulating Fgl2 is derived from a radioresistant source and influences the rate of T cell reconstitution. Both radiosensitive and radioresistant sources of Fgl2 affect thymic Treg expression of PD-L1. Our future work will focus on elucidating the mechanisms Fgl2 utilizes to regulate Tregs' developments.

AUTOMATED DIAGNOSIS OF LIVER ALLOGRAFT FIBROSIS USING DEEP LEARNING APPLIED TO CONVENTIONAL ULTRASOUND IMAGES

STUDENT: MADHUMITHA RABINDRANATH
SUPERVISOR: MAMATHA BHAT

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Background & Purpose: Liver transplantation (LT) is currently the only treatment for patients with end-stage liver disease. However, LT recipients can succumb to graft-related pathologies which include new or recurrent disease and cancer, contributing to graft fibrosis (GF). Progression of GF is determined to be faster compared to native livers and result in cirrhosis, compromising the long-term outcomes of LT recipients. Providing early interventions for GF is dependent on timely diagnosis. Current methods to diagnose GF are limited: Liver biopsy is the gold standard but is an invasive tool associated with complications, and other less invasive methods have lower accuracy, unable to replace liver biopsy. With recent advances in machine learning (ML) and deep learning (DL), automating non-invasive methods by leveraging radiological images is increasingly seen as an alternative. We aim to develop non-invasive diagnostic and prognostic tools using ultrasound (US) images that can determine GF grade and the risk of developing significant GF, respectively.

Hypotheses: In-house developed DL tool for GF diagnosis will perform comparably to liver biopsy results. The predictive ML tool will provide superior risk scores and predictions of significant GF compared to conventional biostatistical modeling.

Methods: Using 2,485 anonymized US images from 1,384 patients which are matched to biopsy results, the cohort was split into 70% for training and 30% for testing sets. Training US images will be annotated with regions-of-interest and subsequently inputted into a DL model, U-Net, to classify fibrosis grade (METAVIR F0-F4), developing a non-invasive diagnostic model. Additionally, demographic, clinical, and serum data were collected for all patients and combined with transformed imaging features extracted from the U-Net will be inputted into a support vector machine, a ML model, to calculate the risk of developing GF. Both models will be validated using the testing set and specifically, the prognostic tool's performance will be compared to statistical models.

Anticipated Results: Based on previous studies that have leveraged non-invasive diagnosis and prognosis using ML in non-LT populations, we anticipate that both models will perform well and provide accurate fibrosis grade and risk scores.

Conclusion: Developing accurate non-invasive tools can enable longitudinal monitoring of grafts and assist clinicians in administrating early interventions, improving the long-term outcomes of LT recipients.

BIOENGINEERING VASCULARIZED FLAP ALLOGRAFTS FOR RECONSTRUCTION OF LARGE SOFT TISSUE DEFECTS

STUDENT: MICHAEL XU
SUPERVISORS: SIBA HAYKAL, TOM WADDELL

Authors: Michael Xu, MD, Golnaz Karoubi, PhD, Thomas K. Waddell, MD, PhD, Siba Haykal, MD, PhD

BACKGROUND – Large volume soft tissue defects impose substantial effects on patient quality of life, cause loss of function, and can result in permanent disability. Engineered tissues using decellularization/recellularization methods can circumvent issues of donor site morbidity or immunosuppression associated with conventional autologous free flap reconstruction or vascularized composite allotransplantation (VCA). We describe the decellularization and recellularization of vascularized soft tissue flaps (omentum, tensor fascia lata, radial forearm, and latissimus dorsi muscle) in a porcine model using an *ex vivo* perfusion bioreactor.

OBJECTIVES/HYPOTHESIS - We seek to decellularize and recellularize vascularized soft tissue flaps (omentum, tensor fascia lata, radial forearm, and latissimus dorsi muscle) in a porcine model using an *ex vivo* perfusion bioreactor. We hypothesize that perfusion seeding of human umbilical vein endothelial cells (HUVECs) can be used to recellularize the flap vascular compartment in an *ex vivo* perfusion bioreactor.

METHODS – Soft tissue flap scaffolds based on their main vascular pedicle were surgically procured from Yorkshire pigs (~35-40kg), cannulated, then placed in an *ex vivo* perfusion bioreactor. Flaps were immediately flushed with heparinized saline (15 U/mL) then perfused at 2mL/min in a perfusion bioreactor with a sequence of 0.05% sodium dodecyl sulfate (SDS), DNase (0.1 mg/mL), phosphate-buffered saline, followed by sterilization with 0.1% peracetic acid/4% ethanol. Recellularization was performed using 2 x 10^7 HUVECs suspended in EGM2 growth media (Lonza) introduced into the scaffold arterial inlet by manual syringe seeding. Scaffolds were statically cultured for 4 hours to allow cell attachment and then perfused with EGM2 for 1 day. Biochemical analysis for DNA and glycosaminoglycan (GAG) content was performed to quantitatively assess decellularized scaffolds. Decellularized and recellularized scaffolds were also assessed histologically by hematoxylin & eosin staining.

RESULTS – Four vascularized soft tissue flaps in a porcine model were procured and perfusion decellularized in a bioreactor. The omentum, tensor fascia lata, and radial forearm flaps were decellularized with 0.05% SDS for two, three, and five days, respectively and demonstrated absent nuclear staining on H&E. However, latissimus dorsi decellularization is incomplete even after 14 days. DNA content in the acellular scaffolds was significantly lowered in the omentum, tensor fascia lata, and radial forearm flaps although no significant change in GAG content was observed. Preliminary experiments to recellularize the omentum and tensor fascia lata with HUVECs showed evidence of cell attachment on the vascular lumen after one day of culture.

CONCLUSIONS – Acellular omentum, tensor fascia lata, and radial forearm flap scaffolds can be successfully decellularized with low concentration SDS, although latissimus dorsi decellularization is heterogenous and requires further optimization. Scaffold recellularization with HUVEC cells is also feasible with evidence of cell attachment onto the acellular omentum and tensor fascia lata. Future work will expand recellularization conditions for each porcine flap model and characterize the vascular networks in recellularized flaps using microtomography. We will also examine recellularization using endothelial cells co-cultured with human mesenchymal stem cells.

Group M: Endocrine-Gastroenterology/Other

BONE HEALTH IN YOUNG ADULTS WITH TYPE 1 DIABETES AND PROGRESSIVE EGFR DECLINE

STUDENT: OLUWAFUNMBI (FUNMBI) T. BABALOLA
SUPERVISOR: FARID H. MAHMUD

Background: Bone disease is an emerging complication of type 1 diabetes (T1D) with higher fracture rates observed and associations with microvascular complications, such as diabetic nephropathy, contributing to increased bone fragility.

Purpose: To determine if progressive eGFR decline in youth with T1D is associated with changes in bone structure and bone biomarkers. Secondarily, to assess the impact of demographic and diabetes variables on bone health.

Hypothesis: We hypothesize microarchitectural changes and altered bone biomarkers in the progressive eGFR decliner group compared to the group with stable eGFR decline. We hypothesize certain variables such as vitamin D status, blood glucose control and sex would have an effect on bone health.

Methods: Linear mixed effect modeling was used to generate subject specific eGFR slopes using CKiDU25 eGFR measurements at 4-time-points. Progressive eGFR decline was defined as decline $\geq$ 3ml/min/1.7m^2/year. Bone health was assessed in adulthood using High Resolution Peripheral Quantitative Computed Tomography (HRpQCT) and serologic bone biomarkers: osteocalcin, procollagen-type-1-N-terminal-propeptide (P1NP), bone-specific-alkaline-phosphatase (bALP), and C-terminal-cross-linked-telopeptide (CTX). Linear regression analysis with adjustment for covariates was performed.

Results: 99 participants, 45% male, were assessed over 7.4 ± 1 years, from 14 ±1.7 years to 21.3 ± 2.1 years. Mean A1C was 8.3%. Mean eGFR was 108.1 ± 13.1ml/min/1.73m^2/year and 44% of study participants showed progressive eGFR decline. 26% of study participants had optimal 25 hydroxy-vitamin D $\geq$75nmol/L. eGFR decliners had higher tibia cortical porosity diameter than non-decliners ($p = 0.035$). Increase in 25 hydroxy-vitamin D was associated with decrease in tibia trabecular separation ($p = 0.01$). Increase in diabetes duration was associated with increasing tibia trabecular separation ($p = 0.004$) and decreasing trabecular number ($p = 0.01$). Increasing A1C was associated with reduced levels of P1NP ($p = 0.0008$). Higher BMI was associated with decreasing osteocalcin ($p = 0.009$).

Conclusion: Progressive eGFR decline in youth with T1D was associated with early signs of altered bone microarchitecture, highlighting a potential at risk group. Low vitamin D status and longer duration of diabetes contributed to microarchitectural changes while higher BMI and suboptimal glycemic control was associated with impairments in bone formation.

CYP2C19 PHARMACOGENETIC TESTING IN PAEDIATRIC PATIENTS WITH EOSINOPHILIC ESOPHAGITIS INFLUENCES DOSING OF PROTON-PUMP-INHIBITORS AND RESPONSE TO THERAPY

STUDENT: KRISTEN BORTOLIN
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Background: Eosinophilic esophagitis (EoE) is a chronic inflammatory disorder that can be treated with a proton pump inhibitor (PPI). Pharmacogenetics (PGx) is the study of how variations in an individual's genome influences drug response. Genetic variation in the metabolism gene CYP2C19 can produce differences in enzyme activity which is known to be a contributing factor for therapeutic failure with PPI treatment. Use of 2nd-generation PPI (rabeprazole) can be beneficial in some as this PPI is less effected by CYP2C19 metabolism. PGx has been studied in PPI therapy for peptic ulcer disease but has not been demonstrated in patients with EoE.

Aims: To describe the CYP2C19 metabolism in patients with EoE on PPI and to estimate the clinical utility of PGx testing in directing subsequent changes in therapy with improvement in remission rates.

Methods: Interim analyses of a single centre, non-interventional, ongoing descriptive pilot study investigating CYP2C19 metabolism in patients with EoE, as part of a larger PGx pilot study and EoE- AHEAD Registry Study at SickKids. Patients with EoE that were newly diagnosed and started PPI or those not in remission on current non-PPI therapy or not in remission on dose PPI (2 mg/kg/day, max 30 mg lansoprazole BID) were included. Active disease was defined as a peak eosinophil count >15/hpf.

Results: 51 patients met the inclusion criteria with completed PGx test; mean age was 11 years, 43(84%) were male, and 42(82%) had concurrent atopic disease. PGx testing showed that 16(31%) and 4(8%) were rapid (RM) and ultrarapid metabolizers (URM) respectively. Of this subgroup, 9 started rabeprazole, 1 had a lansoprazole dose increase, and 6 had no changes. Overall, changes in therapy based on PGx testing were made in 38(75%) patients, 13 are awaiting follow-up. Patients with available repeat biopsy results after PGx test-guided therapy changes is limited due COVID-19 related delays in endoscopies.

Conclusions: The preliminary findings of our study using PGx to guide PPI dosing in pediatric patients with EoE demonstrate that PGx test results lead to a change in clinical management in most patients. In RM and URM, PGx results trigger an adjustment of PPI dose or type could lead to earlier disease remission in PPI-responsive patients, thereby optimizing PPI efficacy. PGx may support dose reduction in poor metabolizers aiming to avoid long-term adverse events.

Further correlation with endoscopy and histology findings of patients after PGx-guided therapy changes will follow. Furthermore, it is important to examine if CYP2C19 variant information available before PPI therapy further streamlines an initial phase of the treatment.

ENDOTHELIAL REGULATION OF PLATELET ACTIVITY IN *DGKE*-DEFICIENCY DEPENDS ON PHOSPHOINOSITIDE-MEDIATED VEGF SIGNALLING

STUDENT: ERGI DULI
SUPERVISOR: MATHIEU LEMAIRE

Ergi Duli, Vincent So, Jing Wu, **Mathieu Lemaire**

Introduction: Atypical hemolytic uremic syndrome (aHUS) is a rare pediatric thrombotic disease affecting the glomerular capillaries. Mutations in diacylglycerol kinase epsilon (*DGKE*), a lipid kinase of the phosphatidylinositol (PI) cycle, cause a novel and aggressive form of aHUS. Sequestration of vascular endothelial growth factor (VEGF) or blocking of its receptor triggers thrombus formation and glomerular injury in humans and mice that parallels the phenotype of *DGKE*-aHUS. VEGF promotes the production of endothelial cell (EC) derived platelet antagonists via metabolism of $PI(4,5)P_2$, a critical signalling PI-cycle lipid.

Objective and Hypothesis: My goal is to establish a connection between EC phosphoinositides and the regulation of platelet activity. I hypothesize that *DGKE*-deficiency alters $PI(4,5)P_2$ levels in ECs, thereby increasing the risk of glomerular thrombosis.

Methods: Thrombotic potential was measured by assessing the binding of fluorescent-labelled donor platelets to EC monolayers treated with VEGF for 15 minutes. Lipid levels of immortalized human umbilical vein EC, primary blood-derived ECs, and mouse glomeruli isolated with a graded-sieving strategy were analyzed using mass spectrometry lipidomics. Protein expression was assessed via Western Blotting.

Results: *DGKE*-deficiency was associated with low levels of $PI(4,5)P_2$ containing stearic and arachidonic acid acyl chains. Knockout EC monolayers displayed significantly increased platelet binding which was restored to normal upon VEGF treatment. *DGKE*-deficient ECs contain significantly reduced levels of endothelial nitric oxide synthase and activated protein kinase B, enzymes downstream of $PI(4,5)P_2$ metabolism which generate the potent platelet antagonist nitric oxide. VEGF treatment increases activation of protein kinase B and endothelial nitric oxide synthase.

Conclusion: *DGKE*-deficient ECs exhibit a pro-thrombotic phenotype stemming from decreased availability of $PI(4,5)P_2$ and downregulated expression of enzymes involved in the production of platelet antagonists. VEGF transiently attenuates the effect of low $PI(4,5)P_2$ and may be a suitable first-line treatment for *DGKE*-aHUS and other renal thrombotic disorders.

DOES PHOTOTHERAPY EXPOSURE IN NEONATES PREDICT AN INCREASED RISK OF CANCER?

STUDENT MARIA GHOBRIAL
SUPERVISOR DR. MICHAEL SGRO

Background: Phototherapy is an established method of managing neonatal hyperbilirubinemia (jaundice), caused by high bilirubin levels in the blood. Untreated infants face severe neurological damage and decreased quality of life. The goal of therapy is to prevent kernicterus brain injury by decreasing serum bilirubin levels. Phototherapy, a blue LED lamp unit, assists in changing bilirubin's conformation, allowing its elimination. Since its conception, phototherapy has been known to be safe and effective. Many clinicians often initiate phototherapy at levels lower than those recommended by the American Academy of Pediatrics. The rise in phototherapy use may be a result of a general belief that phototherapy is safe, increased identification of neonates with hyperbilirubinemia through universal screening protocols, and fear of kernicterus. However, since its first use, phototherapy has not been studied for potential long-term side effects. Some studies have shown interference with maternal-infant bonding during breast feeding and development. Other side effects include oxidative stress, and bronze baby syndrome. Although phototherapy is generally thought to pose minimal risk, questions of cancer risk have been raised. Previously, two large epidemiologic studies have found a trend toward increased likelihood of cancer risk and of subsequent cancer, but none have been large enough to show conclusive evidence.
Purpose: To assess if blue light phototherapy exposure in neonates can predict an increased risk of cancer.
Hypothesis: H_A: Phototherapy exposure predicts an increased risk of cancer.
Methods: This is a systematic review and meta-analysis of available literature with two phases of screening: title and abstract, then a full-text review of studies within predetermined inclusion criteria. Each phase has two independent reviewers, and two others to resolve any inclusion decision conflicts. Quantitative and qualitative assessments will be performed. Extraction and presentation of data will include summary tables of demographic characteristics, incidence and type of cancer, logistic regression analysis results, hazards, and confidence intervals. Figures of cumulative incidence and risks will be included. Confounding variables will be adjusted by propensity adjustment as well as traditional logistic regression models. Regression models for hazard ratios and confidence intervals may be used.
Results: Initial review of MEDLINE has generated 600 results, notwithstanding other databases.
Conclusions: If a significant correlation is found, a recommendation to modulate and carefully utilize phototherapy nomograms will be advised for. However, as phototherapy is a safe and effective treatment, no significant results are anticipated.

THE ROLE OF A HOMOZYGOUS PROTEIN CODING VARIANT OF EPS8 IN THE PATHOGENESIS OF PEDIATRIC IBD

STUDENT: KELVIN LONG
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Background: The rate of onset for Inflammatory Bowel Disease (IBD) is rising worldwide—most significantly in the pediatric population. The pathogenesis of the disease involves a complicated interaction between the environment and genetics. Recent findings suggest that there is a broad range of rare, single-gene mutations that correlate with the IBD phenotype in children. Some of these single-gene defects can disrupt the epithelial barrier, which alters intestinal immune homeostasis. Epidermal Growth Factor Receptor Kinase Substrate 8 (EPS8) is crucial for the growth and elongation of F-actin in epithelial microvilli. Previous literature shows that loss of expression of EPS8 disrupts the polymerization of F-actin in intestinal microvilli resulting in shortened brush borders. We identified a pediatric IBD patient with a homozygous missense *EPS8* mutation (c.2099C>T, p.Ile700Thr) in the actin-binding domain. The patient presented with pancolitis, colonic strictures and other IBD-like symptoms at 6 weeks of age. Currently, the functional role of EPS8 in the pathogenesis of very early-onset IBD remains elusive.

Purpose:

1. To investigate if the patient with EPS8 mutation has microvilli disorganization.
2. To determine EPS8 localization in patient samples and cell models.
3. To clarify how the EPS8 mutation affect F-actin *in vitro.*

Hypothesis: the EPS8 mutation disrupts microvilli organization, localizes less with F-actin and alters F-actin structure length and density *in vitro*.

Methods: A rare, damaging mutation in *EPS8* was identified in a very early-onset IBD patient by Whole Exome Sequencing. From patient-derived colon biopsy samples and *in vitro* models, the localization of EPS8 and F-actin was visualized by immunofluorescence microscopy. The morphology of the patient's intestinal microvilli was assessed using transmission electron microscopy. EPS8 expression was measured using western blot.

Results: Immunofluorescence microscopy of the colonic sections revealed lower co-localization of EPS8 and actin on the apical surfaces, compared to IBD and normal controls. Closer examination of the cell structure using electron microscopy showed disruption of the microvilli in the mutant EPS8 patient, but not in IBD or normal controls. Western blots showed no differences in protein expression between wildtype and mutant EPS8 in HEK293T cells. Immunofluorescence microscopy of cos7 cells showed lower F-actin density and length in Eps8 mutant vs wildtype.

Conclusion: The patient with EPS8 mutation had microvilli disorganization. EPS8 localized differently in the patient colon tissues compared to normal and IBD control samples. The EPS8 mutation may affect F-actin polymerization, which may lead to disruption of the microvilli.

CHARACTERIZATION OF THE EFFECT OF A NOVEL HYPERVALENT ANTIMICROBIAL AGENT ON SKIN CELLS

STUDENT: VIDA MAKSIMOSKA
SUPERVISOR: KATALIN SZASZI

Background: Chronic complex non-healing wounds are difficult to treat and frequently debilitate affected patients. Vulnerable patients, such as those with diabetic foot ulcers, are in constant pain as wounds tend to worsen and are stalled in an infected state. When chronic wounds become infected, high morbidity and mortality are common. Despite widespread efforts by researchers and industry to advance therapeutic strategies for effective treatments of chronic wounds and associated infections, antibiotic resistance of pathogens found within wound beds remains of significant concern. Additionally, few therapeutics promote cell migration of key skin cells, such as keratinocytes, that are known to aid in the re-establishment of a skin barrier. Developing new therapies requires a better understanding of cellular processes that are key for wound healing, including the collective migration of skin cells (keratinocytes) and their regulation.

Antibiotic treatments often fail to clear wound infections due to antibiotic resistance. Unresolved inflammation due to infection can lead to biofilm formation by the microbes within chronic wound beds, reducing immune cell function and inflicting patients with persistently infected wounds. A novel therapeutic called Ag373K is a promising new drug being developed to treat these resistant microbes. Ag373K was tested against a variety of microbes and was shown to clear even the most persistent microbes. Ag373K is a promising candidate drug for the treatment of chronic wounds however there is a gap in our knowledge regarding its effects on skin cells.

Methods: Novel Ag373K is a stable chelate complex that has shown promising preliminary in-vitro evidence against microbes. In this study, we assessed the impact of Ag373K on human keratinocytes (HaCat cells). We assessed viability, and cytotoxicity using an MTT assay, and cell migration using an imaging-based migration assay. Analysis of the cytokines present in the medium of migrating cells and qPCR analysis of migrating cells at various time points was conducted to assess the difference between treated and untreated conditions.

Results: MTT assay results showed an optimal and non-toxic Ag373K concentration to fall within 10-5µl. Interestingly at the 5µl, a heightened metabolic response was observed. Migration assays showed that the cell monolayers treated with Ag3K3K at 5µl showed increased cell movement and a faster wound closure time than compared with untreated controls. Analysis of the cytokine levels present in the medium of migrating cells showed that the Ag373K treated cells had increased levels of soluble VEGFA and EGF compared to the untreated control. Lastly, the qPCR analysis of migrating cells showed increased expression of VEGFA, ROHA, and RAC1 mRNA.

Conclusion: Ag373K is a novel drug showing a lot of promise in clearing infections and aiding with collective cell migration. At an ideal concentration, this drug is shown to be bactericidal and safe to treat on human cells and has been shown to promote wound closure in an in-vitro migration assay. Ag373K was shown to increase the soluble levels of VEGFA in the supernatant of migrating cells which may facilitate cell migration. The role of the altered ROHA and RAC1 genes and the released factors in augmenting migration will be established in future studies. Our studies verify that Ag373K is a promising new drug in the field of chronic wound healing.

EMERGENCY GENERAL SURGERY CONDITIONS IN KIDNEY TRANSPLANT PATIENTS: HIGH-RISK DISORDERS IN A HIGH-RISK COHORT

STUDENT: DR. JORDAN NANTAIS
SUPERVISOR: DR. NANCY BAXTER

Background: Emergency general surgery (EGS) conditions have substantially higher rates of morbidity and mortality than similar elective events. Kidney transplant (KT) recipients represent a growing demographic presumed to be high-risk surgical candidates. However, knowledge of EGS outcomes in these patients is limited.

Purpose: To quantify the differences in the clinical outcomes of KT patients admitted with EGS disorders compared to the non-transplant population.

Hypothesis: We hypothesized that patients with a previous KT would have higher odds of mortality and complications in comparison to non-transplant patients.

Methods: We performed a population-based retrospective study, using databases housed at ICES, of all adults (≥18) admitted for the first time with an EGS condition in Ontario. We included admissions from April 1, 2002 to December 31, 2019. A descriptive analysis of KT recipients compared to non-transplant patients was performed to examine patient characteristics, diagnoses, and clinical outcomes including mortality and complications.

Results: 1,273 KT and 679,492 non-transplant EGS admissions were identified over the period. Patient age, income, and rurality were similar, but fewer KT patients were female, and KT patients had a higher comorbidity burden. Those with a KT were less frequently admitted with cholecystitis or appendicitis but more often had diverticulitis. The KT patients had higher odds of complications (odds ratio, OR 4.0), readmission (OR 1.9), and death (OR 1.7).

Conclusions: Previous KT status is associated with higher rates of complications, readmission, and death compared to non-transplanted patients, verifying their high-risk nature.

EFFECT OF ANTIRETROVIRAL SWITCH FROM TDF TO TAF ON ALT, LIPID PROFILE AND RENAL FUNCTION IN HIV/HBV-COINFECTED INDIVIDUALS IN A NATIONWIDE CANADIAN STUDY

STUDENT NAME: ARIF SAROWAR
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Introduction: Compared to tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) shows increased renal safety, with studies noting changes in lipid profile and ALT normalization for patients switching to TAF.

Purpose: We aimed to study changes in these three parameters after switching from TDF to TAF therapy in a real world setting in HIV/HBV co-infected patients.

Hypothesis: We hypothesize that TAF switch will demonstrate higher renal function and ALT normalization in HIV/HBV co-infected populations than those on TDF.

Method: HIV/HBV patients who switched from TDF based antiretroviral therapy (ART) to TAF based ART from 6 academic institutions from the Canadian Hepatitis B Network (CanHepB) were included. Changes in lipid profile, eGFR, and ALT were evaluated using linear mixed effect model regression.

Results: 82 HIV/HBV co-infected patients switched from TDF to TAF with mean 103 week follow up duration. At time of TAF switch, 19/82 had elevated ALT levels, 80/82 were virally suppressed, and 62/82 had eGFR > 60 mL/min/1.73 m^2. 26/82 had pre-existing renal comorbidities. There were no significant changes in total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels 2 years after TAF switch. Those with elevated ALT levels (>40 U/L) showed increased ALT normalization after TAF switch (-0.008 [-0.003— -0.012] log_{10}U/L/month, P<0.005). eGFR decline while on TDF (-0.65 [-0.23— -1.08] mL/min/month, P=0.003) was halted after switching to TAF (-0.03 [-0.17— 0.11] mL/min/month, P=0.7). Those with eGFR <60 ml/min experienced increased eGFR levels after TAF switch (0.22 [0.03— 0.87] mL/min/month, P=0.04).

Conclusion: Switching from TDF to TAF positively influenced long-term overall renal function and ALT normalization in HIV-HBV coinfected individuals. Limited lipid changes were observed.

Group N: Cancer

DEVELOPING LUNG CANCER DATA CURATION PIPELINES WHILE INCORPORATING NATURAL LANGUAGE PROCESSING

STUDENT: RAMI AJAJ
SUPERVISOR: GEOFFREY LIU

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BACKGROUND: The widespread uptake of electronic health records (EHRs) has made the creation of custom, real-world datasets for research more feasible. As a result, multiple research datasets with overlapping populations are often generated, using different methodologies, and frequently siloed within and between research groups, limiting the scope of the data's use. Using existing lung oncology datasets, we developed an approach to determine optimal methods of combining and curating clinical data from different sources.

METHODS: Two separate study datasets containing data for lung cancer patients diagnosed and/or treated within Princess Margaret Cancer Centre (PM, Toronto) were investigated. Study 1 manually abstracted clinical data for 1,990 patients, first seen at PM between 2014-2016; Study 2 leveraged the artificial intelligence engine, DARWEN™, to extract clinical data directly from EHRs for 4,466 patients, diagnosed between 2014-2018. Each dataset was individually assessed for internal consistency before comparing the overlapping population (Test Group, n=1892) to identify, investigate, and resolve differences. Patterns of data extraction performance were evaluated to define optimal methods for combining datasets and informing future data collection. Herein, epidermal growth factor receptor (EGFR) mutation status is used as an illustrative example.

RESULTS: Study 1 and 2 had similar distributions of clinicodemographic data and frequency of EGFR mutations. The Test Group had 100% agreement for date of birth, and >99% agreement for sex, with all discrepancies resulting from human error in Study 1. The Test Group had an 86.2% agreement for overall EGFR status and 85.3-85.4% agreement for specific exon mutations status. Of the 351 conflicting values for specific mutations, 42.45% and 54.52% were correctly labelled in study 1 and study 2 respectively. Study 2 prioritized specificity over sensitivity for biomarker extraction, resulting in more missing values (186 vs 50 for study 1). As DARWEN™ only extracted EGFR data from pathology reports, 25% (n=88) of discrepancies were due to lack of access to relevant information captured elsewhere in patients' EHRs. Both databases were 100% accurate for 5% sample of the congruent results.

CONCLUSIONS: By comparing overlapping datasets, the strengths and weaknesses of each study design and extraction methodology were identified. This process demonstrated the effectiveness of artificial intelligence for extracting accurate patient-level clinicodemographic and mutation status data from EHRs, and the value of targeted manual chart review. Our approach provides a roadmap for leveraging existing clinical datasets to their fullest potential, which is relevant across diverse data extraction methods and study designs.

FIRST-IN-CLASS P38α MITOGEN-ACTIVATED PROTEIN KINASE PET RADIOTRACERS FOR IMAGING NEURODEGENERATION IN ALZHEIMER'S DISEASE

STUDENT: MELISSA CHASSÉ
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Background: Alzheimer's Disease (AD) diagnostics rely on biomarkers of beta-amyloid accumulation, hyperphosphorylated tau protein, and neurodegeneration which form the "A/T/N" classification scheme. Positron emission tomography (PET) imaging agents targeted towards β-amyloid and tau currently dominate AD PET, despite showing limited correlation with cognitive impairment. Biomarkers of neurodegeneration, on the other hand, have shown comparatively strong correlations with cognitive impairment, making these targets of interest for novel PET probes. The p38 mitogen-activated protein kinase (MAPK) family converts extracellular stress stimuli into cellular responses through the activation of signal transduction pathways. Increased p38α subtype expression and activity has been implicated in numerous neurodegenerative diseases, including AD, Parkinson's disease, Huntington's disease (HD), amyotrophic lateral sclerosis and traumatic brain injury. Recent phase II clinical trials suggest that selective p38α inhibitors yield significant cognitive improvements in patients with AD, HD, and dementia-related neuroinflammation.

Objectives: This study sought to develop novel radiotracers for imaging neurodegeneration based on the selective p38α inhibitors SCIO-469 and VX-745 as potential drug discovery tools.

Methods: Proposed radiotracer structures were assessed for likelihood of brain penetrance by calculating the predictive CNS-MPO and BBB scores. [^{11}C]SCIO-469 was produced by [^{11}C]CH_3I methylation of the mono-desmethyl precursor. Radiolabelling of the VX-745 nitro, stannane, and boronic acid precursors is being attempted with various radiofluorinating agents. Radiotracers were characterized by high performance liquid radio-chromatography and shake-flask $LogD_{7.4}$ experiments. Immunohistochemistry, immunofluorescence, and autoradiography are being performed in 10 μm fresh frozen tissue sections. Preclinical PET was conducted to assess brain penetrance, imaging kinetics, and radiotracer metabolism.

Results: [^{11}C]SCIO-469 was synthesized with radiochemical yields of 3.41% ± 0.54, molar activities of 38.71 ± 9.8 GBq/μmol, and radiochemical purities of >99% (n = 9). Despite adequate $LogD_{7.4}$, CNS-MPO and BBB scores of 2.97 ± 0.05 (n = 4), 3.9 and 3.71, respectively, healthy rodents displayed no brain uptake with [^{11}C]SCIO-469. Uptake was observed after Elacridar administration, indicating that SCIO-469 is a rodent P-gp substrate. Due to known species differences in P-gp efflux activity, an MDCK-MDR1 permeability assay is ongoing. Self-blocking experiments with non-radioactive SCIO-469 during PET scans caused an increase in brain uptake due to blocking of binding in the periphery. Further imaging studies are underway to assess signal displacement and blocking with structurally dissimilar compounds. Attempts to synthesize [^{18}F]VX-745 from the nitro-precursor have been unsuccessful due to insufficient ring activation, but other methods of radiofluorination are underway.

Conclusions: This study marks the first attempt to develop *in vivo* PET agents for imaging p38α. While [^{11}C]SCIO-469 was identified as a rodent P-gp substrate, it is not known if this tracer is a substrate for human P-gp. Further work will focus on radiolabelling of [^{18}F]VX-745 for brain imaging.

THE ROLE OF ICAM1 IN GLIOBLASTOMA TUMOR ASSOCIATED MACROPHAGES IN HYPOXIA

STUDENT: KAVIYA DEVARAJA
SUPERVISOR: GELAREH ZADEH

Background: Glioblastoma (GBM) is an aggressive and highly fatal brain cancer in adults. Existing treatment methods are ineffective as the average survival time of patients following treatment is fewer than 15 months. Given the terrible prognosis of patients with GBM, we need treatment methods that extend the overall survival, reduce the chance of tumor recurrence, as well as improve the quality-of-life in these individuals. Glioblastoma affects males and females differently, with males having higher incidence and poorer prognosis. Previous studies have examined molecular alterations between male and female GBM patients, revealing sexual dimorphism as a factor contributing to differences in disease outcome. Cell adhesion molecules (CAMs) are proteins that are expressed on the surface of cells and enable them to communicate and interact with one another and the surrounding environment. They, in part, enable tumor cell invasion and migration. Intracellular adhesion molecule 1 (ICAM1) is a CAM expressed by TAMs in GBM. Tumor associated macrophages (TAMs) make up more than 40% of the GBM tumor mass and are thought to enhance tumor growth and proliferation, particularly within the characteristic hypoxic tumor microenvironment (TME) of GBM.
Purpose: Determine if ICAM1 expression in TAMs contributes to GBM tumorigenicity, uncover the mechanism of this behavior and if there are biological-sex related differences associated with its function in macrophages.
Hypothesis: The expression of ICAM1 on the surface of TAMs contributes differently to male and female GBM cell invasiveness, especially in the hypoxic TME by enhancing the interaction between tumor cells and macrophages, thereby facilitating the migration and invasion of the tumor cells.
Methods: I will approach my investigation by first assessing the expression of ICAM1 in primary and immortalized human and mouse macrophages under hypoxic conditions. Then I will analyze the effect of silencing and over-expressing ICAM1 on macrophage behavior, including migration, proliferation, phagocytosis, and adhesion to tumor cells. Lastly, I will use a commercially available ICAM1 knockout mouse model, and intracranially inject them with GBM tumor cells, followed by analysis of tumor growth, overall survival of the mice, and the composition of the tumor microenvironment in males and females by RNA sequencing and serial IHC using antibodies specific markers for macrophages, cell proliferation, angiogenesis, and apoptosis.
Results: ICAM1 is highly expressed in different cell types within the GBM microenvironment, including TAMs. The expression is particularly enhanced when primary or immortalized macrophages are treated with tumor cell-conditioned medium in vitro and is further exacerbated upon incubation of these cells in a hypoxic chamber at 1% and 0.2% oxygen levels and treatment of HIF-stabilizing drug IOX4. The migration levels of bone marrow derived macrophage mouse cell type is higher in wild type cells than in ICAM1 deficient cells. Migration levels are higher when wild type and ICAM1 deficient cells are co-cultured with tumor cell conditioned media then without and upon incubation of these cells in a hypoxic chamber at 1% and 0.2% oxygen. ICAM1 deficient mice succumbed more quickly to GBM than wild type mice.
Conclusions: It is evident that the hypoxic tumor microenvironment increases the expression of ICAM1 in macrophages. ICAM1 increases macrophage migration levels when co-cultured with and without tumor cell conditioned media. The tumor microenvironment increases migration levels of macrophages. The expression of ICAM1 in TAMs in hypoxic TME promotes GBM cell invasiveness and migration. ICAM1 deficiency contributes to poorer overall survival in GBM patients.

EVALUATING NOVEL BIOMARKERS FOR OVARIAN CANCER DIAGNOSIS

STUDENT: ARDALAN MAHMOODI
SUPERVISOR: MOHAMMAD R. AKBARI

Ovarian cancer is the second most common gynecological neoplasm in Canada and accounts for 6% of all cancer deaths worldwide. Most cases remain asymptomatic until late stages, at which point the tumour has extended in the peritoneal cavity, and optimal cytoreduction (tumour removal) is rarely possible. Currently, ovarian cancer has a five-year survival rate of 29%, while detection at an early stage increases this number to over 90%. Therefore, downstaging of the diagnosed ovarian cancers is crucial in improving patients' survival.

Existing ovarian cancer biomarkers, including CA125 and HE4, are used for distinguishing between benign and malignant masses but are not valid for early detection. We are examining two novel biomarkers for ovarian cancer, potentially allowing screening or diagnosing ovarian cancer in earlier stages. These potential biomarkers are arresten and ovarian cancer-specific glycoform of CA125 (OCSG-CA125). Arresten is a protein segment from the C-terminal of the Collagen IV alpha chain, which is released during the exposure of the extracellular matrix during the peritoneal invasion of ovarian cancer cells that starts at stage II. OCSG-CA125 results from the altered glycosylation pattern of CA125 in ovarian cancer cells compared to normal cells.

We propose to measure the concentrations of arresten and OCSG-CA125 as well as CA125 in 407 healthy individuals and 421 ovarian cancer patients. Concentrations of CA125 and arresten will be measured using sandwich ELISA, and the concentration of OCSG-CA125 will be measured by lectin sandwich assay. The significance of the association between each biomarker concentration and ovarian cancer cases will be determined and the composite scores will be calculated by logistic regression from standardized biomarker values. Each marker's best cut-off point will be calculated using the area under the curve (AUC). The specificity and sensitivity of each biomarker and all possible combinations of biomarkers will be calculated.

Our study is still in progress and results have not been fully obtained; however, we expect that using arresten, OCSG-CA125, or a combination of the two, in addition to CA125, could help ovarian cancer diagnosis at earlier stages. Most ovarian cancer mortality is due to high-grade serous tumours, which are mostly diagnosed in stage III and IV. By diagnosing patients in earlier stages, we provide the opportunity for optimal cytoreduction of the high-grade serous tumours when they have not extended too much in the peritoneal cavity. Since optimal cytoreduction is the most promising prognostic factor in ovarian cancer patients, this could be a significant step forward towards the improvement of ovarian cancer patients' survival.

UTILIZING MACHINE LEARNING CLASSIFIERS TO DIFFERENTIATE MOLECULAR MARKERS OF PEDIATRIC LOW-GRADE GLIOMAS BASED ON MRI

STUDENT: KHASHAYAR NAMDAR
SUPERVISOR: FARZAD KHALVATI

Background: BRAF status has important implications for prognosis and therapy of pediatric Low-Grade Gliomas(pLGG). Currently, BRAF status classification relies on biopsy, which is not risk-free and might not be feasible depending on the tumor location. Hence, an imaging-based pipeline will have a high impact on pLGG prognosis procedure.

Purpose: Our aim was to train and validate radiomics-based Machine Learning (ML) models to predict BRAF fusion and BRAF V600E mutation. We also investigate the impact of the training data sample size and type of ML model, as they are decisive factors to improve the generalizability of our models.

Hypothesis: We hypothesize the features extracted based on the radiomics definitions from the Regions of Intrest (ROIs), are information-rich enough to differentiate BRAF fusion and BRAF V600E molecular markers. We also believe ML models enable us to map the radiomics feature into molecular marker labels. Finally, we believe our models might not need the whole training data to learn the mapping.

Methods: In this bi-institutional retrospective study, FLAIR MRIs of 251 pLGG patients from 2 children's hospitals (The Hospital for Sick Children and The Lucile Packard Children's Hospital), acquired between January 2000 and December 2018, were included and analyzed. Radiomics features were extracted from tumor segmentations and five models (Random Forest, XGBoost, Neural Network (NN) 1 (100:20:2), NN2 (50:10:2), NN3 (50:20:10:2)) were tested to classify them. To keep the training and testing datasets independent, classifiers were cross-validated on the data from institution 1 and tested on the cohort from institution 2. Starting with 10% of the training data, models were cross-validated using a 4-fold approach at every step with an additional 2.25% increase in sample size. At each step, experiments were repeated 10 times using randomized versions of the respective percentage of the training data, resulting in 10 classifiers per step, per model. At each step, these 10 classifiers were tested on the independent data set. Mean area under the curve (AUC) and 95% confidence intervals (CI) were calculated for every step for both training and independent data sets and the process was repeated for all five models.

Results: The training data consisted of datasets from 220 patients with pLGG (mean age 8.53 ± 4.94 years, 114 males, 67% BRAF fusion) and the independent data set comprised datasets from 31 patients with pLGG (mean age 7.97±6.20 years, 18 males, 77% BRAF fusion). NN1 (100:20:2) yielded the highest AUC. It predicted BRAF status with a mean AUC of 0.85, 95% CI [0.83, 0.87] using 60% of the training data and with mean AUC of 0.83, 95% CI [0.82, 0.84] on the independent test data set.

Conclusions: Radiomics-based prediction of BRAF status in pediatric low grade gliomas appears feasible in this bi-institutional exploratory study. Neural networks have the highest mean AUC and lowest standard deviations to predict BRAF status compared to Random Forest and XGBoost. Using 60% of the training set, the highest AUC for training and independent data and the most stable outcomes in terms of variance were reached.

IDENTIFYING MARKERS OF TREATMENT RESPONSE IN GLIOBLASTOMA

STUDENT: MARINA NIKOLOPOULOS
SUPERVISORS: ARJUN SAHGAL, SUNIT DAS

Background: Glioblastoma is the most aggressive and common form of primary brain cancer, with a median survival of approximately 15 months following diagnosis. Despite multimodal treatment consisting of surgical resection of the tumour followed by concomitant radiotherapy and chemotherapy, prognosis for patients with glioblastoma remains poor. There is a wide variation in response to standard treatment, with nearly 30% of patients experiencing tumour progression during treatment, and nearly 10% of patients surviving more than 5 years.
Although attempts have been made to subtype glioblastoma into distinct genomic and transcriptomic profiles, these have failed to prognosticate differences in patient survival. To date, there are no non-invasive clinical biomarkers to predict response to first – line treatment. Chemical exchange saturation transfer (CEST) MRI may have the potential to fill this gap. CEST MRI is sensitive to treatment-induced changes and changes in tumour metabolism. Our team has obtained CEST data for patients before, during and after the end of standard chemoradiation treatment, and found that CEST provides markers of early response and can identify short-term survivors before treatment initiation.
Purpose: The purpose of this study is to establish genomic and transcriptomic profiles of short, standard, and longer – term survival in patients with glioblastoma who have undergone CEST-MRI.
Hypothesis: We hypothesize that distinct genomic and transcriptomic signatures underlie differential response to treatment and expect to establish profiles of “treatment responsive” and “treatment resistant” patients.
Methods: Adult patients with glioblastoma who consented to the storage and use of their samples were enrolled in the study. In addition to standard treatment, all patients were imaged with CEST – MRI at multiple time points before, during and after the end of chemoradiation treatment. Samples were obtained from patients at initial surgical resection, prior to treatment initiation. Tumour and blood samples were collected from the Michael and Amira Dan Brain Tumour Biobank Network. Histopathological features of each tumour sample were recorded. DNA and RNA were co-extracted from 25 fresh – frozen matched normal and tumour pairs and submitted for gene expression analysis (Nanostring) and whole genome sequencing (WGS). Initial results were validated on 75 formalin – fixed, paraffin -embedded (FFPE) samples from the same cohort, and further validated using data from The Cancer Genome Atlas (TCGA) program.
Results: Our initial discovery cohort included 25 fresh – frozen samples, (20 – 68 years, median 54, 60% male.). Of these, 9 (36%) were short-term survivors with an overall survival (OS) of less than 209 days (6.9 months), 8 (32%) were ‘standard’ survivors with an OS between 209 and 547 days, and 8 (32%) were longer-term survivors, with an OS greater than 547 days. Methylation status of the MGMT promoter was assessed, with 10 unmethylated (40%), 12 methylated (48%) and 3 unknown (12%). When comparing the short-term survival group to the longer-term survival group through a differential expression analysis on Nanostring, the log-2-fold change of known tumour drivers was up to 4 times greater in short-term survivors ($p< 0.000589$). Conversely, the log-2-fold change of known tumour suppressors was higher in longer-term survivors compared to shorter-term survivors ($p< 0.000699$). Results from the genomic analyses and analyses of the validation cohort are pending.
Conclusions: Collectively, this data has the potential to serve as a radiogenomic biomarker to assess treatment response before or within early phases of treatment and allow for individual tailoring of the treatment plan.

IMPROVING PEDIATRIC LOW-GRADE GLIOMA SEGMENTATION THROUGH MULTITASK LEARNING

STUDENT: PARTOO VAFAEIKIA
SUPERVISOR: FARZAD KHALVATI

Background: Brain tumor segmentation is a critical task that enables radiologists and AI models to detect abnormalities and develop treatment plans for patients. However, it is a time-consuming process and requires neuroradiology expertise, which makes it prohibitive due to cost and time constraints. Furthermore, while there has been extensive research focused on optimizing brain tumor segmentation in adult population, studies on AI guided pediatric tumor segmentation are limited. The anatomy and physiology of the general and central nervous systems in children differ from that of adults. Thus, there is a need to develop segmentation algorithms that are designed for brain tumors in pediatric population. **Purpose:** My research bridges this gap by developing a segmentation model trained on magnetic resonance imaging (MRI) scans of pediatric patients with low-grade gliomas (pLGGs) from the hospital for Sick Children (SickKids). **Hypothesis:** In pLGG, it has been shown that frequent alterations in the mitogen-activated protein kinas pathway can be identified from the molecular characterization of the tumors. These alterations have been shown to differ pLGG prognosis, and their identification allows for more focused therapies. In our study, since features obtained from genetic alterations and segmentation of pLGG are interconnected, as an auxiliary task, the genetic markers classifier is added in parallel to the main network with the hypothesis to boost segmentation results. Using dMTL, information learned from related tasks increases the model's capacity to learn a usable representation of the data, reducing overfitting and improving generalization.

Methods: In this research, we propose an MRI-based model that provides segmentation of pLGG and at the same time, classifies genetic alteration of the tumor as an auxiliary task. To achieve this goal, fluid attenuation inversion recovery (FLAIR) MRI sequences of 311 pediatric patients treated at The Hospital for Sick Children between 2000 and 2018 were included. The ground truth tumor segmentations were provided by two neuroradiologists in consensus, and the associated genetic markers were assessed through biopsy. A U-Net based network was trained, which is the core of the state-of-the-art approaches for tumor segmentation. In order to produce classification output, we added a branch of fully connected layers at the bottleneck of the U-Net which generalizes the encoder to both segmentation and classification tasks. **Results:** I trained two models on the same training (70% of patients), validation (15% of patients), and test (15% of patients) datasets. The first model is a single task model optimized for pLGG segmentation, while the second model utilizes the proposed dMTL framework. The same scenario was tried 6 times by only changing data split in the training, validation, and test datasets. On 4 out of 6 runs, the dMTL method improved the performance of pLGG segmentation, on average, by 3% (0.77 to 0.80) and 4% (0.74 to 0.78) in validation and test sets, respectively, and it performed similar to single task training in the 2 remaining runs. Overall, the dMTL method improved the performance of pLGG segmentation, on average, by 2.10% (0.767 to 0.788) and 3.0% (0.743 to 0.773) in validation and test sets, respectively. **Conclusions:** This research presents a method to improve the performance of pLGG tumour segmentation task in MRI using a deep multi-task learning approach. The effect and complementarity of auxiliary representations was assessed by integrating a genetic marker classifier into the main segmentation network. This method improved the segmentation task, on average, by 2% and 3% in validation and test datasets, respectively.

EPIGENOMIC EVOLUTION AND DRIVERS OF MALIGNANT TRANSFORMATION IN GLIOMAS

STUDENT: MATTHEW VOISIN
SUPERVISOR: GELAREH ZADEH

Authors: Mathew R. Voisin, MD, Vikas Patil, PhD, Farshad Nassiri, MD, **Gelareh Zadeh, MD, PhD**

Background: Gliomas are the most common primary malignant brain tumor and inevitably recur after treatment. The majority of recurrent gliomas are treatment-resistant, and there is currently a lack of understanding in the factors that influence glioma recurrence and aggressiveness.

Purpose: To investigate the evolution of gliomas through the epigenome and determine the epigenomic drivers of glioma recurrence and transformation.

Hypothesis: We hypothesize that specific genes and pathways are upregulated in recurrent gliomas, and identification of these biomarkers of recurrence will allow us to better understand and treat recurrent gliomas.

Methods: We analyzed a total of 350 glioma patient samples comprised of 268 tumor samples and 82 plasma samples. This included a unique cohort of 81 patients with paired fresh frozen tumor samples taken at two timepoints: primary and recurrent disease. A multi-omics approach consisting of tumor DNA methylation, gene expression, and cell-free DNA methylation from the plasma was performed.

Results: Tumor and plasma DNA methylation identified epigenetic stability in IDH wildtype samples, and loss of DNA methylation in IDH mutant samples at glioma progression, driven by gliomas undergoing malignant transformation. Samples undergoing malignant transformation at recurrence were associated with decreased tumor purity, increased B cells in the tumor microenvironment, and *CDK6* amplification. An integrated analysis of DNA methylation and gene expression identified key pathways and genes involved in glioma progression and malignant transformation.

Conclusions: In summary, this study identifies the epigenomic changes that occur in glioma progression and malignant transformation through an integrated, multi-platform approach on a unique cohort of paired samples and highlights novel genes and pathways that may serve as future therapeutic targets in the treatment of recurrent disease.

Group O: Cardiovascular-Respiratory-Musculoskeletal

SERUM PENTOSIDINE IN WOMEN IN THE CANADIAN MULTICENTRE OSTEOPOROSIS STUDY

STUDENT: LINSIE BLENCOWE
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Background: Canada's population is aging. By 2030, seniors will represent the largest share of the Canadian population and will be primarily female. Women experience the musculoskeletal changes due to aging to a greater extent than men. Musculoskeletal health research in this demographic needs to be a priority. Advanced glycation end-products (AGEs) have been implicated in many degenerative diseases and aging. In bone, the accumulation of AGEs makes bones brittle and may contribute to fracture incidence. Pentosidine (PEN) is an AGE which has been implicated in bone fragility and may be a potential biomarker of osteoporosis and fracture. **Purpose:** Serum PEN was assessed in women in the Canadian Multicentre Osteoporosis Study (CaMOS) to establish normative data and to characterize this potential biomarker. **Hypotheses:** 1) Serum PEN levels are associated with age and comorbid conditions such as diabetes and chronic kidney disease. 2) Serum PEN levels are inversely associated with duration of bisphosphonate use and BMDs at the total hip, femoral neck, lumbar spine and trabecular bone score (TBS). **Methods:** Total PEN was assessed using liquid-chromatography tandem mass spectrometry in stored serum samples from 897 women at Year 10 in CaMOS. The association of demographic and clinical variables with total serum pentosidine levels were analysed by univariate and multivariate regression analyses. Demographic and clinical variables included; age, menopause status, estimated glomerular filtration rate (eGFR, calculated as per Levey *et al.* 2009), fasting glucose, bisphosphonate use, bone mineral density (BMD) of the total hip, femoral neck, lumbar spine and trabecular bone score (TBS). **Results:** The mean age of women in the cohort was 68.8 years (range 40-92 years) and the median level of PEN was 39.7nM (range 12.0-1752.4nM). 15% of the cohort had moderate-severe (Stage 3-4) chronic kidney disease (CKD) based on eGFR. In univariate analyses, PEN level was significantly associated with age (p<0.001) and eGFR (p<0.001). Subgroup analysis of participants with normal eGFR (>90 mL/min per 1.73 m^2, n=200) revealed that PEN is inversely associated with total hip (p=0.020) and femoral neck (p=0.023) BMD, but not associated with lumbar spine BMD (p=0.218) or age (p=0.418). TBS could not be assessed in this group due to limited data. In multivariate analyses of the total cohort, associations with PEN were explored while adjusting for eGFR. PEN was associated with eGFR (p=0.001) but not associated with age (p=0.757), menopause status (p=0.363), fasting glucose (p=0.196), bisphosphonate use (p=0.826), total hip BMD (p=0.515), femoral neck BMD (p=0.626) or TBS(p=0.523). **Conclusions:** Our results demonstrate that reduced renal function is associated with increased serum PEN, independent of age and BMD in women. Whether serum PEN adds to age, sex and BMD for fracture risk assessment will require further study.

DESIGNING AND SELECTING NUTRIENT ENRICHED PERFUSATE SOLUTIONS FOR EX VIVO LUNG PERFUSION

STUDENT: DEJAN BOJIC
SUPERVISOR: DR. MINGYAO LIU

Lung transplantation procedures are lifesaving interventions that are unfortunately limited by low organ utilization rates. Ex Vivo Lung Perfusion (EVLP) is a technology that restores organ metabolism by maintaining lungs at 37°C though a circulating perfusate and mechanical ventilation, thus allowing clinicians to repair donor lungs which would have been discarded. Extending EVLP support time can further increase organ utilization and has been achieved with total parental nutrition (TPN) supplemented perfusates. However, the cellular mechanisms activated by TPN leading to prolonged EVLP remain unknown. Additionally, protective metabolites (GlutaMAX), and other macromolecule groups (lipids and carbohydrates), could provide complete energy support, but this too remains untested. Therefore, we developed a high-throughput screening pipeline to identify essential nutrients, and their molecular mechanisms, involved in supporting lung cells during prolonged EVLP. We hypothesize that proper compositions of nutrients (TPN, lipids, glucose, and GlutaMAX) will address metabolic demands of lung cells during prolonged EVLP conditions to maintain cellular viability. Mathematical modeling using nutrient solution concentrations from clinical EVLP studies will be used to develop low, medium, and high concentrated nutrient supplemented perfusates. Human pulmonary endothelial cells will then be cultured in the nutrient supplemented perfusates for 48 hours. Using the IncuCyte Sx5 Live-Cell Analysis System, cell viability, confluence, migration, ATP metabolism, and mitochondrial membrane potential will be assessed. Solutions which perform significantly better than the standard perfusate (Steen) will then be tested in an EVLP culture model. Cellular factors including cytoskeletal integrity and adhesion molecules will be evaluated via immunostaining; inflammatory response via ELISA, mitochondrial permeability transition via western blot; and mitochondrial reactive oxygen species production via flow cytometry. Overall, we can use our results to develop the next generation of EVLP perfusates and significantly improve transplant patient outcomes.

EXTRACELLULAR VESICLE-DERIVED MICRORNAS FROM HUMAN ABDOMINAL AORTIC ANEURYSM ASSOCIATE WITH PROANEURYSMAL CELL SIGNALING AND SENESCENCE PATHWAYS

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Background: Abdominal aortic aneurysm (AAA) is associated with significant post-rupture mortality in aging populations. Management of AAAs is limited to watchful waiting and surgical repair, which stems from our limited understanding of AAA disease processes. Current research foci including extracellular vesicles (EVs; nano-sized packages of proteins, RNAs, and lipids that facilitate cell-cell communication) may aid in discovering novel AAA biomarkers and therapies. To characterize this regulatory cargo, we isolated EVs from human AAA tissue or control aortic punch biopsies and profiled EV content with microRNA (miRNA) sequencing to identify pathway dysregulation.

Methods: The study was approved by the University Health Network Research Ethics Board. EVs were isolated from human AAA (excluding infectious and known connective tissue disease etiology) or aortic punch tissue and enriched using size exclusion chromatography (SEC; qEVoriginal columns 70 nm, Izon Science Ltd.) (n=3). EV size and concentration were determined using nanoparticle tracking analysis (NTA; NanoSight NS300, Malvern Panalytical Ltd.). EV-miRNA sequencing was performed with the Illumina NextSeq platform (HTG Molecular Diagnostics Inc.) and analyzed using Partek Genomics Suite (v.10) and MIENTURNET (19-11-25).

Results: Patients were selected for infrarenal AAA requiring surgical intervention (AAA) or coronary artery disease requiring bypass graft surgery (control). EV size and concentration were confirmed with NTA. Principal components and gene set analyses revealed distinct clustering of groups with 901 and 687 miRNAs enriched in AAA and control samples, respectively. Pathway prediction using miRNAs previously associated with AAA identified proaneurysmal gene targets including *MMP14*, *SMAD3*, and *FBN1*. Significant interactions were observed between established AAA miRNAs (e.g., miR-122, miR-146a, and miR-503) and proaneurysmal signaling pathways (e.g., PI3K-AKT, AGE-RAGE, JAK-STAT, and HIF-1) as well as cell senescence processes (e.g., cellular senescence, cell cycle, and longevity regulation) (FDR < 0.05).

Conclusions: EV-derived miRNAs from patients with AAA prominently associate with cell signaling and senescence pathways involved in aneurysm pathogenesis. To our knowledge, this is the first study to profile the EV-miRNA landscape in human AAA tissue. Further investigation will explore EV-miRNAs as mediators of communication between distinct vascular cell populations that contribute to AAA development.

EXERCISE AND PHYSICAL ACTIVITY INTERVENTIONS FOR PEDIATRIC RHEUMATIC DISEASES: A SCOPING REVIEW

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Background: Previous studies have reported impaired physical fitness in children with pediatric rheumatic diseases (PRDs) compared to healthy peers. Exercise prescription and physical activity promotion may have an important role to play in symptom and disease management.

Purpose: This scoping review describes the various types and outcomes of exercise and physical activity interventions for PRDs from published peer-reviewed journal articles. Research findings across four PRDs, namely, Juvenile idiopathic arthritis (JIA), Juvenile dermatomyositis (JDM), Juvenile systemic lupus erythematosus (JSLE), and Juvenile fibromyalgia (JFM) was summarized to identify emergent themes and knowledge gaps.

Methods: A search of literature was conducted with a research librarian on the following electronic databases: Ovid MEDLINE, Ovid Embase, SPORTDiscus (EBSCO Host), CINAHL (EBSCO Host), Cochrane Library, and Ovid PsycINFO. Published peer-review journal articles were included if they had an exercise or physical activity intervention, if they studied the intervention in participants <18 years of age with a diagnosis of JIA, JDM, JSLE, or JFM, and if the article was written in English. Study screening and selection were performed independently by two authors (YL and KS). Data extraction was performed by one author (YL) and checked by a second author (KS).

Results: Based on preliminary analysis of 63 papers, most studies examined the effects of conventional structured rehabilitative exercises to improve strength, range of motion, and aerobic capacity. Several studies examined the effects of exercises such as Yoga, Pilates, Cardio-karate, and Qigong. The duration of interventions ranged from a single 20-minute session to 14 months; most were between 6 to 12 weeks in duration. Pain and physical function were the two most commonly measured outcomes. No study reported significant disease activity exacerbation or persistent discomfort as a result of exercise. Details regarding the efficacy of the interventions will be reported.

Conclusions: This study identifies key themes and knowledge gaps, which helps to inform the design of future research studies.

CHEMOGENETIC ACTIVATION OF GLUTAMATERGIC PREBÖTZINGER COMPLEX NEURONS INCREASES BREATHING IN MICE

STUDENT: NATALKA PARZEI
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Background: Respiratory rhythm is primarily regulated within the ventral medulla, with the preBötzinger Complex (preBötC) critical for inspiration. The preBötC contains a heterogenous group of neurons, with a large portion being glutamatergic and marked by vesicular glutamate transporter 2 (vGLUT2). A subpopulation of preBötC neurons co-express μ-opioid receptors, lending to the role of the preBötC in opioid-induced respiratory depression. We propose that activation of vGLUT2-expressing preBötC neurons can stimulate respiration and potentially alleviate opioid-induced respiratory depression.

Purpose: To identify the activation of the preBötC as a viable method to prevent respiratory depression, and fatality, caused by opioid use.

Hypothesis: Chemogenetic activation of glutaminergic neurons within the preBötC will induce respiration in mice.

Methods: Excitatory hM3Dq receptors were expressed in vGLUT2 preBötC neurons of vglut2-IRES-Cre male mice (Jackson Labs) through cre-lox recombination. Four weeks after insertion, mice were anesthetized and electrodes were implanted into the diaphragm and genioglossus muscles to record respiratory activity. Mice (n=5 per group) were given consecutive intramuscular injections of saline and the hM3Dq ligand, clozapine-N-oxide (CNO; 0.5 and 1 mg/kg), while respiratory responses were monitored. Following recordings, brains were collected for histological analysis. hM3Dq expression was determined through mCherry fluorescence.

Results: Administration of 0.5 mg/kg CNO resulted in significant changes in respiratory patterns 11-20 minutes post-injection. Respiratory rate increased by 30.55% ($p=0.0126$) following glutamatergic activation. Diaphragm amplitude, inspiratory time, and expiratory time all decreased following glutamatergic activation by 33.62% ($p=0.0017$), 10.78% ($p=0.0054$), and 45.12% ($p=0.0059$), respectively. Histology confirmed expression of mCherry in the region of the preBötC.

Conclusions: Preliminary data showed that respiratory rate can be increased through glutamatergic preBötC stimulation during anesthetized conditions. Further studies will determine if stimulation of glutamatergic preBötC neurons is sufficient to overcome opioid-induced respiratory depression.

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EARLY PREDICTION OF MECHANICAL VENTILATION-FREE SURVIVAL IN PATIENTS ADMITTED WITH TRAUMATIC SPINAL CORD INJURY: A REGISTRY-BASED OBSERVATIONAL STUDY

STUDENT: ANNIA SCHREIBER
SUPERVISOR: LAURENT BROCHARD

Background: The need for mechanical ventilation (MV) greatly impacts life expectancy and quality of life of patients with spinal cord injury (SCI), but predictors of weaning from MV have not been systematically assessed. **Purpose:** In this registry-based observational study our aims were: i) to investigate the probability of survival, free of MV (weaning success was defined as ventilator-free breathing at discharge from the primary intensive care unit), ii) to develop and validate a prediction score for weaning success and iii) to estimate the time to weaning and identify its predictors in ventilated patients with SCI. **Hypothesis:** in patients with SCI, we hypothesized that some characteristics of the patients and some characteristics of the spine lesion with potential clinical relevance to the outcome would predict the probability of weaning success and time to liberation, and result in different weaning success probabilities. **Methods:** We obtained data from the Trauma Registry at St. Michael's Hospital. We included adult patients with traumatic SCI requiring MV, admitted to the trauma-neuro intensive care unit (TNICU) of St. Michael's Hospital, between January 2005 and December 2019. Multivariable logistic and competing risk regression analyses were used, respectively, to identify early predictors of weaning success and time to liberation. We developed and internally validated via bootstrap a prediction score based on regression coefficients; its ability to discriminate between weaning success/failure was assessed using ROC curve analysis and compared to the Injury Severity Score (ISS) through pairwise testing. To estimate the time to liberation and its predictors while accounting for the competing risk of death, cumulative incidence curves were plotted, and Fine-Gray competing risk regression models were built. **Results:** Of 257 patients requiring MV after SCI, 178 (69.3%) experienced MV-free survival at TNICU discharge, and 40 (15.6%) died; the remaining 39 (15.1%) patients were transferred, still ventilated, to other acute care or rehabilitation facilities. Factors relevant for weaning success included: presence of **B**lunt injury (OR 7.09, p= 0.005), **I**SS (OR 0.98, p= 0.084), number of **C**omorbidities (OR 0.8, p= 0.045), age in **Y**ears (OR 0.97, p= 0.003), and presence of a **C**ervical level **LE**sion (OR 0.35, p= 0.005). From the linear combination of these variables, we computed a prediction score, named **BICYCLE** after the initials of the relevant predictors. The BICYCLE score showed a much better performance predicting weaning success than the ISS (AUROC= 0.78, 95%CI 0.70 – 0.83, *vs* 0.57, 95%CI 0.50 – 0.66, p< 0.0001). Median crude time to liberation from MV was 15 days; the risk of remaining on ventilation for at least 70 days was significantly higher in patients with a cervical SCI level compared to patients with either a thoracic or lumbar level SCI (p<0.0001 for both comparisons). The risk of death was significantly higher in patients with a C3-or-above level injury compared to C4-or-below, thoracic, or lumbar level injuries (p< 0.045 for all comparisons). The same factors relevant for weaning success, along with the presence of complete SCI (p= 0.004), also predicted time to liberation. **Conclusions:** 69.3% of patients were discharged from primary ICU alive and free of MV after SCI. A newly developed score based on readily available patient characteristics on admission could predict weaning success with good discriminative properties in internal validation. External validation will be conducted during on-going research.

THE METABOLIC ADVERSE EFFECTS OF ANTIPSYCHOTIC USE IN INDIVIDUALS WITH INTELLECTUAL AND/OR DEVELOPMENTAL DISABILITY (IDD): A SYSTEMATIC REVIEW AND META-ANALYSIS

STUDENT: EMILY SMITH
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Background: Individuals with intellectual and/or developmental disability (IDD) are often prescribed antipsychotics (AP). However, despite their known propensity to cause metabolic adverse effects, including weight gain, diabetes, and increased risk of cardiovascular events, there is currently a limited body of literature describing the metabolic consequences of AP use in this patient population.

Purpose: By synthesizing these findings, our review aims to present a comprehensive overview of the metabolic side effects of AP use in patients with IDD across the lifespan.

Methods: We searched MEDLINE, EMBASE, PsychINFO, CENTRAL and CINAHL databases from inception until July 2021 to identify all randomized trials that reported on the metabolic effects of APs in individuals with IDD. Random effects meta-analyses were used to examine weight gain as both a continuous and dichotomous outcome.

Results: Seventeen trials met our inclusion criteria with a total of 1357 patients across a variety of IDDs. AP use was associated with significantly greater weight gain compared to placebo (Continuous: mean difference = 1.11 kg, [0.78, 1.43], $p<0.00001$, $I2=57\%$; Dichotomous: odds ratio = 3.94, [2.15, 7.23], $p<0.00001$, $I2=0$). Sub-group analysis revealed a significant effect of AP, with risperidone having the greatest effect size (continuous only). Insufficient data in the reporting literature precluded analysis of the effects of APs on other metabolic outcomes.

Conclusion: This review demonstrates that use of APs, and particularly risperidone, is associated with significant weight gain among patients with IDD. Concerningly, most reported studies were in children and adolescents, which sets up an already vulnerable population for adverse medical sequalae at an early age. Further studies are required to better understand how AP use affects metabolic parameters beyond weight in these patients.

IMPACT OF A DIGITAL COUNSELLING PROGRAM WITH INTEGRATED SOCIAL SUPPORT NETWORK FOR SELF-CARE ON MENTAL HEALTH IN CHRONIC KIDNEY DISEASE AND CHRONIC HEART FAILURE

STUDENT: FATIMA SYED
SUPERVISOR: DR. ROB NOLAN

Background: Patients with chronic kidney disease (CKD) or chronic heart failure (CHF) demonstrate decreased quality of mental health compared to the non-clinical population. Self-care behaviours are integral in maintaining patient well-being and involve daily actions that promote health and disease management. Providing patients with relevant counselling and peer support for self-care behaviours may increase their psychosocial well-being and improve their prognosis. Digital interventions present an efficient opportunity to administer counselling and peer support for self-care.

Purpose: This research will examine whether a digital counselling program (vCHAT-ODYSSEE) with an integrated social support network (vCHAT) can increase mental health in CKD and CHF patients. It also explores whether the vCHAT component increases overall mental health and perceived social support while also decreasing loneliness and symptoms of depression.

Hypotheses: Primary: Greater engagement with the vCHAT-ODYSSEE program, both counselling and social support components, will result in increased overall mental health for CKD and CHF patients. **Secondary:** Greater engagement with vCHAT will add incremental benefit for increasing overall mental health and perceived social support, as well as decreasing symptoms of depression and feelings of loneliness.

Methods: Design: vCHAT-ODYSSEE is an online digital intervention that provides resources for self-care management (ODYSSEE) in conjunction with a social support network (vCHAT). This single-group study is currently enrolling a sample of CKD and CHF patients. Online assessments will be administered at baseline and 4-months. **Outcomes:** The primary outcome for this study is an improvement in scores of MCS of the SF-36. The secondary outcomes will be assessed by validated questionnaires for overall mental health (MCS), loneliness (RULS-6), depression (PHQ-9), and social support (ESSI), respectively. **Planned Analyses:** Both primary and secondary outcomes will be analyzed using Generalized Linear Models. Statistical significance will be $p < 0.05$ on all analyses.

Results: The expected primary outcome is a greater MCS score indicating an increase in overall mental health. The expected secondary outcome is a greater score on the MCS and ESSI, indicating increased mental health and perceived social support, and decreased PHQ-9 and RULS-6 scores, indicating lower depressive symptoms and feelings of loneliness.

Conclusions: vCHAT-ODYSSEE is a novel digital intervention that aims to improve the mental health of CHF and CKD patients through counselling and peer support, in conjunction with patients' usual care.

THE ROLE OF LONG-TERM GLYCEMIC CONTROL ON THE AUTONOMIC NERVOUS SYSTEM IN HEALTHY INDIVIDUALS

STUDENT: JEFFREY YU
SUPERVISORS: DR. JEAN CHEN, DR. LINDA MAH

Background: Impaired glycemic control, as seen in diabetes mellitus (DM), poses a potential threat to healthy brain aging. Disturbances in the autonomic nervous system (ANS) are also present in DM and may occur prior to a clinical diagnosis of DM. ANS dysfunction is linked directly to glycemia and can be monitored through heart rate variability (HRV). Thus, HRV can be potentially used with blood glucose markers, such as glycated hemoglobin (HbA1c), to better understand the impact of glycemia on the nervous system3.

HRV represents autonomic function related to the cardiac system, and its interpretation may be confounded by blood pressure. To fully evaluate the effects of glycemia on the nervous system, additional markers such as resting-state fMRI functional connectivity (FC) in networks such as the central autonomic network (CAN) and salience network (SN) need to be incorporated.

Purpose/Hypothesis: To investigate the effects of glycemic control on the nervous system through HbA1c and HRV. We hypothesize that HbA1c will be negatively correlated with HRV and FC. Furthermore, HRV will mediate the relationship between HbA1c and FC.

Methods: 141 subjects (48 female, age range: 20-77 years) from the Leipzig Mind-Brain-Body (LEMON) dataset was included in this study Briefly, fMRI and ECG data were acquired simultaneously in a 3T MRI scanner. Seated systolic blood pressure (SBP) was recorded immediately before the MRI scan while blood was drawn following MRI acquisition.

FC analysis was conducted using the CONN toolbox. The root-mean-square of successive differences (RMSSD) were identified from ECG data using Kubios HRV while high-frequency (HF) and low frequency (LF) HRV were calculated using variational-mode decomposition through an in-house MATLAB script. Outliers were identified by Cook's distance and removed. Simple Pearson correlations, partial correlations and mediation analysis were used to examine the relationship between HbA1c, HRV metrics and average SN FC across the cohort in R. As SBP is known to vary with age and influence HRV, it was included as a covariate.

Results: HbA1c and CAN FC were significantly correlated while HbA1c was inversely correlated with RMSSD varying for age. RMSSD was positively correlated with CAN FC and SN FC . RMSSD significantly mediates the relationship between HbA1c and SN FC. The associations among HbA1c, HRV, and SN are independent of SBP.

Conclusion: The positive correlation between HbA1c and HRV metrics and the inverse correlation between RMSSD and SN FC each agrees with findings reported in the literature in DM patients. This work highlights the link between glycemic control and brain health even in healthy adults. Future work involving longitudinal follow-up of these metrics would help elucidate the mechanism of association.

Group P: Neuroscience Brain Health

DEVELOPING V2A AND V2B INTERNEURON-BIASED NEURAL PROGENITOR CELL LINES AS A POTENTIAL TRANSPLANTATION THERAPY FOLLOWING SPINAL CORD INJURY

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Purpose: Surviving host propriospinal interneurons play an important therapeutic role after spinal cord injury (SCI). The transplantation of post-mitotic excitatory V2a interneurons after a C3/C4 hemisection SCI model showed improved behavioural recovery in rats when compared to neural progenitor cell (NPC) transplantation. Our goal is to create an NPC line which is biased to form two populations of ventrally-derived propriospinal interneurons: V2a (excitatory) or V2b (inhibitory) interneurons. As NPCs have improved ability for integration in the injured spinal cord, the expectation is that NPCs biased to a V2a or V2b interneuron fate will better integrate than mature V2a and V2b interneurons. These biased NPCs would more readily reconnect circuits in the injury spine and may restore the excitatory/inhibitory imbalance which occurs after SCI.

Methods: Human NPCs are treated with EC23 to caudalize their regional identities to the cervical region of the spine. The NPCs are also treated with purmorphamine, a Sonic hedgehog agonist, to induce development into p2 progenitors. For V2a differentiation, the NPCs are treated with DAPT to inhibit Notch-1 signaling. For V2b differentiation, the NPCs are treated with Jagged-1, a Notch-1 agonist. qPCR is used to identify a progenitor identity (Nestin, Pax6, Sox2 upregulation), V2a identity (VSX2, Sox14 upregulation) or a V2b identity (Gata3, Foxn4 upregulation). Electrophysiology will be used to confirm the formation of mature, functional gabaergic (V2b interneurons) and glutamatergic (V2a interneurons) synapses. Confocal fluorescent imaging will be used to verify the percentage of Gata2 and VSX2 positive cells which form mature gabaergic (GAD67 positive) and glutamatergic (VGLUT2 positive) interneurons.

Expected Results: Future qPCR experiments are expected to demonstrate upregulation in VSX2 and SOX14 for V2a cultures and upregulation in Foxn4 and Gata2 for V2b cultures. The expression of these markers will be used to determine when the NPCs have been biased to either a V2a or V2b fate.

Conclusions: V2a and V2b biased NPCs would be ideal transplantation candidates following cervical SCI, as they will facilitate improved functional recovery after injury over unbiased NPCs. The interneuron-biased NPCs would more effectively integrate into spinal circuits and aid in the re-establishment of the excitatory/inhibitory balance.

INVESTIGATING FATTY ACID AMIDE HYDROLASE IN COMORBID BORDERLINE PERSONALITY DISORDER AND DEPRESSION: A [11C]CURB POSITON EMISSION TOMOGRAPHY STUDY

STUDENT: MICHELLE DE POL
SUPERVISOR: DR. NATHAN KOLLA

Background. Borderline personality disorder (BPD) is frequently comorbid with major depressive disorder (MDD), however, there is a lack of research investigating biomarkers that are common to both conditions. The endocannabinoid system (ECS) modulates mood and stress circuits within the brain, and its dysregulation has been implicated in psychiatric disorders. Fatty acid amide hydrolase (FAAH), an enzyme in the ECS, plays a vital role in the metabolism of endogenous cannabinoids. There is evidence to suggest that FAAH is elevated in fronto-limbic brain regions associated with BPD and MDD, making it a potential transdiagnostic biomarker. The FAAH protein, however, has never been characterized in humans with comorbid BPD+MDD using *in vivo* imaging techniques.

Aims and Hypothesis. The aim of this study was to evaluate levels of FAAH in adults with comorbid BPD and MDD compared to BPD alone and healthy controls. We hypothesized that brain FAAH would be elevated in the prefrontal cortex, hippocampus and anterior cingulate cortex in humans with BPD+MDD compared to BPD alone and healthy controls.

Methods. Study participants, aged 18 to 65 years, met criteria for a current major depressive episode and/or borderline personality disorder. Exclusion criteria included medication use, a history of manic episodes, psychosis, or a current substance use disorder. Control participants were excluded if they had any history of a psychiatric disorder. FAAH binding was measured using [^{11}C]CURB positron emission tomography (PET). PET images were motion-corrected and co-registered with T1-weighted magnetic resonance (MR) images. Participants also underwent genotype sampling to determine the *FAAH* gene polymorphism. A linear mixed methods model was employed to determine the differences in FAAH levels between the three groups, with genotype as a fixed factor.

Results. A significant increase in FAAH binding was found in the medial prefrontal cortex ($p<0.005$, 95% CI [0.008, 0.041]), ventrolateral prefrontal cortex ($p<0.02$, 95% CI [0.003, 0.037]), anterior cingulate cortex ($p<0.004$, 95% CI [0.008, 0.042], and amygdala ($p<0.0001$, [0.038, 0.068] in the comorbid BPD+MDD group compared to the BPD-only group. No significant differences, however, were found between healthy controls and the comorbid diagnosis group.

Conclusions. This research may lead to a better understanding of the neurobiology of comorbid BPD and MDD, and potentially identify a transdiagnostic biomarker that can be used to develop novel pharmacotherapies.

EVALUATING MOTIVATION AND REWARD MECHANISMS AND BRAIN SUBSTRATES IN ADULTS WITH OBESITY

STUDENT: HARTEJ GILL
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Background: Obesity is a highly prevalent condition within Major Depressive Disorder (MDD) populations, and it is shown to be associated with reward disturbances. In particular, MDD patients have a decreased willingness to exert effort and reduced motivation to work towards a reward. Anhedonia and abnormalities in reward behavior are core features of obesity and MDD. Consequently, this represents a relevant area of research. Herein, we are primarily interested in three overlapping, yet distinct aspects of anhedonia. We are primarily interested in motivation, reward valuation, and reward learning towards addressing the measurement of each of these respective subdomains.

Purpose: My primary interest is investigating motivation, reward valuation, and reward learning towards addressing the measurement of each of these respective subdomains, using validated measures (i.e. the Effort Expenditure for Rewards Task (EEfRT) (reward valuation), Probabilistic Reward Task (PRT) (reward learning), and the Monetary Incentive Delay (MID) task).

Hypothesis: We believe that obesity results in disturbances in reward/motivation and functional disconnectivity in reward networks. It is hypothesized that obesity moderates blood-oxygen-level-dependent (BOLD) signalling in reward and default mode networks.

Methods: A single center, single visit study will recruit 20 participants categorized as overweight/obese (BMI>30 kg/m^2). We evaluated the association between effort-expenditure for monetary reward and neural activation in regions associated with reward-based decision making (i.e., the caudate nucleus and left middle temporal gyrus) in people with MDD and obesity comorbidity. We acquired structural and functional magnetic resonance imaging (MRI) in 12 participants and performed a spherical region of interest analysis (ROI) using previously defined peak MNI coordinates. A one-sample t-test was employed to compare whole-brain blood-oxygen-level-dependent (BOLD) signal change during the task choice selection window (i.e., high-effort vs. low-effort task) of the effort-expenditure for reward task (EEfRT).

Results: Preliminary results are based on tweleve participants. We observed that higher BMI was associated with decreased activation in the left caudate ($p< 0.05$; 95% CI: [(0.11) - (17.67)]), a brain region implicated in effort-based and cost-benefit decision making. In particular, contrasting the 'high effort reward - low effort reward' reward magnitude conditions saw decreased activation in the left caudate with higher participant BMI. The neuroimaging findings are presented in **Figure 1.**

Conclusion: Replicated neuroimaging studies have reported specific morphological alterations in the caudate body in MDD and obesity comorbidity. Consequently, these areas have important implications of reward valuation. We believe that obesity results in disturbances in reward/motivation and functional disconnectivity in reward networks and our study hopes to elucidate this relationship.

PREVALENCE OF PREOPERATIVE COGNITIVE IMPAIRMENT IN OLDER SURGICAL PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

STUDENT: PARAS KAPOOR
SUPERVISOR: DR. FRANCES CHUNG

Background: Older surgical patients with cognitive impairment are at an increased risk for adverse perioperative outcomes, however the prevalence of preoperative cognitive impairment is not well-established within this population.

Study objective: The purpose of this review is to determine the pooled prevalence of preoperative cognitive impairment in older surgical patients.

Hypothesis: In older surgical patients, preoperative cognitive impairment prevalence will differ depending on the type of surgery.

Methods: MEDLINE (Ovid), PubMed (non-MEDLINE records only), Embase, Cochrane Central, Cochrane Database of Systematic Reviews, PsycINFO, and EMCare Nursing were searched for relevant articles from 1946 to April 2021. Inclusion criteria included patients aged ≥60 years old undergoing surgery, and preoperative cognitive impairment assessed by validated cognitive assessment tools. Primary outcomes were the pooled prevalence of preoperative cognitive impairment in older patients undergoing either elective (cardiac or non-cardiac) or emergency surgery.

Results: Forty-eight studies (n = 42,498) were included. In elective non-cardiac surgeries, the pooled prevalence of unrecognized cognitive impairment was 37.0% (95% confidence interval [CI]: 30.0%, 45.0%) among 27,845 patients and diagnosed cognitive impairment was 18.0% (95% CI: 9.0%, 33.0%) among 11,676 patients. In cardiac surgeries, the unrecognized cognitive impairment prevalence across 588 patients was 26.0% (95% CI: 15.0%, 42.0%). In emergency surgeries, the unrecognized cognitive impairment prevalence was 50.0% (95% CI: 35.0%, 65.0%) among 2389 patients.

Conclusions: A substantial number of surgical patients had unrecognized cognitive impairment. In elective non-cardiac and emergency surgeries, the pooled prevalence of unrecognized cognitive impairment was 37.0% and 50.0%, respectively. Preoperative cognitive screening warrants more attention for risk assessment and stratification.

HEALTHCARE WORKER MENTAL HEALTH DURING THE COVID-19 PANDEMIC: LEARNING FROM PEER SUPPORT FACILITATORS

STUDENT: MELISSA B KORMAN, MSC CANDIDATE
SUPERVISOR: DR. ROBERT MAUNDER

Background: Healthcare Workers are at high risk of long-term negative mental health effects (e.g., depression, anxiety, and substance abuse) resulting from the COVID-19 pandemic. Mental wellness programs have been implemented for staff in healthcare work environments worldwide, with initial reports of rationale and program structure recently published. Reports on outcomes, lessons learned and the experience of offering support to healthcare workers (including impact on the supporters' own mental health) are lacking. The evidence-informed Social Support, Tracking Distress, Education and Discussion Community (STEADY) Staff Wellness Program was implemented in select units at Sunnybrook Health Sciences Centre to support healthcare worker mental health during the COVID-19 pandemic. The multidisciplinary team of Peer Supporters, or "Knowledge-Users," who implemented STEADY have a unique understanding of the process and experience of using a structured program to provide support to healthcare workers during a pandemic.

Purpose: To gather the knowledge created by Peer Supporters over their time facilitating STEADY, and to learn about their experiences.

Hypothesis: The inductive qualitative research design used requires objectives, not hypotheses, which are as follows:
1. To elucidate Knowledge Users (Peer Support Facilitators) experience in implementing peer support programming for HCWs amidst the COVID-19 pandemic.
2. To gain insight into lessons learned and future directions for the implementation of peer support programming into HCW work environments.

Methods: This study uses an interpretive description methodological approach and a post-positivist ontological perspective, leaning towards constructivism, to illuminate the experience of implementing STEADY in a chaotic healthcare environment. Two open-ended semi-structured focus groups were held with the multidisciplinary staff who implemented STEADY at Sunnybrook Health Sciences Centre. Question guides were developed considering knowledge translation and implementation science framework. Preliminary findings from the first focus group were presented during the second meeting, so that feedback could be gathered, while illuminating areas that were not deeply explored during the first meeting. Qualitative thematic analysis of de-identified focus group transcripts is currently underway.

Results: Emerging themes describe the benefits and challenges of acting as a peer supporter, as well as lessons learned during implementation. Stories regarding important barriers and facilitators to implementation are emerging, including description of the importance of building trust and relationships between Supporters and those being supported, as well as the importance of having management or leadership buy-in.

Conclusions: Though many groups have begun to study healthcare workers experiences amidst COVID-19, this is a rare opportunity to learn from peer supporters who actively implemented mental wellness programming over multiple waves of the pandemic. Lessons learned can be shared with knowledge users and policy makers to better support HCWs.

VISUALISATION OF A QUIESCENT NEURAL STEM CELL POPULATION WITHIN THE VENTRICULAR-SUBVENTRICULAR ZONE

STUDENT: SABINE LOVEJOY
SUPERVISOR: DEREK VAN DER KOOY PHD, FRSC

The ventricular germinal zone of the lateral ventricle is niche to two notable populations of adult stem cells, termed primitive and definitive neural stem cells (pNSCs and dNSCs respectively). pNSCs are distinct in that they are a naturally quiescent population with infrequent division and low cell cycle activity, and are cited as the first NSCs in the developing embryo. They give rise to dNSCs later in embryogenesis and postnatally sink into quiescence. This is interrupted to repopulate dNSCs after injury or experimental ablation throughout the life of the animal, an ability that persists into adulthood. After repopulating, the pNSCs return to quiescence after a couple weeks. Further investigation has found that pNSCs express the marker Oct4 but not GFAP, which allows us to differentiate them from dNSCs, which express the astrocytic marker GFAP but not Oct4. Our work aims to visualise the niche of the pNSCs ex vivo by imaging wholemount dissections of the lateral ventricle in a double fluorescent reporter mouse. We hypothesise that Oct4+ pNSCs are present within the subventricular zone, owing to its history as the germinal zone in embryogenesis. We administer IP tamoxifen injections over three days to label cells possessing our Oct4-cre genotype. Our double fluorescent reporter mouse by default expresses TdTomato in all tissues except for those that also possess an active cre-recombinase. Tamoxifen administration thereby causes Oct4+ cells to uniquely excise their TdTomato and reveal an EGFP. After tamoxifen treatment, brains are collected and carefully dissected, the medial and lateral walls of the lateral ventricle being isolated and trimmed from the rest of the brain by hand. Once mounted on slides, the complete sections of tissue can be imaged under a confocal microscope to screen for GFP expression against TdTomato. As per previous estimates of 100 Oct4+ pNSCs per brain, consistent non-autofluorescent GFP expression was rare within the lateral wall of the lateral ventricle. The dorsal and anterior pockets of the lateral wall demonstrated the highest expression of consistent GFP expression that would indicate an Oct4+ cell. More prolific expression was seen in the medial wall of the lateral ventricle, corroborated by neurosphere assays that indicated the medial wall's increased likeliness to form Oct4+ spheres. GFP-expressing Oct4+ cells were predominantly found in the ventromedial region of the medial wall, a finding that is consistent with the ventromedial region being developmentally derived from the region of the neural plate which is the first area of the nervous system to be induced in development. In conclusion we visualise a quiescent cell population integral for the development and regeneration of the central nervous system and isolate regions of the ventricular-subventricular zone where they are most prominent.

ROLE OF PRIMITIVE NEURAL STEM CELLS IN NEURAL REPAIR AFTER NEONATAL BRAIN INJURY

STUDENT: HUMNA NOMAN
SUPERVISOR: CINDI M. MORSHEAD

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Background: Perinatal hypoxic/ischemia (HI) is an important cause of brain injury. Children who suffer from HI exhibit long-term neurological complications and/or physical impairments. A promising therapeutic to regenerate damaged tissue after HI is the activation of endogenous neural stem cells (NSCs) in the brain. Our lab has previously demonstrated that the administration of metformin, commonly used to treat type-II diabetes, is sufficient to promote sensorimotor and cognitive recovery in a mouse model of neonatal HI.

Purpose: Although it is known that metformin activates NSCs by inducing proliferation and promoting neurogenesis, it is not known if these processes underlie the observed functional recovery as metformin has pleiotropic effects in the brain. My goal is to understand the cellular mechanism of metformin-mediated recovery and will ask whether activated NSCs are directly contributing to tissue repair and functional improvement.

Hypothesis: NSCs comprise a heterogenous population of cells and I hypothesize that rare, resident primitive NSCs (pNSCs) and their progeny are activated in response to HI and metformin treatment, migrate to the site of injury and contribute to neurogenesis and oligogenesis.

Methods: I used the *in-vitro* colony-forming assay to quantify and visualise stem cells and their progeny. Additionally, a neonatal mouse model of H/I injury was used to assess stem cell response to injury and treatment.

Results: I found that pNSCs are activated in response to injury as well as *in-vitro* and *in-vivo* metformin treatment. These cells also present regional heterogeneity in the postnatal brain, and these spatially distinct populations respond differentially to injury and treatment.

Conclusions: Having established the response of these cells to injury and treatment, our next step is to perform *in-vivo* lineage tracking experiments that will allow us to assess the role of these cells in the injured brain. These results together will help us establish the mechanism of metformin-mediated recovery, providing insight into this promising therapeutic for neonatal brain injury.

DIGITAL COUNSELLING PROGRAM WITH SOCIAL NETWORK SUPPORT FOR QUALITY OF LIFE IN KIDNEY DISEASE

STUDENT: RACHEL GRACE PEIRIS
SUPERVISOR: DR. ROB NOLAN

Background. Chronic kidney disease (CKD) impedes on health-related quality of life (HRQOL), which is improved by adhering to clinical and task-force guidelines for self-care behaviours (e.g., following medication regimes, engaging in physical activity, and maintaining dietary restrictions). Despite their prognostic significance, self-care rates remain low among patients. Counselling methods such as cognitive-behavioural therapy (CBT), motivational interviewing (MI), and peer support may address this concern by fostering long-term behavioural change. Furthermore, packaging such approaches into a user-friendly digital program could potentially optimize efficiency and practicality for patient usage, especially given the age of COVID-19.
Purpose. ODYSSEE-vCHAT (Open Access Digital Community Promoting Self-Care, Peer Support and Health Literacy – A Virtual Community Promoting Mental Health, Psychosocial Adjustment, and Peer Support) is a novel digital intervention targeting CKD self-care. The ODYSSEE aspect administers counselling resources using elements of CBT and MI while vCHAT provides peer support through social networking. The purpose of this thesis is to examine the incremental benefit of ODYSSEE-vCHAT on overall wellbeing and behaviours for living well in patients with CKD.
Hypotheses. Primary: Program usage – defined as low ODYSSEE and vCHAT ($<$ 1 hour), high ODYSSEE only ($\geq$ 1 hour), and high ODYSSEE and vCHAT ($\geq$ 1 hour) – will reduce the effect of CKD on daily life, measured by a subscale of the 36-Item Kidney Disease Quality of Life instrument (KDQOL-36) at 4 months. **Secondary:** Program usage will improve physical and mental functioning (remaining KDQOL-36 subscales), psychological wellbeing (Flourishing Scale), self-efficacy in managing CKD (6-Item Self-Efficacy for Managing Chronic Diseases scale), and engagement in activities for living well (Eval**u**ation of Goal-Di**r**ected Behaviours to Promote Well-Being and Health; EUROIA) at 4 months.
Methods. Sample: A target sample of N = 78 advanced CKD patients are enrolled ($\geq$ 18 years; $>$ 10% risk of requiring dialysis in 2 years or end-stage renal disease and on dialysis). **Procedures:** ODYSSEE-vCHAT provides access to counselling materials (educational pages, videos, tools, and trackers), chatrooms, and webcasts (presentations and discussions) on themes of CKD self-care and HRQOL. Subjects are contacted weekly via email to promote program adherence and participation. Hourly program usage is tracked in real time and pre- and post-study self-reports are administered online. **Analyses:** The effect of program usage on primary and secondary outcomes will be analyzed using separate Generalized Linear Models ($p < 0.05$). All tests will be adjusted for baseline scores of corresponding assessments and sociodemographic factors (i.e., gender, ethnicity, household income, education level, dialysis status) will be treated as potential confounders.
Results. Results are not yet available because the estimated sample size has not been on the program for 4 months. Planned analyses will occur in early May.
Conclusions. ODYSSEE-vCHAT has the potential to improve the HRQOL of CKD patients in conjunction with usual care. ODYSSEE-vCHAT may be adapted for other chronic illnesses and for use by caregivers. Its accessibility may be improved by transferring the program onto an application for mobile devices.

EXAMINING THE ROLE OF FRACTALKINE ON FUNCTIONAL RECOVERY AFTER DEGENERATIVE CERVICAL MYELOPATHY

STUDENT: CINDY ZHOU
SUPERVISOR: MICHAEL FEHLINGS

Cindy Zhou, Sydney Brockie, Mandana Movahed, James Hong**, Michael Fehlings**

Background: Degenerative cervical myelopathy (DCM) encompasses several age-related degenerative conditions that cause a compression of the cervical spinal cord. The functional deficits experienced by DCM patients may be caused by a maladaptive elimination of synapses. Recently, the fractalkine receptor (CX3CR1), which is found on microglia, has been shown to be involved in microglial-mediated synaptic elimination. Further, deletion of the receptor results in improved functional outcomes and synapse formation after traumatic spinal cord injury (SCI).

Hypothesis and Purpose: The main objective of this study is to investigate the role of fractalkine (CX3CR1-CX3CL1) signaling on functional outcomes and synaptic elimination following DCM. It was hypothesized that *i)* fractalkine-mediated synaptic engulfment occurs after DCM, and that *ii) Cx3cr1* deletion will attenuate the synaptic loss whilst improving functional recovery.

Methods: DCM was induced in C57BL/6 and *Cx3cr1*$^{-/-}$ mice by inserting a polyether aromatic material under the C5-C6 lamina. Synaptic elimination and functional recovery were characterized at three timepoints (4, 8, and 12-weeks post-DCM) using synaptic markers and CatWalk Gait analysis.

Results: Compared to naïve animals, preliminary immunostaining revealed that after 17-weeks of DCM, the dorsal horns of C57BL/6 mice exhibit a greater synapse to neuron ratio due to less NeuN^{+} cells but no change in the number of synapses (PSD95^{+} and Homer-1^{+} cells).

Conclusions: Current preliminary data suggests that fractalkine-mediated synaptic engulfment does not occur after DCM, but further experiments using *Cx3cr1*$^{-/-}$ mice will provide better insight.

Group Q: Neuroscience Brain-Health

USING QUANTITATIVE MICROSTRUCTURAL MRI TO PREDICT OUTCOMES IN DEGENERATIVE CERVICAL MYELOPATHY

STUDENT: MUHAMMAD ALI AKBAR
SUPERVISOR: DR. MICHAEL FEHLINGS

Background

Degenerative cervical myelopathy (DCM) is the most common spinal cord dysfunction in adults and can lead to devastating neurological impairment including paralysis. The definitive management of DCM is surgical decompression, however mild cases are initially managed non-operatively. Predictors of neurological deterioration are greatly needed for this population to aid in early interventions. Advancement in magnetic resonance imaging (MRI) of the spinal cord has helped validate several techniques that provide metrics correlating with pathophysiological changes in the spinal cord such as demyelination and axonal loss. These techniques are collectively known as quantitative MRI (qMRI).

We hypothesize that qMRI metrics extracted from the cervical spinal cord of a DCM patient, can reliably predict the likelihood of deterioration and surgical intervention needed within one year.

Methods

58 DCM patients were included in the analysis. All patients underwent a multiparametric qMRI protocol including diffusion tensor (DTI), magnetization transfer (MT) and T2* weighted imaging. Template based image analysis was performed using Spinal Cord Toolbox©. Cross sectional area (CSA), fractional anisotropy (FA), magnetization transfer ratio (MTR), and T2* white matter to gray matter ratio (T2*WI WM/GM) were measured in the most compressed level and rostral cervical cord.

Multivariable logistic regression was performed using CSA at the most compressed level, and FA, MTR and T2*WI WM/GM in the rostral cord as predictors for surgical intervention. Model discrimination and reliability was measured using c-index and Brier's score respectively. Validation and calibration was performed using bootstrap method with 100 repetitions.

Results

29 patients required surgery at 1 year and 29 remained in the non-operative group. Baseline qMRI was able to identify patients requiring surgery within 1 year of presentation, with an accuracy of 81.6% (AUC or c-index). The model showed good reliability with a Briers score of 0.18 and Somers Dxy rank correlation of 0.63. Calibration using bootstrap method showed overall good predictive ability with slight over prediction at higher predicted probability of surgery.

Conclusion

Baseline quantitative MRI can accurately and reliably predict the likelihood of deterioration and surgical intervention in mild to moderate DCM patients. It can potentially play an important role in screening patient populations for surgical candidates or those that are at high risk of deterioration.

IN VIVO DETECTION OF MISFOLDED ALPHA-SYNUCLEIN IN CORTICOBASAL SYNDROME

STUDENT: CHLOE ANASTASSIADIS
SUPERVISOR: DR. MARIA CARMELA TARTAGLIA

Background Corticobasal syndrome (CBS) is a heterogenous clinical syndrome that features atypical parkinsonism as well as language and/or behavioral symptoms. Alzheimer's disease and the 4-R tauopathy known as corticobasal degeneration (CBD) are the two main underlying pathologies responsible for this syndrome. More rarely, other forms of frontotemporal lobar degeneration such as progressive supranuclear palsy or TDP-43 proteinopathies are found to be involved.
Adding to that complex picture, recent post-mortem studies have revealed that comorbidities, especially Lewy body disease, are a frequent finding in pathologically confirmed AD, and less so in CBD cases. The implications of these findings for the clinical syndrome of CBS, however, remain unclear. Moreover, these findings are also in contradiction with those from in-vivo studies that assessed misfolded alpha-synuclein levels using the real time quaking-induced conversion (RT-QuIC) assay in the cerebrospinal fluid of CBS patients and found no sign of positivity among them. In the in-vivo studies, the RT-QuIC assay used was unable to detect positivity in subjects with multiple system atrophy, an alpha-synucleinopathy, suggesting that it could lack the sensitivity to detect misfolded alpha-synuclein in CBS as well.

In this study, we aimed to: 1. assess the frequency of misfolded alpha-synuclein pathology in-vivo in CBS, using an optimized version of the RT-QuIC assay; 2. compare CBS with and without AD positive biomarkers; and 3. examine how RT-QuIC positivity is associated with clinical features or neuroimaging markers.

Methods The CSF of 40 patient with CBS (mean age 65.88 ± 9.48 years old, 19 males) was collected to be assessed using the following assays: real-time quaking-induced conversion (RT-QuIC) for misfolded alpha-synuclein aggregates, neurofilament light-chain (NFL), chitinase 3-like 1 YKL-40, and AD biomarkers (p-tau, t-tau, Abeta42). MRI data (T2-w FLAIR, T1-w) were also processed to investigate white matter hyperintensities and total brain volume.

Results 14/40 (35%) of the CBS patients tested positive for RT-QuIC alpha-synuclein using a strict cut-off, and 25/40 (62.5%) were at least borderline positive. In preliminary analyses, the association between RT-QuIC alpha-synuclein outcome and AD status trended towards significance ($p=0.062$, Fisher's exact test). Subjects with positive RT-QuIC were significantly older (mean age 71.92 years old) than those testing negative (mean age 63.20 years old) ($t(35)=-2.8693$, $p<0.01$). There was no difference in NFL, a marker of neurodegeneration, or YKL-40, a marker of altered glial function, between the RT-QuIC alpha-synuclein positive and negative groups. Finally, while half of the RT-QuIC alpha-synuclein positive had a motor onset for their disease, that was the case for only a third of the the RT-QuIC alpha-synuclein negative group.

Conclusions The presence of misfolded alpha-synuclein is frequent in CBS and can be assessed in-vivo. Age is the main contributor to the presence of alpha-synuclein but there was a trend for its increased prevalence in the CBS-AD group. Further analyses are needed to elucidate the contribution of alpha-synuclein pathology to the clinical presentation of CBS, although preliminary data suggest it may contribute to the early symptomatology.

PREBÖTZINGER COMPLEX GABA NEURON ACTIVATION USING *IN VIVO* OPTOGENETICS DEPRESSES BREATHING IN MICE

STUDENT: KAYLA BAKER
SUPERVISOR: GASPARD MONTANDON

Kayla S. Baker, Carolina Scarpellini, **Gaspard Montandon**

Background: Breathing is controlled by the rhythmic activity of brainstem breathing centers to generate respiratory rhythms. The preBötzinger Complex (preBötC) is a brainstem structure and is involved in inspiration. The preBötC contains a heterogenous population of cells that are interconnected, including the excitatory neurotransmitter glutamate and inhibitory neurotransmitter γ-aminobutyric acid (GABA). The role of the preBötC glutamate neurons in inspiration is well studied but how the preBötC GABA neurons contribute to maintaining respiratory rhythms is not known.

Purpose: Approximately half of the neurons in the preBötC are inhibitory, but how these cells contribute to maintaining respiratory rhythms is unknown. We aim to identify the role of the preBötC GABA cells in breathing using *in vivo* optogenetics.

Hypothesis: Optogenetic activation of preBötC GABA neurons will depress breathing.

Methods: To activate preBötC GABA neurons, using cre-lox approaches we stereotaxically injected a cre-dependent adeno-associated virus expressing channelrhodopsin in cells expressing the vesicular GABA transporter (vGAT). We then recorded diaphragm and genioglossal muscle activities in anesthetized mice and used focused 470 nm light to stimulate preBötC vGAT cells. We performed similar experiments while inhibiting glutamate neurons (554 nm focused light) by expressing the inhibitory archaerhodopsin in vesicular glutamate transporter 2 (vGlut2) cells to validate the role of glutamate in respiratory rhythms.

Results: Our preliminary data indicate that vGAT excitation depresses breathing depending on the point in the inspiratory cycle that the laser stimulation occurred. Photostimulation of vGAT cells increases the period between inspirations and the expiratory time but does not alter the inspiratory time or the diaphragm amplitude. Photoinhibition of vGlut2 cells depresses breathing in similar ways to vGAT excitation.

Conclusions: vGAT excitation and vGlut2 inhibition depress breathing by increasing the expiratory time but do not alter inspiration. Determining the role of GABA and glutamate preBötC cells in coordinating respiratory rhythms will allow us to better understand what is occurring in the brain when breathing is depressed such as in opioid-induced respiratory depression. By identifying the cells involved in respiratory depression by opioids, we may be able to develop new and safer analgesics that do not target these sensitive breathing areas.

CLINICIAN PERSPECTIVES ON ACCEPTABILITY OF PEDIATRIC AUTISM THERAPIES

STUDENT: ISABELLE CAVEN
SUPERVISOR: DR. MELANIE PENNER

Background: There are a variety of therapies available to autistic children in Canada, including but not limited to, occupational therapy (OT), applied behaviour analysis (ABA)-based therapies, speech-language pathology (SLP) services, and physiotherapy (PT). These services are commonly accessed by autistic children and their families; however, there are varying levels of evidence supporting their effectiveness. Alongside concerns of the methodological rigour of this research, many autistic individuals have raised objections to the purpose and ethics of certain approaches. There is limited information about the perspectives of clinicians, who may be involved in service delivery or helping families select a therapeutic approach. The overall aim of this study is to explore stakeholder perspectives on acceptability of pediatric autism therapies. These data were collected as part of a larger mixed methods study exploring the perspectives of multiple stakeholders (autistic individuals, parents of autistic individuals, and clinicians).
Purpose: The aim of this study is two-fold: 1) to explore how perceived acceptability of pediatric autism therapies varies amongst clinicians; and 2) to explore what clinician demographic factors are associated with greater perceived acceptability. **Methods:** This study is an online, cross-sectional survey exploring perspectives of physicians, occupational therapists, physiotherapists, speech-language pathologists, behaviour analysts, nurses, social workers, and psychologists on the acceptability of pediatric autism therapies. Using the Theoretical Framework of Acceptability, a survey was built with feedback from an advisory group comprised of autistic individuals, parents of autistic individuals, and clinicians. Recruitment took place on social media and via autism and clinician organizations. Respondents answered questions about their demographic information, experience in their clinical role, and perspectives on the therapies. Likert questions about the therapies were repeated for each therapy type. A composite acceptability score was produced for each therapy, with scores above a neutral point of 32 indicating higher acceptability, and those below indicating lower acceptability. These scores were combined into an overall score, ranging from 0 to 256 with a neutral point of 128. Multiple linear regression was conducted to explore which demographic variables predicted higher acceptability. **Results:** One hundred and twenty-one clinicians completed the survey, including 32 OTs, 26 SLPs and 24 physicians. Most respondents identified their racial/ethnic group to be white (74%) and identified as women (93%). Respondents had been in their current clinical role for a median of 14.5 years (range 1 – 43) and their client populations consisted of a median of 63% of autistic individuals (range 1 – 100). The median OT subscore was 46 (range 16 – 59) and the median SLP subscore was 47 (range 25 – 59). The median ABA subscore was 32 (range 9 – 53) and the median PT subscore was 40 (range 19 – 60). Combined, the median overall score was 164 (range 113 – 211). Linear regression demonstrated that percent of autistic clients was negatively associated with the overall score and having a clinical role of either OT, SLP, PT or behaviour analyst was positively associated, and both variables significantly predicted the overall score ($p < 0.05$). Qualitative analysis of open-ended survey questions is ongoing. **Conclusions:** Delivering an autism therapy and working with a higher proportion of autistic clients may influence clinicians' perspectives on the acceptability of therapies available to autistic children. Integration of qualitative and quantitative findings will further explore clinician acceptability. A second round of data collection will take place in Spring 2022 to make more robust comparisons between the perspectives of autistic individuals, parents of autistic children, and clinicians. Research exploring perspectives of key stakeholder groups is critical to ensure services provided to autistic children and their families are informed by lived experiences.

ON THE ROLE OF MU-OPIOID RECEPTORS IN FENTANYL-INDUCED RESPIRATORY DEPRESSION AND LOCOMOTOR HYPERACTIVITY

STUDENT: ANDREEA FURDUI
SUPERVISOR: DR. GASPARD MONTANDON

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Background: Opioid drugs bind to mu-opioid receptors (MORs) and can exert both inhibitory effects, leading to respiratory depression (a severe decline in breathing rate), and excitatory effects, characterized by locomotor hyperactivity in rodents.

Purpose: Here, we aimed to characterize the dual effects of the opioid fentanyl on breathing and behaviour.

Hypothesis: We hypothesized that fentanyl induces respiratory depression combined with a simultaneous increase in locomotor activity by acting on MORs.

Methods: *In situ* hybridization was used to map MOR mRNA expression in brainstem regions involved in breathing or behaviours. Respiratory depression and motor hyperactivity were induced by an intraperitoneal injection of fentanyl (0.3mg/kg) in male wildtype and MOR knockout (MOR-/-) mice. Respiratory rate was recorded using whole-body plethysmography and video recordings were captured to assess behaviour.

Results: We found that MORs were expressed in multiple brainstem regions involved in breathing or behaviours. We also found that fentanyl injection induced a significant respiratory rate depression in wildtype mice ($P<0.0001$, $n = 8$), while no such change was observed in MOR-/- mice ($P=0.9833$, $n=7$). Similarly, fentanyl injection increased locomotor activity in wildtype mice but not in MOR-/- mice.

Conclusions: We conclude that MORs are expressed in brainstem respiratory and locomotor regions and that fentanyl induces its effects on breathing and behaviours by acting on MORs.

ENHANCING TISSUE REPAIR, FUNCTIONAL AND LOCOMOTOR RECOVERY WITH NX210C PEPTIDE ADMINISTRATION IN A CERVICAL SPINAL CORD INJURY RAT MODEL

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Background: Spinal cord injury (SCI) is a debilitating condition that limits sensation and motor function below the level of the injury. Initial physical trauma in SCI is followed by secondary cascades which involve further cell death in the central nervous system (CNS), upregulation of inflammatory cytokines, and scar formation. The restoration of lost neural networks is inhibited due to the low regenerative capacity of the CNS, thus preventing functional recovery. NX210c is a 12 amino acid peptide derived from the sub-commissural organ (SCO)-spondin, a protein proposed to be involved in vertebrate spinal cord regeneration.

Purpose: To evaluate the efficacy of NX210c to promote repair and functional recovery in a translationally relevant traumatic cervical SCI rat model. Aim 1: assessing forelimb and hindlimb locomotor recovery and bladder function and Aim 2: determining cellular anatomical changes at the lesion site through histological assessments of myelination, apoptosis, scar formation, and the distributions of neurons, oligodendrocytes, and astrocytes.

Hypothesis: NX210c will enhance neural repair and regeneration at and across the injury site, thus improving neurobehavioural recovery.

Methods: Female adult Wistar rats will receive a clip compression-contusion SCI at the C6/C7 level of the spinal cord, which is a clinically relevant model of traumatic SCI in humans. 66 injured rats will be randomized into 4 groups, in a blinded manner, to receive one daily dose of either NX (8mg/kg) or sterile water, starting 4 hours (h) or 8 h post-SCI, until 8 weeks post-SCI. 12 sham rats will only receive a laminectomy with no clip-induced SCI, and water treatment beginning at 4 h post-surgery. Neurobehavioral assessments will be performed until 8 weeks post-SCI, where animals will be sacrificed for histological assessments.

Results: Early administration of NX210c increased forelimb strength (grip strength) and improved several aspects of locomotion including interlimb coordination, (i.e., regularity index or base of support of the forelimbs; CatWalk). When delaying first administration to 8h post-injury, NX210c promoted weight gain, accelerated bladder control recovery from 14 to 9 days post-injury, and reduced sensorimotor deficits (inclined plane). Preliminary histology (n=3/group) demonstrates greater white matter preservation and reduced cavity size at the injury site with NX210c treatment beginning at 8h post-injury compared to vehicle.

Conclusions: NX210c improves motor function and bladder control, while also contributing to improved white matter preservation. We anticipate that this study will provide a strong proof of concept for its use as a treatment for acute SCI patients.

DOES COGNITION AFFECT ADAPTATION TO NEW GAIT PATTERNS IN PARKINSON'S DISEASE?

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SUPERVISORS: DR. ALFONSO FASANO; DR. ANTONIO STRAFELLA

Background: Parkinson's disease (PD) related gait and balance disorders are challenging to treat because they cannot be optimized with pharmacological intervention alone. This treatment gap is important to address because gait asymmetry and incoordination are associated with increased falls in this population, which can be functionally debilitating and lead to increased morbidity and mortality. Freezing of gait (FOG) has also been associated with reduced quality of life independent of its association with impaired mobility. Gait disorders therefore represent an unmet need in the treatment of PD. A split-belt treadmill (SBTM) can be used to adjust the speed of each leg separately and individuals can be prompted to 'adapt' to an asymmetric gait and 're-adapt' with return to symmetrical gait in a phenomenon known as 'after-effect'. Feasibility trials have previously shown that individuals with PD, including those with FOG, can adapt to SBTM conditions.

Purpose: Inter-limb coordination and temporal aspects of gait control are challenged in SBTM walking. However, automaticity of motor control is impaired in PD, especially in individuals with FOG, so there is increased cognitive control of gait. The purpose of this study was to determine whether baseline cognitive status affects gait adaptation in PD with FOG.

Hypothesis: In PD with treatment-resistant FOG, cognitive impairment reduces 1) gait adaptation and 2) after-effects induced by SBTM walking.

Methods: Twenty participants with idiopathic PD and treatment-resistant FOG were recruited. Inclusion criteria required at least 3 months of stable clinical response to medications or stimulation parameters, in case of deep brain stimulation (DBS) and the ability to walk on a motorized treadmill. A comprehensive cognitive assessment was performed using the Toronto Cognitive Assessment (TORCA) and the median percentile was used to distinguish between cognitively 'intact' versus 'impaired'. The velocity of the treadmill was adjusted to the over-ground speed of the participant and during the 20 minutes of SBTM training, the belt velocity on the least affected side was reduced by 25%. Virtual reality technology was used to mimic natural gait as closely as possible in a simulated setting. A composite score was developed to reflect gait adaptation and duration of after-effects, with higher scores reflecting faster adaptation and prolonged after-effects. The primary outcome measure was the effect of cognitive status on the composite score. Secondary objectives assessed the relationship between composite scores and FOG frequency, severity of gait instability, Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) score, levodopa-equivalent daily dose (LEDD) and DBS. Exploratory objectives included step length asymmetry during adaptation and reduction in gait asymmetry after SBTM training.

Results: Fourteen participants adapted to SBTM walking. Of the six who did not adapt, five had normative TORCA scores in the $\leq 5^{th}$ percentile (impaired). Cognitively intact participants demonstrated higher composite scores ($p=0.01$), which was primarily driven by the working memory sub-score ($p=0.02$). Fifty percent had deep brain stimulation at the time of recruitment. The composite score was not influenced by FOG frequency, severity of gait instability, MDS-UPDRS motor score, LEDD or DBS status ($p<0.05$). The step length asymmetry during adaptation and gait asymmetry after SBTM training did not vary between fast and slow adaptors.

Conclusions: Cognitive impairment, particularly impaired working memory, reduces gait adaptation and after-effects in PD with FOG, independent of disease and FOG severity, LEDD and gait instability.

NEURAL STEM CELLS PAIRED WITH AUTOREGULATED CHONDROITINASE (ChABC) AS A THERAPEUTIC OPTION POST SPINAL CORD INJURY

STUDENT: OLIVER ZHANG
SUPERVISOR: DR. MICHAEL FEHLINGS

Purpose: Post-traumatic inflammation and ischemia leading to the formation of a microcavity and a glial scar in the chronic phase of spinal cord injury (SCI). A glial scar forms around the site, partially composed of CSPG which *in vitro* are inhibitory to axon growth. Bradbury et al. (2002) found that using intrathecal ChABC promoted regeneration of ascending sensory projections and descending corticospinal tract neurons. Previous work from Fehlings lab (Karemi-Abdolrezaee, S. et al, 2010) found that intrathecal administration of ChABC optimizes neural progenitor cell (NPC) transplantation in chronic SCI, resulting in extensive migration, promoted axonal integrity and plasticity of corticospinal tract, improved neurobehavioural recovery. This project focuses on the comparison between two promoters for ChABC – a more traditional tet-on promoter in comparison with an autoregulated DDN promoter.

Methods: Rats will receive a spinal cord injury though a clip-contusion protocol. Various behaviour tests will be used to gage recovery and effectiveness of both promoters, including catwalk, inclined plane, grip strength and Montoya reaching test. Twelve weeks after transplantation, the animals will be sacrificed and immunohistochemistry will be performed.

Results: We have yet to have experimental results, however we are in the midst of processing our data.

Group R: Infection-Immunology/ Other

VALIDATION OF THE PROMIS DEPRESSION COMPUTER ADAPTIVE IN LIVER TRANSPLANT RECIPIENTS

STUDENT: TIBYAN AHMED
SUPERVISOR: DR. ISTVAN MUCSI

Background: Depressive symptoms are frequently reported among LTR and are associated with poor health-related quality of life and clinical outcomes. Patient reported outcome measures can be used to identify patients who may benefit additional psychosocial support and clinical assessment. The NIH Patient Reported Outcome Measures Information System Depression Computer Adaptive Test (PROMIS-D CAT) provides precise, tailored assessment of depressive symptom level. However, evidence of PROMIS-D CAT validity has yet to be established in LTR.

Purpose: To provide data to support the validity of PROMIS-D CAT in LTR.

Hypothesis: I hypothesize that PROMIS-D CAT will demonstrate very good to excellent measurement properties, including reliability and validity in LTR.

Methods: A cross-sectional convenience sample of adult LTR completed PROMIS-D CAT and legacy Patient Health Questionnaire-9 (PHQ-9) using an electronic data capture platform. Sociodemographic and clinical data were also collected. Reliability was assessed using the item response theory approach, defined as 1-standard error of measurement [SEM] and plotted over the depression level (T score). Test-retest reliability was assessed using intraclass correlation coefficient (ICC2,1). Construct validity was assessed using Spearman's rho correlation between PROMIS-D CAT and PHQ-9, and known-group comparisons. Discrimination of PROMIS-D CAT was assessed using receiver operating characteristic (ROC) curve analysis with PHQ-9 ≥ 10 as referent for moderate/severe depressive symptoms.

Results: Of 206 participants, mean(SD) age was 56 (15), 66% were male, and 72% were White participants. Based on a PHQ-9, 20% of participants had moderate/severe depressive symptoms. Excellent reliability (> 0.90) was found across levels of depression (T score) for 91% (n=188) of participants. Additionally, ICC2,1 was 0.89 indicating excellent test-retest reliability. PROMIS-D CAT T scores were strongly correlated with PHQ-9 scores (r=0.68, p <0.001). Construct validity was further confirmed by known-groups comparisons: higher mean (SD) PROMIS-D CAT T scores were reported in participants < 50 years of age vs. those 50-65, and > 65 [53 (8) vs. 50 (10) vs. 50 (8); p = 0.005] respectively. Higher PROMIS-D CAT T scores were also observed in those with higher global symptom distress, Edmonton Symptom Assessment System-revised ≥ 30 compared to < 30 [(59 (9) vs. 48(8); p < 0.001)] and in those with significant fatigue, PROMIS-Fatigue CAT ≥ 60 compared to < 60 [(58 (9) vs. 48 (9), p < 0.001)]. A cut-off of 55 for PROMIS-D CAT had good discrimination against PHQ-9 ≥ 10 with an area under ROC curve of 0.82 (95% CI: (0.72, 0.92).

Conclusions: The data contributes new evidence of the reliability and validity of PROMIS-D CAT for measuring depressive symptoms in LTR and supports its use in clinical care. Future studies could assess the responsiveness of PROMIS-D CAT in LTR.

SUSTAINED IMPACT OF LACTIN-V (LACTOBACILLUS CRISPATUS CTV-05) ON GENITAL IMMUNOLOGY FOLLOWING STANDARD BACTERIAL VAGINOSIS TREATMENT: RESULTS FROM A RANDOMIZED PLACEBO-CONTROLLED TRIAL

STUDENT: ERIC ARMSTRONG
SUPERVISOR: DR. RUPERT KAUL

Background: Bacterial vaginosis (BV) increases HIV acquisition risk, likely by eliciting genital inflammation and epithelial barrier disruption, while vaginal *Lactobacillus crispatus* is associated with immune quiescence and HIV protection.

Purpose: The purpose of this study was to evaluate the impact of LACTIN-V, a novel, *L. crispatus*-based live biotherapeutic, on genital immunology and the vaginal microbiota following standard antibiotic treatment for BV compared to matched placebo.

Hypothesis: We hypothesized that LACTIN-V administration would be associated with sustained reductions in vaginal levels of the proinflammatory cytokine IL-1a and soluble E-cadherin (sE-cad), a marker of epithelial disruption, compared to placebo.

Methods: Soluble immune factors and the absolute abundance of bacterial taxa were assayed by multiplex ELISA and qPCR for 66 participants nested within a phase 2b randomized double-blind placebo-controlled trial of LACTIN-V to prevent BV recurrence (NCT02766023). All participants in the clinical trial received topical metronidazole for five days and were randomized to receive LACTIN-V (n=32) or matched placebo (n=34) for 11 weeks. Co-primary endpoints for this immunology sub-study were vaginal levels of IL-1a and sE-cad) at 24 weeks, or 3 months after the last dose of LACTIN-V or placebo; the BV recurrence endpoints have been described elsewhere.

Results: Three months after the end of the treatment period, LACTIN-V was associated with a sustained reduction in vaginal levels of IL-1a and sE-cad. Secondary analyses demonstrated that levels of the chemokine IP-10 were elevated among women who received LACTIN-V. The immune impact of LACTIN-V was mediated by a reduction in BV-associated bacteria and, to a lesser extent, an elevated abundance of *L. crispatus*. Elevated IP-10 was linked to non-CTV-05 *L. crispatus* abundance, rather than colonization by CTV-05.

Conclusions: Vaginal administration of LACTIN-V following standard BV therapy resulted in a sustained reduction in genital inflammation and a biomarker of epithelial integrity, which were mediated primarily by BV-associated bacteria. These findings have important implications for the use of LACTIN-V as a potential HIV prevention strategy.

BARRIERS TO PLASTIC AND RECONSTRUCTIVE SURGERY CARE FOR INDIVIDUALS WITH CONGENITAL CONDITIONS AFFECTING THE HEAD/FACE

STUDENT: KARIYM JOACHIM
SUPERVISOR: DR. CHRISTOPHER FORREST

Background: Orofacial Cleft (OFC) – also known as cleft lip and cleft palate – refers to conditions where a child's top lip, and/or the roof of their mouth does not fully form before birth. Each year in Ontario, 1.12 in 1000 children are born with OFC, making it the most common facial difference. Surgical and non-surgical interventions are required to mitigate the impacts of OFC on appearance and facial function. Impediments to surgical care access have been shown to have implications for child development targets and socialization. Though no studies have assessed Canadian inequities in OFC surgical access, care access inequities have been identified in other populations within Canada.

Purpose: The objective of this four-stage project is to identify all barriers to OFC care in Ontario and (2) subsequently assess their impacts. The described scoping review is the first stage.

Hypothesis: Healthcare provider anecdotes have suggested that low socioeconomic status, extensive travel distance to care, and indigenous status may be among a number of predictors of poor OFC surgical care access described in the literature.

Methods: A scoping review with qualitative synthesis will identify pertinent barriers faced by individuals with congenital craniofacial conditions (including OFC) requiring plastic and reconstructive surgery. Extracted barriers will inform the subsequent stages of the project.

Results: Data extraction is currently in-progress.

Conclusions: Preliminary conclusions will be presented.

THE ROLE OF IL-17RD IN THE PATHOGENESIS OF JUVENILE IDIOPATHIC ARTHRITIS

STUDENT: RENUKA RAMLOGAN
SUPERVISOR: DR. RAE S. M. YEUNG

Background Data from a Canada-wide new onset JIA cohort indicates that single nucleotide polymorphisms (SNPs) for *IL17RD* are associated with high proinflammatory cytokine levels in a cluster of patients with Juvenile Idiopathic Arthritis (JIA). The association of *IL17RD* SNPs with elevated proinflammatory cytokine levels suggests a role for IL-17RD in regulating proinflammatory cytokine levels in JIA. Evidence supports a regulatory role for IL-17RD in receptor tyrosine kinase signaling through localization to the golgi apparatus, where it binds and inhibits nuclear translocation of activated ERK and p65 (NF-κB). The exonic risk variant for rs17057718 encodes a Valine to Methionine substitution in the transmembrane domain of the protein and is hypothesized to affect IL-17RD localization and function. Since NF-κB is a master regulator of proinflammatory cytokine production, and the rs17057718 risk allele is associated with elevated proinflammatory cytokine expression in JIA patients, the functional effect of the SNP genotype on IL-17RD regulation of proinflammatory cytokine production will be investigated.

Purpose To understand of the role of IL-17RD in regulating proinflammatory cytokine production in JIA.

Hypothesis IL-17RD regulates proinflammatory cytokine production JIA.

Methods Experiments were conducted using two complementary approaches in order to test the functional significance of the SNP genotype in cellular function - in Epstein-Barr Virus Transformed B cell lines (EBV-transformed B cell lines) and also in directed mutants using the CRISPR/Cas9 system in HeLa cells. EBV-Transformed B cells from healthy donors were genotyped for the exonic SNP of interest (rs17057718). SNP genotypes include: CC, TC, and TT; where T represents the polymorphic risk allele. The HeLa cell line used for directed mutagenesis is triploid, and resulting SNP genotypes of the edited clones are CCC and TCC; where T represents the risk allele. The effect of the SNP genotype on NF-κB and p38 activity was examined in EBV-transformed B cell lines at baseline by western blot analysis of phospho-p65 and phospho-p38 respectively. IL-6 levels were examined under stimulated (HeLa mutants) and unstimulated (EBV-transformed B cell lines) conditions by ELISA.

Results Preliminary findings indicate that the IL-17RD risk genotype (TT) is associated with a trend toward increased NF-κB activation, and decreased p38-MAPK activation under unstimulated conditions in EBV-transformed B cell lines. The risk genotype (TT) is also associated with a trend toward increased IL-6 expression at baseline in EBV-transformed B cell lines, and a corresponding trend toward increased IL-6 expression under stimulated conditions in directed HeLa mutants.

Conclusion Preliminary findings suggest a functionally significant role for the *IL17RD* SNP, rs17057718 in the regulation of proinflammatory cytokine production. The risk genotype is associated with differential NF-κB and p38 activation in EBV-transformed B cell lines and a corresponding trend toward increased IL-6 production under stimulated and unstimulated conditions using two complementary models. Future experiments will investigate the mechanism of action of IL-17RD in directed HeLa mutants and transient transfections.

CELL-BASED PASSIVE IMMUNIZATION FOR LONG-TERM PROTECTION AGAINST SARS-COV-2

STUDENT: EVAN SAWULA
SUPERVISOR: ANDRAS NAGY

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Background: Immunologically impaired individuals respond poorly to COVID-19 vaccines, which underscores the need for alternative strategies to protect these vulnerable populations from infection by SARS-CoV-2. While passively administered monoclonal antibodies can confer pre-exposure prophylaxis, their short lifespan in patients limits the duration of immune protection.

Purpose: Combining cell and antibody engineering offers a unique opportunity to provide lasting passive immunity with long-lived cell implants. We aim to genetically engineer cells to produce potently neutralizing SARS-CoV-2 antibodies. We then aim to demonstrate the ability of these engineered cells to act as *in vivo* delivery vehicles, supplying neutralizing antibodies over long-term as cell implants to protect mice from SARS-CoV-2 infection.

Hypothesis: We hypothesize that SARS-CoV-2 neutralizing antibodies produced from a cell implant will provide passive immunity to COVID-19 by preventing SARS-CoV-2 infection.

Methods: We first developed genetically engineered mouse embryonic stem cells that secrete potent neutralizing antibodies to SARS-CoV-2. Upon development, we then implanted these engineered stem cells subcutaneously into C57BL/6 mice.

Results: Here, we show that the engineered cells can supply protective levels of functional SARS-CoV-2 neutralizing antibodies *in vivo* for at least 150 days as a long-lived and stable subcutaneous implant.

Conclusions: Given the plasma neutralizing titers observed in mice harboring cell implants, we expect that this approach will protect mice from SARS-CoV-2 in upcoming infection studies. Altogether, these findings demonstrate the promise and potential of using “off-the-shelf” cell products that secrete neutralizing antibodies to long-term protect against current viral threats, including SARS-CoV-2, and other viruses that may emerge in the future.

THE EFFECTS OF ADA2 ON MONOCYTE FUNCTION AND PHENOTYPE

STUDENT: REETI SHARMA
SUPERVISOR: DR. RAE S. M. YEUNG

Background: Deficiency of adenosine deaminase 2 (DADA2) is a vasculitis involving a loss of function mutation in the adenosine deaminase 2 (ADA2) gene. ADA2 is an enzyme secreted by myeloid cells whose function is still being elucidated. Its newly discovered wide range of functions include monocyte to macrophage differentiation. Stimulated DADA2 patient whole blood and ADA2 knockout U937 macrophages have been shown to produce an increase in cytokine production. Furthermore, DADA2 patients have been seen to have skewed monocyte subtypes compared to healthy controls. Here, we are investigating the effects of ADA2 knockdown on human monocyte cytokine secretion, gene expression profile, and monocyte and macrophage marker expression.

Purpose: To understand the role of ADA2 on monocyte function and phenotype in response to various stimuli.

Hypothesis: ADA2 knockdown will alter monocyte gene expression profile and cytokine production and decrease macrophage marker expression.

Methods: Monocytes were isolated from healthy human peripheral blood mononuclear cells (PBMCs) via a negative selection classical monocyte kit. The purity of the monocytes was assessed by flow cytometry. ADA2 was knocked down in the cells via transfection with siRNA. The percentage of ADA2 knockdown was determined using western blotting. Liposaccharide (LPS) and Pam3CysSerLys4 (Pam3CSK4) were used to stimulate cytokine production in the knockdown cells via the antagonization of toll-like receptor (TLR) 4 and 1/2 respectively. Additionally, monocyte colony-stimulating factor (M-CSF) was added to the knocked down cells to assess the differentiation of macrophages in absence of ADA2. Cytokine production and macrophage marker expression post-stimulation with LPS, Pam3CSK4, and M-CSF will be investigated using Luminex and flow cytometry respectively. To determine differences in gene expression profiles post-stimulation in the presence or absence of ADA2, RNA will be extracted from cells and sent for RNA-sequencing.

Results: The purity of the isolated monocytes was found to be approximately 95% and was determined based upon CD14 expression and the lack of CD16 expression. By day three post-transfection, ADA2 was successfully knocked down, on average, by 86%. This knockdown lasts until six days post-transfection. Day three untransfected cells were analyzed via flow cytometry for macrophage and monocyte marker expression. Macrophage marker expression was found to be significantly increased and monocyte marker expression was decreased in these cells in comparison to day zero cells.

Conclusions: Optimal transfection conditions for maximal ADA2 knockdown in primary human monocytes have been determined. Additionally, day three untransfected monocytes were found to have acquired macrophage-like phenotypic markers. Understanding the role of ADA2 in monocyte cytokine secretion, gene expression profile, and macrophage and monocyte marker expression, in our system, will provide more insights into the role of ADA2 in DADA2 disease pathogenesis.