Centre for
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HLI RESEARCH WEEK

AUGUST 16-20, 2021

20 YEARS
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HLI Research Week 2021
August 16-20, 2021
Zoom Meeting ID: 651 5111 4464
Passcode: 195826

MONDAY, AUGUST 16	
10:30 – 11:00 am	Opening Remarks – Dr. Janice Leung
11:00 – 12:00 pm	**Bruce McManus Lecture** "Bioprinted Therapeutics: Next Generation Cell Therapies" Dr. Sam Wadsworth
12:00 – 12:30 pm	Overview of Gather Platform Rapid-Fire Poster Invitations

TUESDAY, AUGUST 17	
11:00 – 12:30 pm	Trainee Oral Presentations – First Dose
Jinelle Panton *Tebbutt lab*	Using Blood and Sputum Cell Count Parameters for Identification of Occupational Asthma Due to Western Red-Cedar (Thuja plicata)
Fione Yip *Yang lab*	Reduced Expression of IFNB1 and CXCL10 in NFAT5 Deficient Cardiomyocytes Weakens Host Immune Response to Coxsackievirus B3 Infection
Maria Caray *Thamboo lab*	Unified Airway Model: Comparison of the Microbiome of the Upper and Lower Airways in Chronic Rhinosinusitis and Asthma Patients
Ana Hernandez *Leung lab*	The Epigenetic Biomarker of Aging GrimAge is Associated with Lung Function in Patients Living with HIV
Haojun (Margaret) Huang *Brunham lab*	RARG Regulates Doxorubicin-Induced Stress Response in iPSC-Derived Cardiomyocytes
Judy Song *Quon lab*	The Impact Of Elexacaftor/Tezacaftor/Ivacaftor On Nebulized Maintenance Therapy Adherence In People With Cystic Fibrosis (CF)

WEDNESDAY, AUGUST 18	
11:00 – 12:30 pm	Trainee Oral Presentations – Second Dose
Katrina Besler *Francis lab*	Increasing Circulating Lysosomal Acid Lipase in a Mouse Model of Atherosclerosis Using mRNA-Containing Lipid Nanoparticles
Keith Wu *Hackett lab*	Pathobiology of Vascular Remodeling in Chronic Obstructive Pulmonary Disease and Idiopathic Pulmonary Fibrosis
Chris Yuen *Bernatchez lab*	Activation of endothelial function and NO release by losartan: in vitro correlation with custom, UBC-synthesized EXP3179 losartan metabolite activation of AKT
Madison Hung, Emily Gubskaya, William Gervasio *Tebbutt lab*	Optimization of Neonatal BCG and HBV Immunization Using Multi-Omics Data Integration
Genevieve Rocheleau *Russell lab*	Angiotensin Receptor Blockers and ACE Inhibitors Improve Clinical Outcomes in Males Hospitalized with COVID-19 in ARBs CORONA I
Meng Wang *DeMarco lab*	ImmunIQ: Quantitative Isotyping and Subtyping of Antibodies Generated Against SARSCoV-2 Infection and Vaccination

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THURSDAY, AUGUST 19

11:00 – 12:30 pm	Trainee Oral Presentations – Booster Shot
Lauren Forgrave *DeMarco lab*	Identifying TDP-43 pathology via co-aggregating proteins
Coco Ng, Felicia Liu-Fei *McManus lab*	*Digital Spatial Profiling of COVID-19-associated Cardiac Injury in an Autopsy Cohort: A Comparison to Cardiotropic Viruses & Delineating the Mechanisms of Cardiac Injury in COVID-19-Positive Autopsy Patients: A Comparison of Cardiotropic Viruses
Cara Kovacs *Sin lab*	The Effect of IgG Subclass Deficiencies on Mortality Risk in COPD
Carleena Ortega *Francis lab*	The Influence of Different Plasma Lipoprotein Fractions and Macrophages on Smooth Muscle Cell Foam Cell Formation
Daniel He *Tebbutt lab*	Are There Sex-Specific Gene Expression Patterns In Idiopathic Pulmonary Fibrosis Patients?
Aileen Hsieh *Hackett lab*	The role of the dynamic lung environment on fibroblast morphology and inflammation
1:00 – 3:00 pm	Poster Session on Gather Platform
1 Abhinav Kumar Checkervarty *Tebbutt lab*	Impact of Sickle-cell Disease Carrier Status on Immune Ontogeny of Neonates
2 Andy Wang *Daley lab*	The Canadian Asthma Primary Prevention Study: Background and 15 Year Follow-up
3 Ardin Sacayanan *Laksman lab*	Electro-mechanical Stimulation and Functional Characterization of Engineered Heart Tissue
4 Chun-man (Germain) Ho *Sin lab*	Eosinophils in Bronchoalveolar Lavage Predict COPD Exacerbation: Results from DISARM
5 David Yang *DeMarco lab*	Aptamer-based Enrichment of TDP-43 from Human Cells and Tissues with Quantification by HPLC-MS/MS
6 Denitsa Vasileva *Daley lab*	Epigenetic Age Prediction in Large-Scale Methylation Sequencing Project
7 Elodie Sauge *Bernatchez lab*	Protection Against Cardiopulmonary Disease with Losartan and Its Metabolites: Small Chemical Differences, Huge Biological Impacts
8 Firoozeh V.Gerayeli *Sin lab*	The Impact of Chronic Obstructive Pulmonary Disease on COVID-19 outcomes
9 Hacina Gill *Francis lab*	Smooth Muscle Cell Contribution to Atherosclerosis Susceptibility in Familial Hypercholesterolemia
10 Hasan Nathani *Tan lab*	Occupational Exposures and Respiratory Symptoms in the COLD Study

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11	Hattie Luo *Laksman lab*	Differentiation of Patient-Derived Induced Pluripotent Stem Cells into Cardiomyocytes and Expansion to Produce Engineered Heart Tissue for Disease Modeling
12	Irem Yucel *Daley lab*	Assessing The Variability Between Sequencing Centers In Targeted Bisulphite Sequencing
13	Jing Wen *Sin lab*	The Effect of Extracorporeal Application of Radiofrequency on Core Temperature of Emphysematous Lung Tissue in Rats
14	Naomi Potter *Quon lab*	C-reactive protein and Calprotectin to Diagnose Cystic Fibrosis Pulmonary Exacerbations in Equivocal Cases
15	Nicole Coxson *Hackett lab*	Improving and Expanding Digitized Clinical Data for a Lung Tissue Biobank Supporting Respiratory Research
16	Noah Katsuno *Tan lab*	Participant retention status in the CanCOLD initiative: A special emphasis on the CanCOLD Vancouver site.
17	Olivia Canavan *Bernatchez lab*	The Effects of Losartan and its Metabolites on Endothelial Function in an Atherosclerotic Mouse Model
18	Pinhao (Eric) Xiang *Francis lab*	Understanding cross-talk between macrophages and smooth muscle cells in foam cell development.
19	Raveen Badyal *Ryerson lab*	Regulation Of MicroRNA Expression In Scleroderma And Idiopathic Pulmonary Fibrosis
20	Ravneet Hansi *Leung lab*	Interactions between HIV and the Airway: HIV Receptor and Co-Receptor Expression in the Airway Epithelium
21	Reid Mitchell *Guenette lab*	Cardiopulmonary Exercise Testing in an Individual Four Years After an Extra-Pleural Pneumonectomy
22	Shannon Edie *Daley lab*	Investigating a Shared-Control Study Design for Methylation Profiling
23	Shayan Soleymani, William Betzner *Tebbutt lab*	A Meta Analysis of Potential Serum Biomarkers for Diagnosing Eosinophilic Esophagitis: The Quest for a Minimally Invasive Alternative
24	Tony Guo *Dorscheid lab*	Modulation of ACE2, TMPRSS2, and Furin by Dexamethasone in the Airway Epithelium: Implications for COVID-19
25	Yuki Tajima *Sin lab*	Development Of A Novel Porcine Model To Study Radiofrequency Treatment Of Pulmonary Emphysema
26	Yun Li *DeMarco lab*	TDP-43 Stability in Post-Mortem Brain Tissue
27	Zelin He *Daley lab*	A Review: What Does DNA Methylation in Cord Blood Tell Us?

28	Julia Zhang *Guenette lab*	Multidimensional Dyspnea Characterization of Patients in a Respirology Outpatient Clinic
29	Kate Huang *Laksman lab*	Investigating the Effects of Titin Truncating Variants on Sarcomere Integrity in Arrhythmia using Patient Induced Pluripotent Stem Cell-Derived Cardiomyocytes
30	Rylan McCallum *Brunham lab*	Investigating the Biological Underpinnings of HDL-Cholesterol and its Involvement with Sepsis and Patient Outcomes

FRIDAY, AUGUST 20	
10:30 – 10:45 am	Acknowledgements
10:45 – 11:45 am	**Peter Paré Lecture** TBD Dr. Mireille Ouimet
11:45 – 1:00 pm	Closing Remarks – Dr. Don Sin Awards Presentation
1:00 pm	Social on Gather Platform

Sponsors:

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SPEAKER BIOS & ABSTRACTS

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HLI Research Week 2021 Bruce McManus Lecture

Bioprinted Therapeutics: Next Generation Cell Therapies

Dr. Sam Wadsworth, Ph.D.
Chief Scientific Officer and Co-Founder of Aspect Biosystems

Monday, August 16th 11 – 12:00 p.m.
Meeting Link:
https://ubc.zoom.us/j/65151114464?pwd=azNIWFRpREVMUk9wTXNBOUxpUkM1UT09
Meeting ID: 651 5111 4464
Passcode: 195826

Biography

Sam has spent two decades driving developments in human tissue engineering. In 2013, Sam co-founded the UBC spin-out, Aspect Biosystems with Konrad Walus, Simon Beyer and Tamer Mohamed. Aspect Biosystems is a biotechnology company creating bioprinted therapeutics as medicines of the future. Aspect is applying its microfluidic 3D bioprinting technology internally to develop these advanced cell therapies and partnering with leading researchers and industry innovators worldwide to tackle the biggest challenges in regenerative medicine. Sam will be discussing Aspect's proprietary bioprinting technology and will describe how it has the potential to revolutionize the field of regenerative medicine by enabling a new generation of allogeneic cell therapies.

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HLI Research Week 2021

Closing Remarks

Don D. Sin, MD
Director and DeLazzari Chair,
Centre for Heart Lung Innovation
Department of Medicine
University of British Columbia

Friday, August 20th 11:45 AM – 1:00 PM
Meeting Link:
https://ubc.zoom.us/j/65151114464?pwd=azNIWFRpREVMUk9wTXNBOUxpUkM1UT09
Meeting ID: 651 5111 4464
Passcode: 195826

Biography

Don Sin is the Director of the Centre for Heart Lung Innovation (HLI), a Professor of Medicine at University of British Columbia (UBC) and respirologist at St. Paul's Hospital (SPH). He holds a Tier 1 Canada Research Chair in COPD and the De Lazzari Family Chair at HLI. He has published more than 550 peer-reviewed papers and has an H-index of 104. Expertscape.com ranks him as the top COPD expert in North America and 2nd in the world. He obtained his medical degree at University of Alberta in 1991, completed a residency in general medicine and a fellowship in respiratory medicine at University of Alberta. This was followed by a Master's of Public Health from Harvard University (1997) and a research fellowship at University of Toronto. He returned to University of Alberta as an Assistant Professor of Medicine in 1999 where he became a CIHR New Investigator (2001) and an Alberta Heritage Foundation Population Health Investigator (2002). He was recruited UBC/SPH in 2004 as a Tier 2 Canada Research Chair in COPD and the inaugural GlaxoSmithKline/St. Paul's Foundation Professor of COPD. He became a Professor of Medicine at UBC in 2009. During his career, he has received $30M in research funding as a Principal Investigator and $50M as a co-investigator, mostly from Tri-Council agencies, Genome Canada, Canada Foundation for Innovation (CFI), and the US National Institutes of Health. Over the past 10 years, he has directly supervised 13 master's and 3 doctoral students and 29 postdoctoral fellows (PDFs). He currently serves on the Global initiative for chronic Obstructive Lung Disease (GOLD) scientific committee and is the section editor for the European Respiratory Journal and an editorial board member of the American Journal of Respiratory and Critical Care Medicine. His research focus is using "omics" data to discover novel biomarkers of disease activity and new therapeutic targets to reduce hospitalization and mortality in patients with COPD.

Using Blood and Sputum Cell Count Parameters for Identification of Occupational Asthma Due to Western Red-Cedar (*Thuja plicata*)

J. Panton, C. Carlsten, S.J. Tebbutt

Background: Western red-cedar asthma (WRCA) is a common form of occupational asthma in British Columbia. It is due to sensitivity to plicatic acid (PA), a low weight molecule found in western red-cedar wood dust. It occurs in about 5-13% of exposed workers in the sawmill and carpentry industry. Diagnosis of WRCA is conducted through a two-day inhalational challenge test. On the first day, patients are challenged with methacholine to confirm asthma and PA on the second day to confirm WRCA. If patients have WRCA, they are called PA positive and if their tests are negative, they are called PA negative. To mitigate this invasive and time-consuming procedure, a 2017 study performed gene expression analyses on the blood of WRCA patients and identified a two gene biomarker panel associated with WRCA. To further understand the usefulness of blood parameters in diagnosing WRCA, we assessed the peripheral blood cell counts and cell type ratios that have been useful in other diseases. We also assessed the relationship between blood and sputum cell counts as a surrogate for airway phenotype.

Hypothesis: Peripheral blood and sputum white blood cells and their derived ratios can be used to differentiate between people who have asthma due to PA and those with asthma due to other causes.

Methods: 33 participants (23 with WRCA, 10 without WRCA) were recruited for the study. On day one, participants underwent the methacholine challenge while on day two, participants were challenged with PA. Peripheral blood was collected at 0hr (baseline) and 2 and 6 hours post challenges on both days. Induced sputum samples were collected 6 hours post challenge on both days. Quality slides for sputum cell count were obtained from only 13 patients (6 with WRCA, 7 without WRCA). Sputum was induced using aerosolized inhaled hypertonic saline. Differential sputum cell counts were counted by two observers.

Results: No significant differences were found in blood cell counts between PA positive and PA negative subjects on either day. Blood eosinophil count was significantly elevated in PA positive subjects over the 6-hour period on day 1 ($p= 0.02$). Blood lymphocyte count increased significantly ($p = 0.02$) during the 6-hour period on day 1 but did not differ between PA-positive and PA-negative subjects. On day 2, white blood cells ($p = 0.004$) and lymphocytes ($p= 0.002$) increased significantly in blood but did not differ between groups. The platelet/lymphocyte ratio in day 1 blood was lower in patients with WRCA but this was marginally significant ($p = 0.046$). No significant differences were identified when comparing sputum cell counts and cell type ratios in PA positive and PA negative subjects. Weak correlations were identified between sputum lymphocytes and eosinophils with blood neutrophils though statistical significance was not achieved.

Conclusion: Blood eosinophil count is higher in western red-cedar asthmatics than other asthmatics after the methacholine challenge. There is very little correlation between the sputum and blood cell counts in our sample. Complete blood count and sputum differential data have low diagnostic utility for WRCA. Other biomarkers may prove more useful in diagnosing WRCA.

This research was funded by the Mitacs Accelerate program.

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*Jinelle Panton is a second-year student in the Experimental Medicine PhD program. In her spare time, she enjoys writing poetry and learning foreign languages.

Reduced Expression of IFNB1 and CXCL10 in NFAT5 Deficient Cardiomyocytes Weakens Host Immune Response to Coxsackievirus B3 Infection

F. Yip*, G. Zhao, M. Zhang, D. Yang

Background: NFAT5 is a transcription factor belonging to the rel-family, which contains several factors that regulate inflammatory and immune response-related genes. While NFAT5 was first identified to regulate the expression of osmoprotective genes critical in the renal medulla during osmotic stress, its ubiquitous expression in the body suggests additional biological roles important for survival. NFAT5 has been demonstrated to regulate cardiac electrophysiology, and NFAT5 knockdown can result in non-beating ventricles during embryonic development. NFAT5 has also been reported to regulate the expression of toll-like receptor-induced genes in macrophages and inhibit coxsackievirus B3 (CVB3) replication, the predominant pathogen associated with myocarditis. As studies of NFAT5 are often segregated into studies of the heart, immune system, and CVB3, but rarely simultaneously, we aim to study how NFAT5 deficiency affects immune responses in the heart during CVB3 infection.

Hypothesis: NFAT5 upregulation induces the transcription of several immune response genes during viral infection. Inhibition of NFAT5 exacerbates CVB3-induced myocarditis.

Methods: HL-1 cardiomyocytes were maintained in Claycomb medium supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, and 1% norepinephrine. HL-1 cardiomyocytes were transfected with NFAT5 siRNA or overexpression plasmids for 48 hours, followed by transfection with poly(i:c) at the concentration of 1 μg/ml medium. Cells were collected at different time points post poly(i:c) transfection for RT-qPCR. To induce NFAT5 knockout in the heart, control and knockout mice were injected with 40 mg/kg body weight of tamoxifen three times over the span of 5 days. The mice were then infected with 10^4 pfu of CVB3 and the organs were collected 7 days post infection for IHC staining.

Results: Interferon beta 1 (IFNB1) and C-X-C motif chemokine ligand 10 (CXCL10) mRNA expression were significantly greater at 5 hours post transfection (hpt) compared to that at 3 hpt in both control and NFAT5 deficient HL-1 cardiomyocytes. While IFNB1 and CXCL10 mRNA expression were not significantly different between the two groups at 3 hpt, NFAT5 deficient cells showed significantly ($P \leq 0.01$) lower IFNB1 and CXCL10 mRNA expression, about a 1/3 reduction, compared to control cells at 5 hpt. Consistent with these results, NFAT5 overexpressing cells expressed double the amount of IFNB1 and CXCL10 mRNA at 4 hpt compared to control. Heart sections from control mice showed greater staining for IFNB1 compared to NFAT5 knockout mice.

Conclusion: Our results suggest that NFAT5 deficiency is associated with significantly lower expression of IFNB1 and CXCL10 during the early stages of simulated conditions of infection. These results have been observed for IFNB1 *in vivo* during CVB3 infection but work on CXCL10 is still in progress. Further experimentation is required to clarify the mechanism by which NFAT5 regulates the transcription of these immune-related genes and the effect on viral pathogenesis.

This research is funded by Canadian Institutes of Health Research.

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* Fione Yip is a third year UBC undergraduate student in the Microbiology & Immunology Co-op Program and is completing her first work placement at HLI in Dr. Decheng Yang's laboratory.

Unified Airway Model: Comparison of the Microbiome of the Upper and Lower Airways in Chronic Rhinosinusitis and Asthma Patients

M. Caray*, A. Thamboo, A. Javer, J. Leung, D. Sin

Background: The unified airway model posits that the upper and lower airways are intrinsically connected, functioning as one unit. This model has been supported in studies where a relationship between chronic rhinosinusitis (CRS) and asthma severity has been found. In recent years, there has been growing interest in understanding the pathophysiology of CRS inflammation pathways through microbiome interactions. Our goal is to examine the microbiome of the upper and lower airways of participants who have CRS with or without comorbid asthma.

Hypothesis: The sinus epithelium and lung epithelium derived from asthmatic or non-asthmatic participants with CRS will exhibit similar microbiomes.

Methods: Sinus epithelial cells (NEC), lung epithelial cells (LEC), nasal lavage and bronchoalveolar lavage were collected from 18 CRS participants (7 asthmatic, 9 non-asthmatic). Epithelial cells were retrieved with bronchial cytology brushes. From the epithelial cells, DNA was extracted and submitted for 16S rRNA sequencing for microbiome diversity analyses. Nasal lavage and bronchoalveolar lavage were collected through saline washes of the sinuses and lungs, respectively. From the lavages, cell pellets were isolated via centrifuge techniques, stained with Hematoxylin and Eosin and examined for inflammatory cell differentials.

Results: Alpha diversity metrics (Shannon diversity index and Faith's phylogenetic diversity index) were significantly lower in LEC compared to NEC, regardless of asthma status. Beta diversity metrics showed significant overlap in microbiome community compositions between LEC and NEC. Age, type of CRS (with or without polyps), and asthma status did not impact beta diversity distributions. Taxonomy plots of normalized relative abundances for NEC and LEC showed major overlap in microorganisms present. However, *Staphylococcus spp.* and *Corynebacterium spp*. were found to be more frequent in NEC, while *Sphingomonas spp.* was found to be more abundant in LEC.

Conclusion: The results suggest that the lung epithelium and sinus epithelium exhibit similar microbiome community compositions, despite the lung epithelium exhibiting lower microbiome diversity. Optimization of lavage collection and increased sample size are needed to further support the hypothesis.

This research is supported by the St. Paul's Sinus Centre and the Providence Health Care Research Centre Institute.

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* I am a fourth year UBC Science student and it is my first summer at the centre. I will be completing my B.Sc in Biology with a Minor in Psychology. During my spare time, I like to go on hikes and play ultimate frisbee. I plan to attend Medical School and specialize in Pediatrics or Psychiatry.

The Epigenetic Biomarker of Aging GrimAge is Associated with Lung Function in Patients Living with HIV

A. Hernandez, C. Yang, J. Yang, T. Shaipanich, J. MacIsaac, D. Li, M. Kobor, S. Guillemi, M. Harris, W. Lam, S. Lam, J. Montaner, S. Paul Man, L. McEwen, D. Lin, R. Novak, F. Hudson, H. Klinker, N. Dharan, K. Kunisaki, and D. Sin, J. Leung.

Background: Due to advances in antiretroviral therapy, people living with Human Immunodeficiency Virus (HIV, PLWH) live longer. Therefore, age-related comorbidities such as Chronic Obstructive Pulmonary Disease (COPD) are becoming common in this population.

Hypothesis: We hypothesized that epigenetic age in the airways and peripheral blood of PWLH reflects the lung health status of this population.

Methods: Bronchial epithelial brushings from 34 PLWH (COPD=18, non-COPD=16) were profiled for DNA methylation using the Illumina EPIC array. Similarly peripheral blood from PLWH (n=378) enrolled in The Strategic Timing of Antiretroviral Therapy (START) study cohort were profiled for DNA methylation. Within the START cohort 32 HIV patients had airflow obstruction based on the fixed criteria of FEV_1/FVC ratio<0.70. DNA methylation biomarker GrimAge (DNAmGrimAge) was calculated for all the patients in each cohort according to methods by Lu A et al.. We defined GrimAge acceleration as the resulting residuals from the regression of DNAmGrimAge on chronological age, adjusted for sex and body mass index. We then used linear models to test the association between GrimAge acceleration in the airway epithelial cells and COPD and FEV_1/FVC ratio. Likewise, we tested the association of GrimAge acceleration in blood with airflow obstruction as defined by an FEV_1/FVC ratio less than the lower limit of normal. Significant associations were defined at $P<0.05$.

Results: DNAmGrimAge in the airways of PLWH was strongly correlated with chronological age (R=0.88, $P=4.49x10^{-12}$). We found that the airway epithelium of PLWH with COPD was associated with greater GrimAge acceleration compared to PLWH without COPD ($P=8x10^{-03}$). FEV_1/FVC ratio was inversely correlated with GrimAge acceleration in the airways of PLWH ($P=0.03$). In blood, DNAmGrimAge was also highly correlated with chronological age (R=0.86, $P=1.21x10^{-110}$). Airflow obstruction was associated with greater GrimAge acceleration ($P=0.01$). In addition, similar to airway epithelial cells, FEV_1/FVC ratio was inversely correlated in blood with GrimAge acceleration ($P=1x10^{-04}$).

Conclusion: We found that the DNA methylation age biomarker GrimAge is associated with COPD and airflow limitation in PLWH in both airway epithelial cells as well as blood. As a biomarker that was derived from smoking and inflammatory proteins, GrimAge may reflect both lung and systemic epigenetic changes that occur with advanced airflow obstruction specifically in the HIV population.

This research is supported by the Canadian Institutes of Health Research and the British Columbia Lung Association, the National Heart, Lung, and Blood Institute, MITACS, Providence Airway Centre and Michael Smith for Health Research Foundation

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**I am entering my 3rd year as a postdoctoral fellow under the supervision of Drs Janice Leung and Don Sin. I am part of HLI's computational team, and my current research focus on investigating the epigenetic regulation of COPD.*

RARG Regulates Doxorubicin-Induced Stress Response in iPSC-Derived Cardiomyocytes

H. Huang*, E. Christidi, S. Shafaattalab, M. K. Davis, G. F. Tibbits, L. R. Brunham

Background: Doxorubicin is a commonly used chemotherapy drug that treats both adult and childhood cancers, but its clinical usefulness is limited by doxorubicin-induced cardiotoxicity (DIC). The incidence of DIC increases up to 65% at cumulative doses of 550 mg/m^2, which leads to irreversible heart failure and death. Since some patients suffer from DIC even at low doses, genetic differences may account for some of the inter-individual variability in risk for DIC and several associated genetic variants have been identified. Our preliminary data showed that a missense variant rs2229774 (p. S427L) in retinoic acid receptor gamma (RARG) increases doxorubicin-induced double-strand DNA beaks, reactive oxygen species production and cell death in patient-specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). These direct and causal evidence encourage further investigation into the regulatory roles of RARG and its variant in DIC.

Methods and results: Using molecular dynamics simulations, we determined that RARG p.S427L is predicted to alter the stability of the C-terminus, suggesting that the variant may compromise the ability of RARG to activate its target genes. To test this, we generated isogenic, genome-edited iPSC-CMs from patients with or without DIC that differ only at the RARG p.S427L site. RARE-luciferase reporter assay showed RAR regulation activity was activated under doxorubicin treatment and RARG-WT/WT could better respond than WT/S427L. Then we performed RNA-seq in our isogenic cell lines, followed by parallel analyses of a published DIC-related RNA-seq dataset and a RARG targeted gene list that collected from DNase-seq and ChIP-seq databases. Pathway enrichment analysis showed that S427L disrupted RARG targeted signalling pathways activation, leading to decreased DNA repair functions. Weakened DNA repair function in RARG-WT/S427L compared to WT/WT in DIC was further validated by single-cell electrophoresis.

Conclusion: Our findings reveal a novel role of RARG p.S427L in attenuating the ability of cardiomyocytes to mediate DNA repair after exposure to doxorubicin and provide insight into the molecular mechanism of DIC with implications for its prediction, prevention, and treatment.

This study was funded by the Canadian Institute of Health Research.

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* Haojun (Margaret) Huang is a PhD candidate in Experimental Medicine program of Department of Medicine, UBC.

The Impact Of Elexacaftor/Tezacaftor/Ivacaftor On Nebulized Maintenance Therapy Adherence In People With Cystic Fibrosis (CF)

J. Song*, R. Dagenais, S. Desai, A. Franciosi, B. Quon

Background: Over the past decade, treatment options for CF have expanded tremendously to include cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapies, which target and correct the defective CFTR protein, thus treating the disease at its root. The most recent CFTR modulator, Elexacaftor/Tezacaftor/Ivacaftor (ETI), demonstrated its efficacy in clinical trials by providing significant improvements in lung function and patient-reported quality of life. Prior to the advent of CFTR modulators, nebulized medications to augment mucus clearance and attenuate airway infection are often included as part of the standard CF treatment regime. However, nebulized medications are time-consuming (can take up to 2 hours per day to complete) and thus contribute to high treatment burden. Anecdotally, some patients followed at the St. Paul's Hospital Adult CF Clinic have stopped one or more of their nebulized maintenance therapies following the initiation of ETI due self-reported symptom improvement, despite encouragement from the CF care team to continue all therapies. However, the extent to which patients' adherence to nebulized medications has changed has not been measured. Therefore, the present study aims to evaluate the impacts of ETI on adult CF patients' use of nebulized maintenance therapies.

Hypothesis: There is a decrease in adherence to nebulized medications among people with CF post initiation of ETI.

Methods: A retrospective chart review of 23 patients followed at the St. Paul's Hospital Adult CF Clinic was performed to evaluate the impact of ETI on their use of the following nebulized maintenance therapies: inhaled antibiotics, dornase alfa and hypertonic saline (both mucus-thinning medications). Medication possession ratios (MPR; an estimate of medications filled vs. prescribed) in the year prior to ETI initiation for each of these medications were calculated and compared to the MPRs in the year post ETI initiation. To account for the impact of the COVID-19 pandemic during which patients may have felt discouraged from going to pharmacies to refill their medications, two oral maintenance medications were chosen to serve as positive controls. Their MPRs were also calculated and compared pre- and post-ETI initiation.

Results: The median composite MPR for nebulized medications in the three years prior to ETI initiation was 0.46 [IQR = 0.29]. In the year post ETI initiation, the median MPR was 0.24 [IQR = 0.38] (48% decrease). The decrease in MPR pre- vs. post-ETI for each nebulized medication was as follows: dornase alfa (0.45 [IQR = 0.35] to 0.21 [IQR = 0.33]), hypertonic saline (0.48 [IQR = 0.29] to 0.19 [IQR = 0.17]), and inhaled antibiotics (0.48 [IQR = 0.29] to 0.33 [IQR = 0.39]). For control medications, the median composite MPR in the three years prior to ETI was 0.46 [IQR = 0.40], and in the year post ETI, was also 0.46 [IQR = 0.53].

Conclusion: These results show a substantial decrease in medication adherence to nebulized therapies in the year post ETI initiation suggesting that ETI leads to reduced treatment burden.

This research is supported by the Centre for Heart Lung Innovation and the Faculty of Medicine Summer Student Research Program

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*Judy Song is entering her second year at UBC's undergraduate medical program. This is her second summer with Dr. Quon's lab, after a previous co-op term also working with cystic fibrosis patients.

Increasing Circulating Lysosomal Acid Lipase in a Mouse Model of Atherosclerosis Using mRNA-Containing Lipid Nanoparticles

K. Besler, H. Gill, T. Chan, T. Ponomarev, M-G. Alameh, D. Weissman, G. Francis

Background: We have recently discovered that smooth muscle cells (SMC) make up the majority of cholesterol-overloaded cells in atherosclerotic plaques. SMC contain low levels of lysosomal acid lipase (LAL), a key cholesteryl ester hydrolase, compared to the other major plaque cell type, macrophages. Supplementing SMC with recombinant LAL *in vitro* increases cholesterol removal from these cells, and intravenous administration of recombinant LAL in the LDLR-deficient mouse model of atherosclerosis reduces atherosclerotic plaque area. Delivery of mRNA to the liver using lipid nanoparticles (LNPs) is an emerging strategy for increasing circulating levels of a protein of interest. This approach allows for low to no toxicity and low to no immune activation, and is cost-effective compared to recombinant protein therapy. We aim to study the effect of increasing circulating LAL using LNPs on the ApoE-deficient mouse model of atherosclerosis.

Hypothesis: Intravenous administration of LNPs containing LIPA (LAL gene) mRNA will increase circulating levels of LAL in mouse serum, and cause regression of atherosclerosis in an ApoE-deficient mouse model of atherosclerosis.

Methods: ApoE-deficient mice were fed a high fat diet starting from 8 weeks of age, for 12 weeks, then some mice switched to a regular diet for 1-2 weeks. Wildtype C57/Bl6 mice were fed a regular diet. LNPs containing luciferase or LIPA mRNA were incubated at 4C with or without recombinant human ApoE for 2 hrs, 1 day, or 2 days. HepG2 cells were treated with *** LNP *in vitro* in 10% lipoprotein-deficient serum-containing DMEM for 24 hours. LNPs were administered intravenously via tail vein at 1 or 2 mg/kg mRNA per mouse mass, blood collected at 3 and 6 hours, and blood and tissues collected at 24 hours. Serum, livers, cell lysates, and cell culture media were analyzed for LAL activity via a kinetic fluorometric assay using 4-methylumbelliferyl oleate (4-MUO) as substrate. Livers were analyzed for LAL protein via Western blot, and for luciferase activity using a Promega Luciferase Assay System.

Results: 2 mg/kg, but not 1 mg/kg, of LIPA mRNA LNPs incubated overnight with ApoE is sufficient to cause a 5-10-fold increase in serum LAL activity in ApoE-deficient mice on regular diet 3 hours after injection, and a 2-3-fold increase in ApoE-deficient mice on a high fat diet. 1 mg/kg LIPA LNP gives an increase in liver LAL protein at 24 hours in wildtype mice, but not in ApoE-deficient mice, while 1 mg/kg luciferase LNP gives increased liver luciferase activity in both wildtype and ApoE-deficient mice.

Conclusion: Serum LAL activity is detectable by a fluorometric assay using 4-MUO, with or without high serum cholesterol content. LNPs containing LIPA mRNA are successfully delivered to the liver in ApoE-deficient mice by pre-incubation with ApoE. Kinetics of luciferase vs. LIPA mRNA expression differ in ApoE-deficient mice. LNPs with LIPA mRNA are an effective way to increase circulating LAL protein to investigate an effect on atherosclerosis regression.

This research is funded by Canadian Institutes of Health Research.

Katrina Besler is in her fourth year of the MD/PhD Program at UBC. When Katrina is not in the lab, she can be found taking care of her garden, trying new recipes, or walking and talking.

Pathobiology of Vascular Remodeling in Chronic Obstructive Pulmonary Disease and Idiopathic Pulmonary Fibrosis

K. Wu, S. Ledoux, A. Hsieh, J, Cooper, J.C. Hogg, D.M. Vasilescu, T-L. Hackett

Introduction: Pulmonary hypertension within the lung is often associated with chronic lung diseases. Recent studies have shown that up to 90% of patients with end-stage chronic obstructive pulmonary disease (COPD) and 60% of patients with end-stage idiopathic pulmonary fibrosis (IPF) have pulmonary hypertension. It is still unclear whether pulmonary hypertension is an independent disease or a consequence of parenchymal fibrosis in IPF or emphysematous tissue destruction in COPD. Recent, micro CT studies with a resolution of 7uM have shown that the loss of small airways, specifically the terminal bronchioles, is a common pathological feature in both COPD and IPF. The goal of this study was to investigate if loss of terminal bronchioles is associate with the loss or remodeling of the associated pulmonary arterial vasculature in end-stage COPD and IPF patients.

Hypothesis: The reduction in the number of small arterial vessels is associated with the loss of terminal bronchioles in end-stage COPD and IPF and contributes to the development of pulmonary hypertension .

Methods: Explanted lungs from donor control subjects (n=8), patients with end-stage COPD (n=8), and IPF (n=8) following transplant were air-inflated, and frozen. A total of 108 unbiased, systematic uniform random (SUR) samples (n=4/lung) were scanned with microCT and stereology was used to assess the total number, wall and lumen area, alveolar attachments, and the circularity of terminal bronchioles and associated arterial vasculature.

Results: Preliminary data from 8 subjects indicates that the total number of arterial vessels associated with terminal bronchioles was reduced in end-stage COPD and IPF lungs compared to control lungs. In COPD and IPF lungs, the arterial vasculature had an increase in the wall area and lumen area compared to controls, with the greatest change observed in IPF. The terminal bronchioles in IPF patients had a greater distortion compared to COPD patients and controls, and this feature was not seen in the associated arterial vessels.

Conclusion: This study demonstrates there are significant differences in the remodeling of the arterial vascular associated with loss and remodelling of terminal bronchioles in COPD and IPF patients, and indicates that different disease pathologies may be involved in the development of pulmonary hypertension.

Activation of endothelial function and NO release by losartan: in vitro correlation with custom, UBC-synthesized EXP3179 losartan metabolite activation of AKT

C. Yuen, D. Pechkovsky, P. Bernatchez

Background: Basal nitric oxide (NO) generated in the vascular endothelial by endothelial nitric oxide synthase (eNOS), plays critical roles in regulation of vascular tone. Previous studies from our lab have established that losartan, an angiotensin type 1 receptor (ATR1) blocker may display various other vascular protective effects in addition to the lowering of blood pressure, including increased NO release. How this occurs is currently unknown. Losartan is a pro-drug that has been shown to be able to metabolize into EXP3179 (which contains the ATR1 blocking activity) and EXP3179. It is believed that treatment with losartan metabolite EXP3179 could lead to activation of the AKT/eNOS pathway, which could rationalize our published observations on losartan on in vivo NO release.

Hypothesis: Administration of a custom, water soluble EXP3179 analogue, a losartan metabolite can lead to activation of AKT in vascular endothelial cells. If confirmed, this biological response could be used to investigate the off-target effects of losartan on endothelial function and NO release.

Methods: Cultured bovine aortic endothelial cells (BAECs) were used to study the effects of EXP3179 on the vascular endothelium. Stimulation for 0-60 minutes using EXP3179* at 15 uM were used to detect levels of phosphor-Akt (pAkt) in comparison to total AKT. Stimulation using vascular endothelial growth factor (VEGf) was used as a positive control. Western blots were performed to characterize pAkt and tAkt levels, alongside positive and negative controls. Phospho- and total AKT-specific antibodies were used to measure and quantify immunofluorescence.

Results: In vitro stimulation of BAECs with EXP3179 (15 uM) results in a 50% (n=5 experiments) increase of Akt activation in comparison to vehicle controls (water). Activation of pAkt occurs within 10 minutes of stimulation, with its peak activation reached between 30-45 minutes. pAkt effects shortly taper off at the 45-minute mark, with no effect on total AKT.

Conclusion: The results support the hypothesis that losartan metabolite, EXP3179, can activate Akt which may lead to NO release. Results suggest that in vitro, the effects of EXP3179 are not sustained for a long period of time, with peak activation at 30 minutes after stimulation. Future experiments will focus on further studying the mechanism to understand whether activation of Akt is ATR1 independent, with use of receptor tyrosine kinases such as Axitinib and Sunitinib while an ATR1 siRNA is introduced.

Christopher Yuen is entering his first year of his M.Sc. program at UBC, in the Department of Anesthesiology, Pharmacology and Therapeutics within the Faculty of Medicine. He recently completed his B.Sc. in Life Sciences with a double specialization in Cardiopulmonary Sciences and Drug Development and Human Toxicology at Queen’s University at Kingston.

Optimization of Neonatal BCG and HBV Immunization Using Multi-Omics Data Integration

M. Hung*, E. Gubskaya*, W.P. Gervasio*, A.K. Checkervarty, C.P. Shannon, V. Chen, S.J. Tebbutt and G. Cohen-Freue on behalf of the EPIC Consortium

Background: In 2019, more than 2 million neonatal mortalities were reported globally. Neonates, newborns in their first month, are among the most disease-susceptible yet least researched groups. Our goal is to extract predictive biomarkers of immunogenicity given unbiased molecular and cellular data from neonates undergoing immunization with hepatitis B vaccine (HBV), with or without Bacille Calmette-Guérin (BCG).

Hypothesis: We will perform integrated analysis of multi-omics data generated from blood samples of neonates vaccinated during the first week of life. Visualizations of the data will reveal blood-based biomarkers predictive of vaccine response mechanisms 30 days after birth.

Methods: Next generation sequencing, mass spectrometry, and other methods measured thousands of features - proteomics, transcriptomics, flow concentration, and cytometry - from 720 neonates on different vaccine schedules. Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO) built and optimized integrated multi-omics regression models on features from the data subsets. Various visualizations including loading plots, pairwise associative bionetworks, and REACTOME and KEGG pathway enrichment were conducted on the models and compared in an R-Shiny application to gain further mechanistic insights into immunogenicity.

Results: DIABLO generated 15 antibody titer regression models on a subset of 5353 features obtained from 22 neonates vaccinated on day 3 with the BCG vaccine. The model's predictive performance ranges from 56% to 88% in accuracy. Features selected by DIABLO suggest over 300 biomarkers of immunogenicity with varying importance to regression. Pathway enrichment via overrepresentation analysis of the selected biomarkers propose over 1000 significant pathways (FDR < 0.05). Bionetwork clustering of important biomarker panels reveals various mechanisms with strong pairwise associations ($|r| > 0.9$).

Conclusion: Results identified numerous biomarkers with known tuberculosis (TB) determinants such as IFNG and EGF as some of the most predictive features. Overrepresentation analyses of certain biomarkers are consistent with literature reviews on TB-related mechanisms of autoimmune disease and chemokine bindings. Bionetwork clusters such as IL3, IL17A, PpiA, and CD56+ CD16+ NK cells demonstrate known regulatory relationships with other features and antibody titers. As such, preliminary results suggest that multi-omics data integration reveals plausible immunogenicity biomarkers.

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*Madison Hung is in her fourth year at UBC studying computer science and immunology. In the future, she plans on finishing her multidisciplinary degree and pursuing work in the life sciences.
*Emily Gubskaya is in her second year of medical school at UBC and intends on utilizing her background in computer science and immunology to pursue health research.
*William Gervasio is in his third year at UBC Computer Science. He plans on pursuing machine learning and human computer interactions research to advance medical technologies.

Angiotensin Receptor Blockers and ACE Inhibitors Improve Clinical Outcomes in Males Hospitalized with COVID-19 in ARBs CORONA I

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Background:
SARS-CoV-2 spike protein binds to and down-regulates the angiotensin-converting enzyme 2 (ACE2) receptor to gain cell entry. ACE2 converts angiotensin II to angiotensin I-7, ACE2 down-regulation may increase angiotensin II (a vasoconstrictor with pro-inflammatory effects) and ACE2 is on the X chromosome suggesting sex-based differences in renin angiotensin (RAS) system dysregulation in COVID-19. Globally, males have higher hospitalisations (54%), ICU admissions (64%) and deaths (57%). We propose sex-differences in RAS system proteins contribute to sex disparity in clinical outcomes.

Hypothesis: Angiotensin II type 1 receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACEi) improve clinical outcomes more effectively in COVID-19 males than females.

Methods: ARBs CORONA I (ARBS I) is a multisite observational study of hospitalized COVID-19 patients. We recorded baseline characteristics, co-morbidities associated with ICU admission in COVID-19, pre-hospital treatment with ARBs or ACEi, use of ventilation, vasopressors and renal replacement therapy (RRT) and mortality and, to address mechanisms, we measured RAS peptides (duplicate ELISAs) and proteomics at baseline (hospital admission=day 0) and days 2, 4, 7 and 14 in an ARBs I subgroup (n=46) and compared males with females. Multivariable Cox regression was used to determine adjusted hazard ratio for time to outcomes. Multivariable logistic regression was used to determine adjusted odds ratio of outcomes according to sex and ARBs/ACEi treatment.

Results: We included PCR-confirmed COVID-19 patients who were admitted at 10 Canadian hospitals between 28/02/2020 and 14/04/2021 (n=1687). Males had significantly greater adjusted odds of ICU admission (aOR=1.42, p=0.008), ventilation (aOR=1.45, p=0.006), and need for vasopressors (aOR=1.46, p=0.005) compared to females. Males on ARBs had decreased need for ventilation (aOR=0.52, p=0.007), use of vasopressors (aOR=0.55, p=0.011) and shorter time to discharge (aHR=1.35, p=0.009) compared to males not on ARBs. No significant ARBs effects were observed in females. Among males who were on either ARBs or ACEi, there was also a significant decrease need for ventilation (aOR=0.62, p=0.012), vasopressors (aOR=0.67, p=0.032) and renal replacement therapy (aOR=0.52, p=0.041), compared to males not on ARBs or ACEi. Angiotensin II was significantly higher in males (median=109, IQR=76-168) compared to females (median=66, IQR=58-89) on day seven (p=0.048), as was baseline ACE (adjusted difference in median=78.1 ng/ml, p=0.042). After adjusting for multiple testing (>200 proteins) thrombospondin-1 (p=0.00025) and matrix metalloproteinase-9 (p=0.012) levels were significantly higher in females than in males.

Conclusion: Males hospitalized with COVID-19 in Canada experience worse clinical outcomes compared to females, however, males had significantly better responses to ARBs and ACEi. Sex-based dysregulation of RAS may contribute to sex-based differences in outcomes and responses to ARBs and ACE inhibitors.

This research is funded by Canadian Institutes of Health Research (CIHR #439993)
BSc in Biology, MSc in Experimental Medicine, and currently a Medical Student at University of Limerick. I enjoy ice hockey, beach volleyball, and (apparently) being a student forever.

ImmunIQ: Quantitative Isotyping and Subtyping of Antibodies Generated Against SARS-CoV-2 Infection and Vaccination

M. Wang *, T.D. Pobran, M.L. DeMarco

Background: Traditional serological immunoassays used in clinical care, and deployed for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, detect one antibody isotype at a time, most commonly IgG, or “total” immunoglobulins. Shortcomings of traditional serology including the inability to simultaneous detect, differentiate immunoglobulin isotypes and subclasses, and the semi-quantitative nature of the assays, where results are most commonly reported as simply “reactive” or “non-reactive”. Therefore, in order to expand the utility of serology, the analytic approach must first be modernized.

Hypothesis: Quantitative profiling of the immunoglobulin isotypes and subtypes in blood using mass spectrometry can monitor the immune response and predict the disease severity. It can thus be used as a diagnostic/prognostic tool in clinical care.

Methods: We developed a multiplex clinical mass spectrometry assay for absolute quantification of all 11 immunoglobulin isotypes and subclasses (IgG, IgA, IgM, IgD, IgE, IgG1-4, IgA1-2) against SARS-CoV-2, using isotopically-labeled peptide standards/calibrators.

Results: Using 4.2 µL of plasma, the assay enabled quantification of all anti-SARS-CoV-2 immunoglobulin isotypes and subtypes in a single analysis. The method was linear to at least 190 mg/L and had a total precision of 22% for IgG. In clinical samples, a robust IgM or IgA response in concert with a mild IgG response was found to confound a semi-quantitative total Ig serology assay. In patients hospitalized with COVID-19, IgG1 and IgA1 dominated the IgG and IgA repertoire.

Conclusion: In conclusion, this mass spectrometry assay enables the multiplexing analysis of immunoglobulin isotypes and subclasses, with a more accurate reporting of the immune response compared to traditional serological assays. Moreover, the design of this diagnostic tool is easily adaptable to accommodate serological analysis for other emerging pathogens.

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* Meng Wang is a postdoctoral fellow working at the DeMarco research lab. She uses mass spectrometry as a tool to develop protein biomarker assays.

Identifying TDP-43 pathology via co-aggregating proteins

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Background: Clinically, it can be difficult to distinguish frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) from other dementias; as such, biofluid diagnostics and imaging tracers for FTLD-TDP are highly desired. While TDP-43 is not an ideal biomarker for many reasons, including its extensive expression in and outside of the central nervous system, proteins that co-aggregate with TDP-43 may reveal additional potential biomarker targets. With this goal in mind, we performed quantitative proteomic analysis of frontal lobe brain tissue in cases of FTLD-TDP, three types of tauopathies, Alzheimer's disease without TDP-43 deposits and cases with no neuropathology.

Hypothesis: Proteins that co-locate within the TDP-43 insoluble fraction in brain tissue can differentiate FTLD-TDP from controls.

Methods: The proteomic composition of brain tissue from the frontal lobe of immunohistochemically confirmed FTLD-TDP (n=13), related dementias (n=10) and neuropathologically-unaffected controls (n=3) was obtained by high-resolution mass spectrometry. Label free quantification was used, whereby normalized peak area is used as a proxy for protein concentration to enable comparison between samples. In order to identify proteins other than TDP-43 that differentiated the cases studied, the quantitative data was analyzed via unsupervised (principal component analysis [PCA]) and supervised (partitioning analysis) clustering. These results were confirmed using immunoblotting.

Results: Unsupervised clustering revealed 77% of the FTLD-TDP cases could be differentiated from controls using the normalized peak area of a single protein. This protein was significantly increased in the FTLD-TDP group ($p= 0.003$). Via supervised analysis, three proteins were found to differentiate FTLD-TDP cases from controls with 100% specificity, two with 80-85% specificity, and one with 77% specificity. Immunoblot analysis supported the candidate protein enrichment or depletion in the FTLD-TDP cases relative to both sets of controls.

Conclusions: This biomarker discovery research identified seven proteins that differentiated FTLD-TDP cases from both related dementias and unaffected controls with high accuracy, and several with direct functional relevance to the pathological cascade in FTLD. Notably, the proteins that had the best discriminatory power from both types of clustering analysis, are proteins known to be involved in astrogliosis, a process stimulated by the destruction of neurons.

Speaker bio sketch: Lauren is a PhD Candidate in the Pathology and Laboratory Department at UBC. My research interests focus on biomarker discovery to improve clinical trial enrollment, drug development and clinical care.

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Digital Spatial Profiling of COVID-19-associated Cardiac Injury in an Autopsy Cohort: A Comparison to Cardiotropic Viruses

C. Ng*, P.J Hanson, F. Liu-Fei, B.M McManus

Background: As the global COVID-19 pandemic attributable to the novel coronavirus, SARS-CoV-2, has progressed, efforts to investigate and understand viral pathogenesis have intensified. Studies have shown that the heart is a central target for disease and that infection can lead to severe cardiac complications such as myocarditis, acute myocardial infarction, and thrombosis. Research has indicated that COVID-19-induced heart failure results from inflammation-mediated injury, but the underlying mechanisms have not been fully elucidated. Although dysregulated innate immunity has been observed in hospitalized patients, the genes driving this phenomenon have yet to be examined. Our goal is to delineate the mechanisms of COVID-19 myocardial injury by implementing novel digital spatial profiling technologies uniquely suited to analyze gene transcripts expressed in COVID-19 positive patient cardiac tissues. Our findings were compared to other forms of virus-related heart failure and healthy controls.

Hypothesis: Genes involved in innate immunity are significantly overexpressed in COVID-19 tissues relative to healthy individuals. Compared to other forms of virus-associated heart failure, we hypothesize that our analysis will reveal a unique transcriptomic signature for COVID-19.

Methods: Pathological regions of interest (ROI) from formalin-fixed paraffin-embedded cardiac tissues at the time of transplantation or autopsy from 21 consecutive COVID-19 positive decedents, 12 cardiotropic viral infection patients (Adeno-associated virus 2, Hepatitis C virus, Influenza B virus, and Coxsackievirus B3; 3 of each), and ten healthy controls were identified by an expert cardiac pathologist. Tissue cores from the ROI's were extracted and developed into a tissue microarray (TMA), which was then probed via *in situ* hybridization to detect virus-positive regions. Morphology markers, CD31, CD45, and troponin were used to spatially map the vasculature, leukocytes, and cardiomyocytes. Subsequent transcriptome analysis were performed using the Cancer Transcriptome Atlas, and COVID-19 spike-in Panel Plus using the NanoString GeoMX platform. Gene expression between COVID-19, viral myocarditis, and normal controls was compared. A P-value of <0.001 was considered statistically significant.

Results: Phospholipase A2 (PLA2G2A), a gene involved in regulating innate immunity/inflammation was enhanced in COVID-19 tissues relative to normal controls (1.11 log2fold change, $p<.0001$) and in comparison to the other cardiotropic viruses (1.54 log2fold change (mean), $p<0.001$). Desmoplakin (DSP), a cardiac structural protein, was depleted in COVID-19 tissues (-1.39 log2fold change, $p<.0001$). Additionally, S100A9, involved in cell differentiation (1.07 log2fold change (mean), $p<0.001$) was enhanced in COVID-19 tissues. The transcriptomic signatures of COVID-19 tissues were most similar to HCV gene expression.

Conclusion: A reduced expression of DSP in COVID-19 tissues were indicative of cardiac structural damage. Compared to other cardiotropic viruses, COVID-19 tissues displayed high expression of PLA2G2A, suggesting its' unique potential as a driver of disease. PLA2G2A may also have utility as a diagnostic and prognostic biomarker for prolonged, severe infection and as a therapeutic target. A larger patient cohort is required to validate the experimental findings.

This research is funded by the Providence Health Care Research Institute.

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*Coco Ng is a 4th year UBC Science student studying Nutrition. This is Coco's first summer at the HLI Research Labs. After graduation, she plans to pursue a career in public health.

Delineating the Mechanisms of Cardiac Injury in COVID-19-Positive Autopsy Patients: A Comparison of Cardiotropic Viruses

F. Liu-Fei*, P. Hanson, C. Ng, and B. McManus

Background: As the coronavirus disease 2019 (COVID-19) pandemic has progressed, increasing evidence implicates the heart as a critical target of injury in over 25% of patients. Further studies have demonstrated that almost 80% of recovered hospitalized COVID-19 patients have lasting cardiovascular effects. The mechanisms of cardiac injury have not been fully elucidated, although evidence of direct virus-mediated insult, thromboembolic complications, myocarditis, and cytokine storm have been reported. In this study, we examined cardiac from a cohort of twenty-one consecutive COVID-19 positive decedents from the University of Nebraska Medical Center and compared the pathological features and mechanisms of injury to age, sex, and BMI matched controls along with patients with other etiologies of virus-related heart failure from transplant and autopsy at St. Paul's Hospital.

Hypothesis: The crosstalk between direct viral injury, thrombosis, and immune mediated damage are unique to COVID-19 and are a critical component in the exacerbation of disease.

Methods: We developed a custom, single-slide tissue microarray from regions of pathological interest identified by expert cardiac pathologists. We subsequently probed the tissues via *in situ* hybridization to detect viral RNA, and conducted targeted immunohistochemistry to quantify and localize 16 cardiac injury and inflammatory protein markers.

Results: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), detected in 16 of 21 patients surveyed, was found in cardiomyocytes, lining the endothelium, interstitial spaces, and percolating adipocytes within the myocardium. Viral RNA presence corresponded with troponin depletion, indicating virus-induced cardiac structural damage. Indirect mechanisms of injury, notably thrombosis with associated vasculitis of mixed inflammatory infiltrate, were also detected. Von Willebrand factor (vWF) was highly expressed regardless of the degree of cardiac injury and we documented a positive relationship between neutrophil extracellular traps (NETs) and SARS-CoV-2 spike protein expression in COVID-19 patient tissue, suggesting that these could be potential markers of disease. Borderline myocarditis was noted in 19 of 21 patients and inflammation of percolating adipocytes, lymphocytic lesions, and large septal masses of inflammatory cells and platelets were additionally found to be hallmark features.

Conclusions: Collectively, we observed three interrelated mechanisms of COVID-19-associated cardiac damage in our patient cohort: direct viral injury, thrombosis, and immune-mediated damage. COVID-19 pathogenesis of the heart was multifactorial and multiphasic, with elevated levels of NETs and vWF observed as defining, unifying characteristics of direct and indirect viral injury when compared to other forms of virus-related heart failure. Overall, further elucidating the mechanisms of SARS-CoV-2 cardiac injury will significantly contribute to developing better diagnostics and treatments for COVID-19.

This work is supported by Providence Health Care Research Institute and the Michael Smith Foundation for Health Research.

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* Felicia Liu-Fei is entering her fourth year at UBC in the Bachelor of Microbiology and Immunology + Master of Management Program. She is currently on her first Co-op placement at the Centre for Heart Lung Innovation.

The Effect of IgG Subclass Deficiencies on Mortality Risk in COPD

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Background: Chronic obstructive pulmonary disease (COPD) is the world's third leading cause of death. COPD is a heterogenous disease; thus, efforts are directed at enabling precision medicine for the treatment of COPD. Optimizing treatment regimens for individual patients requires reliable, informative disease biomarkers. We have shown that approximately 1 in 4 patients with moderate to severe COPD have serum IgG levels below the normal range (<7.0 g/L), indicating hypogammaglobulinemia. We also showed that COPD patients with hypogammaglobulinemia are at increased risk for exacerbations, hospitalizations, and mortality. Deficiencies in certain IgG subclasses have been shown to increase risk of exacerbations (IgG1 and IgG2) and hospitalizations (IgG2), yet no studies have examined the relationship between IgG subclasses and COPD mortality. The goal of this study is to elucidate which of the IgG subclasses, if any, are associated with increased mortality in COPD.

Hypothesis: COPD patients with one or more IgG subclass deficiencies experience an increased risk of mortality.

Methods: Data were obtained from the Rapid Transition Program (UBC/Providence Health Care Research Ethics Board #H11-00786/ H13-00790; ClinicalTrials.gov Identifier NCT02050022). Blood was collected from 489 patients hospitalized with acute exacerbation of COPD (AECOPD) and 132 clinically stable patients. Samples were processed with standardized protocols and stored in a -80ºC freezer. Serum IgG subclass levels were measured by liquid chromatography-tandem mass spectrometry. Deficiencies were defined when serum IgG subclass levels were below the lower limit of normal for our laboratory: IgG1 < 2.80 g/L, IgG2 < 1.15 g/L, IgG3 <0.24 g/L, and IgG4 <0.052 g/L. Patient's vital status was tracked for one year following enrollment and validated with death certificates. Multivariate Cox regression modelling was performed to obtain hazard ratios that were adjusted for the covariates of age, gender, group (AECOPD or clinically stable at enrollment), asthma status, and cardiac comorbidity status. Data analysis was carried out via R software version 4.1.0 (The R Foundation for Statistical Computing Platform, Vienna, Austria), and RStudio software version 1.4.1717 (RStudio, Boston, MA).

Results: The mean (±SD) age of 621 patients was 66.9 ±11.6 years, 65.7% were males, 83.4% were Caucasians, and 55.7% were current smokers at the time of enrollment. The total 1-year mortality rate was 17.7% (110/621). Deficiencies in IgG1, IgG2, IgG3, and IgG4 were present in frequencies of 1.6%, 11.3%, 5.0%, and 11.3% respectively. One year after enrollment, mortality rates were 50% (5/10) for IgG1 deficiency, 25.7% (18/70) for IgG2, 19.4% (6/31) for IgG3, and 27.1% (19/70) for IgG4. Adjusted Cox regression analysis revealed significant relationships of IgG1 and IgG4 deficiencies with 1-year mortality in COPD patients. Adjusted hazard ratios of 3.51 (p=0.007) and 1.81 (p=0.02) were obtained for IgG1 and IgG4 deficient patients respectively. The other subclasses were not associated with COPD mortality.

Conclusion: IgG1 deficiency, though rare, significantly increases 1-year mortality risk in COPD by approximately 3.5 fold and IgG4 deficiency, while more common, increases the risk by 81%.

This research is supported by Providence Health Care and St. Paul's Foundation.

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* Cara Kovacs is a 4th year UBC student, studying Human Genetics and Disease Susceptibility. This is her first summer at UBC HLI, working as a summer student in the Sin laboratory.

The Influence of Different Plasma Lipoprotein Fractions and Macrophages on Smooth Muscle Cell Foam Cell Formation

C. Ortega, E. Xiang, T. Chan, J. Dubland, S. Allahverdian, G. Francis

Background: Macrophage and smooth muscle cell (SMC) foam cells in atherosclerotic plaque are formed following unregulated uptake of modified (***e.g.***, aggregated) low density lipoprotein (LDL) and other apolipoprotein B-containing lipoproteins retained in the artery wall. An individual's plasma LDL-cholesterol level obtained following fasting was the previous standard indicator of risk for atherosclerosis. Recent clinical studies indicate that the total non-high-density lipoprotein (non-HDL) cholesterol and the nonfasting plasma are stronger predictors of cardiovascular risk than LDL-C and fasting samples. Most *in vitro* studies of cellular uptake and metabolism of cholesterol have similarly used LDL isolated from fasting plasma as the cholesterol loading vehicle.

Hypothesis: *In vitro l*oading of human aortic SMCs and human macrophages with nonfasting and non-HDL fractions will lead to higher intracellular cholesterol accumulation than incubation with fasting and LDL.

Methodology: SMCs and macrophages were grown to desired confluence, switched to 10% lipoprotein-deficient serum for 24 hours to upregulate LDL receptors, and then loaded with 95 µg/mL cholesterol from either aggregated non-HDL or LDL from fasting or nonfasting plasma for 24 hours. Cells were then analyzed for cholesterol content via mass spectrometry or stained with BODIPY, a neutral lipid dye, for foam cell count in flow cytometry.

Results: Preliminary data suggests that monocultured SMCs accumulate the most cholesterol in the presence of fasting aggregated non-HDL but form the most BODIPY-high cells with the nonfasting aggregated LDL fraction. Monocultured macrophages formed the most BODIPY-high cells with the nonfasting aggregated non-HDL loading. Direct contact between SMCs and macrophages promoted the most BODIPY-high SMCs with nonfasting aggregated non-HDL incubation.

Conclusion: Various lipoprotein loading fractions have different influences on SMCs and macrophage cholesterol accumulation and foam cell formation *in vitro.* Direct coculture of SMCs and macrophages *in vitro* may more accurately model the effects of the nonfasting and the non-HDL lipoproteins on foam cell formation *in vivo*.

Are There Sex-Specific Gene Expression Patterns In Idiopathic Pulmonary Fibrosis Patients?

D He, SA Guler, CP Shannon, CJ Ryerson, SJ Tebbutt

Background: Idiopathic pulmonary fibrosis (IPF) is a debilitating disease that causes patients to slowly lose lung function as a result of excess build-up of fibrosis within the lung. The incidence of IPF is higher in males, and previous research suggests that females have better preserved lung function at presentation, exhibit less severe disease progression, and may have a better overall survival compared to males. Molecular studies into this phenomenon have not yet been performed.

Hypothesis: We hypothesized that female and male IPF patients exhibit sex-specific gene expression patterns.

Methods: We screened 5337 publications and identified 22 studies for inclusion into our meta-analysis. Of these studies, 13 had published metadata regarding the biological sex of the IPF. We used the gene expression data from these studies to develop a classifier of biological sex that was then applied to the data of the remaining studies with missing information regarding biological sex. Data from each included study was integrated using the multivariate integrative (MINT) method, then analyzed with partial least squares discriminant analysis (PLSDA) to identify gene expression signatures within the specified classes.

Results: After removing X- and Y-linked genes from our expression data, we identified 2 genes (*CCN5* and *SPESP1*) important in distinguishing between female and male IPF lung samples. This was in contrast to the 45 genes separating female and male healthy control lung samples, of which only *SPESP1* was included. Examination of female-specific (IPF vs control, 8 genes) and male-specific (IPF vs control, 14 genes) gene expression signatures yielded similar results, with both sexes showing similar expression patterns for 5 genes (*COMP*, *IL13RA2*, *COL17A1*, *MYRF*, *HPCAL1*).

Conclusion: Although there is shared expression of many fibrotic genes between female and male IPF lung samples, there is evidence for sex-specific expression of certain fibrotic mediators such as *CCN5*. Further investigation into these sex-specific genes is warranted to determine their role in IPF clinical management and outcomes.

This research is funded by CIHR and the BC Lung Association.

I am a 4th year PhD candidate in the Department of Experimental Medicine. I am not exactly sure what else to include here, as “I plan to” and “During my spare time” imply different tones for the purpose of this biosketch.

The role of the dynamic lung environment on fibroblast morphology and inflammation

*A. Hsieh, NT.R. Vriesde, M.AL- Fouadi, L. Monstaco-Guidolin, D. Maftoun, N. Coxson, K. Usman, S. Booth, E.T. Osei, T-L Hackett

Background: Fibroblasts are the major cell type involved in the remodelling and repair of the extracellular matrix (ECM) scaffold within the lung. Current 2-dimensional (2D) monolayer cultures fail to mimic the stiffness, 3D environment, and mechanics of the breathing lung. In many chronic diseases, the alveolar ECM environment has been shown to be remodeled and have a significant effect on fibroblast morphology. The goal of this study was to develop a 3D model of the alveolar ECM environment that can be used to assess the response of parenchymal fibroblasts to changes in ECM stiffness and mechanical strain.

Hypothesis: 3D collagen models of the alveolar environment will enable more accurate modeling of ECM repair and fibroblast function within the lung.

Methods: Human fetal lung fibroblasts (HFL1) were cultured in 10% fetal calf serum in Dulbecco's Modified Eagle Medium with added antibiotics/antimycotics. For 2D cell culture experiments, HFL1s were seeded on collagen-1 coated Bioflex plates. 3D alveolar models were established using the Flexcell 5000 Tissue Train System and underwent a regime with and without 48 hours of equibiaxial cyclic strain at 1% amplitude at 0.2 Hz frequency to mimic normal breathing mechanics and compared to unstrained gels. To assess alterations in the HFL cytoskeleton, collagen constructs were stained with a cytoskeletal non-muscle myosin IIB(NMMIIBB) antibody and phalloidin for F-actin, imaged using a Zeiss super-resolution confocal microscope. ImageProPlus was used for staining intensity analysis and CellProfiler software was used to assess alterations in cell morphology over time with strain. The release of inflammatory mediators was measured using ELISA and cell death via LDH assays.

Results: In 2D cultures, fibroblasts had 43% spindle-shape morphology and 57% myofibroblasts, whereas in the 3D collagen gel models 90% had spindle shape morphology, no myofibroblasts were present, and 10% had rounded morphology. The staining analysis revealed 2D cultures had a significantly increased amount of F-actin. Compared to 3D cultures, fibroblasts in 2D culture released higher concentrations of inflammatory IL-6 and IL-8 cytokines ($P<0.05$). HFLs cultured on 2D monolayers also had a higher percentage of cell death compared to HFLs in the 3D alveolar collagen model. In response to mechanical strain, fibroblasts in 3D models had a sequential increase in rounded cells from 30 min until the 48th hour of strain, corresponding to decreased F-actin staining and increased NMMIIB positive cells.

Conclusion: The *in vitro* 3D alveolar model mimics the normal *in vivo* fibroblast morphology and inflammatory mediator release compared to 2D monolayer cultures. Extended periods of mechanical strain caused a loss of dendritic extensions and changes to lung fibroblast morphology and cytoskeletal proteins.

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*Aileen recently received her BSc in Biology from UBC. She will be starting her MSc in the fall.

Impact of Sickle-cell Disease Carrier Status on Immune Ontogeny of Neonates.

A.K. Checkervarty*, C.P. Shannon, V. Chen, S.J. Tebbutt, and Hancock laboratory on behalf of the EPIC consortium

Background: Sickle-cell disease (SCD) is a major genetic haemoglobin disorder with an early-life mortality of 50-90%. Each year, an estimated 300,000 neonates are born with severe haematological diseases, including more than 200,000 cases of SCD in Africa. Furthermore, in some regions of Africa, approximately 30% of the population are reported as having sickle-cell trait (heterozygote carrier status). Our study is investigating blood samples from 720 neonates in The Gambia (west Africa) for both homozygous and heterozygous carrier status for SCD. These samples have been collected during the first week of neonatal life (day of birth and either day 1 or day 3 or day 7) as part of a vaccine immunogenicity study of hepatitis B vaccine (HBV) administered with or without the Bacille Calmette-Guérin (BCG) vaccine.

Hypothesis: 1) Carrier status of sickle-cell disease can be determined by genotyping neonates from their whole blood RNA-sequences; 2) Genetic carriers of sickle-cell disease exhibit differences in immune ontogeny and gene expression profiles.

Methods: High-throughput RNA sequencing with reads mapped to globin transcripts were used for genetic variant discovery. For the genotyping, the RNA sequences were compared to the reference human genome version GRCh38 (Genome Reference Consortium). The sickle-cell carrier status was determined by the presence of a single nucleotide variation (SNV): T >A at locus 5227002 (chromosome 11). For variant calling, we used the Broad Institute's Genome Analysis Toolkit (GATK) and confirmed the results with the Sequence Alignment/Map tools (SAMtools) pipeline. To investigate the gene expression differences between the sickle-cell carriers and non-carriers, we performed unpaired and paired differential gene expression (DGE) analyses using Bioconductor's limma package in R studio.

Results: The genotyping analysis revealed that 122 out of 720 (17%) neonates were carriers of the SCD mutation (57 females and 65 males). The variant calling results indicated that 4 neonates (out of 122) had SCD or homozygous carrier status, while 118 neonates were heterozygous carriers for SCD. These results are consistent with genetic epidemiological assumptions for prevalence of SCD trait in Africa. DGE analysis between neonates with SCD carrier status and non-carriers, using the day of birth samples, revealed 97 statistically significant genes (B.H. FDR < 0.3). This includes downregulated genes such as PPP3R1, MAPK1, NRAS related to programmed cell death and MAPK activation. Paired DGE analysis of day 7 samples with the SCD carrier status revealed differentially expressed genes (B.H. FDR < 0.05) such as RHD and RHCE (blood group synthesis), HMBS and UROD (Heme biosynthesis), downregulated with log fold change of -1.00 (approximately).

Conclusion: The results support our first hypothesis of determining the SCD carrier status of neonates using whole blood RNA-seq data. DGE analysis showed differentially expressed genes that take part in blood group synthesis and formation of heme molecule, responsible for catabolism of haemoglobin. Thus, partially supporting our second hypothesis with differences in gene expression profiles of neonates having SCD carrier status when compared to non-carriers. However, more exhaustive study of pathways and networks is required to better understand the differences in neonatal immune ontogeny.

This research is funded by a Mitacs Accelerate Fellowship, EPIC HIPC Grant: U19-AI118608 (NIH-NIAID) and Precision Vaccines Program (PVP).

* Abhinav Checkervarty is a 2nd year PhD student at the Tebbutt laboratory. He is also working as a trainee at the Prevention of Organ Failure (PROOF) Centre of Excellence.

The Canadian Asthma Primary Prevention Study: Background and 15 Year Follow-up

Z. Wang*, D. Vasileva, A. Becker, A. Sandford, E. Chan, D. Daley

Background: Asthma is a chronic inflammatory disease of the airways. It is the most common chronic condition among children worldwide, affecting 13% of Canadian children (Statistics Canada in 2000/2001). In 1995, the Canadian Asthma Primary Prevention Study (CAPPS) was initiated, aiming to assess the effectiveness of a multifaceted program for the primary prevention of asthma. CAPPS is a prospective, longitudinal, randomized controlled cohort study. A total of 545 high-risk families were identified before birth and randomized to the intervention group (n=279) or the control group (n=266). Intervention measures included encouragement of breastfeeding with delayed introduction of solid food as well as avoidance of house dust mite, pets, and environmental tobacco smoke. Follow-up assessments were conducted at one, two, seven and 15-years of age.

Objectives: Evaluate the effectiveness of a multifaceted intervention program in decreasing the prevalence of asthma and other atopic disorders at 15 years of age.

Methods: Outcomes at the 15-year follow-up were assessed using questionnaires, pediatric allergists diagnoses, methacholine and skin prick allergy tests. Distributions of demographic variables and comparisons between intervention and control groups were assessed using chi-square tests. Logistic regression was used to calculate relative risks (RR) and 95% confidence intervals (CIs) between intervention and control groups for phenotypes of interest. Potential confounding variables in the regression model included sex, first-born status, ethnicity, maternal education, and family history of asthma.

Results: At 15 years, 387 of the 545 (71.0%) families filled out questionnaires. Of these, 335 returned for further assessment by physicians and 327 consented to allergy skin testing. After adjusting for sex and differences in family history of asthma, 26 of the 182 subjects in the intervention group and 27 of the 153 subjects in the control group had physician-diagnosed asthma (14.3% vs. 17.7%; adjusted RR, 1.01; 95% CI, 0.43-2.11). The prevalence of atopy (defined as positive skin test reactions to any common allergen) was 110 of 176 in the intervention group and 93 of 151 in the control group (62.5% vs. 62.8%; adjusted RR, 1.00; 95% CI, 0.71-1.21). Neither asthma nor atopy showed significant differences between the two groups.

Conclusion: The multifaceted intervention program did not show a significant impact on reducing the prevalence of asthma or atopy in high-risk children at 15 years of age. More analyses on potential reasons and other outcomes are needed.

This research is funded by the Canadian Institutes of Health Research (CIHR).

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*Zihan (Andy) Wang is a 4th year Data Science student at SFU and is currently working part-time in Dr. Daley's lab. This is his second time joining HLI's Summer Student Research Event.

Electro-mechanical Stimulation and Functional Characterization of Engineered Heart Tissue

A. Sacayanan*, L. Rohani, M. Ashraf, J. Churko, M. Rodriguez, K. Huang, J. Parker, Z. Laksman.

Background: Several studies have demonstrated the ability to incorporate human induced pluripotent stem cell derived cardiomyocytes into engineered heart tissue (EHT). This model system has the capacity to reproduce patient specific phenotypes and has the potential to be incorporated into higher throughput drug screens. A critical determinant of maturation, and an important element of experimentation is the ability to stimulate these EHTs electrically. We have therefore set out to integrate and test electrical stimulation into our optical mapping rig which is capable of recording voltage changes in the EHT using a high speed, high resolution camera and potentiometric dyes.

Hypothesis: Engineered heart tissues derived from human induced pluripotent stem cells and subjected to an in-house electrical stimulator can efficiently alter the intrinsic force of contraction and beat rate properties.

Methods: Electrical stimulation is developed using an Arduino as a processor in conjunction with regulators and semiconductors to form the user-computer interface, variable power supply and biphasic H-bridge which produces a periodic pulse signal. The EHT samples will then be subjected to varying pulse signals with different voltages and frequencies to analyze the changes in force of contraction and beat rates. Afterwards, the tissues will be stimulated in varying lengths of time to observe the long-term effects of electrical stimulation.

Results: Initial studies show that activated device can alter the beat rate and force of contraction of EHTs. When stimulated, the BPM of each EHT is the inverse of the input frequency. Meanwhile the force of contraction when stimulate is 0.07mN, which is smaller than the intrinsic force of contraction at 0.1mN. Additional observation includes stimulating at higher voltage and frequency for a short period of time can resuscitate non-beating samples.

Conclusion: Preliminary results shows that properties of EHTs can be modified by introducing external energies. Further analysis on varying voltages, frequency and time is required to fully capture the efficiency of the in-house devices on engineered heart tissues.

This research is supported by the Stem Cell Network of Canada and Centre for Blood Research.

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*Ardin Sacayanan is a biomedical engineering student entering his last year at UBC. This is Ardin's first summer at the Laksman Research Laboratories, following his work at Hydrogen in Motion Inc. and several school design teams such as UBC BEST – MINT & M2M.

Eosinophils in Bronchoalveolar Lavage Predict COPD Exacerbation: Results from DISARM

C.G. Ho*, X. Li, C.X. Yang, S. Milne, S.F. van Eeden, T. Shaipanich, J.L. Leung, D.D Sin

Background: Chronic obstructive pulmonary disease (COPD) is the number ONE cause of urgent hospitalizations in Canada. Although COPD is a heterogeneous disorder, it is characterized by airway inflammation. However, to date, there are no biomarkers of inflammation that can reliably predict risk of exacerbation in COPD patients. Notably, most studies have focused on blood biomarkers rather than biomarkers from small airways, which are the primary site of disease of COPD. Furthermore, many previous biomarker studies were confounded by use of inhaled and systemic corticosteroids.

Hypothesis: Immune cell counts in BAL fluid can predict disease burden and risk of exacerbations in stable COPD patients.

Methods: We used data from bronchoalveolar lavage (BAL) fluid collected during baseline (first) bronchoscopies in participants of DISARM (A Study to Investigate the Differential Effects of Inhaled Symbicort and AdvaiR on Lung Microbiota). At the time of bronchoscopy, none of the participants were using inhaled corticosteroids. The final sample size was 58 patients with COPD defined by either FEV1/FVC ratio below 70% or CT-evidence of emphysema in the presence of >10 pack-year history of smoking. Clinical information including the St. George's Respiratory Questionnaire (SGRQ) scores, spirometry, and bloodwork were obtained approximately 4 weeks prior to bronchoscopy. Study participants were followed over time and had their COPD exacerbations documented over 1 year. Exacerbations were defined as increase in symptoms leading to the use of antibiotics and/or systemic corticosteroids. Multiple linear regression was used to adjust for the covariates: age, sex, BMI, and smoking status. BAL eosinophilia was defined using the threshold >1%.

Results: There was no significant relationship between blood neutrophil or eosinophil counts and their respective counterparts in the BAL fluid (R^2=0.015, p=0.39 and R^2=0.037, p=0.17 respectively). BAL eosinophilia was associated with decreased FEV_1 (48.03% vs 58.68% of predicted, p=0.028) and FEV_1/FVC ratio (45.80% vs 53.45%, p=0.029). BAL eosinophilia was associated with increased number of exacerbations over 1 year (mean of 0.806 vs 1.727, p=0.017; RR=1.44 for experiencing an exacerbation within 1 year). BAL eosinophil count was significantly related to the time to first exacerbation (p=0.011). BAL eosinophilia was not related to baseline SGRQ scores (which reflect health status of participants). BAL neutrophilia (defined using a threshold cutoff of >3%) or lymphocytosis (defined using a threshold cutoff of >2.125% for this study) was not significantly related to risk of exacerbation.

Conclusion: BAL eosinophilia is associated with reduced lung function and increases the risk of exacerbation, making it a promising biomarker in COPD. These data also raise the possibility of identifying potential subgroup of patients who may benefit from anti-IL5 therapies, which inhibit the effects of eosinophils in patients with COPD.

The DISARM study was supported by funding from AstraZeneca®, Canadian Institutes for Health Research (CIHR), and the British Columbia Lung Association.

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*I will be going into my 2nd year of medicine at UBC and have been with the Sin Lab since the beginning of the summer. I completed my undergrad at UBC in 2016 and worked for several years in academic research labs prior to medicine.

Aptamer-based Enrichment of TDP-43 from Human Cells and Tissues with Quantification by HPLC-MS/MS

D. Yang*, T.D. Pobran, I.R.A. Mackenzie, M.L. DeMarco

Background: There is great interest in detecting, characterizing and quantifying transactive response DNA binding protein 43 kDa (TDP-43), and its post-translational modifications, due to its association with frontotemporal degeneration (FTD) and amyotrophic lateral sclerosis. However, detailed analysis of TDP-43 in human biological matrices by immunometric methods has been hindered by the relatively low abundance of TDP-43 and poor antibody reagent specificity. With the goal of developing a selective and multiplex method for characterizing TDP-43, we previously developed a high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) assay for relative quantification of TDP-43 in human brain tissue and cells. To improve analytical sensitivity and to perform absolute quantification, we coupled a novel RNA-based aptamer enrichment workflow (and inclusion of a stable isotope-labeled standard) to HPLC-MS/MS.

Hypothesis: The application of aptamer-based enrichment of TDP-43 coupled to HPLC-MS/MS analysis would improve analytical sensitivity and sequence resolution for quantification and characterization of TDP-43 in human cell lines and brain tissue.

Methods: By exploiting the physiological function of TDP-43 to bind oligonucleotides, TDP-43 was enriched from human cells and brain tissue using a TDP-43-binding RNA aptamer and quantified by HPLC-MS/MS. Comparisons were made between aptamer enrichment and immunoenrichment, and TDP-43 concentration and peptide profiles were assessed from cases of FTD with TDP-43 pathology and cases with no neurodegenerative pathology (controls). To demonstrate the application in detecting TDP-43 fragments, *in vitro* cleavage of endogenous TDP-43 in human cells was performed using caspase-3.

Results: The TDP-43 aptamer-enrichment—HPLC-MS/MS assay was linear from 0.37 to 2.55 nmol/L, a range suitable for analysis of both human cells and brain tissue homogenates, and had a total CV of 14.8%. Compared to immunoenrichment, aptamer-enrichment yielded cleaner recoveries of TDP-43. The aptamer-enrichment—HPLC-MS/MS method, compared to our previous method without enrichment, increased analytical sensitivity by 8.7-fold and 11.8-fold for endogenous TDP-43 in human cells and brain tissue, respectively. Quantitative TDP-43 peptide profiles were developed for cases of FTD with TDP-43 pathology and controls. Critically, inclusion of the aptamer enrichment step improved sequence resolution and enabled identification of TDP-43 truncations.

Conclusion: The aptamer-enrichment—HPLC-MS/MS method enabled highly selective quantification, enhanced sequence coverage and structural characterization of endogenous TDP-43.

D.Y. and T.D.P. have nothing to disclose. M.L.D. reports a Scholar Award from the Michael Smith Foundation for Health Research. I.R.A.M. reports personal fees from Prevail Therapeutics, outside of the submitted work.

Speaker Biosketch: David Yang graduated from UBC with a Bachelor of Medical Laboratory Sciences (BMLSc), and is working as a Research Assistant in the DeMarco Lab.

Epigenetic Age Prediction in Large-Scale Methylation Sequencing Project

D. Vasileva*[1], M, Wan[1], A. B. Becker[2], E. S. Chan[3], C. Laprise[5], A. J. Sandford [1], C. M. T. Greenwood[4], D. Daley[1]

[1]Center for Heart Lung Innovation, Faculty of Medicine, University of British Columbia, Vancouver, Canada; [2]Department of Pediatrics and Child Health, University of Manitoba, Manitoba, Canada; [3]BC Children's Hospital Research Institute, Faculty of Medicine, Vancouver, Canada; [4]Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Canada; [5]Centre intersectoriel en santé durable (CISD) de l'Université du Québec á Chicoutimi, Saguenay, Canada, Centre intégré universitaire de santé et de services sociaux (CIUSSS) du Saguenay–Lac-Saint-Jean, Saguenay, Canada

Background: The Horvath epigenetic clock was developed using Illumina array data (27K and 450K) from predominantly adult samples (chronological age > 18) with the purpose of predicting epigenetic age. However, its accuracy in children as well as in methylation sequencing data has not been established.

Purpose: The purpose of this study was to 1) assess the accuracy of the Horvath epigenetic clock in targeted methylation sequencing samples from three Canadian studies and, if necessary, develop a novel algorithm and 2) evaluate the utility of the Horvath age prediction as a quality control (QC) metric in a large-scale targeted methylation sequencing study.

Methods: Targeted bisulfite methylation sequencing using Illumina's MethylCapture sequencing library was completed on 932 samples from three Canadian studies- The Canadian Asthma Primary Prevention Study (CAPPS, n=632 samples); the Saguenay- Lac- Saint- Jean study (SLSJ, n=180 samples) and the Canadian Peanut Allergy Registry (CanPAR, n=120 samples). CAPPS is a longitudinal cohort that follows children at high-risk for developing asthma from birth to year 15, with targeted methylation sequencing done on at least one of three occasions- birth, and/or years seven and 15. Maternal samples were also included in the CAPPS study. SLSJ consists of three-generational triads from families of French-Canadian descent. CanPAR is a registry of children and their families deemed at high risk of developing peanut allergy.
The Horvath algorithm was used to predict epigenetic age. Accuracy was assessed using the metrics of mean absolute error (MAE, MAE=abs(predicted age-reported age)) and relative difference (RD, RD = abs (predicted age-reported age)/reported age)). Samples with RD > mean + 2 SD. for their reported groups were deemed outliers. Additional QCs included principal component analyses to assess age, ethnicity and cell counts deconvolution as well as genotype concordance checks using overlap single nucleotide polymorphisms (SNPs) from GWAS and sequencing studies. Linear and mixed effects regressions were performed on the CAPPS and SLSJ data to identify additional age informative CpG sites.

Results: Following QC of the CAPPS and SLSJ datasets, 915 of the 932 remained in the analysis. Overall, the Horvath epigenetic algorithm had a MAE±SD:7.00 years ($\pm$7.03) in these samples. There was a negative correlation between the RD and the chronological age of individuals: 1.82 ±1.73 (gestational age for cord blood), 0.86 ±0.97 (age seven), 0.44 ±0.36 (age 15), 0.25 ± 0.20 (age >18), 0.33 ±0.39 (SLSJ) and 0.59± 0.70 (CanPAR). The Horvath prediction aligned with the results of other QC metrics when identifying swapped samples. Regression models identified new CpG sites significantly associated with age.

Conclusions: We demonstrated the applicability of the Horvath age algorithm to targeted methylation sequencing studies and its utility as a QC metric. Results of the regression analysis point to an opportunity for the development of a novel, more accurate epigenetic clock.

Funding for this study was provided by CIHR through the CEEHRC consortium.

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*Denitsa is a graduate student in the UBC bioinformatics program.

Protection Against Cardiopulmonary Disease with Losartan and Its Metabolites: Small Chemical Differences, Huge Biological Impacts

E. Sauge, D. Pechkovsky, P. Atmuri, M. Ciufolini, P. Bernatchez

Background: The well-established anti-hypertensive angiotensin II receptor type 1 (ATR1) blocker losartan has been tested in many cardiovascular, pulmonary and neurological disease settings not linked to high blood pressure (BP). We have recently shown that losartan can unexpectedly activate endothelial function in vivo via the endothelial release of vasodilatory nitric oxide (NO), a biological response typically associated with aerobic exercise. Losartan is a prodrug and we suspect its metabolites to be behind some of its unexpected properties like NO release. First-passage metabolism converts losartan to EXP3179, assumed to be devoid of ATR1 antagonistic properties followed by further metabolism into EXP3174, the main ATR1 antagonist. Hence, we tested the contribution of ATR1 blockade to losartan's effect on endothelial NO release by comparing the effects of losartan VS EXP3179 (ATR1-independent) VS EXP3174 (ATR1-dependent) on angiotensin II signaling, BP and endothelial NO release.

Hypothesis: We hypothesized that the non-ATR1-blocking EXP3179 may be responsible for losartan's endothelial NO-releasing properties whereas ATR1-blocking EXP3174 may mediate its BP lowering properties.

Methods: The ATR1 blocking capabilities of losartan and EXP metabolites were tested by Western blotting using aortic smooth muscle cells (ASMC) treated with ANGII. BP lowering effects of losartan, EXP3174 and EXP3179 were studied in vivo using mice treated with one of the 3 drugs (10 mg/kg) for 3 hours. Finally, the endothelial NO release properties of losartan, EXP3174 and EXP3179 were evaluated via vasorelaxation of mouse aorta rings of WT mice using dual-wire myograph systems.

Results: We unexpectedly found that EXP3174, EXP3179 and to a lesser extent losartan inhibit ANGII signaling in ASMC suggesting that each of them have ATR1 blocking activities. In vivo, losartan and both metabolites decreased BP. Ex-vivo experiments revealed a 73.9% increase in NO-dependent vasodilation in mouse aortic rings treated with EXP3179 (15uM) but not with losartan or EXP3174. When mice were treated for 2 weeks with EXP3179, aortic ring contraction was reduced by 28.8%. Finally, we found that the effects of EXP3179 on mouse aortic rings were absent following endothelium denudation.

Conclusion: Our data unexpectedly show that both EXP3179 and EXP3174 can block angiotensin II signaling. Moreover, EXP3179 but not EXP3174 or losartan can directly activate endothelial NO release in blood vessels despite their structural similarities. Therefore, these data suggest that losartan first passage metabolism is necessary to enhance NO release.

This research is funded by MITACS. We also would like to thank the Providence Airway Center.

I have just started my third year at HLI as a PhD Student. My area of research is mainly cardiovascular pharmacology, and this is the second time I participate in the HLI Research Day.

The Impact of Chronic Obstructive Pulmonary Disease on COVID-19 outcomes
F. V.Gerayeli, S.Milne, C.Cheung, X.Li, C.W.T.Yang, A.Tam, L.H.Choi, A.Bae, D.D.Sin

Background: Coronavirus Disease-19 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel virus that is responsible for the current global pandemic. Risk factors for poor outcomes among COVID-19 patients include older age, male sex, obesity and comorbidities. Although chronic obstructive pulmonary disease (COPD) is not a significant risk factor for risk of COVID-19, its impact on severe COVID-19 including hospitalization and mortality remains controversial. To address this issue, here, we conducted a systematic review and a meta-analysis of existing literature.

Hypothesis: COPD is a significant risk factor for poor outcomes in COVID-19.

Methods: This systematic review was performed according to the recommendations of the Preferred Reporting in the Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We adopted a broad search strategy using the keyword, "Chronic obstructive pulmonary disease or COPD and COVID-19 outcomes". The data were collected from studies that were published through digital search of Google Scholar, Pubmed, Ovid Medline and MedRxiv between November 1st 2019 and January 15th 2021. Subsequently, pre-print studies were removed to ensure the integrity of data used for the meta-analysis. Inclusion and exclusion criteria were defined *a priori* to ensure relevant studies were selected for this systematic review without significant bias. Risk of bias for included studies was assessed using the Clinical Advances through Research and Information Translation (CLARITY) group tool. Severe COVID-19 was defined as the endpoint of hospitalization, ICU admission and mortality. Data analysis was conducted using the metaphor (2.1-0) package in R (3.6.0) program and was used to calculate the risk of the aforementioned outcomes. For each meta-analysis we fitted a random effects model using a Restricted Maximum Likelihood (REML) estimator to calculate the odds ratio (OR). Funnel plots were used for assessment of heterogeneity across the included studies using a Cochran's Q test and I2 value.

Results: Out of 1,296 papers that were initially identified, 295 were fully assessed for eligibility. Of these, 59 studies were used for qualitative synthesis and 39 were selected for meta-analysis. In aggregate, COPD increased the risk of hospitalization by 4.23-fold (95% CI 3.65–4.90, $p < 0.0001$), the risk of ICU admission by 1.35-fold (95% CI, 1.02–1.78; $p = 0.03$) and mortality by 2.47-fold (95% CI, 2.18–2.79; $p < 0.0001$).

Limitation: COPD was diagnosed based on physician-report or chart review and not based on physiology or imaging.

Conclusion: COPD is a significant risk factor of severe COVID-19. The mechanisms underlying this relationship are unknown. One possibility is up-regulation of ACE-2 (the putative receptor for SARS-CoV-2) in airway epithelia of COPD patients, which may lead to rapid propagation of the virus in lower respiratory tract. Our current work focuses on molecular changes in airway epithelia of COPD patients to address this hypothesis.

This research is supported by the MITACS Accelerate program. Firoozeh V.Gerayeli is a second year graduate student in the Sin laboratory at the Centre for Heart Lung Innovation.

Smooth Muscle Cell Contribution to Atherosclerosis Susceptibility in Familial Hypercholesterolemia

H. Gill, K. Besler, S. Allahverdian, T. Chan, L. Brunham, G. Francis

Background Familial hypercholesterolemia (FH) is a genetic disorder involving defects in the clearance of low density lipoprotein cholesterol (LDL-C) from the blood. Patients with FH have elevated blood cholesterol, putting them at very high risk of coronary heart disease, and if left untreated, most patients with FH will develop premature atherosclerosis. Remarkably, some FH patients demonstrate zero evidence of atherosclerosis on coronary artery imaging despite never receiving treatment for their elevated cholesterol. The Francis lab has discovered that smooth muscle cells (SMCs) form at least half and likely the majority of lipid overloaded cells or “foam cells” in both human and mouse atheromas, which are critical to the commencement and progression of atherosclerosis. We believe that the key driver of foam cell formation and atherosclerotic lesion progression in humans is retention of atherogenic lipoproteins in the artery wall, induced by SMC-secreted proteoglycans. Therefore, it is plausible that differences in SMC phenotype, such as secretion of proteoglycans (biglycan and decorin are the predominant species inducing lipoprotein retention), could be a major predictor of whether or not a hypercholesterolemic individual develops atherosclerosis.

Hypothesis SMCs derived from induced pluripotent stem cells (iPSCs) of FH patients with atherosclerosis will demonstrate differing gene expression and lipid-loading ability than SMCs derived from FH patients without atherosclerosis.

Methods We will isolate peripheral blood mononuclear cells from the blood of FH patients who either exhibit or show no evidence of atherosclerosis. We will then convert these cells to iPSCs followed by differentiation to arterial SMCs using an established protocol. These iPSC-derived SMCs are expected to retain the full complement and expression pattern of native arterial SMC genes present in the donor patients. We will perform an unbiased microarray analysis of these derived-SMCs, focusing on genes affecting proteoglycan synthesis, lipoprotein uptake, and cholesterol metabolism to identify differences in FH patients who are either protected from or exhibit atherosclerosis. The derived SMCs will then be tested in culture in gene knockdown or overexpression studies to test the effect of manipulating key predictors of atherosclerosis risk, as identified in these gene arrays, on cell functions including lipoprotein binding, lipoprotein uptake, and cholesterol efflux to HDL.

Results We are currently optimizing the differentiation of SMC from iPSCs.

Conclusion Given the emerging role of SMCs in predicting risk for atherosclerosis and the striking observation that some typically high risk patients do not develop atherosclerosis, we expect these studies will identify key targets for preventing atherosclerosis and inducing its regression in FH patients and in the population generally.

We would like to thank the Heart and Stroke Foundation of Canada.

I completed my B.Sc. with a major in biochemistry in 2021 at the University of the Fraser Valley in Abbotsford, BC. This is my first summer at HLI and I will be shadowing other Francis lab members and beginning work on my PhD thesis. In my spare time, I enjoy reading, doing yoga, and spending time with my dog.

Occupational Exposures and Respiratory Symptoms in the COLD Study

H.Nathani*, W. Tan

Background: It has been estimated that 15% of the population burden of chronic obstructive pulmonary disease (COPD) population is attributable to occupational factors. Our aim is to examine the relationship between occupational exposures and respiratory symptoms across different regions in Canada participating in the population-based, cross-sectional COLD study.

Hypothesis: Occupational exposures have a significant impact on the burden of respiratory symptoms in the population.

Methods: We analyzed data from 5176 adults aged ≥40 years who completed respiratory and occupational questionnaires and had acceptable and repeatable post-bronchodilator spirometry measurements. Occupational exposures comprised three categories (organic dusts; inorganic dusts; fumes) and 11 high-risk occupations (farming; flour, feed or grain milling; cotton or jute processing; hard-rock mining; coal mining; sandblasting; working with asbestos; chemical or plastics manufacturing; foundry or steel milling; welding; and firefighting). The associations of respiratory symptoms with occupational exposures were estimated using logistic regression models adjusted for potential confounders for each COLD site and then pooled using meta-analysis.

Results: We found that people working in inorganic dusts and fumes were more likely to report respiratory symptoms than those who do not work in any of those occupations. The most common occupation in inorganic dusts exposure was working with asbestos. Compared to people not working in any high-risk occupation, people working with asbestos for ≥7 years were more likely to have chronic cough (OR=1.66, 95%CI 1.30-2.13), chronic phlegm (OR=1.73, 95%CI 1.42-2.10), and wheeze (OR=1.59, 95%CI 1.30-1.94), but not dyspneoa (OR=1.36, 95%CI 0.99-1.87). The most common occupation in fumes exposure was working with chemical or plastic manufacturing. Compared to people not working in any high-risk occupation, people working with chemical or plastic manufacturing for ≥9 years were more likely to have chronic cough (OR=1.89, 95%CI 1.42-2.50), chronic phlegm (OR=1.39, 95%CI 1.04-1.87), and dyspneoa (OR=1.69, 95%CI 1.32-2.16), but not wheeze (OR=1.28, 95%CI 0.98-1.67). None of the occupations in organic dusts exposure were associated with respiratory symptoms.

Conclusion: In a cross-sectional study, we found respiratory symptoms to be associated with many high-risk occupations. Our findings suggest that working in inorganic dusts and fumes may lead to increased burden of respiratory symptoms. Increased awareness of this should alert enhanced preventive measures and public health surveillance among exposed workers.

We would like to acknowledge the investigators, staff, and subjects that are part of the CanCOLD Study as well as our sponsors: Canadian Institute of Health Research (CIHR/Rx&D Collaborative Research Program Operating Grant – 93326); Astra Zeneca Canada Ltd; Boehringer Ingelheim Canada Ltd; GSK Canada Ltd; Merk; Novartis; Nycomed; Pfizer Canada Ltd; Theratechnologies; the Respiratory Health Network of the FRSQ; and the CRRN.

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*I am a first-year statistics master's student at SFU. This is my first work term at HLI where I am working as the CanCOLD Data Management and Analyses co-op student.

Differentiation of Patient-Derived Induced Pluripotent Stem Cells into Cardiomyocytes and Expansion to Produce Engineered Heart Tissue for Disease Modeling

H. Luo*, L. Rohani, K. Huang, S. Lee, S. Wu, and Z. Laksman

Background: Engineered heart tissues (EHT) have been useful in cardiac disease modeling and regenerative medicine applications. In order to produce functional EHTs, the patient-derived induced pluripotent stem cells (iPSC) need to be differentiated into cardiomyocytes (CM) with high efficiency and in a scalable manner. Reduction of the batch-to-batch effect is also critical in the production of EHTs. Therefore, we have incorporated an expansion protocol, which utilizes Wnt activation and reduced cell-cell contact to induce CM proliferation, into our CM culture procedure. The aim is to obtain a sufficient number of CMs to generate EHTs required for disease modeling.

Hypothesis: Optimization of seeding density and CHIR concentration can increase the differentiation efficiency of disease iPSCs into CMs, while the application of a CM expansion protocol helps achieve a sufficient number of CMs for EHT casting.

Methods: Two disease iPSC lines LN1 and LN2 are cultured in mTeSR1 for four days before starting differentiation with the addition of 10 μM of CHIR in RPMI B27- medium on day 0. On day 1, 7.5 μM of IWP2 is added to inhibit the Wnt pathway. Fresh RPMI B27- medium is changed every two days until day 7. Then RPMI B27+ medium is used to culture the cells. On day 10, the metabolic selection medium is applied to the cells to select the CMs and induce apoptosis of other types of cells. Pure CMs are harvested and seeded for expansion using 2 μM CHIR treatment in RMPI B27+ medium and are passaged when confluent. These CMs are matured before harvested for EHT production.

Results: LN1 and LN2 iPS cells were differentiated into CMs with low efficiency. Therefore, we applied the CM expansion protocol. A 1.25 to 2 fold expansion of CMs was obtained for LN1 using the expansion protocol. The purity of the CMs increased from 60% to 98% during expansion. We will employ qPCR to assess the maturity and cell cycle stage of the CMs generated.

Conclusion: Preliminary data indicates that the CM expansion protocol is capable of inducing the proliferation and increasing the purity of LN1 and LN2 CMs. It is useful for obtaining sufficient CMs when differentiation alone does not generate enough cells for EHT manufacture. However, optimization of seeding density and CHIR concentration is required to improve differentiation for further efficiency and improved fold expansion.

"This research is supported by the Stem Cell Network of Canada."

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*Hattie Luo is a fourth year molecular biology and biochemistry student at SFU. This is Hattie's first summer at the Laksman Research Laboratories, following her work at STEMCELL Technologies and Dr. Edgar C. Young's lab at SFU.

Assessing The Variability Between Sequencing Centers In Targeted Bisulphite Sequencing

I.Yucel, M.Wan, G. Ellis, A. Sandford, C. Laprise, D.Daley

Background: DNA Methylation is an epigenetic mechanism that can influence the expression of a gene. To date methylation arrays have been the primary tool used to assess the methylation profiles, as they are cost-effective and a small amount of DNA is required, however they suffer from low genome coverage and high error rate due to probe cross-hybridization. While arrays continue to be used, a powerful alternative approach is targeted methylation sequencing with the Illumina TruSeq Methyl Capture EPIC Library. Illumina performs the library pull-down first then bisulfate converts the DNA, which results in more efficient and reproducible results as high as 99%. This study aims to compare the reproducibility between two sequencing centres using biological replicates from two Canadian asthma studies.

Hypothesis: Targeted bisulphite sequencing has the same mapping efficiency for two different sequencing centres across technical replicates.

Methods: 12 samples from the Canadian Asthma Primary Prevention Study (CAPPS) and 12 samples from The Saguenay-Lac-Saint-Jean and Quebec City Familial Asthma Collection (SLSJ) were used in this study. These 24 biological replicates were bisulphite treated and sequenced using Illumina TruSeq Methyl Capture EPIC Library at both Genome Science Centre (GSC) and Centre d'expertise et de services Génome Québec (GQ). 16 additional samples were sequenced at GQ, a total number of 40 samples. The samples were then aligned to human genome assembly GRCh38 using NovoAlign’s Bisulphite alignment mode.

Results: A total of 64 alignments were performed, 24 biological replicates sequenced at GSC and 40 sequenced at GQ. The resulting alignment statistics of the samples included the percentage of reads with unique alignment, multi alignment and no alignment. Samples that were sequenced at GQ had a percentage of reads with higher no map (GSC: 1.38% for CAPPS and 1.32% for SLSJ. GQ: 1.64%) and lower unique map (GSC: 90.41% for CAPPS and 90.54% for SLSJ; GQ: 90.13%), than samples that were sequenced at GSC.

Conclusion: The technical replicates were to estimate the variability between the sequencing centers and measure the reproducibility. On average, the alignment statistics for the samples are similar but not the same for both sequencing centers. These results indicate that there is a possible systematic error with GQ samples, which can be further addressed with additional methylation level summary statistics.

Irem is a student at the UBC MSc Bioinformatics program.

The Effect of Extracorporeal Application of Radiofrequency on Core Temperature of Emphysematous Lung Tissue in Rats

J.Wen, M.Tsutsui, C.Y. Cheung, Y. Tajima, D.D. Sin

Background:
Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes of mortality worldwide, and emphysema is a common phenotype of COPD characterized by the destruction of alveoli, enlargement of airspace and loss of lung elasticity. Our previous studies have shown that extracorporeal application of radiofrequency (RF) in rodents with emphysema can improve lung function. However, the exact mechanism by this happens is unknown. One possibility is that RF therapy may selectively increase the core temperature of lung tissue with emphysema owing to reduced blood flow to this area relative to normal parts of the lung. Here, we sought to determine temperature differentials between emphysematous and non-emphysematous lung during RF therapy in rats.

Hypothesis:
Extracorporeal application of radiofrequency (RF) will lead to higher core temperature of emphysematous lung relative to normal lung.

Methods:
Thirty-six male rats (7 to 8 weeks of age) were used and subjected to two different conditions: porcine pancreatic elastase (PPE) (n=12) and PPE+RF (n=24). A unilateral emphysema model was created by intratracheally instilling PPE selectively into the left lung (85U/100g body weight). RF treatment (70 W) was then applied to animals assigned to the PPE+RF group 2 weeks following the initial PPE instillation. Temperature measurement was performed on a subset of animals (n=8) in the PPE+RF group by surgically attaching two thermometer probes onto each lung through a thoracotomy. Temperature changes were monitored over the course of RF treatment. These animals were sacrificed immediately following the temperature measurement; the remaining animals were sacrificed 5 weeks following the initial PPE instillation. Lungs from all the groups were harvested for histological assessment to determine mean linear Intercept (Lm) and the extent of fibrosis based on an Ashcroft scoring scale.

Results:
The temperature increase during RF was greater in the emphysematous lung compared to the normal lung (mean 3.64 °C vs 2.80 °C). Emphysematous changes were observed histologically in the lower left lung, and this was accompanied by an increase in Lm value compared to the lower right lung, but the difference did not reach statistical significance (median 50.38 vs 46.49, $P = 0.0547$). The PPE + RF group demonstrated a higher prevalence of lung fibrosis (≧ Grade 3) in the left lung compared to that in the PPE group (9.10% vs 6.74%, $p < 0.0001$).

Conclusion:
Extracorporeal application of RF therapy induced a slightly higher rise in core temperature in the emphysematous lung compared with normal lung. However, it is uncertain whether the small absolute difference in temperature is responsible for the RF-related improvements in lung mechanics and exercise capacity of rodents with emphysema.

This research is supported by Mitacs and Ikomed.

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* Jing Wen is a fourth year UBC student, major in Microbiology and Immunology. I joined the center in January 2021 as a Co-op student. I enjoy reading and hiking in my spare time.

C-reactive protein and Calprotectin to Diagnose Cystic Fibrosis Pulmonary Exacerbations in Equivocal Cases

N. Potter*, J. Jang, K. Dong, B. Quon

Background: Cystic fibrosis (CF) patients frequently experience pulmonary exacerbations (PEx) characterized by uncontrolled airway infection and inflammation. Without a consensus definition of PEx, these events can be overlooked by clinicians which can impact long-term lung function, quality of life and life expectancy. Innovative approaches to PEx identification are needed to aid current diagnosis which relies heavily on signs of increased respiratory symptoms (e.g., cough, mucus, breathlessness) and decreased lung function, particularly in equivocal cases where the clinical course of action is unclear. Our objective is to evaluate the use of C-reactive protein (CRP) and calprotectin as promising diagnostic biomarkers, to objectively characterize the inflammatory status and aid in the diagnosis of PEx in cases where lung function and respiratory symptoms are discordant.

We **hypothesize** that CRP and calprotectin will effectively differentiate patients with discordant changes in symptoms and lung function who do poorly without antibiotic treatment

Methods: We identified patients who present to the CF clinic with discordant changes in respiratory symptoms and lung function and were not prescribed antibiotics as they were deemed clinically stable. We classified these visits into the phenotypes “silent decline in lung function” or “symptomatic with stable lung function.” Biobanked blood samples collected as part of the St. Paul’s Hospital CF Biomarker (CFB) study will be used to measure serum CRP and calprotectin levels with electrochemiluminescence immunoassays. We will categorize the visits as with versus without systemic inflammation using a stepwise algorithm previously developed by our research group. Using clinical data, we will compare the respiratory outcomes of patients with versus without systemic inflammation and evaluate whether systemic inflammation is associated with worse respiratory outcomes in clinical follow up (higher risk of PEx, lower forced expiratory volume in 1 second (FEV1)).

Results: 53 discordant CF clinic visits where patients were not prescribed antibiotics were identified from 784 CF clinic visits with biobanked blood samples and clinical data available. 12 of these visits are classified as “silent decline in lung function” and 41 of these visits are classified as “symptomatic with stable lung function.” Notably, the silent decline in lung function group is primarily male and in the normal lung function range (precent predicted FEV1 ≥ 90%).

Conclusion: We have identified 53 discordant visits which we will categorize as with versus without systemic inflammation using CRP and calprotectin measurements and compare respiratory outcomes to test our hypothesis. If CRP and calprotectin are found to be discriminative of PEx, use in an objective add-on test could help improve PEx diagnosis and facilitate timely treatment. Further validation of CRP and calprotectin cut-offs will be required in a larger validation cohort, and a randomized control trial is necessary before clinical implementation, even if this study finds CRP and calprotectin to be discriminative of PEx in CF.

This research is supported by the U.S. Cystic Fibrosis Foundation and Canadian Institutes of Health Research CGS-M Award

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* Naomi is a first year MSc student in Experimental Medicine at UBC. This is her first year at HLI following her BSc in honors physiology at the University of Alberta.

Improving and Expanding Digitized Clinical Data for a Lung Tissue Biobank Supporting Respiratory Research

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Background: The James Hogg Lung Registry (JHLR) is an established biobank of human lung tissue that has provided samples for respiratory research since its creation 43 years ago. Each collected sample is assigned a unique registry number and has associated patient demographic, lung function, computed tomography (CT), and blood data. From the inception of the JHLR, these files have been kept as paper records. The goal of this project was to continue the development of a custom, encrypted database by digitizing and cataloguing clinical paper records.

Methods: For all cases in the JHLR, lung tissues were collected for research purposes with informed consent from patients undergoing surgery as standard of care. Sample data from patients includes demographic and clinical data (occupational questionnaire, lung function, CT imaging, pathology, radiology, and blood reports). In this project, the clinical data was verified by two observers using hardcopies of patient records and entered into a custom Oracle® database that can be matched to the sample inventory database. Verification of data ensures that there are no mistakes submitted into the database. The focus for this specific project was digitizing the clinical pathology and radiology paper reports by recording searchable quantitative and qualitative data values from the reports and organizing the information into a database.

Results: The lung registry contains 2,041 cases and includes samples from a range of different lung diseases, including 28 asthma, 19 acute respiratory distress syndrome (ARDS), 461 chronic obstructive pulmonary disease (COPD), 17 cystic fibrosis,116 interstitial lung disease, and 436 lung cancer cases. From the 2,041 cases, there are a total of 38,981 samples, which are composed of 6,563 air inflated cores, 5,656 cryomatrix inflated cores, and 26,762 formalin fixed, paraffin embedded blocks. For this project, 1,200 radiology and pathology reports including 29 qualitative and quantitative data fields were reviewed and inputted into the database. In addition, to previously entered lung function data and tissue samples, the expanded collection of digitized clinical reports can now be used as resources for future research or studies.

Conclusion: Careful phenotyping of patient samples is essential for understanding disease pathology. For continued good practices within the JHLR, all samples, clinical, and demographic data are being efficiently curated and secured on digital databases. The growth of the encrypted database with the addition of pathology, radiology, blood work, and lung function data will ensure the functionality of the biobank for lung research for the foreseeable future.

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* Nicole Coxson is entering her fourth year at McMaster University in the Arts and Science program. This is Nicole's third summer at the Centre for Heart Lung Innovation, following work in the JHLR in 2020 and the Jack Bell Research Centre in the COERD lab in 2019.

Participant retention status in the CanCOLD initiative: A special emphasis on the CanCOLD Vancouver site.

N. Katsuno*, W. C Tan

Background: The Canadian Cohort of Obstructive Lung Disease (CanCOLD) study is a longitudinal prospective study examining the incidence and development of Chronic Obstructive Pulmonary Disease (COPD) and its shared characteristics among the general Canadian population. As a national study, CanCOLD is carried out across 9 Canadian sites, with Vancouver having the highest number (438) of recruited participants. Despite following-up the same cohort with tri-monthly phone interviews, a key challenge for a longitudinal study is the generalizability of data. Over a period of time, retention rates drop as participants become lost to follow-up or dropout. A comparison of the national and local retention rates is needed to evaluate current retention practices at the individual sites and ensure quality assurance in the sample contribution from each site.

Hypothesis: The average retention rate for the CanCOLD Vancouver site will present rates equal to or greater than the average national CanCOLD retention rate.

Methods: To calculate the average retention rate for CanCOLD Vancouver, the Tracking Log for CanCOLD Subjects_Visit 5 was used to determine the year of recruitment and the number of retained participants from 2010 to 2021. First, the total number of retained participants for 2010 to 2021 was calculated by computing the sum of retained participants from each recruitment year for the year that is being evaluated. Each average retention rate for 2010 to 2021 was then calculated by converting the proportion of retained participants from each recruitment year into percentages for the year that is being evaluated. Lastly, the average retention rates were compared to the national retention rates.

Results: According to 2019 retention rates, the average for the national and local level were 76% and 81%, respectively. For 2021, the retention rate in Vancouver is currently at 75%. From 2011-2019, the national average retention rates declined from 92% to 76%, followed by a similar trend in Vancouver with a range of 93% to 81%. Both the national and local retention rates present a declining trend.

Conclusion: In comparison to the national retention rate, CanCOLD Vancouver retains a higher proportion of its recruited participants. However, neither the retention rates from a national nor a local level demonstrate immobility. Rather, both rates indicate a decreasing trend over the study period. With further examination to the CanCOLD dropout code, future work should identify shared patterns across lost participants to help maintain the retention rates for each individual site of the CanCOLD initiative.

We would like to acknowledge the investigators, staff, and subjects that are part of the CanCOLD Study as well as our sponsors: Canadian Institute of Health Research (CIHR/Rx&D Collaborative Research Program Operating Grant – 93326); Astra Zeneca Canada Ltd; Boehringer Ingelheim Canada Ltd; GSK Canada Ltd; Merk; Novartis; Nycomed; Pfizer Canada Ltd; Theratechnologies; the Respiratory Health Network of the FRSQ; and the CRRN.

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*Noah is a third-year Health Sciences student from SFU. This is her first co-op work term as the CanCOLD Study Coordinator in the Tan-Hogg Lab.

The Effects of Losartan and its Metabolites on Endothelial Function in an Atherosclerotic Mouse Model

O. Canavan*, E. Sauge, P. Bernatchez

Background: Atherosclerosis is a cardiovascular disease, occurring due to a buildup of plaque on the walls of blood vessels. The plaque impairs blood flow, damages vessels, and restricts the release of nitric oxide (NO), a vasodilator. Atherosclerosis can be modelled in Apolipoprotein E (ApoE) knock-out (KO) mice. ApoE is a gene responsible for metabolising lipoproteins and, evidently, the lack of the gene allows for a buildup of lipoproteins in blood vessels, producing plaque. Losartan is an angiotensin II receptor blocker (ARB) that is well-known for its antihypertensive effects. Specifically, in vivo experiments have shown that losartan effectively enhances endothelial function by increasing the production of NO. In the body, losartan is metabolised into EXP 3179, then immediately into EXP 3174, which all have similar structures. Recently, we observed that EXP 3179 creates the greatest improvement in endothelial function in wild type (WT) mice. Our goal is to use ApoE KO mice to compare the effects of losartan, EXP 3179, and EXP 3174 and determine whether they are successful at improving endothelial function in a mouse model of atherosclerosis.

Hypothesis: As observed in WT mice, we hypothesize that EXP 3179 will provide an improvement in endothelial function in ApoE KO mice, while losartan and EXP 3174 will not enhance endothelial function to the same extent.

Methods: Aortic rings from 6-month-old ApoE KO mice were put on a dual-wire myography system that assesses endothelial function by measuring the rings' constriction and relaxation abilities. The rings received either 15μM losartan, EXP 3179, EXP 3174, or a DMSO control by direct stimulation, a technique where treatment is applied directly to the vessel.

Results: Our data showed that EXP 3179 improved endothelial function the most compared to losartan and EXP 3174. EXP 3179 had an 84.3% decrease in endothelium-dependent constriction using phenylephrine (PE) compared to DMSO control. EXP 3174 decreased PE-induced constriction by 8.3% whereas losartan treatment showed a 1.6% increase. We did not observe a significant difference between groups for endothelium-dependent relaxation using acetylcholine (Ach). When we inhibited the release of NO with L-Name, we found that the drugs lost their effect, indicating that losartan and its metabolites act in an NO-dependent manner.

Conclusions: EXP 3179 enhances endothelial function more effectively than losartan and EXP 3174 in an atherosclerotic mouse model. This demonstrates that EXP 3179 interacts differently with the endothelium than losartan and EXP 3174 using direct stimulations. Future studies with ApoE KO mice should use intravenous injection, an in vivo technique for administering treatment to blood vessels that is comparable to in vitro direct stimulations. Thus, we could determine whether losartan and its metabolites produce the same effects in both administration methods.

This research is supported by the Centre for Heart Lung Innovation and the University of British Columbia.

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*This is Olivia Canavan's first summer at HLI, and she will be entering her second year at McGill University for the B.Sc. Major in Pharmacology.

Understanding cross-talk between macrophages and smooth muscle cells in foam cell development.

P. Xiang, C. Ortega, G. Francis

Background: Cardiovascular disease is the leading cause of death worldwide, and is mostly caused by atherosclerosis, a progressive narrowing of the blood vessels. The formation of foam cells in blood vessels is the hallmark of atherosclerotic lesions, which is a result of uncontrolled cellular uptake of aggregated low-density lipoprotein (agLDL) and other harmful lipoproteins. Foam cells were previously thought to originate primarily from macrophages derived from circulating blood monocytes.[1] However, our lab has recently reported that the majority of foam cells in humans (~70%) are actually derived from vascular smooth muscle cells (SMCs) in the intima. Although SMCs and macrophages are not in contact in early atherosclerosis, as the atherosclerotic lesion progresses, there is a mixing of macrophages and SMCs after macrophages infiltrate into the artery wall, allowing for significant cross-talk between the two cell types. **We hypothesize that the presence of pro-inflammatory macrophages or SMCs will promote foam cell development in the other cell type through the release of chemical cytokines.** To test this hypothesis, we will use *in-vitro* cell culture models and human coronary artery tissues to determine the effect of pro-inflammatory (M1) or anti-inflammatory (M2) macrophages and SMCs on foam cell development by the other cell type.

Methods: The first stage of the project focuses on the optimization of generating lipid-laden human monocyte-derived macrophages (macrophages) with agLDL. In brief, Human peripheral blood mononuclear cells (PBMC) will be isolated by density gradient centrifugation using Lymphoprep.To differentiate macrophages from monocytes, monocytes will be cultured in RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin for 7 days. Recombinant human (rh) MCSF (10ng/ml) will be added on the 1st and the 5th day after seeding monocytes. Mature M0 macrophages will be obtained on the 7th day. 100 µg/ml of agLDL will be added to the culture media for 24 or 48 hours to assess foam cell formation. Fatty-acid-free albumin will be used in the negative control medium to measure the baseline foam cell formation. Oil Red O Neutral Lipid Staining (ORO staining) and Lipidtox fluorescent staining are used to qualitatively assess the foam cell formation and amount of lipid loaded.

Results: Through ORO staining, the preliminary results confirmed lipid-laden macrophages were generated after incubating the cells with 100 ug/ml aggregated LDL for 24 hrs. 48 hrs incubation lead to a higher amount of lipid loading as indicated by the fluorescent intensity. Future experiments will use FACS to quantify % of foam cells generated. We will also assess the amount of lipid loaded using mass spectrometry. The results from the first stage will lay the foundation for assessing % of foam cells generated when coculturing naïve or polarized macrophages with SMCs.

Conclusion: The experiment in the first stage confirmed the generation of lipid-laden macrophages. More experiments are needed to conclude about the cytokine interaction between SMC and macrophage during atherosclerosis and how the interaction influences atherosclerosis.

This research is funded by Canadian Institute of Health Research

Regulation Of MicroRNA Expression In Scleroderma And Idiopathic Pulmonary Fibrosis

R. Badyal, B.A. Whalen, G.K. Singhera, B. Sahin, K.J. Keen, C.J. Ryerson, P. Wilcox, J.V. Dunne

Background: Scleroderma (SSc) is an autoimmune disorder that is not fully understood and is of unknown cause. SSc involves the thickening and tightening of the skin, connective tissue, and internal organs. The thickening of the skin is associated with excess collagen production due to fibroblast dysfunction and increased skin cell turnover. SSc patients are susceptible to developing interstitial lung disease (ILD), which leads to thick and stiff lung tissue with decreased function. SSc has a prevalence estimated to be 44 per 100,000 in Canada. With such a low prevalence, the pharmaceutical industry would not financially benefit from treatment development; therefore, SSc is considered an orphan disease. In addition to normal controls, this study includes cases with Idiopathic Pulmonary Fibrosis (IPF), which is a form of ILD not associated or secondary to other diseases. IPF is estimated to have a prevalence of 12 per 100,000.

Disrupted levels of microRNAs (miRNAs) are speculated to be part of the driving force behind SSc. The lung involvement of SSc will be studied by observing how disease progression and pathogenesis differ between patients living with SSc and ILD compared to healthy controls and the controls that have IPF. Multiple targets of disease pathogenesis have been identified through miRNA sequencing. For example, the DICER enzyme (encoded by the DICER1 gene in humans) appears promising as a therapeutic target. This enzyme works closely with the protein DGCR8 and the enzyme DROSHA in the RNA interference pathway.

Hypothesis: DICER, DROSHA, and DGCR8 contribute to the progression of ILD.

Methods: Human peripheral blood mononuclear cells (PBMCs) from healthy controls, SSc, and IPF patients were isolated with density gradients. Subcellular fractionation buffer was used to lyse the PBMCs, resulting in a cytosolic and nucleic fraction in western blot analysis of DICER, DROSHA, and DGCR8. Much effort was required to optimize the cell lysis method and western blot protocol for 42 samples.

Results: Preliminary western blot data on four healthy controls revealed that protein concentration of DICER is dependent on age.

Conclusion: The novel protocols that have been developed in this study will be applied in the future for the analysis of the samples from controls and disease subgroups: limited cutaneous scleroderma (lcSSc) with ILD, lcSSc without ILD, diffuse cutaneous SSc (dcSSc) with ILD, dcSSc without ILD, and idiopathic pulmonary fibrosis.

This research is funded by The Scleroderma Association of BC, Saint Paul's Foundation, The University of British Columbia, and The University of Northern British Columbia.

Raveen Badyal is a fourth year Biology student at UBC, and she has been a co-op student with BC Centre on Substance Use at the Centre for Heart Lung Innovation since January 2021.

Interactions between HIV and the Airway: HIV Receptor and Co-Receptor Expression in the Airway Epithelium

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Background: Since the beginning of the epidemic, mortality associated with human immunodeficiency virus (HIV) has drastically decreased through the introduction of antiretroviral therapy (ART). Despite this, people living with HIV (PLWH) still face a higher risk of chronic conditions including chronic obstructive pulmonary disease (COPD) and on average tend to experience worse symptom manifestations of this condition than uninfected individuals.

Hypothesis: The combination of smoking and HIV may augment injury to the airway epithelium by upregulation of important HIV cell receptors.

Methods: Bronchial epithelial cells (BEC) obtained from bronchial brushings from uninfected control (n=6), uninfected COPD (n=6), PLWH without COPD (n=4) and PLWH with COPD (n=6) donors, were cultured as monolayers. Total protein lysates from passage zero and passage one monolayers were used to quantify the expression of HIV receptor CD4 and co-receptors CCR5 and CXCR4, respectively, using immunoblotting. An ANOVA test on receptor expression was performed to determine statistically significant differences between the groups.

Results: Immunoblot analysis demonstrated a trend towards an increased expression of CD4 in PLWH with COPD (1.22 ± 0.46), PLWH without COPD (0.92 ± 0.24) and uninfected COPD donors (1.29 ± 0.56) compared to the control group (0.50 ± 0.63, $p=0.0655$). There was a significantly higher expression of CCR5 in PLWH with COPD (0.93 ± 0.16) compared to the control group (0.46 ± 0.04, $p=0.0131$). CXCR4 demonstrated a significant increase in expression in PLWH without COPD and PLWH with COPD (0.95 ± 0.59 and 1.04 ± 0.16, respectively) vs. control and COPD donors (0.08 ± 0.04 and 0.22 ± 0.25, respectively, $p<0.0001$).

Conclusion: Expression of HIV canonical receptors, particularly CCR5 and CXCR4, is increased in PLWH with COPD compared to control individuals. Although a weaker association was found with CD4, this suggests that greater interaction between the virus and the airway epithelium, potentially causing airway epithelial injury to occur in PLWH with COPD. Since both CXCR4 and CCR5 are important receptors in the inflammatory response, this upregulation may be due to increased inflammation within the airway epithelium.

This research is funded by Canadian Institutes of Health Research (CIHR) & BC Lung Association.

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*Ravneet Hansi is currently a second year Masters Student in Experimental Medicine at UBC. She works under the supervision of Dr. Janice Leung at the Centre for Heart Lung Innovation.

Cardiopulmonary Exercise Testing in an Individual Four Years After an Extra-Pleural Pneumonectomy

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Background: Extra-pleural pneumonectomy (EPP) is a radical surgical treatment for mesothelioma, a cancer of the tissue lining the thoracic cavity. The 59-year-old female participant underwent a left-sided EPP that involved the removal of the cancer-affected lung, half of the diaphragm and its phrenic nerve, as well as the pericardium. To our knowledge, the physiological responses to exercise are expected to be impaired post-EPP; however, this has not been studied.

Hypothesis: The ventilatory response to exercise will be impaired and contribute to exercise intolerance due to the removal of one lung and half of the diaphragm.

Methods: The participant (height=166cm, mass=56kg) completed a symptom-limited incremental cycle cardiopulmonary exercise test (20watts/2min). Metabolic and cardiorespiratory measures were recorded with a commercially available metabolic cart. Operating lung volumes were derived from dynamic inspiratory capacity maneuvers. The presence or absence of expiratory flow limitation was determined by placing expiratory tidal flow-volume curves within the maximum expiratory flow-volume curve. Heart rate and peripheral oxygen saturation were monitored via a heart rate strap and pulse oximetry, respectively.

Results: The participant achieved a peak work-rate of 100watts (88%predicted) and peak oxygen consumption (VO_{2peak}) of 1.07L/min (71%predicted). The slope of VO_2/WR was 7.2ml/min/watt. Minute ventilation was 88% of the measured maximal voluntary ventilation. Tidal volume plateaued early and remained constrained throughout exercise, resulting in a rapid increase in breathing frequency. The ratio of ventilation to carbon dioxide production was elevated at the gas exchange threshold. The participant showed evidence of expiratory flow limitation during the last two stages of exercise. Peak heart rate and oxygen pulse were 80 and 95% predicted, respectively. Peripheral oxygen saturation (SpO_2) remained at resting levels (96%) throughout exercise.

Conclusion: This case study demonstrates that there were mechanical constraints on ventilation during exercise, whereby tidal volume plateaued early and remained constrained throughout exercise and resulted in the adoption of a rapid and shallow breathing pattern. Despite the ventilatory limitation, the achievement of a relatively normal peak incremental work-rate reflects a high level of physical conditioning, emphasizing the importance of maintaining a physically active lifestyle post-EPP.

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Investigating a Shared-Control Study Design for Methylation Profiling

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Background: Bisulphite sequencing is a powerful tool for investigating the epigenetic underpinnings of many diseases. Unlike array technologies, sequencing measures methylation at single base-pair resolution across the genome. However, the high financial and computational investment required for these studies substantially constrain the sample size and consequently the investigative power of these studies. One potential solution is a "shared-control" study design, where several separate studies use the same control population, but incorporate separate case populations. Shared controls have been used in genetics studies, but the higher potential for both technical and biological variability in bisulphite sequencing experiments presents a unique set of problems. This study therefore aims to investigate the efficacy of shared controls in bisulphite sequencing studies.

Hypothesis: We hypothesize that the extent to which biological and technical variability influence methylation profiles will not significantly confound differential methylation attributable to the disease or condition in question.

Methods: 140 case samples were sourced from the Canadian Peanut Allergy Registry (CanPAR); 298 control samples were sourced from the Canadian Asthma Primary Prevention Study (CAPPS). All samples were processed using the same bisulphite sequencing and alignment pipeline to mitigate technical variability. The remaining variability was explored through comparison of quality control metrics as well as through principal components analysis (PCA). A biological and technical replicate study was then conducted to further quantify technical variability through the use of PCA as well as quality control metric comparison.

Results: PCA displayed good mixing of CanPAR cases and CAPPS controls in the first few principal components, indicating no substantial batch effects. Biological variability was clearly separable using the PCA, allowing for effective quantification of biological variability.

Conclusion: There is evidence to indicate presence of biological and technical variability in this shared-control study. PCA has been an effective approach to quantifying biological variability. Current research is focused on quantifying and controlling for this variability in downstream analysis using statistical models. Future directions focus on further advancement of the pilot replicate study to better characterize and quantify technical variability.

This research is supported by the Canadian Institute of Health Research and AllerGen.

* Shannon Edie is a student at UBC pursuing a Master's in Biostatistics. This is Shannon's first summer working at the Centre for Heart and Lung Innovation under Dr Denise Daley.

A Meta Analysis of Potential Serum Biomarkers for Diagnosing Eosinophilic Esophagitis: The Quest for a Minimally Invasive Alternative

W. Betzner (co-first author)*, S. Soleymani (co-first author)**, S. Tebbutt

Background: Eosinophilic Esophagitis (EoE) is a chronic immune system disease affecting children and adults, with ~1 in 1000 people being affected over the last decade. EoE causes an accumulation of white blood cells (eosinophils) in the esophagus which ultimately leads to dysphagia and damaged tissue. Since EoE has several general symptoms, it is commonly confused with other gastrointestinal (GI) diseases resulting in a delayed diagnosis. EoE is currently diagnosed through 4 biopsies taken from the esophagus, followed by a count of the eosinophils within the esophageal tissue. This method is problematic due to the invasive nature of a biopsy, expenses to the healthcare system as the process requires sedation, and the time expenditure of GI specialists and patients. The goal of this project is to verify a serum biomarker (BM) as a potential method, or part of several methods to non-invasively diagnose EoE through a meta-analysis of public data.

Hypothesis: A meta analysis of previously collected data will reveal whether or not the current candidates for serum biomarkers to diagnose EoE are viable means of diagnosing the disease.

Methods: After consultation from the doctor leading the top EoE clinic in Canada (Dr. Milli Gupta), a scope search of Medline Ovid, Pubmed, Web of Science, and Google Scholar was conducted using the keywords “eosinophilic esophagitis”, “serum”, and “biomarkers”, revealing 114 potential articles. Once inclusion and exclusion criteria were applied and duplicates were removed, there were 11 articles identified for potential sources of serum data collected by protein assay.

Results: 3 datasets have been obtained to date. No data analysis has occurred as of yet. Will have an update by mid-August.

Conclusion: The conclusion of this project will help clear up the debate on minimally invasive biomarkers for EoE and potentially serve as a stepping stone for more in depth statistical analysis of available biomarker data. .

This research is funded by Faculty of Medicine Summer Student Research Program and the support from Tebbutt Lab. We would like to thank and acknowledge the consultation of Dr. Milli Gupta of the South Health Campus in Calgary, Alberta.

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*William Betzner is a 4th year UofA Honours Physiology student with this being his first research project at both the Centre for Heart Lung Innovation and PROOF Centre.
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Modulation of ACE2, TMPRSS2, and Furin by Dexamethasone in the Airway Epithelium: Implications for COVID-19

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Background: Coronavirus disease 2019 (COVID-19) is caused by infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical manifestations of this disease can range from a fever, dry cough, dyspnea to more severe presentations such as pneumonia and acute respiratory distress syndrome (ARDS). Certain underlying respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD) alter clinical outcomes of COVID-19. A mainstay treatment for acute exacerbations in asthma and COPD and more recently, in severe COVID-19, is dexamethasone, a systemic corticosteroid with potent anti-inflammatory and immunosuppressive effects. Our goal is to investigate whether dexamethasone can modulate the protein expression of the host viral entry factors ACE2, TMPRSS2, and furin, in the airway epithelium.

Hypothesis: We hypothesize that with dexamethasone treatment, ACE2, TMPRSS2, and furin expression are modulated and can attenuate SARS-CoV-2 entry into the airway epithelium.

Methods: $1HAE_0$ human airway epithelial cell line and primary bronchial epithelial cells (BEC) from control, COPD, and asthma patient donors were grown as a submerged monolayer culture. Viability assays were performed to ascertain cytotoxicity of all concentrations of dexamethasone being tested. The steroid, at half-log concentrations from 0.3 to 10 μM, was added to these cultures, which were treated for 24 hours before being lysed and resolved on SDS-PAGE. ACE2, TMPRSS2, and furin were probed through western blotting and quantified. At least two independent replicates were done for each target. One-way ANOVA with Dunnett's Multiple Comparison test was used to compare each drug-dose group to their respective untreated vehicle control. Statistical significance for all analyses were determined at $p<0.05$.

Results: Dexamethasone treatment on $1HAE_0$ increased ACE2 protein expression in a dose-dependent manner, with only the two highest concentrations being statistically significant. We observed a statistically significant increase in furin expression with all concentrations of dexamethasone tested. 0.3 μM treatment of dexamethasone resulted in a significant increase in TMPRSS2 but a significant decrease at 1 and 3 μM. We observed a decrease in ACE2 and furin expression in dexamethasone treated primary BEC but further optimization is required.

Conclusion: The results may indicate a potential increase in viral interaction due to increased ACE2 expression caused by dexamethasone treatment in our cell line model. However, this modulation of ACE2 may be beneficial after viral clearance due to its protective effects against acute lung injury and ARDS. Further investigations are required in order to ascertain whether the alteration of ACE2, TMPRSS2, and furin expression by dexamethasone affects viral entry and clinical outcomes.

"This research is by the Faculty of Medicine Summer Student Research Program"

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* Tony Guo is entering his fifth year at UBC, majoring in physiology. This is his fourth summer working in the Dorscheid Lab at the Centre for Heart Lung Innovation. He also works at the respiratory clinic at BC Children's Hospital as a research coordinator.

Development Of A Novel Porcine Model To Study Radiofrequency Treatment Of Pulmonary Emphysema

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Background: Emphysema is one of the common pathological features of chronic obstructive pulmonary disease (COPD). Radiofrequency (RF) treatment is a non-invasive method to heat up tissue, which, if applied to emphysematous lung areas, may lead to selective tissue destruction and remodeling. We have previously reported that RF treatment improves lung compliance of emphysematous lungs in rats and exercise capacity in mice with emphysema. The next step is to validate this therapy in larger animals such as pigs. Here, we investigated the dose of porcine pancreatic elastase (PPE) that is needed to establish emphysema in pigs.

Hypothesis: PPE instillation produces emphysema in pigs.

Methods: PPE (850, 750, 725 U/Kg) was instilled into the left bronchus of three one-year–old female Yukatan pigs using a double lumen endobronchial tube under anesthesia. Six weeks after instillation, pigs were sacrificed and lungs were harvested. Lung function was measured by a water displacement method and plethysmography. Lungs were also air inflated, frozen at 10 cmH_2O on dry ice, and imaged using computed tomography (CT). One tissue sample from lower lobes was assessed for emphysema by a combination of micro-CT and histology.

Results: The compliance of the left lung was higher than that of the right lung at $5cmH_2O$ in all three pigs. The results of plethysmography were similar to that of water displacement. CT did not show a significance difference in lung density between the right and the left lung (mean HU; -855.5 vs -863.88, p=0.33), but micro-CT at 7μm resolution revealed that the mean linear intercept length (Lm) of the left lung was larger than that of the right lung in all three pigs (mean Lm; 310.0 μm vs 239.4 μm, p=0.035). On hematoxylin and eosin stain, Lm of the left lung was also larger than that of the right lung in all three pigs (mean Lm; 103.6 μm vs 64.9 μm, p=0.038). There was mild to trivial fibrosis in both lungs based on histology using Gomori Trichrome stain.

Conclusion: PPE in doses provided induces mild emphysematous changes in pig lungs. These data indicate that PPE treated pigs are a reasonable model of COPD.

This research is funded by IKOMED.

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* I am a Postdoc Fellow and it is my first summer at the Centre for Heart Lung Innovation. During my spare time, I like to go to the gym.

TDP-43 Stability in Post-Mortem Brain Tissue

Y. Li*, L.M. Forgrave, C. Guo, I.R.A. Mackenzie, M.L. DeMarco

Background: Transactive response DNA-binding protein 43 kDa (TDP-43) is the main pathological protein in the majority of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) cases. Based on immunoblot analysis of pathological brain tissues, truncated forms of TDP-43 have been associated with pathology. TDP-43 can be cleaved by endogenous proteases, which can be due physiological processes like protein recycling or pathological processes like cellular stress. Cleavage can also occur *ex vivo* during sample collection, storage and processing as the brain tissue specimens are subjected to an array of different temperatures and chemicals that may contribute to activation of endogenous proteases and related degradation pathways. To understand if TDP-43 truncation results from pre-analytical factors, TDP-43 stability in *ex vivo* brain tissue requires assessment.

Hypothesis: Temperature and the use of protease inhibitors will impact TDP-43 proteolytic cleavage during sample processing.

Methods: To evaluate possible TDP-43 degradation during sample handling, mouse brain tissue (endogenous TDP-43) was homogenized and aliquoted. Each aliquot was incubated for 18 hours at different temperatures (-80, -20, 4, 25, or 37 ^{o}C), with and without protease inhibitors. The samples, along with recombinant TDP-43 (45 kDa) as a positive control, were then subjected to anti-TDP-43 western blot analysis. The recombinant TDP-43 has a polyhistidine-tag, resulting in the molecular weight shift. Using ImageJ, the immunopositive TDP-43 bands at 43kDa representing full-length endogenous TDP-43 was quantified. These values were normalized relative to the 45 kDa band from the recombinant TDP-43 to allow comparison between all samples. TDP-43 degradation was defined as a significant decrease in TDP-43 immunostaining relative to the sample stored at -80 ^{o}C. A Mann Whitney U-test was used.

Results: It is expected full-length TDP-43 will be present in all samples and the temperature treatments and use of protease inhibitors with lead to variation in band intensity (i.e., TDP-43 concentration). It is expected that the band intensity will decrease as the temperature increases from -80 to 37^{o}C as endogenous proteases may be activated. Also, the addition of protease inhibitors is expected to reduce the degradation of TDP-43, and therefore preserve the band at 43 kDa.

Conclusion: This study is currently on going.

Y.L. is supported by the UBC Faculty of Medicine Summer Student Research Program.

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*Yun Li is a recent graduate from the Biomedical Laboratory Science program at UBC. This is her first summer research project at HLI, following her COVID-19 biobanking work in the DeMarco lab during the pandemic.

A Review: What Does DNA Methylation in Cord Blood Tell Us?

Z. He*, D. Daley

Background: Over the last five years, multiple studies have investigated the role of DNA methylation at birth to find the relationship between maternal exposure and neonatal growth. The objective of this literature review is three-fold: 1) identify recent association studies on DNA methylation in cord blood; 2) summarize current knowledge about epigenetic mechanisms linking environmental exposures and phenotype differences; 3) provide recommendations for future research.

Hypothesis: Neonatal DNA methylation is influenced by maternal exposures and contributes to neonatal phenotype differences and susceptibility to disease development later in life.

Methods: A comprehensive literature search was conducted in PubMed using the search terms 'cord blood' AND 'methylation' and limited to studies published from January 2016 to July 2021. Titles and abstracts were screened and papers that did not assess cord blood methylation were excluded, as were reviews and commentaries.

Results: The search identified 780 papers, of which 450 were excluded due to not containing association studies on cord blood methylation, resulting in 330 papers included in the review. Of these papers, 213 assessed the impact of maternal exposures on DNA methylation at birth, 99 identified DNA methylation loci that were associated with neonate or childhood phenotype differences, and 25 investigated the inheritance of DNA methylation patterns. The available evidence suggested that 117 types of maternal exposures and 63 types of neonate or childhood phenotype differences were associated with DNA methylation changes in cord blood cells. Moreover, there appeared to be a significant correlation of methylation levels between mother-child pairs exposed to smoking and air pollutants.

Conclusion: Current studies suggest DNA methylation is a key mechanism by which maternal exposure interacts with the genome leading to neonatal phenotype differences and the development of disease. However, the results should be interpreted with caution because of the small sample sizes and modest DNA methylation differences in most studies. Further studies are needed to provide more robust evidence for the hypothesis.

This research is supported by Mitacs. We would like to acknowledge the hard work and dedication of the whole study team.

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* Zelin He is a Mitacs intern from Beijing Normal University in China. He is a third-year student majoring in statistics. This is Zelin’s first summer at the Centre for Heart Lung Innovation.

Multidimensional Dyspnea Characterization of Patients in a Respirology Outpatient Clinic

J. Zhang, K.M. Milne, J.A. Guenette

Background: Shortness of breath (dyspnea) is the subjective experience of breathing discomfort consisting of distinct sensory qualities. Like pain, dyspnea is multidimensional, comprised of several distinct but interconnected domains: sensory-perceptual, affective distress, and symptom impact. It is a frequent cause for presentation to the emergency department and is associated with high levels of resource utilization and hospital costs. Although measurement of dyspnea intensity (i.e. “rate the intensity of your shortness of breath”) is a part of standard care for patients with cardiopulmonary disease, assessment of the specific sensory qualities of dyspnea is an emerging area of research. The combined impact of underlying diagnosis on qualitative dyspnea ratings in the outpatient respirology setting, controlling for disease severity, is unknown. The objective of this study is to evaluate differences in dyspnea quality by respiratory diagnosis (i.e. asthma, chronic obstructive pulmonary disease) in patients presenting to an outpatient respirology clinic for assessment using the Multidimensional Dyspnea Profile (MDP) and a standardized qualitative dyspnea descriptor questionnaire.

Hypothesis: Disease-based differences will exist in the sensory dimension of dyspnea quality assessed using the MDP and qualitative dyspnea descriptors.

Methods: Eight respirology outpatients (6M: 2F, 71±13 years) referred for assessment of dyspnea were recruited from The Lung Centre clinic at Vancouver General Hospital. Interviews were done in person or online via Zoom videoconference. Demographics, anthropomorphic and spirometry data were collected from their electronic medical record. Chronic exertional dyspnea intensity and impact were measured using the modified Medical Research Council (mMRC) dyspnea scale and Baseline Dyspnea Index (BDI). Participants selected qualitative descriptors of breathlessness using an established questionnaire and completed the MDP. Dyspnea assessment was anchored to the most severe episode of dyspnea experienced by the patient in the 6 weeks preceding study participation.

Results: Respiratory diagnoses of the participants were diverse (4 COPD, 2 asthma, 1 pulmonary hypertension, 1 pulmonary sarcoidosis). Two participants had spirometry that showed an obstructive pattern (FEV_1/FVC= 51.99) and two had a normal FEV_1/FVC ratio. Four participants had missing spirometry data. There was moderate impairment due to dyspnea on grading scales (mMRC grade= 1.6±1.2, BDI task=1.8±0.9, BDI effort=2.3±0.9, BDI function=2.8±1.2). Participants selected descriptors in the clusters of “work” and “unsatisfied inspiration” most frequently to describe their dyspnea. On the MDP, participants had moderate unpleasantness of dyspnea (5±2.45, 0-10 scale) and selected the sensory quality “My breathing requires muscle work or effort” most frequently.

Conclusion: Data collection for this study is still ongoing. However preliminary results suggest that work and effort is a common quality used to describe dyspnea in outpatients.

This research is supported by the UBC Faculty of Medicine via the Florence E. Heighway Summer Research Award.

Julia is a medical student at UBC and will be entering her second year in the fall. In her spare time, she enjoys playing volleyball, spikeball and ultimate frisbee.

Investigating the Effects of Titin Truncating Variants on Sarcomere Integrity in Arrhythmia using Patient Induced Pluripotent Stem Cell-Derived Cardiomyocytes

K. Huang*, H. Huang, L. Rohani, M. Ashraf, H. Luo, A. Sacayanan, Y. Lin, J. Roberts, R. Tadros, F. Lynn, G. Tibbits, L. Brunham, Z. Laksman

Background: Atrial fibrillation (AF) is the most common arrhythmia worldwide and is linked to a greater risk of stroke, heart failure, and death. Multiple genetic studies have found a significant association between protein truncating variants in the titin gene (TTNtvs) and increased risk of AF. TTNtvs are a well-established cause of dilated cardiomyopathy and induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) models of structural heart diseases have shown severe sarcomere defects in the presence of TTNtvs. However, the contributions of TTNtvs in the development of arrhythmias is unclear. Our goal is to investigate the effects of a heterozygous TTNtv c.55695_55698delCAGC p.(Ser18566Trpfs*25) that was identified through whole-exome sequencing of patients with unexplained AF.

Hypothesis: iPSC-CMs derived from arrhythmia patients with a heterozygous TTNtv will exhibit sarcomere defects that can be rescued through genetic correction to WT/WT genotype.

Methods: Patient iPSCs were generated in collaboration with the BCCHRI Tissue and Disease Modeling Core. iPSCs were genetically corrected using CRISPR/Cas9 homology-direct repair and differentiated into iPSC-CMs using a small molecule differentiation protocol. iPSC-CMs were cultured on micropatterned coverslips for immunofluorescent staining and confocal microscopy to compare sarcomere organization in the presence or absence of the heterozygous TTNtv.

Results: Isogenic iPSCs were successfully generated through CRISPR/Cas9 gene editing with an efficiency of 6.3%. Isogenic iPSCs displayed high cardiomyocyte differentiation efficiency as assessed through flow cytometric analysis of cardiac troponin T expression. Comparisons of sarcomere organization indicated that WT/TTNtv iPSC-CMs exhibit compromised sarcomere integrity compared to isogenic WT/WT iPSC-CMs.

Conclusion: Arrhythmia-related TTNtvs result in abnormal sarcomere organization in iPSC-CMs which may contribute to disease. Further studies are needed to delineate the mechanistic processes linking sarcomere disarray and arrhythmia.

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*Kate Huang is a 3rd year PhD student in the Experimental Medicine Program at UBC. Kate is investigating genetic variants associated with heart rhythm disorders using cardiac subtype-specific iPSC-CM models. Aside from her work in disease modeling, Kate seeks to understand how genetics influences patient outcomes and disease risk using the UK Biobank.

Investigating the Biological Underpinnings of HDL-Cholesterol and its Involvement with Sepsis and Patient Outcomes

R. McCallum*, E. Deng, L. Brunham

Background: Sepsis is clinically defined as life-threatening organ dysfunction resulting from dysregulated host response to bodily infection. Sepsis is one of the leading causes of patient mortality and morbidity, and a major cause of increased health-care expenditures globally. Aside from the century-old approach of intravenous fluids and antibiotics, there are no targeted therapies that reduce sepsis mortality, reflecting the limitations in our understanding of the key causal pathways in sepsis upon which to intervene. Recently, high-density lipoprotein (HDL) particles have emerged as key players in sepsis development and outcomes, as they are able to sequester and eliminate pathogen-associated lipids (PALs) and possess anti-inflammatory properties. Low concentrations of HDL-cholesterol are associated with more severe sepsis and a lower chance of survival. We recently reported that a gain-of-function single-nucleotide polymorphism (SNP) in the cholesteryl ester transfer protein (CETP) gene is an important contributor to the decline of HDL-C during sepsis. In support of these findings, APOE*3-Leiden.CETP mice (with a humanized lipid profile), that were intravenously exposed to the CETP inhibitor, anacetrapib, displayed preserved plasma levels of HDL-C and increased survival from experimental sepsis. Taken together, these results suggest that strategies to preserve HDL-C levels may represent a new approach to improve outcomes for individuals with sepsis. However, the mechanisms by which CETP regulates HDL during sepsis, and how HDL leads to improved sepsis outcomes remains unknown.

Hypothesis: We have developed three major hypotheses: 1) HDL-bound PALs are cleared via SR-B1 meditated endocytosis; 2) pharmacological inhibition of CETP will significantly improve survival from sepsis via its effect on increasing HDL particle concentration; and, 3) CETP-mediated HDL remodeling is responsible for the regulation of host inflammation.

Methods: The studies experimental framework follows a translational research approach using human and mouse macrophages (*in vitro*). This will allow us to investigate the role of CETP in HDL-mediated hepatic uptake of PALs. We will be using animal models of sepsis to study the role of CETP in lipid metabolism and the potential therapeutic benefit of CETP inhibition. RAW264.7cells and pre-clinical therapeutic evaluation of anacetrapib in a sepsis model will also be used. These techniques will help us explore the mechanism by which CETP inhibition decreases host inflammation during sepsis.

Expected Results and Conclusion: This study will elucidate the emerging roles of HDL in sepsis and host innate immunity. These studies will provide the mechanistic understanding need to develop a new strategy of pharmacological inhibition of CETP for sepsis treatment. This will ultimately improve clinical outcomes of sepsis patients, leading to a significant reduction of morbidity and mortality, and lessen the healthcare burden of sepsis.

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*Rylan McCallum is entering his first year in the Master of Science of Experimental Medicine Program at UBC. He recently completed an honours degree in biology at UBC and is continuing his research in the Brunham Lab at the Centre for Heart Lung Innovation.