CENTRE FOR
HEART LUNG INNOVATION
UBC AND ST. PAUL'S HOSPITAL

4th Annual

HLI RESEARCH DAY

A Joint Event for Trainees and Summer Students

AUGUST 21, 2020
VIA ZOOM AND TWITTER
HTTPS://UBC.ZOOM.US/J/68822262273
10:00 AM TO 2:15 PM

We invite you to attend a one-day event that showcases the breadth of research being conducted at HLI

KEYNOTE SPEAKER: DR. JEREMY HIROTA

TALK AT 1:00 PM: MAKING DO WITH WHAT YOU HAVE – TO GET WHAT YOU WANT

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Centre for Heart Lung Innovation
UBC and St. Paul's Hospital

2020 HLI Research Day Keynote Speaker

Making do with what you have – to get what you want

Dr. Jeremy Hirota, Ph.D.
Canada Research Chair in Respiratory Mucosal Immunology
Assistant Professor of Medicine at McMaster University
Affiliate Professor of Medicine at UBC
Adjunct Professor of Biology – University of Waterloo

Friday, August 21st; 1 – 2:00 p.m.
Hosted by HLI Trainee Association

Photo Credit: Tina Depko –McMaster University

Biography

Dr. Hirota received his PhD in Physiology and Pharmacology from McMaster University in the lab of Dr. Mark Inman at the Firestone Institute for Respiratory Health, where he studied mechanisms of airway remodeling in mouse models of allergen exposure. Dr. Hirota then pursued postdoctoral studies at the University of British Columbia at the James Hogg Research Centre under the supervision of Dr. Darryl Knight, where he studied airway epithelial cell biology and innate immunity. While at UBC, Dr. Hirota transitioned to the supervision of Drs. Chris Carlsten and Don Sin for a CIHR Banting Postdoctoral Fellowship, where he combined his in vivo and in vitro training to address how air pollution can impact chronic respiratory diseases. In January 2015, Dr. Hirota joined faculty at UBC in the Department of Medicine in the Division of Respiratory Medicine. In 2015, Dr. Hirota received the American Thoracic Society Ann Woolcock Memorial Award for future promise in asthma research and a CIHR New Investigator Award. In November 2016, Dr. Hirota returned to McMaster University where he is currently a Canada Research Chair in Respiratory Mucosal Immunology (Tier 2), Assistant Professor of Medicine in the Division of Respirology, within the Firestone Institute for Respiratory Health. Dr. Hirota maintains Affiliate status at UBC and is an Adjunct Professor of Biology at University of Waterloo.

Hirota Lab Research Summary

The Hirota Lab aims to develop an internationally recognized translational research program in respiratory mucosal immunology focused on lung health and disease, with an increasing focus on how to bring these research findings to market in terms of commercialization efforts. The Hirota Lab uses a translational research strategy with in vitro cell culture models, in vivo pre-clinical models, and clinical studies. The lab research is guided by three mutually reinforcing foci: i) patient oriented research on respiratory mucosal immunology in health and disease, ii) biomedical engineering strategies for model, technology, and diagnostic tool development and iii) pure basic science characterization of the biology behind innate immune receptor and related signalling pathways.

UBC a place of mind

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Centre for Heart Lung Innovation
UBC and St. Paul's Hospital
HLI

HLI Research Day 2020
Friday, August 21, 2020, 10:00 am – 2:15 pm
Virtual Zoom Conference

2020 HLI Research Day Schedule

10:00 – 10:15 am	**Opening Remarks**
	Dr. Jordan Guenette, PhD
	Associate Director, Centre for Heart Lung Innovation

SESSION I (Chair: Emmanuel Osei)

10:15 – 10:35 am	**5-Minute Oral Presentations**	**Lab**
Tony Guo	*Therapeutics on the modulation of ACE2 expression in airway epithelial cells*	Dorscheid
Rachel Bugis	*Imaging inflammation in atherosclerotic plaque: analysis of the Use of Ultra-small Superparamagnetic Particles of Iron Oxide (USIPOs) in optical coherence tomography imaging of ApoE-/- aortic plaque*	Sellers/ Leipsic
Julia Zhang	*Reliability of multidimensional tools for the measurement of dyspnea during exercise testing*	Guenette

10:35 – 10:45 am	**1-Minute Thesis Presentations**	**Lab**
Guangze Zhao	*Role of NFAT5 transcription factor in the pathogenesis of coxsackieviral myocarditis*	Yang
Jinelle Panton	*Biomarkers to predict risk of hospitalization in heart failure patients: a sex-stratified analysis*	Tebbutt
Terry Chen	*Identification of intercalated disc proteins regulated by NFAT5 transcription factor in CVB3-induced myocarditis*	Yang
Olivia Hutchinson	*Effects of high-intensity interval exercise on the respiratory muscles and ventilatory response*	Guenette
Srijan Subedi	*Differentiating various asthma subtypes and potential therapeutics using analysis of a serve asthma registry*	Dorscheid
Sophia Shen	*Biologic therapies targeting eosinophilic inflammation for asthma and allergic bronchopulmonary aspergillosis in adults with cystic fibrosis*	Quon
Mehima Kang	*Ethnicity-related differences in risk factors and presentation of premature atherosclerotic cardiovascular disease in UK biobank & SAVE BC*	Brunham
Lauren Choi	*Targeting SARS-CoV-2 viral fragments using CRISPR Cas13d in human airway epithelial cells*	Sin
Carl (Ling Xiang) Zou	*The use of meta-analysis to examine heterogeneity in a multisite study (COLD) on COPD and smoking*	Tan
Simran Samra	*Molecular biomarkers for phenotyping allergic rhinitis*	Tebbutt

10:45 – 10:50 am	**Break**	

SESSION II (Chair: Daniel He)

10:50 – 11:10 am	**5-Minute Oral Presentations**	**Lab**
Annie Bae	*Lung fibrosis assessment in an emphysema mouse model after radiofrequency therapy*	Sin
Huitao Liu	*MiRNA-modified coxsackievirus B3 (CVB3) for treating lung cancer*	Luo

Effimia Christidi	*Variation in RARG gene influences susceptibility to doxorubicin-induced cardiotoxicity in patient iPSC-derived cardiomyocytes*	Brunham
11:10 – 11:20 am	**1-Minute Thesis Presentations**	**Lab**
Marcia Jude	*Multi 'Omics profiling of the HIV airway epithelium: integration of the microbiome and transcriptome*	Leung
Taylor Minato	*Adaptive anatomical and pathological features of the elephant heart and cardiac blood vessels: learning from a case series*	McManus
Raisa Shabbir	*Evaluating peripheral blood eosinophilia and health outcomes in hospitalized cystic fibrosis patients with pulmonary exacerbations*	Quon
Rylan McCallum	*Investigating the prevalence of hypercholesterolemia across global indigenous population: a narrative review*	Brunham
Roy Zhao	*Heterogeneity of angiotensin II receptor blockers as pleiotropic activators of endothelial function*	Bernatchez
Denitsa Vasileva	*Epigenetic age prediction as a quality control step in large-scale methylation sequencing project*	Daley
Mai Tsutsui	*Radiofrequency therapy improves exercise capacity of mice with emphysema*	Sin
Min Hyung Ryu	*Effect of exposure to traffic related air pollution on the airway is modified by the microbiome*	Carlsten
Al Rohet Hossain	*Characterization of epidermal growth factors as key molecular regulators in patients with virus-associated heart failure*	Hanson/ McManus
George Chen	*A pipeline to repurpose drugs and identify novel drug synergies by leveraging computational pharmacogenomics*	Ng
11:20 – 11:25 am	**Break**	

SESSION III (Chair: Simran Samra)		
11:25 – 11:50 am	**5-Minute Oral Presentations**	**Lab**
Yasir Mohamud	*Coxsackievirus B3 cleaves Transcription Factor EB (TFEB) to impair host lysosomal function*	Luo
Ana Hernandez	*Is ABO as a causal risk factor for COVID-19 susceptibility?*	Sin
Mark Trinder	*Genetic and pharmacological inhibition of cholesteryl ester transfer protein improves survival in sepsis*	Brunham
Alessandro Cau	*Acute Kidney injury and renal replacement therapy in COVID-19 versus other respiratory viruses – systematic review and meta-analysis*	Russell
11:50 – 12:00 pm	**1-Minute Thesis Presentations**	**Lab**
Feng Xu	*Chronic Obstructive Pulmonary Disease and Idiopathic Pulmonary Fibrosis mechanism comparison*	Hogg
Emmanuel Osei	*Fibroblast-collagen interactions in the mechanical lung environment and the potential role in asthmatic airway remodeling*	Hackett
Sally Chung	*The effect of COVID-19 pandemic on the Canadian Cohort Obstructive Lung Disease (CanCold) study*	Tan
Carleena Ortega	*The effects of non-fasting and non-HDL on cellular cholesterol uptake and foam cells formation*	Francis
Aileen Hsieh	*Assessment of small airway morphology using micro-computed tomography in patients with idiopathic pulmonary fibrosis*	Hackett

Kate Huang	*Titin gene variants and modifiable cardiovascular risk factors contribute to the risk of atrial fibrillation*	Brunham
Andy Wang	*Accuracy and reliability of the BS-SCPer bisulfite sequencing SNP calling algorithm*	Daley
Nicole Coxson	*Digitizing clinical data for a lung tissue biobank supporting respiratory research for four decades*	Hackett
Abhinav Kumar Checkervarty	*Visualizing heterogenous cell clusters in single-cell analysis using Bioconductor in R*	Tebbutt

12:00 – 12:45 pm **Break for Twitter Competition**

@AnaIsabelHC *Is ABO as a causal risk factor for COVID-19 susceptibility?*
@JinellePanton *Biomarkers to predict risk of hospitalization in heart failure patients: a sex-stratified analysis*
@MH_Ryu *Do bugs in your lung change the way air pollution impact your body?*
@Andy1Wang *Accuracy and Reliability of the BS-SNPer Bisulfite Sequencing SNP Calling Algorithm*
@DenitsaVasilev6 *Epigenetic Age Prediction As A Quality Control Step in Large- Scale Methylation Sequencing Project*
@olivia_h30 *Effects of High-Intensity Interval Exercise on the Respiratory Muscles and Ventilatory Response*
@thedanhe *Using publicly available data to identify disease mechanisms: a meta-analysis of gene expression profiles of ILD*
@RylanMcCallum *Investigating the Prevalence of Hypercholesterolemia Across Global Indigenous Populations*
@carleenaortega *The Effects of Non-Fasting and Non-HDL on Cellular Cholesterol Uptake and Foam Cell Formation*
@DrEmmanuelOsei *Fibroblast-collagen interactions in the mechanical lung environment and the potential role in asthmatic airway remodeling*

	SPEAKER SESSION (Chair: Carleena Ortega)	
12:50 – 1:00 pm	**Peter Paré Scholars Talk**	**Lab**
	Tony Guo and Aileen Hsieh	Dorscheid / Hackett
1:00 – 2:00 pm	**Peter Paré Speaker**	
	Dr. Jeremy Hirota, Ph.D.	
	Making do with what you have – to get what you want	
2:00 – 2:15 pm	**Awards and Closing Remarks**	
	Min Ryu	

Thank you to our volunteers, students, PI's, judges and sponsors:

Therapeutics on the Modulation of ACE2 Expression in Airway Epithelial Cells

T. Guo*, G. K. Singhera, J. M. Leung, D. D. Sin, D. R. Dorscheid

Background: Coronavirus disease 2019 (COVID-19) is caused by infection by severe acute respiratory syndrome virus 2 (SARS-CoV-2), which requires angiotensin-converting enzyme 2 (ACE2) to enter susceptible cells within the respiratory mucosa. ACE2 is an important component in the renin-angiotensin system and activation of the ACE2/MasR axis contributes to protective effects against acute lung injury. Therapeutic agents such as ACE inhibitors (ACEi) and angiotensin II type 1 receptor antagonists (ARB) are being actively investigated in the treatment of COVID-19 and have been attributed with increased ACE2 expression but only in studies with murine models. Other agents include anti-inflammatory drugs such as dexamethasone and hydroxychloroquine, with the former shown to reduce mortality of individuals hospitalized for COVID-19 that required ventilation in a randomized trial. Our goal is to investigate ACE2 expression modulation in airway epithelial cells *in vitro* after treatment with potential therapeutics for COVID-19, including ACEi, ARB, steroids, and hydroxychloroquine.

Methods: *In vitro* monolayer cultures of human airway epithelial (1HAE) cell lines were grown and treated with losartan and telmisartan (ARB), captopril (ACEi), and hydroxychloroquine at half-log concentrations from 1 to 100μM, and fluticasone, ciclesonide, and dexamethasone (steroids) at half-log concentrations from 0.3 to 10μM. Whole cell lysates were obtained 24hr and 48hr post-treatment and ACE2 expression was determined through western blotting. Statistical analysis was performed using one-way ANOVA and Dunnett's multiple comparison test.

Results: Our data demonstrate increased ACE2 expression with losartan treatment compared to untreated control, with 3 and 10μM concentrations being statistically significant. No difference in ACE2 expression was observed with all concentrations of telmisartan. Captopril treatment demonstrates statistically significant dose dependent decrease in ACE2 expression. In contrast, ACE2 expression increases with increasing dexamethasone concentrations, with only 3 and 10μM concentrations being statistically significant. Ciclesonide treatment demonstrates statistically significant decrease in ACE2 expression with all tested concentrations. No difference in ACE2 expression was observed with all concentrations of hydroxychloroquine.

Conclusion: Treatment with losartan, captopril, dexamethasone and ciclesonide may alter *in vitro* ACE2 expression in a cell line model of human airway epithelial cells. Further investigations of these therapeutics in primary human epithelial cells and analysis of ACE2 expression using immunocytochemistry and flow cytometry in 1HAE cells must be performed to validate the observed modulation of ACE2 expression.

*Tony Guo is a third-year UBC student majoring in Cellular and Physiological Sciences doing his first Co-op term in the Dorscheid Lab.

PRESENTATION ABSTRACTS

Imaging Inflammation in Atherosclerotic Plaque: Analysis of the Use of Ultra-small Superparamagnetic Particles of Iron Oxide (USIPOs) in Optical Coherence Tomography Imaging of ApoE-/- Aortic Plaque

RE Bugis, C Vergez, SJ Wilson, C Gray, MR Miller, R Duffin, A Mitchell, DE Newby, P Bagnanichi, NL Cruden, IV Martin, JA Leipsic, MR Dweck, SL Sellers

Background: Inflammation is a pathological component in the development of atherosclerosis with monocyte recruitment and subsequent macrophage accumulation being a significant component of plaque; plaque inflammation is a risk factor for adverse events and anti-inflammatory therapies can reduce the risk of cardiovascular events. However, how to best image and evaluate plaque inflammation is an on-going question. Optical coherence tomography (OCT) utilizes signal variance associated with macrophage accumulation to provide in vivo assessments of macrophage density, but this can be misinterpreted and cannot distinguish activated macrophages. Therefore, we aimed to investigate the use of ultra-small superparamagnetic particles of iron oxide (USPIO)-enhanced OCT imaging to detect macrophage accumulation in plaque.

Hypothesis: We hypothesize that USPIOs will enhance the ability of OCT imaging to detect macrophages in plaque of apolipoprotein E knockout (ApoE-/-) mice, which will correlate with areas of plaque accumulation, inflamed plaque, and USPIO accumulation in macrophages.

Methods: Male 8-9 week old ApoE-/- knockout mice were fed a high fat diet for 16 weeks and treated with USPIOs (N=3) or saline (N=3) by tail vein injection 12 hours prior to sacrifice. Aortas were harvested and imaged en face using an OCT catheter (LightLab/St. Jude Medical) with aortas and livers subsequently fixed in 4% paraformaldehyde and paraffin embedded. Aortic plaque was quantified on histological sections stained with H&E and Movat's pentachrome. Aortas were also assessed by immunohistochemistry (IHC) for CD31, CD68, and CD163 and accumulation of USPIOs was evaluated by Perls prussion blue stain in livers and aortas over 5 fields of view with and without CD68 co-staining.

Results: OCT imaging demonstrated higher signal in areas of plaque in USPIO treated mice compared to untreated controls ($p<0.05$). Validation of CD31, CD68, and CD163 was successful in aortic sections. Perls prussion blue stain demonstrated cellular accumulations of USPIOs; livers show the accumulation of USPIOs in CD68+ cells of treated mice (71 ± 26) compared to saline controls, which showed no USPIO staining. Initial histological analysis of aortic tissue showed similar plaque burden between USPIO treated and saline treated control mice. Quantification of USPIOs and assessment of IHC markers in aortic tissue is on-going.

Conclusion: Treatment of ApoE-/- mice with USPIOs increases OCT signal in aortic atherosclerotic plaque with USPIOs detectable in CD68+ cells in liver sections and on-going histological validation in aortic sections. Overall, USPIOs may enhance OCT imaging of plaque to provide greater insight to the active inflammation within plaque.

I am a third year McMaster Health Sciences undergraduate student and this my first summer doing research at St. Paul's Hospital with Dr. Stephanie Sellers and Dr. Jonathon Leipsic.

Reliability of Multidimensional Tools for the Measurement of Dyspnea during Exercise Testing

J. Zhang, M.R. Schaeffer, R.A. Mitchell, K.G. Boyle, O.N. Hutchinson, J.H. Puyat, J.A. Guenette

Background: Dyspnea is defined as the subjective experience of breathlessness or breathing discomfort. It consists of qualitatively distinct sensations and can be separated into sensory and affective components. To achieve a more comprehensive measurement of this complex symptom, tools such as qualitative dyspnea descriptors and the Multidimensional Dyspnea Profile (MDP) have been proposed. However, the reliability of these tools for describing dyspnea during exercise testing has not been formally tested.

Hypothesis: The selection of qualitative dyspnea descriptors and the MDP questionnaire for the measurement of dyspnea during exercise in healthy individuals would show moderate-to-high test-retest reliability.

Methods: Forty-four healthy participants (24M:20F, 25±5yr) completed 3 identical maximal incremental cycling tests ≥48 hours apart. On all visits, participants selected all applicable qualitative dyspnea descriptors from a list of 15 and completed the MDP questionnaire for dyspnea at peak exercise.

Results: Majority of the 15 qualitative descriptors for dyspnea at peak exercise had good-excellent reliability (ICC>0.60), aside from 2 descriptors with fair reliability (ICC>0.50) and 1 descriptor with poor reliability (ICC=0.27). All the individual items in the MDP had good-to-excellent reliability (ICC>0.60), with the exception of fair reliability (ICC>0.50) for 2 items in the SQ most section and 1 item in the A2 section. The mean MDP scores for the immediate perception and emotional response domains of dyspnea both had excellent reliability (ICC>0.75).

Conclusion: Exercise-induced dyspnea in healthy individuals can be measured reliably using qualitative descriptors and the MDP.

*Julia will be entering her first year in the UBC Faculty of Medicine MD program this fall. This is Julia's second summer working with Dr. Guenette at the Centre for Heart Lung Innovation.

Role of NFAT5 transcription factor in the pathogenesis of coxsackieviral myocarditis

Guangze Zhao, Mary Zhang, Terry Chen, Fengping Wang, Decheng Yang

Background: Coxsackievirus B3 (CVB3) is one of the most common pathogens for viral myocarditis. Our preliminary data have shown that CVB3 infection impairs the NFAT5 (nuclear factor of activated T-cells 5)-regulated cellular stress-responsive pathway. NFAT5 is a cardiac protective transcription factor and plays a crucial role in maintaining cell viability against various stress conditions. However, our previous studies found that NFAT5 is cleaved during CVB3 infection by viral proteases, which benefits CVB3 replication.

Hypothesis: Thus, we hypothesize that the cleavage of NFAT5 transcription factor upon CVB3 infection may contribute to the virus-induced myocarditis.

Methods: To test this hypothesis, we first generated a conditional cardiac-specific NFAT5 KO mouse model by using an inducible Cre-loxp system. Then, the resulting (NFAT5flx/flx-CreαMHC6+) mice were injected with tamoxifen to induce NFAT5 KO in the heart. Two weeks later, mice were further infected with CVB3 at a pfu of 10^5. Mouse heart as well as other organs were harvested to confirm the heart-specific KO of NFAT5 by semi-qPCR and western blot. The replication of CVB in the heart was detected by qPCR, western blot and plaque assay. Meanwhile, H&E staining was used to determine the inflammatory response in different mice organs.

Results: The protein level of NFAT5 in the heart dropped significantly compared to that in other organs. Also, more cardiac damages and inflammation infiltrates were observed in CVB3-infected NFAT5 KO mice than in control. Taken together, our study suggests that the cardiac-specific NFAT5 KO mice are more susceptible to CVB3-induced viral myocarditis.

Biomarkers to predict risk of hospitalization in heart failure patients: a sex-stratified analysis

Jinelle D. Panton, Amrit Singh, Scott J. Tebbutt

Background: Heart failure (HF) affects over 600,000 Canadians and remains a leading cause of hospitalizations. In addition, around 50% of HF patients die within five years of diagnosis. A 2019 study conducted on HF patients aimed to identify predictive biomarkers of risk of hospitalization within three months. An ensemble biomarker panel was identified with an accuracy of 0.88 (area under receiver operating characteristic curve; AUC 0.88) using the following variables: differentially expressed mRNA and proteins, Holter variables as measures of cardiac function, and blood cell types. Of note, the non-hospitalized group had both males and females while the hospitalized group was completely comprised of males. Based on this imbalance, we undertook a sex-stratified re-analysis of the datasets.

Method: We used the Omics BioAnalytics web application (https://amritsingh.shinyapps.io/omicsBioAnalytics/). Classification performance of the sex-stratified (males only) biomarker panels was assessed using AUC.

Results: The AUC of the male-stratified ensemble biomarker panel was 0.76. Using male only data, the protein biomarker panel had the highest AUC of 0.79 compared to the original publication in which the ensemble biomarker panel performed the best (AUC 0.88). In the secondary analysis, the following proteins ANG1, APO A2, CAH1 and CRP were included in the ensemble biomarker panel.

Conclusion: It is likely that the female sex in the non-hospitalized group contributed to the differences found in the original analysis. It is important to account for differences attributed to sex to improve the interpretability of results and translational utility for medical care.

Identification of intercalated disc proteins regulated by NFAT5 transcription factor in CVB3-induced myocarditis

Terry Chen, Decheng Yang

Background: Nuclear factor of activated T cells 5 (NFAT5) is a master transcription factor regulating genes responding to cellular stress conditions and is particularly highly expressed in the heart. Coxsackievirus B3 (CVB3) is a major pathogen of viral myocarditis. In our previous study, we found that CVB3 proteases cleave NFAT5 at a later time point following infection. Intercalated discs (ICDs) are structures connecting neighboring cardiomyocytes in the heart. It is composed of three major complexes, namely the desmosome, which functions as cell anchor, adherens junction, which provides cell strength and gap junction, which couples cells electrically and metabolically. Our previous studies also found that CVB3 infection decreases ICD protein expression in mouse hearts, but the underlying mechanism is unclear.

Hypothesis: We hypothesize that the CVB3-induced cleavage of NFAT5 decreases transcription of ICD protein genes, which leads to cardiomyocyte injury during CVB3 infection

Methods: Using position weight matrices from TRANSFAC and JASPAR databases, the promoter region of 22 key ICD proteins were screened by several programs for the NFAT5 binding motif. Evolutionary conservational analysis was performed to support the predictions. Knockdown experiments with siRNA targeting NFAT5 gene were used to verify the regulation of the target gene.

Results/Conclusion: Program predictions indicated NFAT5 binding motifs in the promoter regions of desmoplakin (from desmosome), N-cadherin (from adherens junction) and connexin 43 (from gap junction). A pilot experiment with NFAT5 knockout in mice produced decreased desmoplakin expression in PCR analyses. Further experiments with luciferase assays and CHIP-seq are needed to confirm NFAT5 regulation of the identified genes.

Effects of High-Intensity Interval Exercise on the Respiratory Muscles and Ventilatory Response

O.N. Hutchinson, A.H Ramsook, J.A Guenette

Background: High-intensity interval exercise (HIIE) is a type of exercise prescription which involves intermittent periods of high-intensity exercise alternating with low-intensity recovery phases. The present case-study aims to describe the acute ventilatory responses to a single bout of HIIE and investigates the presence of diaphragm fatigue (DF) from HIIE. Exploration of the ventilatory stress imposed by HIIE will aid in determining its usefulness as an exercise prescription to maximize physiological adaption in respiratory compromised individuals.

Hypothesis: The ventilatory response [i.e., Ventilation ($\dot{V}E$), tidal volume (VT), breathing frequency (Fb), and work of breathing (Wb)] during HIIE will be less than that of a high intensity constant work rate (CWR) exercise test performed at the same absolute work rate. HIIE will result in less DF compared to CWR exercise.

Methods: A healthy, adult female (24 yrs.) with normal lung function reported to the laboratory for three visits: 1) symptom-limited incremental cycle exercise; 2) high-intensity CWR test; and 3) HIIE test. Visits 2 & 3 used the following methods. The participant had two balloon catheters inserted to measure transdiaphragmatic pressure (Pdi). Pdi twitches (Pdi,tw) were assessed using cervical magnetic stimulation of the phrenic nerve's before exercise as well as, immediately- and 15-minutes post exercise. Peripheral diaphragm fatigue was characterized by a decrease in Pdi,tw by 15% from baseline measurements.

Results: The outcome variables of the ventilatory response were generally greater for CWR than HIIE. Peak $\dot{V}E$ was greater in the CWR (77.6 L·min-1) compared to HIIE (71.9 L·min-1). CWR had a larger peak VT (2.1 L) than HIIE (1.8 L) at termination of exercise. Wb was greater for a given $\dot{V}E$ during CWR in comparison to HIIE. HIIE elicited greater peak Fb (39 breaths·min-1) than CWR exercise (36 breaths·min-1). Cumulative Wb was greater for HIIE (946.5 J) compared to CWR (838.5 J). DF occurred during HIIE, resulting in a 24% reduction in Pdi,tw at 15-minutes post-exercise. CWR did not result in DF resulting in only an 0.11% reduction in Pdi,tw

Conclusion: The ventilatory response outcome variables ($\dot{V}E$, VT, and Wb) were relatively greater during CWR exercise in comparison to HIIE, although cumulative Wb was greater for HIIE. Fb was the only parameter greater in the HIIE. Diaphragm fatigue was present following HIIE, but not CWR.

Differentiating Various Asthma Subtypes and Potential Therapeutics Using Analysis of a Severe Asthma Registry

S. Subedi*, T.R. Bai, D.R. Dorscheid

Background: Asthma is a common illness affecting nearly 300 million people around the world and causing more than 346,000 deaths per year. Although asthma cannot be cured, effective treatment and management would significantly lower the severity of asthma. The primary purpose of Asthma Research Registry is to accumulate a large cohort of individuals in order to answer questions about the nature and treatment of asthma, and to better understand why exacerbations of asthma develop. The Registry will also help researchers identify and recruit subjects who are eligible for participation in future research studies. The registry can also be used to ask specific questions regarding asthma. For instance, we are currently analyzing the response of asthmatic cohorts to various biologics and hope to create an algorithm using biomarkers which indicates the ideal biologics for each patient.

Objective: Creating the Asthma Research Registry will help differentiate asthma subtypes and their pathophysiological mechanisms from cluster analysis and may lead to stratification of patients, therefore enabling more specific therapeutic and prevention approaches. It could also allow researchers to identify, grade the severity, and follow up exacerbations. From the medical information collected, researchers could also be able to answer new research questions.

Methods: Subjects are recruited for Asthma Research Registry from the Pacific Lung Center at St. Paul's Hospital. Upon receiving subject's consent, information such as demography, past and current medical history (asthmatic phenotype, breathing test, sputum results, blood test, cultures and exacerbations), smoking history, ICU admissions, current treatment and relevant family history of respiratory diseases were recorded. This will be used for cluster analysis to potentially find different asthma subtypes in the future. The subject will also be invited to give a vial of blood for collection of serum and plasma, a 30 ml urine sample, and an induced sputum sample for investigative asthma phenotyping and cultures. A subset of registry enrollees will also provide airway brushing and BAL from bronchoscopy.

Results: 600 subjects were enrolled in the registry. Average age: 57 years, 52% males and 48% females, with 81% Caucasian, 12% Asian, 3% Middle Eastern and 4% consisting of Hispanic, First Nations and others. Around 95% of the severe asthmatics also suffer from other chronic diseases – 38% had sinus, 31% had GERD, 22% had COPD, 20% suffered from depression, 19% had high blood pressure, 11% had diabetes 10% had hypothyroidism, and 8% had high cholesterol. Using the subset of patients from Asthma Registry who were treated with omalizumab, the ones who had a positive clinical response had the initial level of IgE and blood eosinophils at 415 and 298, respectively. However, patients who failed on omalizumab, on average had the initial level of IgE and blood eosinophils at 844 and 488, respectively. Patients with positive clinical response also had a significant increase in their FEV1 when treated with omalizumab, compared to patients that failed.

Conclusion: Severe asthmatics have high susceptibility of suffering from other chronic diseases. With the preliminary data, it indicates that patients with high IgE and blood eosinophils tend to fail on omalizumab.

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*Srijan is entering his fourth year in the CMS program at UBC. In his free time, he likes hiking, playing guitar and trying out all kinds of different food.

Biologic Therapies Targeting Eosinophilic Inflammation for Asthma and Allergic Bronchopulmonary Aspergillosis in Adults with Cystic Fibrosis

Z.Y. Shen*, S. Desai, A. Stephenson, B. Quon

Background: Individuals with cystic fibrosis (CF) are at increased risk of developing asthma and allergic bronchopulmonary aspergillosis (ABPA), allergic airway conditions characterized by increased eosinophilic inflammation. The mainstays of treatment for asthma and ABPA exacerbations are systemic corticosteroids, which have the potential to exacerbate airway infection in the CF. Over the past 5 years, a number of new steroid-sparing therapies that block IL-5, referred to as Th2 biologics (e.g. mepolizumab, benralizumab, reslizumab) have been developed to abrogate eosinophilic inflammation. To date, just one small case series has assessed their effectiveness and safety for the treatment of eosinophilic inflammation in the CF population.

Objective: The goal of this study is therefore to evaluate the effectiveness and safety of Th2 biologic therapies targeting eosinophilic inflammation for asthma and ABPA in the adult CF population.

Methods: This will be a retrospective observational study aiming to identify all CF patients with asthma and/or ABPA who have been started on a biologic targeting Th2 inflammation. Effectiveness of treatment will be assessed by comparing clinical characteristics up to one year prior to and after initiation of treatment. Major outcomes of interest are FEV1 (% predicted and variability in measurements), days on antibiotics, hospitalization days, systemic corticosteroid requirement and frequency of pulmonary exacerbations. We will also perform a sub-group analysis to evaluate response based on treatment indication, biomarkers of Th2 inflammation, and sputum culture microbiology. Treatment safety will be investigated by evaluating adverse effects of Th2 biologics and reasons for treatment discontinuation.

Results: TBD

Conclusion: TBD

Sophia Shen is entering her second year of UBC Medicine. This is her first summer at the Centre for Heart Lung Innovation. During her spare time, she enjoys hiking across BC and plans to complete the St. Mark's Summit trail for her next hike.

Ethnicity-related differences in risk factors and presentation of premature Atherosclerotic Cardiovascular Disease in UK biobank & SAVE BC

M. Kang, D. Vikulova, M. Trinder, C. Brown, S. Pimstone, L. Brunham

Background: The global incidence of premature coronary artery disease (PCAD) has increased, and the burden is disproportionally high for South Asians (SA), Canada's largest visual minority. The goal of this study was to investigate the relationship between ethnicity and PCAD risk and presentation.

Hypothesis: SAs will present earlier, with a higher prevalence of traditional risk factors, and with a greater extent of disease compared to East Asians (EAs) and Caucasians.

Methods: We used data from UK Biobank (UKB) and SAVEBC (Study to Avoid Cardiovascular Events in BC) cohorts. PCAD was defined as individuals ≤50 years in males and ≤ 55 years in females with an acute coronary syndrome, revascularization, or angiographically proven disease. Descriptive statistics were stratified by ethnicity.

Results: The prevalence of PCAD in the UKB for SAs, EAs, and Caucasian was; 4.9%, 1.2%, 1.8%, respectively (p<0.001). EAs had highest gensini scores (mean:52.1, SD:28.6, p<0.001), followed by SAs (mean:36.3, SD:27.0), and then Caucasians (mean:31.4, SD:23.9). EAs had the most affected vessels (mean:2.2, SD:0.8), followed by SAs (mean:1.9, SD:0.8), and then Caucasians (mean:1.8, SD:0.8), (p<0.008). Caucasians had the highest BMI (p<0.001) and waist circumference (p<0.001). HDL-C was lowest in SAs (p<0.001). SAs had higher random blood glucose (p<0.001), HbA1C (p<0.001), and Lp(a) (p<0.02).

Conclusion: The prevalence of PCAD in SAs was more than double that in EAs or Caucasians. EAs, followed by SAs, had more clinically extensive PCAD than Caucasians. The high frequency of modifiable risk factors in SA and EAs reflects a need for improvement in PCAD prevention.

Targeting SARS-CoV-2 viral fragments using CRISPR Cas13d in human airway epithelial cells

L Choi*, A Tam, CWT Yang, Witzigmann D, Kulkarni J, CJ Lim, GK Singhera, DR Dorscheid, P Cullis, DD Sin.

Background: As of July 31st, 2020, the COVID-19 pandemic has resulted in over 17 million infected individuals and caused 650,000+ deaths worldwide. COVID-19 is caused by SARS-CoV-2, which invades the host's airway epithelial cells. Upon cellular entry, the viral RNA genome uses the transcriptional and replicative machinery of the host to synthesize viral mRNA, proteins and genomic RNA to produce daughter virions. Previous reports showed that viruses have evolved unique strategies in blocking the eukaryotic RNA interference system in eukaryotic cells using nucleocapsid protein. To overcome this barrier, the bacterial RNA targeting class II – type VI CRISPR Cas13 system can potentially be used to target the RNA genome of SARS-CoV-2. This project aims to model different viral loads by delivering one of its structural mRNAs encoding the nucleocapsid (N) protein in airway epithelial cells using Lipofectamine.

Hypothesis: Transfection of Cas13d/sgRNA using Lipofectamine 3000 efficiently cleaves nucleocapsid mRNA of SARS-CoV-2.

Methods: Cells from a human airway epithelial cell line (1HAEo) were pre-treated with 50nM of N mRNA-targeting guide RNA (gRNA #1 and #2) or scrambled gRNA, and Cas13d mRNA (3ug/ml) for 5h to allow the formation of the gene editing complex. Different concentrations of N mRNA were then transfected in cells for 2 days and RNA was extracted for real time PCR quantification.

Results: A concentration-dependent effect in N mRNA was observed with increasing concentration of N mRNA during transfection (0.003, 0.03, 0.3 and 3ug/ml). The data showed that gRNA #2 significantly knocked down N mRNA by 62% (p=0.0015) compared to scrambled gRNA when transfected at a concentration of 0.03ug/ml.

Conclusion: Our data shows that Cas13d has a potential as a therapeutic in reducing the viral load in subjects positive for SARS-CoV-2.

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* Lauren Choi is entering her fourth year in Biology at UBC. This is her first summer at the Centre for Heart Lung Innovation, working in the laboratory of Dr. Sin and Providence Airway Centre.

The Use of Meta-analysis to Examine Heterogeneity in a Multisite Study (COLD) on COPD and Smoking

L. Zou*, W.C. Tan

Background: Cigarette smoking is a primary risk factor for the rising incidence of chronic obstructive pulmonary disease (COPD) in the population, and is an increasing concern in Canada. Meta-analysis can be an effective method to show the overall risk of smoking for the country and for each site while comparing the site heterogeneity of the risk.

Objective: The purpose of this analysis was to evaluate cigarette smoking as a risk factor for COPD and to assess the heterogeneity of smoking for COPD across 9 Canadian sites.

Methods: Random sampling of 5176 non-institutionalized adults aged 40 years and older were obtained between 2006 to 2011 at 9 sites across Canada. Participants answered an interviewer-administered questionnaire and performed spirometry before and after bronchodilation. COPD was defined by post-bronchodilator spirometry of FEV1/FVC < 5th percentile of the frequency distribution in a healthy population(LLN). Logistic regression models were constructed to evaluate cigarette smoking as a risk factor for COPD with adjustment for age and school year for each site using SAS 9.4 (SAS Institute, NC, USA). Meta-analysis was used to summarize the odds ratio of each site and assess site heterogeneity with the use of I2, which is the variation in study outcomes between studies. The method of meta-analysis used was the random-effect model in which Individual sites were weighted according to an estimate of the mean effect in the range of the studies. These weights were reflected in a forest plot using Stata V.10.0 (Stata Corp).

Results: Participants included 53.5% ever cigarette smokers; 11.2% had COPD. The overall odds ratio [OR, 95% CI] for ever smokers was 2.18 (1.74,2.73). This suggests that the odds of having COPD are 2.18 times higher among cigarette smokers as compared to non-smokers. There was no site heterogeneity of cigarette smoking for COPD, I2 = 20.6% and p=0.26, because a low I2 value of 20.6% with a non-significant p-value indicates homogeneity of the studies.

Conclusion: Meta-analysis effectively estimated the overall risk of cigarette smoking for COPD and assessed the heterogeneity of smoking for COPD in a multisite study. This analysis confirmed that cigarette smoking behaviour is the cause of COPD, and the results are consistent across 9 sites.

*Ling Xiang Zou is a statistics undergraduate student at SFU. This is his first summer at the Centre for Heart Lung Innovation in Dr. Tan's Lab. He is involved in the management of a database of the Canadian Cohort Obstructive Lung Disease (CanCOLD) study.

Molecular biomarkers for phenotyping allergic rhinitis

Simran Samra, Anne K. Ellis, Scott J. Tebbutt

Background: Allergic rhinitis (AR) is a heterogeneous disorder that is associated with inflammation of the upper airways. Several phenotypes have been identified using a controlled allergen exposure model. The nasal symptoms experienced after exposure are utilized to phenotype an individual with AR as either an early responder (ER) or a protracted early responder (PER) or a dual responder (DR). We hypothesize that molecular differences detectable in peripheral blood can be utilized to phenotype AR.

Methods: Baseline blood samples collected from ERs (n=33), PERs (n=26), DRs (n=8) and healthy controls (n=17) were evaluated using a gene expression (RNA) assay. This assay (180 genes) was previously developed to distinguish subtypes (ERs vs DRs) of allergic asthma. A multivariate approach of biomarker discovery was utilized to develop panels that could discriminate between the phenotypes. The threshold for panel selection was an area under the receiver operating characteristic curve (AUROC) greater than 0.70 for two classification algorithms (elastic net and random forest) using leave-one-out cross validation.

Results: ERs and DRs were discriminated using a biomarker panel (AKT3, PIK3CG, SMAD2) with AUROC between 0.71 and 0.76. PERs and DRs were discriminated using a biomarker panel (ATP8A1, CD59, NAPA) with AUROC between 0.76 and 0.77. Additionally, DRs and healthy controls were discriminated using a biomarker panel (FNIP1, GATA3, RPS6) with AUROC between 0.81 and 0.85.

Conclusion: These identified genes could be relevant for diagnostic purposes and may aid in the development of biomarker tools that can phenotype AR patients

Lung Fibrosis Assessment in an Emphysema Mouse Model after Radiofrequency Therapy

A. Bae*, M. Tsutsui, C. Cheung, C.W.T. Yang, D.D. Sin

Background: Emphysema is a common phenotype of Chronic Obstructive Pulmonary Disease (COPD), and is characterized by alveolar wall destruction and airspace enlargement. The current treatment for emphysema is lung volume reduction surgery. However, this procedure is limited in practice due to its peri-operative morbidity and mortality, and high cost. In collaboration with IKOMED, we have been working to develop a minimally-invasive therapy, which uses percutaneous application of radiofrequency (RF) waves to target emphysematous lung regions. Healthy lung tissue is spared from RF-induced thermal injury due to the cooling effect provided by regular blood flow. Meanwhile, emphysematous tissue is subjected to localized heating due to a lack of normal blood perfusion, and thus experience thermal injury and subsequent fibrosis. These fibrotic changes decrease lung compliance and improve lung function. In this study, we investigated the efficiency of RF therapy in promoting fibrosis in a mouse lung model of emphysema.

Hypothesis: Thermal injury caused by RF in the emphysematous regions of the lung will promote fibrosis.

Methods: Emphysema was induced in mice by intratracheal instillation of pancreatic porcine elastase (PPE). Three groups were used in the experiment, mice treated with 1) saline, 2) PPE, and 3) PPE + RF. Upon sacrifice, whole mouse lungs were fixed, sectioned, and stained with Masson's trichome for histological analysis. Each lung section was divided into 1 mm2 squares, and quantitatively assessed for fibrosis using the Modified Ashcroft Scale upon evaluation at 8x and 20x magnification.

Results: In total, 1736 samples were assessed in the saline group, 1590 in the PPE group, and 1435 in the PPE+RF group. In the PPE-treated lungs, only 5.5% of the samples had notable fibrosis (Grade 2 or higher) compared to 16.9% of the samples in the RF-treated lungs. The lungs treated with PPE + RF had a significantly greater proportion of higher fibrosis scores relative to the PPE group ($p<0.001$), which indicates that RF therapy can successfully induce lung fibrosis in emphysematous mice.

Conclusion: RF therapy causes thermal injury and promotes subsequent fibrosis in emphysematous mouse lungs.

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*Annie Bae is entering her fourth year in UBC Biology. This is her first summer working at the Centre for Heart Lung Innovation under the supervision of Dr. Sin.

MiRNA-Modified Coxsackievirus B3 (CVB3) for Treating Lung Cancer

Huitao Liu, Yuan Chao Xue, Haoyu Deng, Yasir Mohamud, Chen Seng Ng, Axel Chu, Chinten James Lim, William W. Lockwood, William W.G. Jia, and Honglin Luo

We recently discovered that coxsackievirus B3 (CVB3) is a potent oncolytic virus against KRAS mutant lung adenocarcinoma. Nevertheless, the evident toxicity restricts the use of wild-type (WT)-CVB3 for cancer therapy. The current study aims to engineer the CVB3 to decrease its toxicity and to extend our previous research to determine its safety and efficacy in treating TP53/RB1 mutant small-cell lung cancer (SCLC). A microRNA-modified CVB3 (miR-CVB3) was generated via inserting multiple copies of tumor-suppressive miR-145/miR-143 target sequences into the viral genome. *In vitro* experiments revealed that miR-CVB3 retained the ability to infect and lyse KRAS mutant lung adenocarcinoma andTP53/RB1-mutant SCLC cells, but with a markedly reduced cytotoxicity toward cardiomyocytes. *In vivo* study using a TP53/RB1-mutant SCLC xenograft model demonstrated that a single dose of miR-CVB3 via systemic administration resulted in a significant tumor regression. Most strikingly, mice treated with miR-CVB3 exhibited greatly attenuated cardiotoxicities and decreased viral titers compared to WT-CVB3-treated mice. Collectively, we generated a recombinant CVB3 that is powerful in destroying both KRAS mutant lung adenocarcinoma andTP53/RB1-mutant SCLC, with a negligible toxicity toward normal tissues. Future investigation is needed to address the issue of genome instability of miR-CVB3, which was observed in ~40% of mice after a prolonged treatment.

Variation in RARG gene influences susceptibility to doxorubicin-induced cardiotoxicity in patient iPSC-derived cardiomyocytes

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

Introduction: Doxorubicin is a potent chemotherapeutic drug used to treat various types of malignancies. However, its use is limited by doxorubicin induced cardiotoxicity (DIC). A genetic variant in the RARG gene (rs2229774, S427L) has been associated with susceptibility to DIC. We used patient specific induced pluripotent stem cell (iPSC) derived cardiomyocytes (CMs) from patients who have been administered doxorubicin to investigate the functional role of RARG-S427L in DIC.

Methods and Results: We generated iPSC-CMs from patients who experienced DIC, as well as from control individuals who were wild type for RARG. Cases showed significantly higher sensitivity to DIC compared to controls (IC50: 0.91 µM and 2.59 µM respectively, p = 0.003). In iPSCs from a case who carried the RARG S427L variant, we performed genome editing to correct the variant to wild type. This resulted in significant protection from the cytotoxic effects of doxorubicin (IC50: RARG-WT/WT= 4.44µM vs RARG-S427L/WT=1.15µM, p=0.04). In iPSCs from a control individual who was wild type for RARG, we performed genome editing to introduce the S427L variant, which lead to increased susceptibility to DIC (IC50: RARG-S427L/WT = 0.54µM vs RARG-WT/WT= 2.14µM and, p=0.02). Presence of S427L also resulted in increased doxorubicin-induced double stranded DNA breaks and reactive oxygen species generation.

Conclusion: These results establish a direct causal role for RARG-S427L in the pathogenesis of DIC.

Multi 'Omics Profiling of the HIV Airway Epithelium: Integration of the Microbiome and Transcriptome

Marcia Jude, Chen Xi Yang, Fernando Studart, Julia Yang, Tawimas Shaipanich, Michael Kobor, Don D Sin, Janice M Leung

Rationale: People living with human immunodeficiency virus (HIV, PLWH) have an increased susceptibility to Chronic Obstructive Pulmonary Disease (COPD) independent of their cigarette smoke exposure. We hypothesize that dysbiosis of the HIV airway epithelium may impact its transcriptomic profile, and may together contribute to more rapid lung function decline in PLWH.

Methods: Cytological brushes of airway epithelial cells were obtained from 76 subjects (COPD+HIV+ (n=18), COPD-HIV+ (n=16),COPD+HIV- (n=19)and COPD-HIV- (n=23)). Microbiome data was measured using 16s amplicon sequencing (Illumina Miseq®) and analyzed using QIIME2® pipeline, with further characterization of alpha and beta diversity. Gene expression was measured using RNA sequencing (NovaSeq 6000®) and analyzed using limma in R. Multiomics' data integration was performed using Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO). Fifty repeats of 10-fold cross validations were used to tune model parameters, and a correlation threshold of 0.7 was set to determine key interactions between amplicon sequencing variants (ASVs) and gene transcripts.

Results: Reduced alpha diversity (Shannon Index, p=0.001) and microbial community shifts (Beta diversity-Weighted Unifrac PERMANOVA, p = 0.002) were seen in COPD+HIV+ subjects compared to the other groups. Linear discriminant analysis Effect Size (LEfSe) analysis (LDA effect size = 2) identified 32 discriminating ASVs between the four groups. RNA sequencing data analysis identified genes FERMT2, NTS, ADAMTSL5, WNT2B and PCDH17 to be the top five differentially expressed genes between the COPD+HIV+ and COPD-HIV- (reference) groups. Integration analysis revealed 38 'omics features that distinguished between the airway epithelium of COPD+HIV+ and COPD-HIV-groups (Figure). This included top ASV-ASV pairs - (i) Firmicutes Streptococcus - Bacteroidetes Prevotella, (ii) Firmicutes Streptococcus - Bacteroidetes Chryseobacterium, and Bacteroidetes Prevotella - Bacteroidetes Chryseobacterium, and top gene-ASV pairs – (i) FGFR3-Bacteroidetes Prevotella, (ii) C11orf95 - Bacteroidetes Prevotella, and (iii) GCSH - Proteobacteria Pseudomonas.

Conclusions and Future Directions: We found that Bacteroidetes Prevotella, associated with HIV infection, was positively correlated with 31 genes involved in processes such as cell growth and development, amino acid and lipid metabolism, transcription regulation, and immune response. Further analysis of microbiome-gene interactions distinguishing all four subgroups will help understand the relative contributions of HIV and COPD to the impact of dysbiosis on airway gene expression.

Adaptive Anatomical and Pathological Features of the Elephant Heart and Cardiac Blood Vessels: Learnings from a Case Series

T Minato, PJ Hanson, O Campbell, A Hossain, GK Singhera, J McManus, P Day, J Shoshani, and BM McManus

In this study, we detailed gross and histological features of the cardiovascular (CV) anatomy and pathology of a small cohort of African savannah elephants (Loxodonta africana) and Asian elephants (Elephas maximus) from infancy to adulthood. We also compared adaptive features and cardiac diseases in the elephant and other mammalian CV systems. Our hypothesis was that certain features of the elephant CV system have evolved to accommodate a large body size and shifting blood volumes. Eight elephant hearts were assessed by an expert cardiovascular pathologist. Sections were taken from formalin-fixed paraffin-embedded samples, stained with hematoxylin & eosin and Movat's pentachrome stains and imaged using an Aperio™ image scanner. Quantitative and qualitative results were garnered from gross observations, histological images, retrieved autopsy reports and appraisal of published reports. Key anatomical features include a bifid cardiac apex, normally dilated cardiac chambers, prominent epicardial fat pads, deeply penetrating Purkinje fibers, abundant fibroelastic tissue, coronary venous valves, muscular arterioles in the atrial appendages and non-pathological myofiber disarray. Certain of these features may function in enabling proper blood circulation. Our study of pathological features revealed that schistosomiasis-induced myocarditis in an elephant calf was characterized by polymorphic leucocyte infiltration and microcalcification. In adult elephants, focal medial calcification in the epicardial coronaries and a cholesterol-laden sclerotic scar in the aortic media were identified. Compositely, this work provides background on the adaptive anatomical features of the elephant's central CV system and renders a foundation for future research into cardiac function and dysfunction of elephants and other mammalian species.

Evaluating Peripheral Blood Eosinophilia and Health Outcomes in Hospitalized Cystic Fibrosis Patients with Pulmonary Exacerbations

R. Shabbir*, P. Wilcox, B. Quon

Background: Cystic Fibrosis (CF) patients suffer from pulmonary exacerbations (PEx), which are occurrences of increased symptoms and worsening lung function and are generally treated with antibiotics that target bacteria colonizing the lungs. However, up to a quarter of patients experience permanent loss of lung function even after antibiotic treatment, which suggests the current course of treatment for PEx is not ideal for everyone. Eosinophils are a type of white blood cell that are present during the Th2, or "allergic" inflammatory response. When eosinophils are activated by the immune system, they release toxic products that cause damage to lung cells and contribute to the inflammatory response already present due to the primary lung infection. Our goal is to determine if PEx driven solely, or in part, by eosinophilic inflammation might represent a phenotype more likely to have worse health outcomes compared to CF patients without this "allergic" inflammation component complicating their PEx. If so, these patients may be more likely to benefit from the addition of systemic corticosteroids or more specific anti-inflammatory agents in treatment.

Hypothesis: CF patients with peripheral eosinophilia at the time of hospital admission will have a slower symptomatic and lung function response to intravenous (IV) antibiotics.

Methods: This will be a retrospective study using clinical data and previously collected blood samples from adult CF patients hospitalized at St. Paul's Hospital as part of the CF biomarker study. Peripheral eosinophilia (elevated eosinophil count in the blood) will be defined as >300 cells/uL and/or >3% of total leukocyte count on admission to hospital. Clinical outcomes, including symptomatic response to IV antibiotics, lung function recovery, and requirement for antibiotic switches and/or systemic corticosteroids use resulting in longer hospital stays, will be evaluated in patients with vs. without peripheral eosinophilia.

Results: TBD – We are looking at a data set from 77 exacerbations from 53 CF patients. In 29 of these 77 PEx (38%), the patients had peripheral eosinophilia at the time of hospital admission.

Conclusion: TBD

*Raisa Shabbir is entering her second year of medical school at UBC. This is Raisa's first summer at the HLI. During her spare time, she enjoys battling her allergies in order to experience the great outdoors, catching up on Netflix, and attempting doomed DIY projects.

Investigating the Prevalence of Hypercholesterolemia Across Global Indigenous Populations: A Narrative Review

R. McCallum*, L. Brunham

Background: Hypercholesterolemia is widely classified by elevated levels of low-density lipoprotein cholesterol (LDL-C) and is an important risk factor for cardiovascular disease. Elevated levels of LDL-C may be indicative of severe hypercholesterolemia (SH) or familial hypercholesterolemia (FH), each commonly found in approximately 1 in 250 people. SH and FH are critically understudied and the prevalence within Indigenous communities around the world is widely unclear. FH specifically, goes undiagnosed in roughly 90% of the general population, and this percentage may be greater throughout Indigenous communities due to a lack of resources invested towards them. Statistics are overgeneralized to include Indigenous Peoples without considering the intersectional health experiences they face historically and culturally. Genetic ancestry plays an important role in health outcomes and therefore developing a clear understanding of the prevalence of hypercholesterolemia, as well as other hereditary disorders, is critical in advancing underdiagnosed minoritized communities.

Hypothesis: Increasing the understanding of FH prevalence across Indigenous communities will sanguinely lead to development of proper treatment strategies and adopting ethical practices to improve patient outcomes and reduce health care expenditures.

Methods: We conducted a systematic search of the literature using MEDLINE, EMBASE, PubMed, Cochrane Register of Controlled Trials, and Cochrane Database of Systematic Reviews. This allowed us to identify peer-reviewed articles reporting on the prevalence of hypercholesterolemia in Indigenous populations. In completing this study, we will also be investigating the prevalence of hypercholesterolemia using sub-analyses according to demographic data and self-reported status collected from the analyzed articles.

Results: Our search yielded 118 articles for which abstracts were reviewed resulting in 27 papers that are currently undergoing full text review. Based on early analysis of studies conducted on global Indigenous Peoples, there is a clear need for increased understanding regarding heritability of disorders as well as increased identification and betterment of patient care throughout Indigenous communities.

Conclusion: General underdiagnoses of common disorders is dangerous across all communities especially Indigenous ones, which are already subjected to a lack of health care accessibility and poor treatment strategies. Reviewing previous articles aids in increasing identification and characterization of hypercholesterolemia across Indigenous communities. This work will highlight the importance of developing personalized treatment and access to standard-of-care therapeutic approaches, thereby reducing cardiovascular risk across populations.

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* Rylan McCallum is entering his final year at the University of British Columbia in the Faculty of Science majoring in Biology. This is Rylan's second NSERC USRA but first summer conducting research at the Centre for Heart Lung Innovation; following his work with both the Angert and Martone labs at UBC and the Henriquez lab partnered with Agriculture Canada.

Heterogeneity of Angiotensin II Receptor Blockers as Pleiotropic Activators of Endothelial Function

R. Zhao*, A. Tehrani, P. Bernatchez

Background: Endothelial nitric oxide (NO) production is strongly associated with vascular health and is a key marker of endothelial function. Our lab has previously shown that losartan, an angiotensin II receptor type 1 (ATR1) blocker (ARB), is capable of increasing endothelial function independent of ATR1 blockade and blood pressure (BP) lowering. However, which ARB is most efficacious at increasing endothelial function is not known.

Objective: To determine which ARBs can best increase endothelial function independent of their BP lowering effects.

Methods: 6-week old wild-type mice were randomized into experimental groups and treated with different ARBs in drinking water. Drug doses were titrated to EC5 of BP lowering. After 4 weeks of treatment, freshly excised thoracic aortas were mounted on a wire myograph and subjected to pharmacological agents in order to investigate changes in endothelial function.

Results: 4-week pre-treatment with telmisartan led to a 53% inhibition of vascular contractility, in a fully L-NAME sensitive fashion, whereas valsartan and azilsartan showed no endothelial function enhancement at a similar BP lowering dose. There was no observable effect on acetylcholine-induced relaxation between treated aortic vessels and vehicle controls. Four other ARBs are currently being investigated.

Conclusion: At a low BP lowering dose, ARBs show heterogeneity in enhancing endothelial function independent of their BP lowering effects. This may have clinical implications as to the consideration of which ARB should be prescribed for conditions where endothelial function enhancement may be necessary.

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* Roy Zhao is entering his last year at UBC and will be completing his B.Sc. Pharmacology Program. This is Roy's first summer at the HLI.

Epigenetic Age Prediction as A Quality Control Step in Large- Scale Methylation Sequencing Project

Denitsa I. Vasileva *, Ming Wan, Allan B. Becker, Edmond S. Chan, Celia M. T. Greenwood, Catherine Laprise, Andrew J. Sandford and Denise Daley

Introduction: The Horvath epigenetic clock consists of 353 age-informative CpG sites identified from methylation arrays using mostly adult subjects, and is used to compare epigenetic vs biological age. It. The goal of our study is to: 1) Evaluate the efficacy of the Horvath algorithm using targeted methylation sequencing data from both adults and children and 2) assess the utility of the epigenetic age prediction as a quality control (QC) measure.

Study Design: The Canadian Asthma Primary Prevention Study (CAPPS)- is a longitudinal high risk asthma birth cohort, consisting of 549 children. DNA samples were collected at three-time points: birth (cord-blood), at ages 7 and 15 allowing investigators to evaluate changes in methylation as a function of time/disease/exposure. Methylation was assessed at multiple time points with 89 children having all 3 time-points and 110 at two time points. Maternal samples (n=115) were also included in the study to evaluate correlations between maternal/fetal methylation and assess the effect of intrauterine exposures (maternal asthma, smoking, medication) and imprinting. Sequencing was performed using Illumina's TruSeqMethyl Capture sequencing library. Epigenetic age prediction was conducted using the Horvath algorithm. Multiple other QC checks were performed including sex, Principle Component Analysis (PCA) to assess ethnicity, deconvolution for cell count heterogeneity and genotype concordance checks.

Results: The relative difference between predicted vs. reported age decreased as the biological age increased: 2.80±6.27 (cord blood samples), 0.95 ±1.09 (age seven), 0.46 ±0.39 (age 15) and 0.26 ± 0.22 (age >18). Following PCA and genotype concordance checks, four cord blood samples were suspected as possible sample swaps and their relative differences in their age prediction were two standard deviations higher than the mean (Table1).

Conclusion: The Horvath age prediction algorithm is highly accurate in older children (age>=15) and adults and has demonstrated utility as a QC metric in large scale methylation projects. However, a more robust algorithm is needed for epigenetic age prediction in cord blood and young children.

*Denitsa is a Master's student in Bioinformatics.

Radiofrequency therapy improves exercise capacity of mice with emphysema

Mai Tsutsui, Chung Yan Cheung, Takeyuki Wada, Jen-erh Jaw, Cheng Wei Tony Yang, Pascal Bernatchez, Zoe White, Dan Glebart, Eran Elizur, Kim Wolf, Evan Goodacre, Marek Lipnicki, Denny Wong, Don D. Sin

Background: Emphysema is a common phenotype of Chronic obstructive pulmonary disease (COPD). Although the resection of emphysematous tissue can improve lung mechanics, itis invasive and fraught with adverse effects. Meanwhile, radiofrequency (RF) treatment is an extracorporeal method that leads to tissue destruction and remodeling, resulting in "volume reduction" and overall improvement in lung compliance in emphysema. Whether these changes lead to improved exercise tolerance is unknown. Here, we investigated the effectiveness of RF treatment to improve exercise capacity of mice with emphysema.

Hypothesis: Treatment of emphysema with RF improves exercise capacity of mice.

Methods: A bilateral emphysema model was made by intratracheally instilling porcine pancreatic elastase (PPE). RF treatment was performed extracorporeally two weeks later and mice were sacrificed after another three weeks. The exercise capacity of mice was measured by using a treadmill. Treadmill runs were performed just before PPE instillation (baseline), before RF treatment and before sacrifice. Following sacrifice, lung compliance and mean linear intercept (Lm) were measured and fibrosis was assessed using the modified Ashcroft score.

Results: The maximum velocity achieved for each animal from the treadmill was found to be significantly higher in the PPE + RF compared to the PPE without RF group (p=0.01) at the end point. PPE + RF group also has significantly higher distribution of clear-cut fibrosis (p<0.0001) and decreased in Lm (p=0.03). Lung compliance of the PPE+RF group was lower, albeit not statistically significant.

Conclusions: Extracorporeal RF treatment improved exercise capacity of emphysematous mice and may provide functional improvement in COPD.

Effect of exposure to traffic-related air pollution on the airway is modified by the microbiome

Min Hyung Ryu, Illiassou Hamidou Soumana, Fernando Studart, Juma Orach, Julia Yang, Corey Nislow, Janice Leung, Chris Carlsten

Rationale: Human-microbial interaction is thought to be an important determinant of health and disease. Little is known about the influence of lung microbiome on exposure to air pollution in human.

Hypothesis: The effects of exposure to diesel exhaust (DE) on airflow and airway cytokines are modified by the diversity of airway microbiome.

Methods: We recruited twenty-five participants in this randomized, double-blinded, crossover, controlled human exposure study to DE. Each participant was exposed for 2-h to DE (diluted to 300 µg/m3 PM2.5) and filtered air (FA) in random order with 4-week washout. 24 h after each exposure, bronchoscopy was performed to collect microbial DNA from endobronchial brush and bronchoalveolar lavage (BAL). PCR was performed to generate amplicons of V4 hypervariable region of 16S rRNA gene. Amplicons were sequenced using Illumina Miseq, and Qiime2 pipeline was used for microbiome analyses. BAL was also analyzed for cytokines using multiplex electrochemiluminescence. Statistical analysis was performed using linear mixed-effects model (nlme) on R.

Results: There was significant exposure-by-amplicon sequence variants (ASV) interaction on FEV1 (p=0.006) and interleukin-6 (IL-6) (p=0.03). Participants who had lower alpha diversity (values ≤ median of Richness) experienced greater airflow obstruction and increase in IL-6 following the DE exposure compared to those with higher diversity (> median). In the low diversity group, DE significantly increased IL-7 (p=0.03) and IL-15 (p=0.03) in BAL. In contrast, neither COPD status, ex-smoking status, nor age were effect modifiers.

Conclusion: Individuals with low microbial diversity in the lower respiratory tract appear to be more susceptible to the harmful effects of air pollution.

HLI Research Day 2020
Friday, August 21, 2020, 10:00 am – 2:15 pm
PRESENTATION ABSTRACTS   **Virtual Zoom Conference**

Characterization of Epidermal Growth Factors as Key Molecular Regulators in Patients with Virus-Associated Heart Failure

Al Rohet Hossain, Paul J. Hanson, Gurpreet K. Singhera, Bruce M. McManus

Virus-associated heart failure is a complex multi-phasic syndrome that affects all demographics. Our limited understanding of the molecular underpinnings which drive disease pathogenesis severely impacts diagnosis and treatment. To address these challenges, we studied epidermal growth factors (EGFs), a family of key modulators with critical roles during infection and pathogenesis. Previously, we demonstrated that coxsackievirus B3 (CVB3), a pathogen associated with heart failure, induces tissue and isoform-specific expression of EGF family members, Nrg1β and erbB4, in murine models. Therefore, we hypothesized that CVB3 infection induces isoform specific expression of EGFs in the heart, a process distinct to the pathogenesis of virus-associated heart failure in the human condition. Tissue from patients with diagnosed or suspected virus-associated heart failure (n=18), coronary artery disease (CAD; n=5) or healthy controls (n=8) were analyzed using a tissue microarray (TMA) and probed via RNAScope™ (in situ hybridization) for CVB3 replicative strand RNA. Expression of NRG1, EGFR, ERBB2 and ERBB3 were assessed via immunohistochemistry (IHC) and signals were quantified via Aperio™ ImageScope. CVB3 RNA was detected in 5 of 18 patients (27.8%). Initial observations of IHC indicate NRG1, EGFR and ERBB2/3 are upregulated in CVB3-induced heart failure as compared to CAD and healthy controls. Thus, our preliminary impression suggests a central virus-specific role for EGFs in the pathogenesis of CVB3-associated heart failure, although further analysis is required.

A pipeline to repurpose drugs and identify novel drug synergies by leveraging computational pharmacogenomics

George Chen, Casey Shannon, Raymond Ng

Advances in sequencing technology has created an explosion of genomics data, enabling research that studies the relationship between drugs, genes, and diseases to be conducted. Here, we present a data pipeline that identifies synergistic drug combinations that are predicted to treat diseases. Our tool integrates data from Connectivity Map, ArrayExpress, and DrugBank. In brief, given an input dataset Accession Code, it outputs a user-specified number of synergistic drug combinations that are predicted to most reverse an input disease gene expression signature while minimizing drug-drug interactions.

Once the user provides an Accession Code, our tool downloads genomics data from ArrayExpress. It then identifies differentially expressed genes from the input dataset using the R limma package with the help of a few user-specified parameters. With the differentially expressed gene files, it automatically submits a query to Connectivity Map and loads the results when analysis completes. Finally, it computes orthogonality scores, as adapted from the SynergySeq tool, to assess drug synergy. Drug interactions are taken into consideration using the DrugBank drug interactions dataset.

Our tool encapsulates a long analysis process — including differential gene analysis, Connectivity Map querying, and drug synergy prediction — into a convenient, easy-to-use pipeline for biologists. No programming knowledge is required and the user only needs to provide the Accession Code and a few parameters for the analysis to take place. This enables researchers to rapidly identify potential therapeutic combinations for reversing any disease signature they input to potentially take to in vivo or in vitro experiments

PRESENTATION ABSTRACTS

Coxsackievirus B3 cleaves Transcription Factor EB (TFEB) to impair host lysosomal function

Yasir Mohamud, Hui Tang, Junyan Shi, Pinhao Xiang, Yuan Chao Xue, Huitao Liu, Chen Seng Ng, Amirhossein Bahreyni, Honglin Luo

Background: Coxsackievirus B3 (CVB3) is a prevalent etiological agent for viral myocarditis -induced heart failure for which effective therapy is lacking. Virus-encoded proteinases have emerged as cytopathic factors that contribute to pathology in part through targeting of the cellular recycling machinery of autophagy. Autophagosomes normally collect cellular waste inside vesicles and facilitate recycling upon fusion with lysosomes. However, the mechanisms by which viral proteinases disrupt autophagy remain unclear.

Hypothesis: Here we hypothesize that viral proteinases cleave autophagy factors to ultimately disrupt this recycling process and promote viral pathogenesis.

Methods/Results: Using an established CVB3 infection model of cultured cells (HEK293 & HeLa), we identified transcription factor EB (TFEB), a master regulator of lysosome biogenesis, as a novel target of CVB3. Time-course infections revealed a significant loss of full-length TFEB and the emergence of a lower molecular weight fragment at ~63kDa. Cellular and *in vitro* cleavage experiments identified the involvement of viral proteinase 3C in this proteolytic process while site-directed mutagenesis confirmed the site of cleavage after glutamine 60 (Q60). Functional studies that compared full-length TFEB with truncated Δ60-TFEB using a reporter construct of TFEB transcriptional activity discovered a loss of function despite electrophoretic mobility shift and immunoprecipitation assays showing Δ60-TFEB fragment retains DNA and protein binding.

Conclusions: Our study reveals that viral proteinase 3C indeed targets the critical host factor TFEB to disrupt lysosomal function.

Significance: Lysosomes are emerging as central regulators of many important cellular processes. Understanding how CVB3 circumvents the functions of lysosomes may reveal novel targets for anti-viral development.

Is ABO as a causal risk factor for COVID-19 susceptibility?

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Background: The genes that influence the pathophysiology of COVID-19 have yet to be identified. Association analysis has found genetic loci for COVID-191. We used integrative genomics (IG) to combine gene expression and proteomic information with COVID-19 phenotypes to identify candidate genes for this disease.

Methods: For this analysis we used the COVID-19 Host Genetics Initiative meta-analysis, the Lung eQTL study2 (n=1,038), eQTLGen3 study (n=31,784) and the INTERVAL4 study (n=3,301). We conducted two IG methods (Bayesian Colocalization [coloc] and Summary Based Mendelian Randomization) to link gene and protein expression in lung and blood tissues to COVID-19 susceptibility. We identified the most consistently colocalized gene and conducted a Mendelian Randomization (MR) to assess the causal association of its protein ('exposure') with COVID-19 susceptibility ('outcomes'). Significant MR was set as $P < 0.05$.

Results: The expression of 16 genes in lung and 25 genes in blood was associated with COVID-19. Out of those, three genes (LZTFL1, SLC6A20 and ABO) were within previously identified loci. Interesting first-time associations identified included genes involved in interferon pathways (IL10RB, IFNAR2 and OAS1). In addition, COVID-19 was also associated with plasma protein levels of ABO. Based on the MR, ABO demonstrated a significant causal association with COVID-19 with increased levels of this protein in plasma associated with an increased risk of COVID-19 (Figure 1).

Conclusion: This multi-omics approach led to the discovery of novel genes associated with COVID-19. Our analysis indicates that the ABO protein may be a causal risk factor for COVID-19.

Genetic and pharmacological inhibition of cholesteryl ester transfer protein improves survival in sepsis

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Background: Sepsis is a dysregulated host response to an infection that is responsible for up to 1/5 of deaths globally. Numerous clinical trials have failed to identify drugs that improve outcomes from sepsis, suggesting the need for therapies that target pathways causal to the pathogenesis of sepsis. High-density lipoprotein (HDL) particles, although best-known for their inverse association with coronary artery disease, possess properties that are relevant to the resolution of sepsis. Here, we tested the hypothesis that genetic or pharmacologic inhibition of cholesteryl ester transfer protein (CETP), which facilitates the metabolism of HDL-C, would decrease mortality from sepsis in humans and mice.

Methods and Results: Firstly, we performed targeted resequencing of HDL-related genes in 200 patients that were admitted to an emergency department with sepsis and had HDL-C levels measured. We identified a rare missense variant in CETP (rs1800777, p.Arg468Gln) that was associated with significantly lower HDL-C levels during sepsis. A fixed-effect meta-analysis of 7 sepsis cohorts composed of 10,427 patients found that the CETP gain-of-function variant was associated with increased risk of 28-day sepsis mortality (hazard ratio [95% confidence interval]: 1.44 [1.22-1.70], $p < 0.0001$). Secondly, we examined the effect a genetic score, comprised of 8 independent CETP variants associated with decreased CETP function, on 28-day sepsis mortality in the UK Biobank (n=5,949) and Identification of SNPs Predisposing to Altered Acute Lung Injury Risk (iSPAAR; n=882) cohorts that had genotyping array data available. The genetic score for decreased CETP function was associated with significantly decreased sepsis mortality in both the UK Biobank (hazard ratio [95% confidence interval]: 0.77 [0.59-1.00] per 1 mmol/Lincrease in HDL-C) and iSPAAR cohorts (hazard ratio [95% confidence interval]: 0.60 [0.37-0.98] per 1 mmol/L increase HDL-C). Thirdly, we performed functional validation by administering a single intravenous dose of a CETP inhibitor (anacetrapib) to adult, female CETP transgenic mice at 6 hours post-onset of experimental sepsis. CETP inhibitor treatment preserved plasma levels of HDL-C post-sepsis and reduced mortality relative to placebo (3-day mortality: 29.4% vs 64.7%, Log-rank p=0.03).

Conclusions: Genetic or pharmacologic inhibition of CETP improves survival from sepsis in humans and mice, respectively.

Acute Kidney Injury and Renal Replacement Therapy in COVID-19 Versus Other Respiratory Viruses–Systematic Review and Meta-Analysis

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Background: Acute kidney injury (AKI) is a potentially fatal complication of Coronavirus Disease-2019 (COVID-19). SARS-CoV-2 binds to and down-regulates Angiotensin Converting Enzyme 2 (ACE2) which may cause AKI.

Hypothesis: Frequencies of AKI and RRT in COVID-19 are higher in COVID-19 than in other respiratory viruses.

Methods: We synthesized the literature and performed a meta-analysis of the frequencies of AKI and renal replacement therapy (RRT) in hospitalized adult COVID-19 patients and compared them to those frequencies in patients infected by respiratory viruses that also bind to or downregulate ACE2 and patients infected by non-ACE2-associated viruses.

Results: In our meta-analysis (55 studies) of 32,261 hospitalized COVID-19 adults, AKI frequencies (11%) were significantly lower in COVID-19 than in ACE2-associated (38%, $p < 0.001$) and non-ACE2-associated viruses (47%, $p < 0.001$). Similarly, RRT frequencies were also significantly lower in COVID-19 (4%) than in ACE2-associated (15%, $p < 0.001$) and non-ACE2-associated viruses (18%, $p < 0.001$). After adjusting for shock and vasopressor use by meta-regression, AKI and RRT rates were not significantly different between viral groups.

Conclusions: AKI appears less common in COVID-19 than in other ACE2-associated or non-ACE2-associated respiratory viruses. However, after adjusting for shock and vasopressor use, AKI and RRT rates were not significantly different between viral groups. Thus, lower rates of shock and use of vasopressors in COVID-19 versus other viruses could explain lower rates of AKI and RRT.

* Alessandro Cau is entering his second year of medical school at UBC. This is Alessandro's first summer at HLI, following his work at the Djavad Mowafaghian Centre for Brain Health.

Chronic Obstructive Pulmonary Disease and Idiopathic Pulmonary Fibrosis mechanism comparison

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Rationale: The purpose of this study is examining the similarities and differences between COPD and IPF. The results of this project would disclose the relationships between the two diseases, which might coexist in a patient.

Methods: Explanted lungs from patients with either COPD(n=10), or IPF(n=9), and unused donor lungs(n=9) that served as controls were collected. Gene expression profiling and Quantitative histology were used to compare the lung tissues from COPD and IPF patients.

Results: IFNG, key regulator of immune response, is upregulated in both COPD and IPF. Furthermore, the receptor of IFNG, namely IFNGR1, is downregulated, which is the symbol for down-regulation of the T cell blocker, PD-L1. These expression patterns account for the activation of the immune response, which is confirmed by the increased infiltration of immune cells in both COPD and IPF. Besides the consistency, we also observed the differences between COPD and IPF. The expression level of TGFBI, a transcription factor that regulate collagen production, is significantly up-regulated in IPF, but significantly down-regulated in COPD. The components of NF-kB pathways, including NFkB1, NFkB2, TRAF3, PDK1, and CYLD are all up-regulated in IPF samples but not in COPD tissue. These differences are also observed in TGF-beta pathway and Treg cell signature genes.

Conclusions: There are significant differences in gene expression between COPD and IPF in terms of the expression profile of TGF-beta and NF-kB pathways. Meanwhile, some T cell blocker and T cell co-stimulators share the similar expression patterns, which explain the lung tissue destruction in both diseases.

Fibroblast-collagen interactions in the mechanical lung environment and the potential role in asthmatic airway remodeling

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Rational: Asthma is a chronic lung disease associated with airway remodeling that involves an increased deposition of extracellular matrix (ECM) proteins which is unaffected by current therapeutics. Human lung fibroblasts (HLFs) are the main structural cells involved in the production and repair of ECM such as fibrillar collagen I. However, the ECM can also greatly affect the phenotype of HLFs. Although this fibroblast-ECM interaction occurs in an abnormal airway mechano-environment in asthma, the effect of lung-mechanics on fibroblast repair-function is unknown. Hence, we assessed the effect of mechanical loads using 3D collagen-gel constructs which are able to effectively mimic the in vivo environment compared to 2D-cell models. We also assessed HLF phenotype in increasing collagen I concentrations representative of airway remodeling.

Methods: HLFs were plated on 2D collagen-coated culture-plates with flexible membranes or mixed in 3D collagen I gels and placed into culture-plates in a Flexcell®5000 tissue-train bioreactor using a trough-loading system to make 3D linear gels. Collagen gels were also made at 0.4mg/ml and 2.1mg/ml to mimic fibroblast-collagen remodeling in a normal and fibrotic ECM environment respectively. Fibroblast-seeded 2D plates and 3D gels were left unstrained (controls) or subjected to a 1% equibiaxial mechanical strain of 0.2Hz at different time points to mimic the mechanical forces experienced by fibroblasts in the airways. The effect of cell-strain in the 2D and 3D environment and different collagen concentrations on HLF-morphology was assessed by staining for non-muscle myosin IIB (NMIIB) and F-actin. HLF-ability to remodel hydrolyzed collagen into fibrillar collagen was assessed with second harmonic generation non-linear microscopy (SHG-NLOM).

Results: Mechanical strain on 3D fibroblast-seeded collagen I gels led to the loss of fibroblast dendritic extensions as shown by the loss of NMIIB and F-actin staining compared to unstrained conditions. These morphological changes were only visible in HLFs in 3D gels and not in cells on 2D culture-plates. Added to this, HLFs in the 2.1mg/ml 'fibrotic' gel were inhibited in their ability to remodel collagen as demonstrated by a lower intensity signal for fibrillar collagen and had lower cell numbers compared to 'normal' 0.4mg/ml gels indicating lower proliferation rates, as demonstrated by SHG-NLOM and confocal microscopy.

Conclusion: We show for the first time that in a 3D ECM environment, alterations in mechanical strain caused by asthmatic episodes may affect the cytoskeletal structure of lung fibroblasts through the reduction of fibroblast dendritic extensions. In addition, increased collagen concentrations in fibrotic airways may affect lung fibroblast proliferation and inhibit their ability to repair collagen I fibers. This abnormal fibrillar-collagen remodeling phenotype may promote an abnormal repair environment that may contribute to airway remodeling in asthma. These findings provide new avenues for therapeutic research into airway remodeling.

The Effect of the COVID-19 Pandemic on the Canadian Cohort Obstructive Lung Disease (CanCOLD) Study

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Background: CanCOLD is a prospective multi-center cohort study following a random sample of Canadians in Vancouver and eight other sites across Canada. The objective of the study is to characterize and identify the incidence and development of chronic obstructive pulmonary disease (COPD) and investigate its impact on the general population. The study provides an in-depth phenotyping of participants through multiple tests conducted during each of five visits that are spaced 18 months apart. During these visits, participants complete two face-to-face standardized questionnaires (CRF) and various tests including cardiopulmonary exercise test (CPX), full pulmonary lung function test (PFT), spirometry test, blood draw, fractional exhaled nitric oxide measurement (FeNO), 6-minute walk test, and CT scan. Due to the COVID-19 pandemic, patient recruitment has been put on hold for the CanCOLD study. Our current aim is to continue phenotyping participants in absence of physical meetings.

Objective: To develop alternate methods for data collection which comply with the COVID-19 restrictions in place.

Methods: We first reviewed each component of the CanCOLD study and determined which tests required in-person visits and which could be completed virtually. All testing such as CPX, PFT, spirometry test, blood draw, and CT scan were put on hold; however, we executed what could be done amidst the restrictions. Aside from the usual 3-monthly phone exacerbation questionnaire, the study implemented a new monthly survey regarding the participants' possible exposures, risk factors, and symptoms related to COVID-19. The CRF was solely conducted over the phone due to security and logistics problem for our older adult participants. Lastly, signage of the data linkage study consent form was carried out by means of mailing out letters to obtain physical signatures.

Results: A total of 87 participants have enrolled in the COVID-19 questionnaire administration thus far; 56 have been followed up for month 2, and 24 have been followed up for month 3. As for the data linkage consent form, a total of 64 participants have signed the consent form either by mail or electronically. Nevertheless, complications include time constraints with executing the lengthy 60-paged CRF over the phone as well as the delay of obtaining physical signatures as some participants preferred to sign the consent form in-person after the virus settled down.

Conclusion: Although in-person visits were deferred and major portion of the CanCOLD study were put on hold, we were still able to collect data despite the restrictions through administering questionnaires over the phone, implementing a new questionnaire relating to COVID-19, and communicating with participants to keep them updated on the current study process amidst the pandemic.

*Sally is a third-year health sciences student at SFU. It is her second Co-op work term at HLI where she is working as the CanCOLD Study Research Coordinator.

The effects of non-fasting and non-HDL on cellular cholesterol uptake and foam cells formation

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Background: Macrophage and smooth muscle (SMC) foam cells in atherosclerotic plaque are produced following unregulated uptake of modified (oxidized, aggregated) low density lipoprotein (LDL) and other apolipoprotein B-containing lipoproteins retained in the artery wall. Previously, the standard indicator of lipoprotein risk for atherosclerosis has been considered to be an individual's plasma LDL-cholesterol (LDL-C) level obtained following fasting. Recent clinical studies indicate that total non-high-density lipoprotein (non-HDL) cholesterol levels obtained in non-fasting plasma are stronger predictors of cardiovascular risk than LDL-C from 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: Loading human aortic SMCs and human monocyte-derived macrophages with non-fasting and non-HDL will lead to higher intracellular cholesterol accumulation than treatment with fasting and LDL in vitro.

Methodology: Monocultures of SMCs and macrophages were grown in medium containing 10% fetal bovine serum, switched to10% lipoprotein-deficient serum for 24 hours to upregulate LDL receptors, and loaded with 95 µg/mL cholesterol from either fasting or non-fasting plasma in the aggregated form of non-HDL or LDL 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 mass spectrometry data suggests that fasting and the non-HDL fraction result in the highest intracellular lipid accumulation. This is confirmed by flow cytometry as foam cells are most abundant in SMCs loaded with fasting non-HDL.

Conclusion: This study may provide insight into the cellular mechanisms of non-fasting and non-HDL as predictors of atherosclerosis risk. Further studies using co-culture of SMCs and macrophages may more accurately model the effects of the non-fasting and the non-HDL lipoproteins on formation of foam cells of both types in vivo.

Assessment of Small Airway Morphology using Micro-computed Tomography in Patients with Idiopathic Pulmonary Fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF) is an aggressive interstitial lung disease (ILD), characterized by chronic, progressive fibrosis. ILDs are initially diagnosed by qualitative, radiological assessment of distinct CT patterns. A recent study (Verleden et al., Lancet Respr. Med. 2020) showed regions of minimal fibrosis have significantly reduced numbers of terminal bronchioles compared to controls. What remains unknown is how airway morphometry changes and how airway remodeling present in IPF is reflected in the clinical CT scans.

Hypothesis: Radiological CT patterns in IPF are associated with distinct pathological changes of the small airways and surrounding parenchyma.

Methods: Explanted lung specimens from severe IPF patients (n=10) and age-matched donor controls (n=5) were air-inflated and frozen in liquid nitrogen vapor. Up to 5 samples per IPF case were obtained from each radiological CT pattern using targeted sampling. Systematic uniform random samples (n=8) were obtained from each control case. All samples were scanned with micro-computed tomography (microCT) at a resolution of 11-13 μm. Image processing was performed to segment all terminal bronchioles. The segmentations are used to extract cross-sectional images and to quantitatively assess airway wall thickness, cross-sectional area, branch length, curviness, and twistiness. Airway dimensions were compared to radiological CT patterns and to control lungs.

Results: A total of 140 targeted tissue samples have been scanned with microCT from 5 different radiological CT patterns. All segmentations for targeted samples have been completed with cross-sectional analysis pending.

Conclusion: Image segmentation is tedious. We will quantitatively assess morphological measurements of the airways and perform correlations with clinical radiological CT patterns.

Titin gene variants and modifiable cardiovascular risk factors contribute to the risk of atrial fibrillation

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Background: Variants in the cardiac structural gene titin (TTN) have been implicated in the risk of developing atrial fibrillation (AF). However, the effect of TTN variants on AF risk compared to established modifiable risk factors is unclear. The objective of this study was to evaluate the risk of AF and associated cardiovascular complications in TTN variant carriers and examine interactions between TTN variants and modifiable AF risk factors.

Methods: We used whole exome sequencing data and medical histories of 49,882 individuals from the UK Biobank study to examine the association of TTN variants and four risk factors (hypertension, diabetes, obesity, and smoking) with AF. Adjusted hazard ratios (aHR) were calculated using Cox proportional hazard models.

Results: TTN variants were associated with a higher risk of AF (aHR, 3.04 [95% CI, 2.23–4.14]; P = 1.95×10^{-12}). In participants that developed AF, TTN variants were associated with an increased risk of dilated cardiomyopathy (aHR, 12.37 [95% CI, 5.72–26.8]; P = 1.7×10^{-10}) and heart failure (aHR, 2.32 [95% CI, 1.35–3.97]; P = 2.27×10^{-3}) but not of stroke or death. We identified additive effects between TTN variants and hypertension, diabetes, obesity, and smoking on the risk of AF (log-rank trend tests P < 0.0001).

Conclusion: TTN variants and modifiable cardiovascular risk factors exhibited additive effects on the probability of developing AF. Our findings highlight the potential role for TTN sequencing to further inform AF risk stratification and aggressive management of modifiable cardiovascular risk factors.

Accuracy and Reliability of the BS-SNPer Bisulfite Sequencing SNP Calling Algorithm

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Background: DNA methylation is an epigenetic mechanism that plays an important role in regulating gene expression. Bisulfite sequencing (BS-Seq) is the gold standard for determining methylation patterns but is often cost prohibitive for large studies. Recently, more cost-effective targeted libraries designed to interrogate only the variable portion of the methylome have been introduced. It is unclear whether computational methods designed for whole-genome bisulfite sequencing (WGBS) can be utilized for targeted sequencing libraries without adaptations. For example, identifying single nucleotide polymorphisms (SNPs) using BS-Seq data is essential for the study of allele specific epigenetic events. Several BS-Seq specific SNP calling programs have been recently developed. However, SNP calling from BS-Seq data has been shown to be complicated and time consuming. In this project, we will utilize a SNP calling program: BS-SNPer and use BS-Seq data to call genotypes. We will also conduct a preliminary evaluation of the effectiveness and accuracy of the genotyping.

Objectives: The primary objective of this research is to implement BS-SNPer, which is a representative SNP calling program designed for WGBS to call genotypes. We aim to determine its accuracy and reliability of the algorithm when applied to targeted BS-Seq libraries.

Methods: Once genotypes were obtained by BS-SNPer using BS-Seq data, we conducted a concordance analysis of the genotype calls derived from subjects with samples obtained at multiple time-points: birth (cord-blood), 7 and 15 years of age. To evaluate the accuracy and reliability of the BS-Seq derived genotypes, we extracted the overlapped SNP sites and compared their genotype calls from samples with multiple time-points as these samples are biological duplicates. Additionally, we compared genotype calls from BS-SNPer to genotypes obtained from a genome-wide association study (GWAS) performed in 2009.

Results: The concordance rate was 98.5% for all subjects with multiple time-points (two and three time-points). We used the concordance rates across all samples to identify outliers. As a representative example, a child subject "Az1071-1" had samples from three time-points. The concordance rate between samples from year 7 and year 15 was 98.78%. However, the concordance rate between cord-blood and year 7 samples was much lower (77.24%) and a similar lower rate was observed between cord-blood and year 15 samples (77.14%), indicating a potential issue (plating or sample preparation errors) with the cord-blood sample. This observation was further confirmed by additional quality control checks such as gender, sample age and/or ethnicity.

Conclusion: We found that genotype correlations from samples with repeated measures had high concordance rates (98.5%) across multiple time-points. Furthermore, we demonstrated how concordance rates can be used in experimental quality control. These observations provided us with confidence in the accuracy and utility of genotypes derived from BS-Seq data. Future plans include combining genotypes derived from multiple studies (GWAS and BS-Seq) to develop methylation haplotypes.

*Andy Wang is a 4th year Data Science student at SFU and a Co-op student in Dr. Daley's lab.

Digitizing Clinical Data for a Lung Tissue Biobank Supporting Respiratory Research for Four Decades

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Background: The James Hogg Lung Registry (JHLR) is an established biobank of surgically-resected human lung tissue that has been providing samples to researchers since its inception 42 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 increase the efficiency and capabilities of the registry by translating the clinical paper records into a custom, encrypted database.

Methods: For all cases within the JHLR, lung tissue was collected from patients undergoing surgery as standard of care with informed consent. Sample data includes demographic data (age, sex, height, weight, ethnicity, co-morbidities, smoking history exposure and occupational exposure history) and clinical data (lung function, CT scans, pathology, radiology and blood reports). In this project, the sample data was verified using hardcopies of patient records and entered into a custom clinical demographic database (using Oracle® database management system software) that can be matched to the sample inventory database.

Results: The lung registry contains 2,041 cases and includes samples from a range of different lung diseases, including 38 asthma, 22 acute respiratory distress syndrome (ARDS), 461 chronic obstructive pulmonary disease (COPD), 17 cystic fibrosis, 116 interstitial lung disease, and 444 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. During this project, 1,200 lung function and blood data forms, including 72 data points, were reviewed, validated, and inputted into the database. In addition to the use of well-phenotyped tissue samples for research, the clinical data can now also be used as a resource. For example, when analyzing smoking history between the dates of 1980-2006, it was determined that, on average, the number of pack years smoked is greater for males (2.80×10^{-8}), and the age of smoking initiation is earlier in males compared to females (4.20×10^{-6}).

Conclusion: To ensure best practices within the lung registry, it is important that samples and clinical data points are curated efficiently and data is secured. The development and validation of the JHLR clinical data and sample repository within an encrypted database will ensure the functionality of the biobank for lung research for the years to come.

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

Visualizing heterogeneous cell clusters in single-cell analysis using Bioconductor in R

Checkervarty AK, Tebbutt SJ

Single-cell RNA-seq analysis is used to determine expression of genes in individual cells unlike the traditionally used bulk RNA-seq analysis which focuses on average gene expression throughout the population. The cell level resolution provided by single-cell analysis can be used to understand the heterogeneity of cells present in a sample. In this study, we explore the general steps for reading in the single-cell data files to forming clusters that enables the visualization of cell population heterogeneity using R studio. The datasets used in this study were generated from the Primary Human Bronchial Epithelial Cells (HBEpC) taken from two participants; one asthmatic and another non-asthmatic. Before performing the single-cell sequencing using the 10x genomics technology, these cells were grown in a sub-merged monolayer culture and incubated with the *Aspergillus Fumigatus* fungus conidia spores. The cells from each participant (biological replicate) were sequenced in two separate batches, generating two replicates for each individual i.e. four technical replicates in total. We used the "SingleCellExperiment" class provided by Bioconductor to perform the analysis. The data was processed by removing any damaged or low expression cells and normalized to remove any technical biases before further downstream analysis that leads to dimensionality reduction and clustering to visualize heterogeneous cells.

Keywords: single-cell, clustering, Bioconductor, RNA-seq, HBEpC