Poster Session One

Moderator: Matthias Görges
Participants: Chris Calvin  Jenny Chen
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Title: Alternatively processed forms of proinsulin and pro-islet amyloid polypeptide as markers of beta cell stress

Author(s): Chris Calvin, Jaques Courtade, Bruce Verchere

Abstract:
Introduction: Diabetes is characterized by reduced function and mass of insulin-producing beta cells, leading to disrupted blood glucose homeostasis. Processing of proinsulin, the insulin precursor, is defective in type 2 diabetes. Proinsulin is co-processed with another beta cell propeptide, pro-islet amyloid polypeptide (proIAPP); the mature peptides IAPP and insulin are then co-secreted from the beta cell. IAPP also undergoes post-translational modifications, including C-terminal amidation and O-linked glycosylation. We propose that changes in circulating levels of alternatively processed and post-translationally modified forms of proinsulin and/or proIAPP are markers of beta cell stress and impending diabetes.

Methods: Using enzyme-linked immunosorbent assays (ELISAs) that we have developed for amidated, glycosylated, and N-terminally unprocessed forms of human IAPP, we measured alternatively processed forms of this peptide in stressed beta cells. Islets isolated from mice with pancreatic beta cell expression of human proIAPP were treated with 0.01 or 0.1 µM thapsigargin, a potent inducer of ER stress, or the pro-inflammatory cytokine IL-1ß for 16 hours. Islet lysates and incubation media were tested for alternatively processed forms of proIAPP. qPCR was performed to assess expression of ER stress and beta-cell survival genes.

Results: We detected amidated and precursor IAPP forms in mouse islets expressing human proIAPP. Preliminary results indicate that incubating islets in thapsigargin for 16 hours did not yield significant changes in expression of ER stress-related genes or beta cell pro-survival genes. There was a trend for increased levels of amidated proIAPP over total proIAPP with increasing concentration of IL-1ß.

Conclusion: We have developed novel ELISAs to detect amidated, glycosylated, and N-terminally unprocessed forms of the beta-cell peptide proIAPP and our preliminary data suggest an effect of IL-1ß on the amidation of IAPP intermediates. These assays will be further adapted for use in human plasma as possible biomarkers of beta cell stress and the prediction of diabetes.
Abstract:
Hyperglycemia, the diagnostic feature of Type 1 and Type 2 Diabetes, inherently results from β-cell death and dysfunction. Understanding how β-cells respond to immune attacks and the complex transcriptional networks that regulate β-cell survival is crucial to diabetes prevention. To this end we have identified the transcription factor Myt3. Myt3 is highly expressed in mature pancreatic islets and is down regulated by cytokine. Several papers show that similar to the Myt3 down regulation, cytokine also down regulates cell adhesion genes and molecules.

Preliminary evidence suggests that Myt3 may regulate cell spreading and this project aims to verify this relationship. We want to determine if the loss of cell adhesion following cytokine exposure is due to the reduction of Myt3. We hypothesize that overexpression of Myt3 will prevent cytokine-induced loss of β-cell adhesion and spreading. To test our hypothesis, we will confirm Myt3’s involvement in spreading with the knockdown and then use the overexpression to see if spreading can be induced. We will also treat islets with cytokine to determine if the spreading observed in the previous experiments is negatively affected by cytokine exposure. We predict that Myt3 overexpression will prevent cytokine-induced loss of cell spreading. Furthermore, we hope to explore the mechanisms behind these effects by examining gene expression and protein levels for genes involved in cell adhesion. The conclusions made from this project will help us understand cytokine-induced cell spreading, apoptosis, and the function of Myt3 in these pathways. This research will ultimately lead to new and improved therapeutic options to treat and prevent diabetes.
Abstract:
Allogenic hematopoietic stem cell transplants (HSCT) aim to cure a patient of a disease, such as leukemia, by transplantation of blood-forming tissues like bone marrow. When a patient undergoes HSCT, a common complication arises called graft-versus-host disease (GVHD). GVHD is a potentially life-threatening condition that occurs when the effector T cells from the graft recognize the patient (host)’s organs as foreign and begins attacking the host. Through the past decade, regulatory CD4+CD25+FoxP3+ T Cells have been studied extensively for their ability to suppress many types of immune cells including B Cells, NK Cells, and effector T Cells.

Literature has shown that cell-therapy with regulatory T cells (Tregs) in combination with immunosuppressive therapy (IST) such as anti-thymocyte globulin in a murine model reduces the effects of GVHD but has minimal effect on a graft-versus-tumor effect. Currently, clinicians are unable to pinpoint exactly when IST should be withdrawn because it is difficult to distinguish immune-suppression from the development of tolerance. Because we suspect Tregs have a central role in mitigating graft tolerance, we seek to create a gene signature specific to Tregs in GVHD. We hypothesize that discovery of this gene signature will provide insights into the induction of graft tolerance and the function of Tregs in HSCT.

Our lab has previously developed a unique gene signature for regulatory T cells using Nanostring nCounter technology, a multiplexed quantitative mRNA assay that allows for accurate, high-throughput sample screening of hundreds of genes simultaneously. Using bioinformatics and statistical analysis, we hope to examine differences in the gene signature found in Tregs in GVHD patients and healthy donors to help identify potential biomarkers for patients who are at higher risk for GVHD.
Investigating the matrix localization signal of tyrosyl-DNA phosphodiesterase I (TDP1)

Lauren Fougner, Hok Khim Fam, Kunho Choi, Cornelius F Boerkoel

Tdp1 is a DNA repair protein that cleaves 3’tyrosyl-DNA overhangs or lesions caused by stalled topoisomerase I (TopI), phosphoglycolates or reactive oxygen species. Given our previous finding that the H493R mutation in Tdp1 causes spinocerebellar ataxia (SCAN1), and that SCAN1 patient tissue express Tdp1 in the cytoplasm; we determined the localization of Tdp1 in peripheral human tissues. Most tissues had a mixture of nuclear and mitochondrial localization. However, Tdp1 was almost exclusively localized in the mitochondria of skeletal muscle. Since skeletal muscle tissue cells produce more ATP and endure a higher oxidative stress burden than most other tissues, the mitochondrial DNA (mtDNA) of these cells are more prone to damage. To prove this, we have shown that Tdp1 migrates into the mitochondria of human fibroblasts in response to oxidative stress, and that the absence of Tdp1 causes an increase in mitochondrial DNA damage.

To investigate the mechanism of Tdp1 translocation into the mitochondria, we set out to determine the location of the matrix localization sequence (MLS) of Tdp1. This was accomplished by truncating the TDP1 gene using a set of primers and fusing the truncated section to a GFP tag. This was completed using TOPO® cloning followed by Gateway® cloning. The plasmids were then transfected into Tdp1 sufficient mouse embryonic fibroblasts (Tdp1 +/- MEF), human fibroblasts (HF) and Tdp1 deficient mouse embryonic fibroblasts (Tdp1-/- MEF). These cells were then treated with hydrogen peroxide, stained with a mitochondrial marker and imaged using fluorescence microscopy. We show that mitochondrial translocation is abolished in the Tdp1 construct lacking 295 amino acids at its C-terminal, but not with the construct lacking 158 amino acids at the C-terminal end, indicating that the MLS lies within this 137 amino acid region. To pinpoint the location of the signal, we will truncate portions of Tdp1 within this region. Once the exact site of the signal is located, we will confirm the ability of our MLS-GFP fusion to translocate into the mitochondria.
Abstract:
Human Respiratory Syncytial Virus (RSV) is the most frequent cause of acute lower respiratory illness in early life. The underlying reasons why some infants and young children are more susceptible to severe RSV infection than others are poorly understood. In this study we examined whether severe outcomes of RSV infection in early childhood are associated with common single nucleotide polymorphisms (SNPs) in genes coding for immune signaling components that have been implicated to play an important role in innate immune recognition and in the immunopathophysiology of RSV infection. Specifically, we examined common SNPs in the genes encoding RIG-I (rs17217280 and rs10813831), MDA-5 (rs1990760 and rs3747517), MAVS (rs17857295 and rs7269320), IL-4 (rs2243250), and IL-4Rα (rs1801275). We genotyped 140 children who required admission to BC Children’s Hospital due to severe RSV bronchiolitis (inpatients), 100 children who were visiting the BC Children’s Hospital Emergency Room and had a confirmed RSV infection but did not require hospitalization (outpatients), and a general population control group of 285 children. The genotype frequencies in the inpatient group were compared with that in the outpatient and general population control groups using Pearson Chi-Square tests (or Fisher Exact Probability tests for low genotype frequencies), applying dominant and additive models. In addition, allele frequencies were compared using Pearson Chi-Square tests. Neither of the models and tests revealed a statistically significant association between a particular genotype or allele frequency and hospitalization due to RSV bronchiolitis, except for a modest overrepresentation of the minor allele of the IL-4Rα SNP among the general population control group (P = 0.049; OR 0.69; 95%CI 0.480-0.999). These results indicate that the variants tested here do not play a significant role in the outcome of pediatric RSV infection.
The effects of a novel liposomal therapy on type I diabetes in non-obese diabetic mice

Abstract:
Type I diabetes (T1D) is an autoimmune disease with an increasing incidence that affects a vast number of Canadians. T1D is caused by the destruction of insulin-secreting pancreatic beta cells, key regulators of blood glucose levels. This destruction may be a result of impaired function of regulatory immune cells that protect the body from autoimmunity, such as regulatory T (Treg) cells and natural killer T (NKT) cells. Tregs can suppress autoimmunity in the pancreas upon presentation of insulin and NKT cells can be activated by the lipid antigen, alpha-galactosylceramide (a-GalCer). We believe that by encapsulating insulin and a-GalCer together in a liposome, antigen-presenting cells (APC) will phagocytose and present insulin to Tregs and a-GalCer to NKT cells thus stimulating their regulatory function. Previously, we demonstrated that subcutaneous administration of the liposomal therapy in the non-obese diabetic (NOD) mouse model prevents the onset of T1D (p=0.044) in comparison to other routes of administration. We hypothesize that subcutaneous injections of the liposomal therapy may alter the pharmacokinetics and pharmacodynamics of the therapy, which ultimately protects NOD mice from T1D compared to other routes of administration. We show after a single subcutaneous injection, there is a-GalCer presentation by macrophages and dendritic cells in the adipose tissue and spleen but not in the blood or pancreatic lymph nodes. Paradoxically, hematoxylin and eosin staining of pancreases show there is no significant difference in the average degree of insulitis between the subcutaneous and control injections (p=0.2746). We believe this may be due to the increased presence of Tregs in subcutaneously injected NOD mice, protecting the islets from cytotoxic lymphocytes; to confirm this, we will use fluorescent microscopy to identify the presence of Tregs in the islets. We believe this research will provide further rationale for developing liposomal therapies to effectively treat T1D and other autoimmune diseases.
Abstract:
Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infections in infants and young children, and also the leading cause of viral death in children under 5 years of age. Despite its global burden, there is still no licensed vaccine against RSV. The bacterium Listeria monocytogenes (Lm) has in recent years emerged as a promising neonatal vaccine vehicle, and attenuated strains of Lm have been developed that are both safe and potent. Lm vaccines generate a strong antigen-specific cytotoxic CD8 and Th1-type CD4 T cell response – precisely the type of immune response needed for protection against primary RSV infection. In this study, we aim to create a neonatal vaccine against RSV by utilizing Lm as a delivery platform for RSV antigens. Based on the presence of CD8+ and CD4+ T-cell epitopes, select regions of the F-, M-, M2- and N-antigens of RSV were cloned into our Lm vaccine vector. Potency of these Lm vaccine strains is defined as the capacity for antigen expression and secretion, as well as intracellular replication. These attributes are verified by western blot and in vitro macrophage infection assay, respectively. An Lm strain containing the RSV M2 antigen- has been generated, and potency has been confirmed by both assays. Strains expressing the other RSV antigens are in development. Following potency testing, these Lm-based RSV vaccines will progress to immunogenicity and efficacy for protecting against RSV infection. Success in this endeavor will be a significant step towards development of an RSV vaccine, which is a pressing need considering the millions hospitalized in addition to the hundreds of thousands of lives lost each year as a result of RSV infection and disease.
Abstract:
The chromatin structure is more than a packaging tool for DNA; it embodies modifications that are essential in regulating transcription, chromosome segregation, DNA replication and repair. One process chromatin can be altered is by the incorporation of histone variants, which replaces core histones within the nucleosome. H2A.Z is a highly conserved histone variant that occupies 5-10% nucleosomes and replaces the canonical histone H2A. Structurally H2A.Z and H2A are almost indistinguishable; however, their protein sequences are only 60% identical. Furthermore, H2A.Z cannot be complemented by H2A, highlighting the significant role H2A.Z has in the cell, including functions in gene regulation, chromosome segregation, and heterochromatin boundary formation. The complex that deposits H2A.Z into chromatin, SWR1-C, is exclusively associated with H2A.Z. Both H2A.Z and SWR1-C are highly conserved evolutionarily from yeast to human and have important implications for human disease. Human H2A.Z is associated with breast and colon cancer progression while human SWR1-C is mutated in a rare childhood disease called Floating-Harbor Syndrome. How H2A.Z is able to perform its unique functions has yet to be resolved. Here we set out to reveal specific amino acid regions that are contributing to the unique function of H2A.Z. To do this, we constructed hybrid histones of H2A.Z/H2A that contain specific regions of H2A.Z on an H2A backbone. The N-terminus, Loop 1, Loop 2, M6, and C-terminus regions of H2A.Z are investigated, as they possess the highest sequence dissimilarities between the two histones. It has been shown that the C-terminus of H2A.Z can be exchanged for H2A without impairing H2A.Z function, and the M6 region is required but not sufficient for H2A.Z functions. Therefore, we tested if the other regions of H2A.Z are contributing to H2A.Z function. A full hybrid containing the N-terminus, Loop 1, Loop 2, and the C-terminus illustrated a greater resistance to genotoxic stress but surprisingly not as well as wild type H2A.Z. All hybrids were able to bind chromatin similar to wild type, which implies these regions are contributing to a partially functional H2A.Z and that additional amino acids of H2A.Z are responsible for overall function.
Abstract:
Type 2 diabetes is a chronic hyperglycemic condition caused by the failure and death of pancreatic beta-cells, and it is often associated with obesity and sedentary lifestyles. Previous studies in our lab suggested that both apoptotic and non-apoptotic cell death mechanisms contribute to beta-cell loss in type 2 diabetes. This project followed up with the study of the specific mechanisms of pancreatic beta-cell death under glucolipotoxic conditions. Our lab has generated a mouse model with genetic deletions of pro-apoptotic proteins Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist/killer (Bak) for the study of beta-cell death. By culturing dispersed wild type and Bax/Bak double knockout (BaxBakDKO) pancreatic islet cells in 25mM glucose and 1.5mM palmitate with or without zVAD-fmk and necrostatin-1, we found that the elimination of Bax and Bak provided some protection against glucolipotoxicity-induced cell death, but the use of zVAD-fmk, which inhibits caspases and thus apoptosis, increased cell death in both genotypes. Necrostatin-1, which blocks the necroptotic pathway, seemed to protect wild type cells but not BaxBakDKO. These results suggest that necroptosis may contribute to beta-cell loss under nutrient stress when Bax and Bak mediated apoptosis is permitted, and that the inhibition of apoptosis by blocking caspase actions may potentiate caspase-independent cell death under nutrient stress. Identification of the different mechanisms by which pancreatic beta-cells die may reveal putative pharmacological targets for preventative treatments of type 2 diabetes.
Abstract:

Noonan syndrome is an autosomal dominant congenital disorder that affects 1 in 2000 newborns. Previous literature has determined the major cause of Noonan syndrome to be a missense mutation in the PTPN11 gene. This gene encodes for the SHP-2 protein tyrosine kinase that plays an important role in the signal transduction of the RAS pathway. The RAS pathway functions to transduce signals from the extracellular region to the cell nucleus where specific genes are activated for cell growth, division, and differentiation. Our research focuses on a family where a different mutation in a RAS pathway gene is the cause of Noonan syndrome. We hypothesize that the affected siblings have inherited a rare genetic mutation from the father. Three of the four children as well as the father have been diagnosed with Noonan syndrome; however, previous genetic testing has shown they do not have a mutation in the common Noonan syndrome genes. We have used exome sequencing to determine common mutations in the exons of the affected father and one of the affected children. We have found a total of 148 common mutations, which can be narrowed down to candidate genes specific to the RAS pathway. Furthermore, we have used SIFT and Polyphen libraries to examine the damaging effects of the candidate genes. This allows us to further narrow down the list of genes to identify damaging mutations that are of interest. Subsequently, we use PCR and Sanger sequencing to confirm the mutation of the candidate genes. Upon identification of the harmful mutations, we believe this research can provide further knowledge of contributing factors to the development of Noonan syndrome.
Student: Steven Zbarsky
Board #: 11
Session #: 1
Supervisor: Bruce Vallance
Title: Exploring the role of tricellulin in the pathogenesis of EPEC infection
Author(s): Steven Zbarsky, Vijay Morampudi, Bruce Vallance

Abstract:
One of the most important functions of the epithelial cell layer is to serve as a physical barrier, allowing for the establishment of distinct compartments within a complex organism. This barrier function is mediated largely by proteins that link cells to adjacent cells or to components of the extracellular matrix (ECM). Tight junctions (TJs), one class of intercellular interactions, are able to seal cells closely together thereby preventing the intercellular leakage of solutes. A group of proteins called claudins have been identified as key players in bicellular tight junctions, however less is known about the molecular interactions at the junction of three cells, such as in tricellular tight junctions (tTJs). In 2005, a transmembrane protein, later named tricellulin, was identified as being concentrated at tTJs, suggesting a role in maintaining tJ integrity. In this present investigation, murine intestinal epithelial cells (CACO2) were transfected with an expression vector encoding the tricellulin gene, and compared to the wild-type (WT) cell line in an in-vitro assay of epithelial barrier integrity. A parameter known as transepithelial electrical resistance (TEER) was measured, and upon infection of the murine cells with enteropathogenic Escherichia coli (EPEC), we observed a reduction in TEER in WT cells at 4h post infection whereas tricellulin transfected cells maintained TEER values throughout the course of infection. Consistent with low levels of TEER in WT cells, we observed a correspondent increase in fluorescein isothiocyanate absorbance (FITC) levels (a marker for barrier leakage) and decreased tricellulin protein expression. To further investigate possible EPEC-tricellulin interactions, EPEC strains were generated lacking specific genes involved in the type III secretion system (T3SS), a structure crucial to EPEC pathogenesis. We found that EPEC lacking the ESPG gene were able to maintain TEER and FITC levels to the levels observed in uninfected CACO2 cells, suggesting a possible role of this gene in interacting with tricellulin during pathogenesis. Overall, our results indicate a promising role of tricellulin as a key protein in maintaining epithelial barrier integrity during EPEC infections, and highlight a potential therapeutic approach for the treatment of gastrointestinal diseases reliant on a functioning epithelial barrier.
Poster Session Two

Moderator: Niels Skotte

Participants:
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In eukaryotic cells, gene expression is intimately linked with the remodeling of over lying chromatin. One form of chromatin remodeling is the post-translational modification of histone proteins. For example, histone H3 is methylated at lysine 4, 36 and 79 (H3K4me, H3K36me and H3K79me), by the histone methyltransferases Set1, Set2 and Dot1 respectively. These enzymes are recruited to chromatin and form distinct patterns along protein-coding regions. Whole genome mapping of these marks have suggested a transcription-activating role for H3K4me and H3K36me and an inhibitory role for H3K79me. Despite these correlations, recent mRNA microarray studies revealed that loss of SET1, SET2, and DOT1 activities resulted in minimal changes to transcript levels in the cell. Here we investigate the role of these histone modifications and how they affect gene expression. By genetically fusing Set1, Set2 or Dot1 to RNAP, in budding yeast, we have created a gain-of-function approach, targeting these marks to novel regions. Our fusion constructs of Set2 and Dot1 are functional, as H3K36me and H3K79me are readily detected in strains having the fusion proteins as their only source of enzymatic activity. Genome-wide mapping of H3K79me in yeast strains containing the Dot1-RNAP fusion protein revealed marked levels of this modification at highly transcribed genes, regions which previously lacked this mark. Having characterized the novel H3K79me patterns, we will determine if regions with altered methylation have altered expression patterns. Ultimately, this approach will allow us to assay directly the functional consequence of these marks on transcription.
Abstract:

**Background:** We have recently characterized a novel deleterious mutation in MALT1, a paracaspase essential for canonical NF-κB signaling, in a 15-year old girl presenting with a combined immunodeficiency. Nothing is known about the effect of this mutation on the development of B cells. It has previously been shown in murine models that MALT1 is essential for B cell activating factor (BAFF) signaling through specific interaction with its principal receptor, BAFF-receptor (BAFF-R). BAFF plays an essential role in the normal development of B cells. The first signaling step of BAFF-R ligation is the degradation of TRAF3 leading to non-canonical NF-κB signaling through subunit p100. The inherited absence of either BAFF or BAFF-R leads to a profound arrest in B cell development at the transitional stage.

**Hypothesis:** The absence of MALT1 function in our patient results in a B cell phenotype that resembles inherited BAFF and BAFF-R deficiencies.

**Methods:** We evaluated both primary B cells and EBV immortalized B cells from our affected patient and siblings/parents who are heterozygous for mutation and clinically unaffected. B cell immunophenotyping and analysis of BAFF signaling was determined by flow cytometric analysis and Western blotting.

**Results:** B cell immunophenotyping demonstrated a profound arrest in B cell development at the transitional stage and absence of mature B cells. There was also a significant down-regulation in surface expression of BAFF-R. We are currently evaluating BAFF-signaling by investigating TRAF3 degradation and p100 phosphorylation.

**Conclusion:** Our patients B cell profile supports the role for MALT1 in BAFF-mediated B cell development.
Abstract:
Mutations in the Nuclear receptor binding SET Domain protein 1 (NSD1) have been shown to result in Sotos syndrome, an overgrowth disorder that affects children. This syndrome is characterized by distinctive facial features, developmental delay and learning disabilities. NSD1 is a methyltransferase involved in development and it is known to play a role in chromatin remodeling. Mutations in NSD1 have been linked to increased risk of developing childhood cancer, as well as heart, lung and urogenital anomalies.

The phenotype of individuals affected with Sotos syndrome overlaps with other overgrowth disorders, especially Weaver syndrome. Weaver syndrome results from mutations in Enhancer of Zeste Homolog 2 (EZH2).

We have a cohort of 21 patients with features of overgrowth that have tested negative for EZH2 mutations. The goal of this study is to develop a research-based diagnostic test for NSD1 in order to identify if any of the patients in our cohort have Sotos syndrome. We performed PCR on patient samples to amplify exons 8 to 23 of NSD1 followed by Sanger sequencing to identify variants within the exons. Thus far we have developed our PCR protocol and are currently Sanger sequencing patient DNA samples.

Future project directions include performing a more thorough characterization of both genes, NSD1 and EZH2, using functional studies. This will allow for a better understanding of the pathways involved, with the goal of generating a stronger genotype-phenotype correlation. Ultimately this will help to better diagnose and treat those affected with overgrowth syndromes.
Student:  Cyrus Chehroudi
Board #:  15
Session #:  2
Supervisor:  Bruce Verchere
Title:  Blockade of IL-1 signalling improves islet amyloid-induced glucose intolerance and prevents early amyloid formation
Author(s):  Cyrus Chehroudi, Clara Westwell-Roper, Janice Pang, Bruce Verchere

Abstract:
Introduction: Islet amyloid polypeptide (IAPP) is co-secreted with insulin by beta cells. Aggregation of IAPP to form amyloid contributes to beta cell dysfunction in patients with type 2 diabetes, a condition also characterized by elevated islet expression of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) that impair the processing and secretion of insulin. Because proIAPP processing occurs in parallel with that of proinsulin and accumulation of partially processed proIAPP may contribute to its aggregation, we sought to determine the effects of IL-1 on glucose homeostasis, islet amyloid formation, and proIAPP processing.

Methods: Wild-type or transgenic mice with beta cell expression of human IAPP (hIAPP^Tg/o, either lean or obese due to A^vy expression at the agouti locus) were injected daily with IL-1 receptor antagonist (IL-1Ra, 50 mg/kg) or PBS for 8 weeks starting at 16 weeks of age. Glucose tolerance was assessed by intraperitoneal glucose tolerance test and islet amyloid prevalence was determined using thioflavin-S. MIN6 beta cells were incubated with recombinant IL-1β and IAPP was detected by western blot. mRNA expression in MIN6 cells was measured by qRT-PCR.

Results: IL-1Ra significantly improved glucose tolerance in both lean and obese hIAPP^Tg/o mice (lean: AUC=710±180 vs. 1150±450; obese: AUC=1360±110 vs. 880±70; p<0.05) and reduced amyloid prevalence in lean (35% vs. 2%; p<0.05) but not obese mice, suggesting that IL-1 signalling may be important in modulating early amyloid formation. Treatment of MIN6 cells with 100 pg/ml IL-1β for 24 h induced expression of the chemokines Ccl2 and Cxcl1 but had no effect on expression of Ins2, IAPP, or the genes encoding the prohormone processing enzymes PC1/3 or PC2. IL-1β impaired proIAPP processing as determined by a 50% reduction in the ratio of mature to total IAPP. IL-1β also caused a dose-dependent decrease in the proportion of active PC2 protein relative to total PC2, suggesting a possible mechanism for impaired processing of proIAPP.

Conclusions: Our data suggest that IL-1 mediates IAPP-induced islet dysfunction and promotes early amyloid formation by a mechanism that may involve impaired IAPP processing. Anti-IL-1 therapies may improve islet function in type 2 diabetes in part by reducing amyloid formation.
Abstract:
Oligodendrocyte progenitor cells (OPCs) differentiation into oligodendrocytes (OLs) is important for myelination of cells in the central nervous system. Dysregulation of this process has been implicated in many neurological disorders, including multiple sclerosis. Recent findings have demonstrated that two independent pathways involving Wnt and PTPα differentially regulate OL formation. Wnt signaling has been identified as an inhibitor of OPC differentiation. Wnt signaling is initiated upon binding of a Wnt ligand to Wnt receptors which prevents degradation of β-catenin and allows β-catenin to migrate to the nucleus and attach to TCF/LEF transcription factors. The binding of TCF/β-catenin complexes to specific sites in the promoter leads to the expression of Wnt targeted genes. In contrast to Wnt signaling, our lab has shown that protein tyrosine phosphotase alpha (PTPα) promotes OPC differentiation. Although the individual roles of PTPα and Wnt signaling in OPC differentiation have been studied independently, no studies have looked at the interactive effects of these regulatory pathways. This study aims to examine the role of PTPα in Wnt signaling by detecting changes in Wnt-mediated gene transcription.

To investigate the effect of PTPα on Wnt signaling in vitro, a rat CG4 oligodendrocyte cell line was used to understand the underlying signaling processes that occur during differentiation. Work in our lab has shown that β-catenin levels tend to decrease while PTPα levels increase when OPCs differentiate. To study the extent of Wnt activation, we have also obtained and amplified reporter plasmids TOPFlash and FOPFlash. TOPFlash contains three repeats of the β-catenin/TCF binding sites driving expression of a firefly luciferase gene. FOPFlash contains mutant constructs of those same binding sites, thereby serving as the negative control. Luciferase expression emits light that can be subsequently detected and quantified. Current efforts are focused on transfecting TOPFlash and FOPFlash into wild type and PTPα knockout mouse embryonic fibroblasts. Once optimized, this assay can be used to assess the effect of PTPα on Wnt signaling and vice versa.
Abstract:
Type 1 diabetes (T1D) results from the autoimmune destruction of insulin producing beta-cells (β-cells). A critical component in the initiation of autoimmune mediated attack on β-cells, is the increased expression of pro-inflammatory genes in such cells resulting from elevated immune system facilitated cytokine secretion. In response to such events, β-cells secrete cytokines of their own, recruiting additional immune cell types, resulting in near complete β-cell loss through increased inflammation and apoptosis. Chromatin state alterations at regulatory regions within the genome are likely responsible for the cytokine-induced expression of these pro-inflammatory genes. Therefore, better understanding the chromatin state changes that promote increased pro-inflammatory gene expression may allow for the identification of chromatin modifying enzymes as novel therapeutic targets to help prevent T1D.

Preliminary work in the Hoffman Lab has identified the increased expression of pro-inflammatory genes, such as Ccl2, Ccl20, and Cxcl10, upon the treatment of islets with a cytokine mixture (IFN-γ, TNF-α and IL-1β). Through chromatin immunoprecipitation (ChIP) analysis, regulatory loci of such pro-inflammatory genes display H3K4me1 enrichment upon cytokine exposure. Additionally, loci displaying H3K4me1 enrichment, signifying an active chromatin state, tend to be associated with the loss of H3K27me3 mediated repression. Furthermore, such loci also appear to be enriched for transcription factors not regularly expressed within islet cells. Such a pattern displays the ability of cytokines to alter chromatin structure from a repressive nature, to an activated state.

As the bulk of such preliminary data was produced using islets, which are heterogeneous in cell composition, validation of results must now occur in β-cells. The purpose of my study is to examine whether the increased expression of pro-inflammatory genes, and identified H3K4me1 and H3K27me3 changes at regulatory regions, truly occurs within β-cells. I will also be assessing if other types of cell stress, such as oxidative stress, appear to mimic epigenetic and gene expression alterations seen with cytokine exposure. I will then investigate the utility of repressing chromatin remodeling factors to prevent cytokine induced alterations in pro-inflammatory gene expression, while identifying other possible concurrent chromatin remodeling events (ex. H4ac and H3K27ac), which may promote β-cell death. Such research will help elucidate therapeutic targets involved in epigenetic modifications to improve β-cell transplantation success, alongside functional β-cell mass in T1D patients.
Abstract:
Alzheimer’s disease (AD) is a progressive neurodegenerative disease and the most common form of senile dementia. The accumulation of neurotoxic molecules such as amyloid beta and hyperphosphorylated tau in the brain parenchyma has traditionally been the focus of research in AD pathology. However, increasing evidence suggests that cerebrovascular dysfunction precedes and contributes to neuronal dysfunction and subsequent neurodegeneration. Blood vessels constantly deliver oxygen, essential nutrients and energy metabolites to neurons and promote the clearance of neurotoxic molecules such as amyloid beta. The blood-brain barrier (BBB), consisting of tight junction-bound endothelial cells, restricts free passage of plasma solutes and provides selective permeability to the brain. Physical breakdown of this important interface leads to leakage of serum proteins and dysregulation of transport into and out of the brain, causing a cascade of problems in the neurovascular milieu including reduced blood flow, neurodegeneration, inflammation, and disrupted clearance of amyloid beta. As part of a larger project, our group is investigating the therapeutic potential of a recombinant protein for AD, with the first step being a biodistribution study in wild-type mice. As the recombinant protein is of bacterial origin, the risk of endotoxin (LPS)-induced damage to the BBB warranted a detailed assessment of cerebrovascular integrity in treated animals. In this study, we therefore explored histological methods to detect potential blood-brain barrier damage using exogenous and endogenous markers. To visualize leakage of serum proteins across the BBB in mice, we performed injections with Evans blue, an exogenous dye with high affinity to serum albumin, and immunohistochemical stains against endogenous serum IgG. Both Evans blue and immunohistochemistry results showed no indications of serum protein extravasation in the brain parenchyma, suggesting there was no acute neurovascular damage associated with our potential recombinant protein therapeutic.
Abstract:

Cystic Fibrosis (CF) is the most prevalent fatal genetic disease affecting children in Canada. CF is caused by a mutation in the gene coding for the CFTR protein, which regulates sodium and chloride ion movement across epithelium. One symptom of CF is a large buildup of mucous in the lungs. This makes CF patients extremely susceptible to recurrent infections by opportunistic pathogens; the chronic inflammation from these infections eventually results in irreparable damage to lung tissue. One of the most feared pathogens among Cystic Fibrosis patients is the Burkholderia Cepacia Complex (BCC). BCC infections are notoriously difficult to treat due to their intrinsic resistance to antibiotics, and are associated with a high mortality rate.

It has been observed that BCC isolates are either mucoid (producing vast amounts of polysaccharides, resulting in a plate with a “slimy” appearance) or non-mucoid; furthermore, it has recently been shown that infections with a non-mucoid morphology are most often far more virulent than mucoid infections. This increase in virulence is not fully understood; we propose that one factor accounting for this increased aggression is the establishment of more biofilms at the site of infection by non-mucoid bacteria.

Our goal was to characterize the relationship between mucoidy and the quantity of biofilm production in an experimental strain panel of BCC isolates. To do so, 96-well plates were inoculated with the chosen isolates and incubated for 48hrs. Growth was measured, and the plates were stained with crystal violet, which was then solubilized with ethanol. The plate was finally read for amount of CV per well, and normalized to growth. The reading gathered for biofilm formation was then compared to each isolate's mucoidy phenotype. Thus far, data has not shown a consistent relationship between mucoidy and biofilm formation.
Altered glial cell function in the YAC128 mouse model of huntington’s disease

Huntington’s disease (HD) is a neurodegenerative disorder which is characterized by the loss of medium spiny neurons in the striatum. The YAC128 mouse model of HD expresses mutant huntingtin protein in all cells. There are three major cell types in the brain, neurons, microglia and astrocytes. Microglia are immune cells in the central nervous system which secrete growth factors and cytokines to regulate the growth and survival of cells in the brain. Astrocytes, another type of glial cell, also secrete neurotrophins and proteins such as the brain derived neurotrophic factor (BDNF) to protect neurons. The two cell types, microglia and astrocytes, interact together to maintain homeostasis in the brain. In this study, I will investigate the effect of mutant huntingtin expression on the function of both microglia and astrocytes. Previously, our lab has demonstrated increased cytokine secretions from both cell types in response to a pro-inflammatory stimulus (LPS). Altered neuro-immune interactions may play a role in neurodegeneration in HD. My primary aim is to assess cytokine and trophic factor production in primary microglia and astrocytes from YAC128 mice. Using control endotoxin standard (CSE) stimulated astrocytes and microglia derived from the YAC128 mouse model, transcript and extracellular levels of BDNF and various cytokines were quantified. Real-time PCR results indicate significant dysregulation of cytokine transcript levels at an early time point in both cell types. The differential changes in cytokine levels may lead to feedback mechanisms which may further contribute to HD pathogenesis.
SIGIRR (single immunoglobulin IL-1R-related receptor) is a negative regulator of the toll-like receptor (TLR) signaling pathways and plays a critical role in regulating the induction of the innate immune response to intestinal enteric pathogens. However, the roles of SIGIRR in host defense mechanisms and TLR responses are not yet fully understood. Campylobacter jejuni is the leading cause of human gastroenteritis in the world, however it does not pathogenically colonize common animal model organism such as mice. Our objective was to validate the use of SIGIRR-deficient mice as a model of C. jejuni infection, and to employ this model to further characterize the role of TLRs and SIGIRR in C. jejuni colonization and the host response to infection.

C57BL/6 wild type (B6) and the knockout strains (SIGIRR-/-, TLR2-/- TLR2/SIGIRR -/-, TLR4-/-, TLR4/SIGIRR -/-) were subjected to infection with C. jejuni over the course of 1 week. The bacterial burden of these mice was determined three and seven days post infection. Quantitative PCR was used to quantify the relative expression of the pro-inflammatory cytokines: IL-1β, IL-6, TNFα, KC-1, IL-17, IL-22, while microscopy of fixed intestinal tissues was used to determine the extent of inflammation in the infected mice. We found (1) that mice lacking SIGIRR suffered from significantly increased intestinal inflammation when compared to WT and single TLR knockout mice; (2) TLR4/- mice demonstrated delayed colonization and lower overall inflammation and (3) TLR2/- and TLR2/SIGIRR-/- mice exhibited severe inflammation and a heightened Th17 response during C. jejuni colonization.

These data provide strong evidence that SIGIRR-/- mice can serve as a murine model for pathogenic C. jejuni infection and that TLRs serve as the primary mediators for the induction of inflammation during the course of C. jejuni infection. Our findings demonstrate that TLR2 drives mucosal integrity, protection from injury, and pathogen clearance. In contrast, TLR4 appears to be driving excessive, damaging inflammation, confinement of infection, and microbicidal activity, which is likely directed from a Th1 or Th17 polarized immune response. Understanding the differential roles of TLR signaling, and how they integrate to generate a host immune response will aid in further research toward better treatment strategies for gastrointestinal infections.
Vac14 is a novel regulator of protein transport to the yeast vacuole

Abstract:
Phosphoinositides are intracellular markers that play a functional role in regulating organelle identity. The unique phosphoinositide found on lysosomes, PI(3,5)P2, is crucial for maintaining proper lysosomal morphology and acidification. Mutations in proteins that regulate PI(3,5)P2 synthesis, including the PI 5-kinase, Fab1, and its activators Vac14 and Fig4, have been linked to neurodegeneration in mouse, and to human diseases including Amyotrophic Lateral Sclerosis and Charcot-Marie-Tooth disease. Both diseases are neuropathies characterized by atrophy and progressive muscle weakness. However, the upstream regulatory pathway that activates Vac14/Fig4 has not been identified, and the downstream targets of PI(3,5)P2 relevant for neurodegenerative disease are not known.

Using a genome-wide screen, we have uncovered a role for Vac14 in the AP-3 transport pathway, which is highly conserved from yeast to humans and is important in delivering cargo to the yeast vacuole and lysosome, respectively. The screen also implicated a number of kinases and phosphatases in the regulation of AP-3 transport. We hypothesize that Vac14 requires phosphorylation in order to regulate the AP-3 pathway, and thus some of these kinases and phosphatases may be upstream regulators of Vac14.

The first aim of this project was to confirm the role of Vac14 in the AP-3 pathway. The AP-3 pathway was assessed using the reporter, GNSS (GFP-nyv1-snc1-suc2), which localizes to the vacuolar membrane only if the AP-3 pathway is functional. A defect in the AP-3 pathway results in GNSS being rerouted to the plasma membrane. The localization of GNSS was detected using live cell fluorescence microscopy. Vac14, as well as Vac7, are both enzyme regulators of PI(3,5)P2 synthesis in yeast and defects in either lead to abnormal vacuolar morphology and acidification defects. However, our results showed that only Vac14 was important for AP-3 dependent transport.

We are currently examining the role of kinases and phosphatases in regulating AP-3 transport and Vac14 function. Since the misregulation of PI(3,5)P2 is linked to human neuropathies, investigating and better understanding the mechanistic nature of this pathway could lead to the discovery of novel targets for future therapies.
Abstract:

**Background:** Schimke immuno-osseous dysplasia (SIOD) is a rare autosomal recessive disorder characterized by immunodeficiency, renal disease, and bone dysplasia. The cause of SIOD is a loss-of-function mutation in the SMARCAL1 gene that encodes a chromatin remodeling enzyme. However, the specific mechanisms by which SMARCAL1 deficiency leads to SIOD remain undefined.

Among other symptoms, this pleiotropic disorder is marked by arteriosclerosis and emphysema. We have found reduced elastin (ELN) expression and fragmentation and splitting of elastin fibres in SIOD aorta. Elastin is a structural protein required for arterial integrity. This suggests that impaired elastogenesis is the cause of the vascular and pulmonary pathology associated with SIOD.

**Objective:** In this study, we investigate DNA methylation and transcription factors as possible developmental or pathological mechanisms in reduced elastin expression. Specifically, we studied the effects of ELN promoter methylation and expression of transcriptional regulators on elastin expression across development and in SIOD patients. Since ELN is highly expressed in human fetal aorta and decreases with age, we hypothesized that ELN promoter methylation increases and expression of transcriptional regulators alters with developmental age. We also hypothesized that ELN promoter methylation is increased in SIOD patients.

**Methods:** We quantified DNA methylation using bisulfite pyrosequencing and analyzed RNA expression using quantitative PCR in a developmental series of human aorta samples (n=23 and n=19, respectively), and two SIOD patient aorta samples for the former. For statistical analysis, we used the Spearman’s Rho test, the Mann-Whitney U-test, and Kruskal-Wallis nonparametric ANOVA.

**Results:** We found a significant increase in ELN promoter methylation in SIOD patient aorta compared to age-matched controls, but no trend across development. We confirmed that ELN expression is correlated with age. Further, transcriptional regulators analyzed showed varied patterns of expression across development. In particular, positive regulator transforming growth factor β-1 (TGFB1) was positively correlated with ELN expression.

**Conclusions:** Methylation of the ELN promoter is not correlated with reduced elastin expression during normal development. However, it may be a pathological mechanism contributing to the decreased elastin expression in SIOD. Further, transcription factors may play a role in elastin expression.
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Student: Muhamed Amirie

Board #: 24

Session #: 3

Supervisor: Michael Kobor

Title: Understanding the role of chromatin remodeling complexes in the DNA damage response of *Saccharomyces cerevisiae*

Author(s): Muhamed Amirie, Phoebe Lu, Michael S Kobor

Abstract:
Precise and efficient repair of DNA lesions via the DNA damage response (DDR) is essential to the cell’s ability to maintain genomic stability. Access of the DNA repair machinery to sites of DNA lesions is dependent on the local chromatin conformation, as determined in part by the activities of chromatin remodeling complexes. Defects in the DDR have been associated with various cancers including lymphomas, head and neck carcinomas, and breast cancer, making a thorough understanding of chromatin remodeling activities invaluable to the study of cancer biology.

For this project we use *Saccharomyces cerevisiae* as a model organism to study the DDR, in which chromatin remodeling is mediated by the SWR1-C and NuA4 complexes. In humans, the TIP60 complex represents a functional fusion of SWR1-C and NuA4, and regulates many of the same processes. Previous studies have shown that the activity of both SWR1-C and NuA4 at the site of DNA damage initiates chromatin reorganization around the lesion, which ultimately results in a structure accessible to the DNA repair machinery. In *S. cerevisiae*, SWR1-C and NuA4 share a functional module composed of four subunits (Yaf9, Swc4, Arp4, and Act1) that may play a crucial role in their coordinated activities during the DDR. Preliminary data suggest that Yaf9 may be required for the recruitment of the two complexes to sites of DNA damage. To explore the role of the shared module in the recruitment of SWR1-C and NuA4, we use a galactose-inducible system to create a single double-stranded break in the yeast genome, at which we are able to assay for the levels of the two chromatin remodeling complexes using chromatin immunoprecipitation (ChIP) and qPCR. To accomplish this, we have tagged various subunits of SWR1-C and NuA4 with specific epitopes, to which we can use antibodies for targeted immunoprecipitation. Comparison of the relative amount of SWR1-C and NuA4 in wild-type cells versus cells harbouring mutations in the shared module around the break site will give valuable insight to the role of the shared module in chromatin remodeling, and ultimately contribute to our understanding of the DNA damage response.
Student: Daniel Choi

Board #: 25

Session #: 3

Supervisor: Stefan Taubert

Title: Investigating the role of a conserved transcriptional co-activator in organelle homeostasis

Author(s): Daniel Y Choi, Nicole S Hou, Stefan Taubert

Abstract:
The maintenance of organelle homeostasis is critical to animal and human health. Perturbation of the endoplasmic reticulum (ER), for example, is implicated in the pathogenesis of diseases such as cancer, diabetes and obesity. Our lab recently found that inactivation of mdt-15, a conserved transcriptional co-activator, causes chronic ER stress in the nematode worm C. elegans. As mdt-15 plays a central role in the transcriptional regulation of lipid metabolism, and lipids are fundamental for organelle integrity and linked to ER homeostasis, I hypothesize that mdt-15 is required for ER homeostasis through its impact on lipid metabolism. To validate that mdt-15 maintains ER homeostasis, I crossed an mdt-15 hypomorphic mutant worm strain with a strain carrying a GFP reporter that indicates ER stress. In agreement with my hypothesis, mdt-15 mutants strongly upregulate the GFP reporter compared to wild-type worms. Using immunoblot analysis, I also found that the mdt-15 mutation increases the expression of another ER stress marker, phospho-eIF2α. Interestingly, dietary supplementation with unsaturated fatty acids substantially but incompletely suppresses the ER stress in mdt-15 hypomorph worms, suggesting that mdt-15 maintains ER homeostasis through regulating unsaturated fatty acid biosynthesis and through other, unidentified pathways. Finally, using markers indicating protein homeostasis in the mitochondria, another protein-processing organelle, I found that mdt-15’s role in organelle homeostasis is ER specific, as the mdt-15 hypomorph mutation does not disrupt mitochondrial protein homeostasis. In conclusion, mdt-15 is essential specifically to maintain ER homeostasis through regulating fatty acid composition and other unknown processes. These new insights into a conserved transcriptional co-activator and ER homeostasis add new evidence to recent findings that lipid metabolism is tightly linked to the ER and therefore expand our knowledge on the pathogenesis of various metabolic diseases.
Identification of potential melanoma vaccine epitopes using current screening methods is both inefficient and costly. A novel method to enhance the specificity for epitope screening is through the use of CD8+ T-cells from mice induced with melanoma and then treated with chemo-immunotherapy. We hypothesized that these in vivo generated T-cells are able to recognize melanoma protein epitopes and would become activated by an effective peptide, and can hence be used as a screening tool for potential peptide vaccines.

Activated T-cells can then be detected using an ELISPOT, an assay that allows the detection of low frequencies of cells secreting IFNg. Based on the same "sandwich" immunochemical principles as enzyme-linked immunosorbent assay, ELISPOT is easy to perform and quantify the results. At the same time ELISPOT remains a state-of-the-art technique that requires procedural refinements before widespread use as a screening tool for melanoma vaccines.

Designing and troubleshooting an ELISPOT procedure for clear results and well-defined spots required test experiments to determine the concentrations of reagents (capture antibody, detect antibody, alkaline phosphatase) and cell number to use. Using splenocytes from mice expressing a T cell receptor specific to ovalbumin (OVA), and the OVA peptide (SIINFEKL) as the stimulant, the optimal conditions for detecting IFNg expressing cells were determined. A melanoma cell line genetically engineered to express the OVA protein will be used to induce melanoma in mice. Using splenocytes from chemo-immunotherapy treated melanoma mice as a screening tool, we are currently optimizing the conditions to detect stimuli that can activate melanoma-specific cytotoxic T cells found in the splenic population, with the positive control being irradiated melanoma cells and the OVA peptide.

Following the determination of optimal assay conditions, the actual experiment will involve using the native melanoma cell line (B16) and screening for melanoma peptides derived from proteins that are overexpressed in melanoma cells but not in benign nevi.
Title: Assessing the efficacy of a potential Alzheimer’s disease vaccine therapeutic in the APP/PS1 mouse model

Abstract:
Alzheimer’s disease (AD) is a neurodegenerative disorder that affects 500,000 Canadians every year and is predicted to double its prevalence with every generation (Health Canada, 2012). AD pathology includes severe gray matter atrophy throughout the brain, with prominent losses in the cortical and hippocampal regions. The defining neuropathological hallmarks of AD include neurofibrillary tangles and amyloid β (Aβ) plaque deposition. We hypothesize that plaque formation is seeded by the presence of toxic Aβ oligomers that act to nucleate growth of Aβ fibrils and plaques. Recently, we identified a unique tri-peptide epitope present exclusively on Aβ oligomers, and designed a cyclical peptide to mimic its structure, which we call cSNK. A mouse monoclonal antibody raised against cSNK, called 5E3, was found to specifically recognize oligomeric Aβ, but not its monomeric or fibrillar counterparts. Preliminary studies also indicate that 5E3 antibody blocks the neurotoxicity of Aβ oligomers in neuronal cell cultures, thus making 5E3 and its corresponding peptide strong candidates for therapeutics against AD.

In this study, we will treat the APP/PS1 transgenic mouse model of AD prior to disease onset with either 5E3 antibody or cSNK peptide to elicit passive and active immune responses, respectively, followed by behavioural and neuropathological analysis at 12 months of age. Mice undergoing passive immunization will receive weekly infusions of 5E3 beginning at 12 weeks of age. Animals undergoing active immunization will receive cSNK peptide beginning at 12 weeks of age, with monthly boosters thereafter. As opposed to other immunotherapy attempts at targeting fibrillar and plaque Aβ that worsened pathology by causing fragmentation and further oligomer-induced nucleation of plaque growth, our cSNK and 5E3 approach holds great promise by specifically inhibiting oligomers alone.
Student: Tracey Hinder

Board #: 28

Session #: 3

Supervisor: Elizabeth Conibear

Title: Finding and characterizing new components of the protein quality control machinery in yeast

Author(s): Tracey Hinder, Matt Tinney, Elizabeth Conibear

Abstract:
Protein quality control is important for human health. Misfolded proteins are a factor in many human diseases, including cystic fibrosis, Parkinson’s, Huntington’s, Alzheimer’s, and alpha 1-antitrypsin deficiency. Our group uses yeast as a model to study protein quality control. These pathways are highly conserved and most components have direct human homologues. However, some components of this protein quality control machinery are still unknown and the mechanisms of quality control are poorly understood in some cases. Our goal was to discover and characterize new components of the protein quality control machinery.

When soluble proteins that enter the lumen of the endoplasmic reticulum (ER) are misfolded, they can be recognized and degraded within the ER: either by the ER-associated degradation (ERAD) pathway, or after transport to the Golgi, by recognition and retrieval in COPI-coated transport vesicles. Knocking out either degradation pathway can cause secretion of a misfolded protein (or “client protein”) that is normally degraded in the cell. Different client proteins use different elements of the quality control machinery: some are general, whereas some rely mainly on ERAD or COPI. Therefore, we are using several client proteins to identify and classify new components of the quality control machinery.

High-throughput robot screening allows us to work with a large number of different mutants simultaneously. Our lab previously performed a genome-wide screen of 5000 mutants in which each mutant had a different gene knocked out. This was condensed into the 350 strongest hits. We screened this collection of 350 mutants for secretion of several different client proteins in order to identify and classify new components of the protein quality control machinery.

We are currently characterizing some of the hits and investigating their potential role in protein quality control through either ERAD or COPI retrieval to the ER. We are also working on further high-throughput work with different client proteins to classify the mutants based on which step of quality control they act on. By filling in the unknown components and mechanisms of protein quality control, we can increase our understanding of misfolded proteins and eventually apply this knowledge to treating human disease.
Interleukin (IL-1β) is a key modulator of inflammation in humans. An excess level of IL-1β has been implicated in several human autoimmune and inflammatory diseases, such as inflammatory bowel disease, and rheumatoid arthritis. On the other hand, an insufficient IL-1β response underlies high risk of infections. Due to its critical role in regulation inflammation, the IL-1β response requires tight regulation by a dual mechanism. Initially, stimulation of pattern recognition receptors (PRRs, e.g. TLR4) by pathogen associated molecular patterns (PAMPs, e.g. LPS) results in transcription of the IL1B gene and translation into its precursor protein form, pro-IL-1β. Upon the recognition of danger associated molecular patterns (DAMPs, e.g. ATP), the NLRP3/caspase-1 inflammasome causes the cleavage and secretion of mature IL-1β.

Susceptibility to infection or inflammatory diseases in humans has been attributed to genetic variations within the IL-1β pathway. However, the contribution of common genetic variants to individuals’ responses are poorly understood. We hypothesize that common genetic variants modulate IL-1β responses in humans.

In order to estimate the degree of stability, we analyzed the IL-1β response to LPS and ATP in 12 healthy adults twice within a 1-month period. Although responses along the pathway are highly variable within the population, they are similar over time for a given individual. Furthermore, we detect a significantly greater variation within women, while men are more stable over time. We have also observed that the amount of IL-1β secreted is primarily determined by the extent of caspase-1 activation in monocytes and by the proportion of the monocytes within the peripheral blood mononuclear cell population.

We plan to conduct a whole-genome association study to identify single nucleotide polymorphisms (SNPs) associated with rate-limiting components of the IL-1β responses and to confirm the influence of SNPs on gene expression. We expect that our study will provide a new insight into the importance of SNPs in determining susceptibility to IL-1β-mediated diseases in humans.
Abstract:

Introduction: Vitamin D is a group of fat soluble molecules that include the physiologically relevant form VD3 which the body metabolizes into a hormone, 1,25(OH)2D3 or calcitriol. It is known that calcitriol modulates immune responses and may play a role in protecting the host during infection. Recent studies indicate that calcitriol signaling through the vitamin D receptor plays a role in modulating bacteria-host interactions in the gut; however, the exact mechanism is unknown.

Hypothesis: Dietary VD3 will alter host susceptibility to infection and regulate host response to bacteria.

Methods: Weanling C57Bl/6 mice were fed a diet differing in vitamin D content (0 IU, 1000 IU and 20000 IU) for 5 weeks, after which they were infected with Citrobacter rodentium. At day 10 post infection the mice were euthanized and tissues collected. Bacterial loads were determined by plating tissues. Intestinal tissues were analyzed for damage by macroscopic and histological scoring.

Results: Macroscopically, VD3 deficient mice had shrunken ceca with less formed cecal contents and shorter colons with less formed stool, compared to VD3 sufficient and VD3 supplemented mice, at day 10 pi. Tissue histology showed that VD3 deficient infected mice had more overall edema, hyperplasia and inflammatory cell infiltrate, compared to other groups. Interestingly, in the cecum we found that both VD3 deficient and VD3 supplemented mice had higher pathogen burdens, compared to VD3 sufficient mice in the cecum. Furthermore, we observed that VD3 supplemented mice had higher pathogen burdens in the spleen and liver compared to other groups, indicating bacterial translocation.

Conclusions: These results show that dietary VD3 is protective during C. rodentium infection, however too much vitamin D, i.e. supplementation, is not necessarily protective, since mice carried higher bacteria burdens, compared to VD3 sufficient mice. Further research is required to understand the underlying mechanisms involved.
During labor, the fetus is at risk of reduced oxygen delivery due to intermittent reductions in uterine and umbilical blood flows. As a result, some fetuses are born with hypoxic organ damage that can compromise survival or long-term health. Consequently, diagnostic methods have been developed to detect fetal hypoxia during human labor, most commonly electronic fetal heart rate recording. However, this approach is associated with a significant false positive rate and an increase in operative deliveries because of suspected fetal hypoxia. A more direct indication of fetal hypoxia is to measure the pH of blood samples collected from the fetal scalp once the cervix is dilated. However, this technique only provides intermittent estimates of pH, and is associated with many complications, including excessive fetal bleeding from the scalp, CSF leakage, and scalp abscess. To improve on the current methods of practice, pH ProbeInc has developed a new medical device that continuously measures pH, and is minimally invasive. The objective of this preclinical study was to assess the efficacy of the pH ProbeInc device in an animal model, by comparing the pH signal obtained from the pH probe with the measurements of pH in blood.

Under general anesthesia, heparin-bonded polyurethanel catheters were implanted in the carotid artery and the jugular vein during the first postnatal week in 5 lambs. For the experiment, the pH ProbeInc device was attached to the scalp of the newborn lamb and its output was recorded. Also, the arterial pressure and the heart rate were recorded continuously throughout the experiment. The pH estimates from the pH ProbeInc device were compared with the measurements of blood gas and pH measurements in arterial blood samples, under (1) normal circumstances, and during and after (2) induced acidosis via IV administration of lactic acid, and (3) induced alkalosis via IV administration of sodium bicarbonate. The experiment is still in the early stages, so there are limited data available. So far, the pH probe has not been very sensitive in detecting pH in vivo, and we suspect that the insertion point is too short to penetrate the skin of the animal.
Student: Jacqueline Siu

Board #: 32

Session #: 3

Supervisor: Megan Levings

Title: Flagellin specific chimeric antigen receptor: a novel approach to immunotherapy for Crohn’s Disease

Author(s): Jacqueline Siu, Rosa Garcia, Kate MacDonald, Jens Vent-Schmidt, Tessa Van Tol, Megan Levings

Abstract:
Crohn’s Disease (CD) is an inflammatory bowel disease that causes chronic inflammation in the gastrointestinal tract. Evidence suggests that the inflammation in CD patients is mediated by skewed T cell responses to commensal bacteria components such as flagellin. In other words, flagellin seems to be a key antigen in the establishment of intestinal inflammation and initiation of CD. Normally, regulatory T cells (Tregs) are present to regulate T cell mediated reactions in an antigen-specific manner. By making a population of Tregs respond specifically to flagellin and activating Tregs’s ability to downregulate T cells, we hope to selectively suppress inflammation in CD patients.

In this study, we aim to generate and test Tregs that recognize flagellin by adding a genetically engineered protein known as Chimeric Antigen Receptor (CAR). We believe this protein will help redirect a polyclonal population of Tregs into Tregs that activate specifically to flagellin in the absence of a classical T-cell receptor signal and only suppress intestinal inflammation. Our CAR consists of an extracellular domain from the variable regions of an antibody that recognize flagellin, a transmembrane “spacer”, and an intracellular signalling domain that activates the cell. The recognition of an antigen via the extracellular part of the CAR triggers the intracellular part that then activates the Treg.

From our mouse monoclonal antibodies that specifically recognize epitopes of E. coli flagellin, FliC, we have generated three different flagellin CARs that recognize a conserved domain of FliC. Specifically, when we transiently transfect 293T cells with the CARs, we found that all three CARs are expressed on the surface of cells by flow cytometry.

We will continue to confirm the specificity of the CARs and then express the CARs in Tregs and test if this will re-direct the cells to flagellin-specific cells. These findings will help pave the way to develop the first antigen-specific cell based therapy and provide a feasible way to develop a novel Treg based cellular therapy for patients with Crohn’s Disease.
Student: Akie Watanabe

Board #: 33

Session #: 3

Supervisor: Francis Lynn

Title: Investigating the length of the G1 phase of the cell cycle in progenitor populations during pancreatic development

Author(s): Akie Watanabe, Nicole A J Krentz, Francis C Lynn

Abstract:

**Background:** Type 1 diabetes is characterized by chronic hyperglycemia and is caused by the destruction of insulin-producing beta cells by the immune system. Islet transplantation, a form of treatment for type 1 diabetes, involves the transplantation of donor islet cells to restore glucose homeostasis; however, transplant rejection and lack of available donor tissue prevents the widespread application of this treatment. Therefore, focus has been placed on using human embryonic stem cells (hESC) to produce large quantities of transplantable beta cells. Currently, hESCs can be differentiated into pancreatic progenitors, but we are unable to produce functional islet cells in vitro. Based on studies in neural development, we hypothesize that the G1 phase of the cell cycle is lengthened during pancreatogenesis. In our study, we characterized the cell cycle length of both multipotent progenitor cells and endocrine/ductal progenitor cells in E11.5 and E12.5 mouse embryonic pancreases.

**Objective:** To understand if there is a difference in cell cycle length of multipotent progenitor cells (Pdx1+ Cpa1+ EdU+) and endocrine/ductal progenitor cells (Pdx1+ Cpa1- EdU+) during embryogenesis that could be harnessed for the efficient in vitro generation of insulin-secreting beta cells.

**Method:** We injected CD-1 pregnant dams at E11.5 and E12.5 every 1.5 hours with the thymidine analog EdU. Using immunofluorescence and confocal microscopy, we quantified the number of Pdx1+Cpa1+ EdU+ and Pdx1+Cpa1-EdU+ cells from embryonic pancreases collected after various lengths of exposure to EdU. From this data, we will mathematically determine the lengths of the G1-, S- and G2/M-phases of the cell cycle.

**Results:** We determined that there is a significant difference between the cell cycle length of endocrine/ductal progenitor cells and multipotent progenitor cells at E11.5 and E12.5 and we are currently determining the lengths of G1, S and G2/M phases of the cell cycle for these two progenitor populations.

**Conclusion:** Using CD-1 pregnant dams at E11.5 and E12.5 injected with EdU, we concluded that the cell cycle length of endocrine/ductal progenitor cells is significantly longer than that of multipotent progenitor cells in the embryonic pancreas. This signifies the potential to manipulate the length of the G1 phase to induce endocrine cell differentiation in hESCs in the future.
Eukaryotic gene transcription is a highly complex process, governed by the interplay between thousands of transcription factors—sequence-specific DNA-binding protein complexes that can modulate the rate of mRNA synthesis. Mutations that fall within transcription factor binding sites (TFBSs) are potentially disruptive to gene expression, and consequently, an organism’s phenotype. Thus far, however, genome analysis techniques have largely been limited to the exome—the “coding” portions of the genome. With the advent of whole-genome sequencing techniques, analyzing the noncoding portion of the genome is now feasible. High-throughput regulatory variant analysis tools are needed to leverage the availability of these techniques to identify causal mutations in various genetic disorders.

Triple negative breast cancer is a type of breast cancer characterized by the lack of expression of typically overexpressed therapeutic targets—estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2). The underlying genetic alterations responsible for inducing triple negative breast cancer phenotypes have not been identified and are likely nonhomogeneous. Consequently, genome analysis tools are needed to discover important, and possibly causal, variants in triple negative breast cancer patients in order to enable verification of new therapeutic targets.

In order to assign relative importance to the millions of noncoding variants in the genome, we first reduced the size of our working set by looking at the variants lying within ChIP-Seq data—regions of the genome that are bound by transcription factors in vivo. We further reduced our set of variants by restricting to those located in predicted transcription factor binding sites—short 6-30bp binding motifs. We used position weight matrices (PWMs) to determine the affinities of transcription factors for modified transcription factor binding sites in breast cancer patients. We identified a small set of noncoding variants in which a significant transcription factor binding affinity decrease correlated with a drop in gene expression of a potential regulatory target.

With the results, we plan to experimentally validate the biological importance of these variants in triple negative breast cancer. It is our hope to further refine our analysis pipeline and make it widely available for clinical use.
Title: Bortezomib circumvents multi-drug resistance in acute lymphoblastic leukemia conferred by enhanced integrin signalling

Author(s): Jenny Zhao, Chi-Chao Liu, Eva Yap, Chinten James Lim

Abstract:
Multiple drug resistance is one of the factors preventing the achievement of a complete cure rate in pediatric acute lymphoblastic leukemia (ALL). Via integrin cell adhesion proteins, leukemic cells attached to certain components in the bone marrow stroma are protected from conventional chemotherapeutics, a phenomenon termed cell adhesion mediated drug resistance (CAMDR). Due to the intrinsic drug resistance, children with relapsed ALL often present with a poor prognosis, leading to the need to find novel chemotherapeutics. Bortezomib is a novel proteasome inhibitor currently approved for the treatment of multiple myeloma, relapsed multiple myeloma, and mantle cell lymphoma. Previous studies have failed to address the role of bortezomib in its ability to circumvent enhanced integrin signaling mediated chemoresistance in T-cell ALL. In order to study this, a Jurkat T-ALL derived cell line with a gain of function (GOF) mutant integrin conferring adhesion-independent chemoresistance and a control cell line were used to examine the in vitro susceptibility to bortezomib. Preliminary results indicated that both the GOF and control cell line were equally susceptible to this novel drug. Significantly, this suggests that bortezomib may be an effective chemotherapeutic by innervating novel pathways to bypass drug resistance in T-ALL cells exhibiting increased integrin activity.
Moderator: Leigh Gabel
Participants: Mazy Abulnaga, Elaine Chan, Michael Hay, Tiffany Lau, Tanjot Singh, Erick Carreras, Quinten Clarke, Lewis Kurschner, Samin Liaghat, Ricky Zhang
**Student:** Mazy Abulnaga  
**Board #:** 36  
**Session #:** 4  
**Supervisor:** Guy Dumont  
**Title:** Do I Wheeze? Utilizing respiratory sound recordings to develop an algorithm for detecting wheeze  
**Author(s):** Mazy Abulnaga, Walter Karlen, Matthias Görges, Chris Peterson, Guy A Dumont, J Mark Ansermino

**Abstract:**

**Background:** Current WHO case management strategies for pediatric pneumonia are based on the assumption that the presence of fever and cough with tachypnea require an antibiotic. Many of these symptoms however are shared with wheezy (a form of adventitious breathing) diseases such as asthma and bronchiolitis. As a result, many children in the developing world with wheezy diseases are often misdiagnosed as having pneumonia and are receiving wrong and unnecessary antibiotics. We sought to develop an algorithm to detect the absence and presence of wheeze in children to improve the treatment and diagnosis of pneumonia.

**Methods:** This study is designed to investigate whether a computerized lung sound analysis algorithm can detect the difference between wheezy or normal breathing. We will record lung sounds from 100 children aged <17 years, 50 with normal breathing and 50 with clinically diagnosed wheeze. The subjects will be recruited during their routine care at the BC Children’s Hospital. The recordings will be used to develop an algorithm to detect the presence or absence of wheeze with a specificity >80%. To perform the recordings, an electronic stethoscope powered by the audio port of a mobile phone has been developed.

**Discussion:** We are currently recruiting subjects for the study. The subjects’ recordings are analyzed in MATLAB using signal processing techniques to develop the wheeze detection algorithm. Additionally, a low-cost lung sound recording device is being developed to be used with a mobile phone in the developing world.

**Future Work:** The algorithm will be used to detect the presence and absence of wheeze with a specificity >80% as compared to clinical diagnoses. The finalized algorithm will then be implemented into a mobile phone application to be used in the developing world. This study is a starting point for the development of low-cost and accurate systems to improve the diagnosis and treatment of pneumonia.
Student: Erick Carreras
Board #: 37
Session #: 4
Supervisor: Andrew Campbell
Title: Cardiac strangulation following epicardial pacemaker implantation: A rare pediatric complication
Author(s): Erick Carreras, Andrew I M Campbell

Abstract:
Purpose of Study: Cardiac strangulation may occur following epicardial pacemaker implantation if its lead becomes adherent to the epicardium and wrap around the heart. With progressive somatic growth this lead may constrict the underlying cardiac structures causing mechanical complications and potentially death. The aim of our study was three-fold: To determine the incidence of the pathology, improve the implantation protocol of epicardial pacemakers and develop a protocol for follow-up that included regular reassessment and potential imaging.

Methods Used: All patients who underwent implantation of an epicardial pacemaker from January 1992 to March 2012 were included with hospital health records being used to gather retrospective data including basic demographics, surgical details and cardiac related check-up information for the first 2 check-up dates post-implant, and for every year thereafter. Any post-operative complication that occurred in between the yearly-recorded follow-ups were included. Prospectively, the patients that had not received a chest x-ray within the previous 2 years were approached for imaging with a standard two film chest x-ray to assess the leads' potential for causing cardiac strangulation - reviewed by a blinded radiologist. The primary outcome was mortality related to cardiac strangulation or reoperation for replacement of the epicardial pacemaker system due to mechanical dysfunction. Specific symptoms were also recorded as secondary outcomes, including: syncope, chest pain, arrythmias and atrioventricular valve regurgitation. A multivariate analysis determined interdependency between the variables and primary and secondary outcomes.

Summary of Results: This study included 87 patients with a 2.2% incidence, and a 1.1% mortality related to this pathology. A characteristic pattern of posterior looping of the ventricular lead was seen in chest radiographs of both patients with cardiac strangulation presenting acutely in both cases.

Conclusions: Our institutional incidence of cardiac strangulation is significantly greater than the previously reported approximation of 0.01%. Our data supports that the 2 cases of cardiac strangulation were not caused by a lack of follow-up but by a lack of effective imaging for diagnosis. This finding is supported by the 7 cases of cardiac strangulation found in the English literature (May 2012).
Abstract:
Obsessive-compulsive disorder (OCD) is a common neuropsychiatric illness that often begins in childhood and has been shown to have complex genetic causes. Siblings of affected youth have a 10 to 30-fold increased risk for OCD and share 50% of the same genes. However, despite many gene study approaches, specific risk genes for OCD have yet to be found. Studies have shown that brain structure and functioning are highly influenced by an individual’s genetic makeup and that neural abnormalities are present in OCD. Measurement of brain structure and functioning associated with genetic risk is potentially more straightforward for identifying genes contributing to OCD than examining disease association as neural differences may act as endophenotypes (heritable quantitative traits associated with increased genetic risk for a disorder).

This cross-sectional study will investigate neural correlates of OCD by comparing three groups aged 10-18 years with different levels of genetic risk for OCD: OCD-affected subjects, at-risk siblings of OCD patients, and age- and sex-matched healthy controls (N=50 per group). Brain structure, connectivity, and functioning will be analyzed using a 3.0 Tesla magnetic resonance imaging (MRI) scanner. Two structural runs, one diffusion tensor imaging scan, and one resting state scan will be conducted in addition to three functional MRI paradigms – a symptom provocation task, a stop signal task, and the Tower of London paradigm. The stability of these profiles will be examined for correlations with OCD severity.

This study aims to enhance early identification of children at risk for OCD via heritability risk markers, increase knowledge of the neurobiology of OCD, and improve treatment strategies for affected individuals.
Student: Quinten Clarke
Board #: 39
Session #: 4
Supervisor: Douglas Courtemanche
Title: An appraisal of the OPSEI academic rounds evaluations
Author(s): Quinten Clarke, Damian Duffy, Douglas Courtemanche

Abstract:
Background/Purpose: The Office of Pediatric Surgical Evaluation and Innovation (OPSEI) hosts monthly academic rounds for the surgical community at BC Children’s Hospital. The purpose of this study is to evaluate the effectiveness of the current OPSEI Academic Rounds evaluation form and consider the possible design of a new form.

Methods: Data was obtained from OPSEI for completed evaluation forms from January 2010 – May 2013 and was collated. As two versions of the evaluation form were used during this period analysis was completed on a dichotomous basis where possible. (1) A distribution of scores from the two forms was constructed with the mode of each form being calculated. (2) Comments from new forms were analyzed qualitatively based upon four scoring categories (Good, Fair, Poor, and Incomplete). Comments were labelled as critical of content, project or presentation and presentation skills or, as positive, ambiguous, or absent. (3) Regression analysis was conducted for form completion as a function of rounds attendance.

Results: (1) The modes for the old and new form were 4/5 and 9/10 respectively. Scores were found on both forms to be spread primarily over four ratings, on the “old” form 2, 3, 4 and 5; and on the “new” form 7, 8, 9 and 10. (2) Comments were found to be largely positive regardless of score. Critical comments in any of the scoring categories were infrequent, ranging from 5.2% to 23.5% of comments. Comments were found to be more frequently included on forms where scoring sections were not completed. (3) Regression analysis found paradoxical trends between the two forms. “Old” forms were found to have a positive relationship between attendance and form completion (R2 = 0.582) while “new” forms had a negative relationship (R2 = 0.157).

Conclusions: The large scoring scales, used by attendees to evaluate presenters at OPSEI Academic Rounds, have become superfluous as few scores are consistently used by evaluators. Comments on OPSEI evaluation forms are frequently omitted or generally neutral, lacking in constructive criticism or meaningful feedback inhibiting the educational potential for presenters. The currently used evaluation form appears to be less comprehensible to infrequent rounds attendees.
Student: Michael Hay
Board #: 40
Session #: 4
Supervisor: Anne Synnes

Title: Canadian Neonatal Follow Up Network: Parental education level and cognitive development of canadian preterm infants

Author(s): Michael Hay, Anne Synnes, Marlee McGuire

Abstract:
Introduction: Cognitive development is associated with parental education level in both babies born term and preterm but has not been studied in a national Canadian cohort. The Canadian Neonatal Follow Up Network (CNFUN) has collected data on a cohort of survivors born 2000-present at < 29 weeks gestational age; this data was collected at a clinic visit when the child was 18-24 months corrected age. The educational level of the primary caregiver and a developmental evaluation of the child using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) are both included in this data. Therefore we can use the CNFUN database to address the following question: Is there an association between the primary caregiver’s education level and cognitive development in Canadian infants born at less than 29 weeks gestation?

Methods: Subjects are evaluated at the 27 Neonatal Follow-Up Programs (NFUs) using a standardized assessment protocol conducted at 18-24 months corrected age; this assessment includes education level of the primary caregiver by parent report and the Bayley-III language, motor and cognitive subscores calculated for the child. Results are uploaded into a web based centralized database. Data on subjects born between April 1, 2009 and September 30, 2011 were extracted into an Excel spreadsheet on June 12, 2013. A statistical comparison of mean or median cognitive scores by education level is planned.

Results: Of the 2574 subjects in the CNFUN database, 2056 or 80% have Bayley-III cognitive scores; of those children that were tested the education levels of 106 or 5% of primary caregivers were unknown. Children with caregivers with High school or less education had median Bayley-III scores of 90 whereas those with parents who had some post secondary education had median scores of 95.

Discussion: Very few caregivers have < 6 years education. Mean and median cognitive scores differ; likely not normally distributed. Education beyond high school is associated with higher scores but the statistical significance needs to be tested. Potential confounding factors need to be evaluated.
Abstract:

Background: The adipocytokine leptin is a signalling protein that functions to increase satiety and energy expenditure. Leptin is secreted by adipocytes and exerts its biological effects by binding multiple receptors expressed abundantly in the hypothalamus. In addition, leptin negates the effect of the neurotransmitters: neuropeptide y and anandamide, and promotes the expression of alpha-melanocyte-stimulating-hormone, a protein that suppresses appetite. Although, circulating leptin concentrations are positively associated with suppressing appetite, previous studies have shown leptin resistance in obese individuals. Despite higher circulating leptin concentrations in obese subjects, these individuals do not respond to a satiety signal appropriately by decreasing energy intakes.

Individuals of South Asian decent are at greater risk for cardiovascular disease (CVD). The Multicultural-Community Health Assessment Trial (M-CHAT) found that South Asians have greater visceral adipose tissue (VAT) than Europeans, despite having similar BMIs. Since BMI measures are similar between Europeans and South Asians, ethnic-specific differences such as VAT distribution and a unique phenotype may be contributing to the increased cariometabolic risk. To date, the molecular mechanisms underlying ethnic-specific differences in VAT deposition are unknown. The goal of this study is to investigate ethnic-specific differences in the relationship between serum leptin and adiposity, body composition, body fat distribution, and CVD risk factors.

Methods/Results: European (n=164), Aboriginal (n=142), Chinese (n=163), and South Asian (n=165) subjects from the M-CHAT cohort were assessed for demographics, serum leptin concentrations, and CVD risk factors. A CT scan was used to assess VAT and subcutaneous adipose tissue (SAT). Serum leptin concentrations were quantified by using a commercial leptin sensitive ELISA (Alpco). General linear models will be used to analyze data. Serum leptin concentrations have been determined in this cohort.
Student: Tiffany Lau
Board #: 42
Session #: 4
Supervisor: Graham Sinclair
Title: Retrospective screening of BCKD-kinase deficiency, a potentially treatable cause of autism
Author(s): Tiffany Lau, Graham Sinclair, Hilary Vallance, Suzanne Lewis, Kristina Calli

Abstract:
Autism Spectrum Disorder is a life-long, neurodevelopmental disorder, affecting 1 in 88 children in Canada and is characterized by deficits in social interaction, impaired communication, and stereotyped behaviour. The etiological heterogeneity of autism causes wide ranges in severity of symptoms. Recently, Novarino et al. identified mutations in the branched-chain ketoacid dehydrogenase kinase (BCKD-kinase) gene in six patients, leading to an increased breakdown of branched-chain amino acids (BCAA). Empirical results reveal that BCAA share a common transporter system with large neutral amino acids (LNAA), some of which are precursors to neurotransmitters; therefore, a perturbation in brain concentrations of LNAA results in neurodegeneration. In a BCKD-kinase deficient mouse model, replacement of BCAA reversed the clinical phenotype.

The purpose of this study is to retrospectively evaluate plasma amino acid profiles to detect BCKD-kinase deficiency in a cohort of 575 autism patients and to establish a multiple-analyte algorithm as a screening tool for BCKD-kinase deficiency.

Methods: Inclusion criteria: patients who received multidisciplinary assessment, a diagnosis of autism and provided consent through the BC Autism Spectrum Interdisciplinary Research Program (n=99). Exclusion criteria: patients who did not have amino acid testing as part of their routine clinical workup or received poor sample handling (n=476). Controls were extracted retrospectively by a LIS analyst from May 5, 2006-April 30, 2013 (n=8232). Principal component analysis (PCA) and receiver operating characteristic (ROC) curves were conducted using MetaboAnalyst software. Published BCKD-kinase cases were included in the study population to establish a theoretical sensitivity and specificity using XLStat.

Results: PCA showed no significant difference between autism cohort and healthy control; however, literature values of BCKD-kinase cases were independently clustered. ROC analyses of autism patients and age-matched controls validated that an increase in the number of variables showed an increase in AUC percentage. Ratio of (Val+Ile+Leu)/Lys has 100% sensitivity and specificity in detecting BCKD-kinase, using a cut-off of the highest value among the published cases.

Conclusions: In this retrospective study, preliminary data suggest that our cohort does not contain any cases of BCKD-kinase; however, we have confirmed that a multiple-analyte algorithm has 100% sensitivity and specificity in the detection of BCKD-kinase deficiency-related autism.
Abstract:

**Introduction:** Developmental dyslexia is a reading disability with a neurological origin. There are many intervention programs designed to improve reading skills in dyslexia, however, few have empirical evidence to support them. A longitudinal study is planned to measure the effectiveness and neurological correlates of a new intervention program. In preparation for this longitudinal study, we have conducted a pilot study to develop the reading tasks to be used in a functional MRI paradigm pre- and post-intervention. Our goal is to develop lists of words for dyslexic and average reader groups that can be read with high accuracy during a fixed time of 3 seconds.

**Method:** We began with a behavioural study that involved 21 healthy children between the age of 7 and 11 years. The children were presented with four different computerized tasks where their reaction time and accuracy were measured. The tasks included: deciding if a word was spelled correctly (orthographic 1-word), deciding which of two words was spelled correctly (orthographic 2-words), deciding if a letter string sounded like a real word (phonological 1-word), or deciding whether two words rhymed (phonological 2-word). Children were placed in their respective reading groups for data analysis based on their performance on the KTEA II Reading Ability Tests. There were 13 children with average reading ability and 8 with dyslexia.

**Results:** Mean reaction times for both reader groups combined were significantly faster on the orthographic than on the phonological tasks (p<.001), but not significantly different between 1- and 2-words tasks (p>.25). Importantly, average reaction time was less than 3 seconds on all four tasks, and reaction times did not differ significantly between the two groups (p>.25).

Mean accuracy was significantly higher on the orthographic than on the phonological task (p<.01). The dyslexic group showed significantly lower accuracy compared to average readers on the phonological 2-word and both orthographic tasks (p<.001). Performance on several components of the KTEA II Reading Ability Tests was significantly correlated with accuracy of the same 3 tasks.

**Next Steps:** We are currently selecting the specific words to be used to achieve similar mean accuracy between orthographic and phonological tasks and between average and dyslexic readers. We will next scan a subset of these children to confirm the feasibility of these tasks for MRI.
**Student:** Tanjot Singh  
**Board #:** 44  
**Session #:** 4  
**Supervisor:** Wendy Robinson  
**Title:** Differential methylation approach to quantify cell-free fetal DNA in maternal plasma  
**Author(s):** Tanjot K Singh, Irina Manokhina, Wendy P Robinson  

**Abstract:**

Prenatal diagnoses allow patients and healthcare providers to monitor the health of mother and fetus during pregnancy. Currently, to study the fetus from a genetics point of view, invasive procedures are used. However, invasive procedures pose a small, yet tangible risk to mother and fetus. The development of a diagnostic tool that has minimal risk to mother and fetus is a major goal in this field. One such method that has been proposed is the use of circulating cell-free DNA. Cell-free DNA is found in the blood of healthy individuals and is a result of cells emptying their contents into the bloodstream. Pregnant women have been found to carry not only their own cell-free DNA, but also DNA of their fetus (placental) in their blood stream, called cell-free fetal DNA (cffDNA). This cffDNA can be used in the detection and/or prediction of different fetal and pregnancy related characteristics such as triploidy, preeclampsia, and fetal sex. However, the majority of research done with cffDNA has targeted male pregnancies through the use of the SRY (Sex-determining region, Y-chromosome) gene.

In this study, the feasibility of a methylation based approach to detect and quantify cffDNA in both male and female fetus bearing pregnancies will be evaluated using assays based on the promoter regions of ACTB (Beta-Actin) and RASSF1A (Ras association domain-containing protein 1) genes. The ACTB promoter is normally hypomethylated in all cell types; the RASSF1A promoter is hypermethylated in placental tissue and hypomethylated in maternal blood cells.

Methylation-sensitive restriction enzymes will be used to cut the hypomethylated regions, leaving behind only hypermethylated RASSF1A of placental origin. The uncut RASSF1A and ACTB (as a control of restriction) fractions are quantified using qRT-PCR. These will be analyzed in cfDNA extracted from the blood serum of 72 pregnant women taken during the 14 to 40 weeks gestational ages. Levels of cffDNA will be compared to pregnancy variables including placental insufficiency (such as preeclampsia), maternal weight, and maternal serum screen results. Once validated, this approach can then be tested in a larger series of patients to evaluate positive predictive value of such screening.
Abstract:
Extensive evidence from literature has documented the importance of play in promoting optimal growth, learning and development throughout childhood. More recent research has indicated the need for risky play for healthy child development. This need can be at odds with current approach to playground design, which emphasizes safety over child-centric design. Combined with increased fears of litigation and a change in parental attitudes towards child safety and risk, this has dramatically reduced children’s access to risky play opportunities. As part of a body of research, we are investigating whether children process risky play differently than non-risky play. Previous research in EEG has generated promising results in the recognition of temperament and emotion states of adults and children. In spite of an abundance of research on childhood development and play, only a scarce number of studies have included physiological measurements such as EEG in its procedures. This study aims to assess whether the possibility of measuring detectable differences in EEG in childhood risk appraisal measurements.

30 participants, aged 4-6 years, will be recruited through flyers, online ads and snowball sampling. Participants will be shown 45 short video clips of children engaged in 5 different outdoor activities with measures of low, medium, and high risk for each clip. Measurements will be taken through EEG and a self-report survey. Preliminary results from the pilot study have shown promise in detecting differences in the anterior cortical hemispheres in terms of EEG power and in amplitude. Further data collection will examine the significance of this trend. Our findings will contribute towards the development of future studies exploring the importance of risky play to children’s development.
Poster Session Five

Moderator: Gillian Hanley
Participants: Yi Qi Chen, Johnathon Gorman, Angela Han, Christopher Koo, Hannah Staniszkis, Eileen Fan, Emily Hamel, Lingsa Jia, Vincent Ng, Sarah Wang
Student: Yi Qi Chen
Board #: 46
Session #: 5
Supervisor: Sheila Innis
Title: Genetic differences in response to diet: Implications for maternal and child health
Author(s): Yi Qi Chen, Kelly Mulder, Alejandra Wiedeman, Roger Dyer, Sheila Innis

Abstract:
Throughout human history, many examples of genetic adaptations to the environment exist. Skin pigmentation involves differences in melanin production due to geographic location and sun exposure. The persistence of the lactase gene into adulthood in some populations is due to continuous exposure to dairy products. Choline is an essential nutrient with a wide range of biological functions. Choline acts as a precursor to the neurotransmitter acetylcholine, the major cell membrane phospholipid phosphatidylcholine, and the important methyl-group donor betaine. The major dietary sources of choline include milk and eggs. Choline can also be produced endogenously in the liver from phosphatidylethanolamine via phosphatidylethanolamine N-methyltransferase (PEMT). Choline deficiency has been associated with neural tube defects during fetal development and non-alcoholic fatty liver disease and muscle damage later in life. It is known that genetic variations, specifically single nucleotide polymorphisms (SNPs), in the PEMT gene alter endogenous synthesis of choline. However, the effect of SNPs on the risk of choline deficiency has not been studied with regards to ethnic background. Therefore, we hypothesized that women of Chinese descent would have higher plasma concentrations of choline and choline metabolites compared to women of Caucasian descent consuming the standard Western diet. This difference would be explained by the unequal presence of SNPs between these two ethnic groups. Blood samples from healthy non-pregnant women were collected. Plasma was used to analyze choline concentration using liquid-chromatography mass spectrometry. White cells were used to determine the presence of SNPs in genes involved in choline metabolism using Sequenom. Preliminary results of 140 participants showed a significant difference in plasma betaine concentration (p=0.029) between women of Chinese and Caucasian descent. Further investigations into the presence of SNPs in genes related to choline metabolism will potentially help to elucidate ethnic groups at risk of choline deficiency.
Study of the sympathetic nervous system activity during urination in children with non-neuropathic voiding disorder

Eileen Fan, Mir Sohail Fazeli, Kourosh Afshar, Jean Paul Collet

Abstract:
Background: Non-neuropathic voiding disorder (NVD) is the most common reason for referral to pediatric urology and affects nearly 5% of children between the ages of 5 to 18. NVD includes several conditions such as dysfunctional voiding, overactive bladder, and underactive bladder. Normally, the activity of the parasympathetic nervous system (PNS) increases while the sympathetic nervous system (SNS) decreases during voiding. The role of Autonomic Nervous System (ANS) dysfunction in pathogenesis of NVD has not yet been fully investigated.

Objectives and Hypothesis: Main objective: to compare the changes in the SNS activity (measured by Pre-ejection period (PEP)) during voiding in patients with NVD and healthy children (controls). We expected a smaller change in PEP in children with NVD compared to controls.

Materials and Methods: We conducted a cross-sectional analysis. Cases and controls (without NVD) aged 5 to 18 were referred by the urologist in BC Children’s Hospital Urology clinic. We assessed the SNS function through impedance cardiography techniques to measure PEP, which is the time interval from beginning of the electrical stimulation of the ventricles to the opening of the aortic valve (electrical systole). SNS is inversely correlated to PEP. For this study, a sample size of 40 cases and 20 controls was selected.

Results: Among the cases, the percentage change of PEP during voiding varied from 2.6% to 31.0% (mean=13.0, median=12.2) while in the controls, the percentage change of PEP ranged from 13.6% to 73.9% (mean= 40.8, median=34.8). This difference was significant (Wilcoxon Rank Sum Test, p=0.048).

Discussion: This study shows that the SNS function during voiding is deeply affected in children with NVD. More studies are warranted to a) assess possible interventions (drug or no drugs) to improve bladder functions and b) whether SNS assessment during voiding can become a non-invasive screening test to diagnose children with NVD.
Student: Johnathon Gorman
Board #: 48
Session #: 5
Supervisor: Douglas Courtemanche
Title: Outcomes of sclerotherapy for the treatment of venous vascular malformations
Author(s): Johnathon Gorman, Marija Bucevska, Jugpal Arneja, Douglas Courtemanche

Abstract:

Background: Venous vascular malformations (VVMs) are the second most common vascular anomaly, with a prevalence of approximately 1.5%. VVMs are congenital, slow-flow lesions which grow in proportion to the patient and do not spontaneously involute. The natural history of these lesions is degenerative. The most common anatomical location of these lesions is the head and neck and common presentations include pain, swelling, discolouration and disfigurement. The preferred treatment of VVMs is sclerotherapy, commonly utilizing detergents or ethanol. This study reviews the outcomes and complications of sclerotherapy for the treatment of VVMs in patients managed by the Vascular Anomalies Clinic at BC Children’s Hospital.

Materials and Methods: A 10 year retrospective chart review with minimum 2 year follow-up was conducted for patients with VVMs who presented to the Vascular Anomalies Clinic at British Columbia’s Children’s Hospital since May 1, 2001. Data collected included demographic data, VVM characteristics, sclerotherapy treatment details, outcomes, recurrence rates, and follow-up course.

Results: A total of 63 patients (44 females and 19 males) were identified for the study, with 65 VVMs treated. The most common indications for treatment included swelling (83%), pain (62%), discolouration (60%), and recurrent thrombophlebitis (40%). There were a total of 145 sclerotherapy procedures conducted during the study period. There were complications associated with 21% of the procedures (31 in total) and the most common complication was significant post-procedural swelling. Of the 65 VVMs treated, 49 had a positive outcome compared to baseline, 4 were unchanged, 4 had a negative outcome, and 8 were still undergoing treatment at the end of the study period.

Conclusions: Sclerotherapy is a relatively safe and effective treatment for VVMs. The complication rate of sclerotherapy procedures was 21%, with the majority of complications being minor and transient.
Introduction: Maternal mortality remains one of the leading causes of death of women worldwide. When critically ill obstetric patients are admitted to an intensive care unit (ICU), there are no pregnancy-specific risk prediction models for adverse outcomes. At present, the current ICU models used to assess patients tend to overestimate adverse outcomes, which is partly due to the unique physiology of pregnancy and the puerperium. Healthcare providers are in need of models that accurately assess which patients obstetric are at high risk for adverse outcomes.

Objective: The aim of this study is to determine which factors contribute most to an increased risk of maternal mortality for obstetric patients admitted to the ICU.

Methods: The CIPHER study is a multicentre international study which is currently collecting data on ICU-admitted women that are either pregnant or within 42 days postpartum from 2000-2013. We used some preliminary data from the CIPHER study to examine the factors contributing to poor maternal outcomes during pregnancy and up to 42 days postpartum. Data was analyzed by univariate analysis, cluster analysis and multivariate logistic regression using SPSS Version 21.

Results: There were 8 maternal mortalities (2.6%) of the 311 women included in analysis from the preliminary CIPHER database. 162 variables (including demographic, clinical, laboratory and intervention-based variables) were assessed for their association with maternal mortality. The four most predictive variables for maternal mortality were maternal age, one or more organ failures as an indication for ICU admission, the need for mechanical ventilation during ICU admission and the occurrence of pregnancy loss. This four-variable model was analyzed using logistic regression, the receiver operating characteristic (ROC) and area under the curve (AUC). We found the AUC ROC to be 0.944 [95% CI 0.891 to 0.997].

Conclusion: We found that increased maternal age, organ failure as indication for admission to ICU, the need for mechanical ventilation and pregnancy loss were moderately predictive of an increased risk of maternal mortality. Future directions will include reassessing the model during the data collection period and refining the model to be applied in clinical practice.
Abstract:

**Background:** When Youth with chronic health conditions and/or disabilities (CHC/Ds) age out of pediatric care, they often face challenges in navigating the different clinical practice and culture of adult services. Gaps in continuity of care during transition are associated with poor health outcomes. Establishing the “medical home” and family physician (FP) attachment prior to transfer are recommended to ensure ongoing managed and coordinated care in adult services.

**Objective:** This study aims to determine the role of the FP in providing care and who manages specific general health care issues for youth with CHC/Ds.

**Method:** Outpatients 14-18 years of age at B.C’s Children’s Hospital (BCCH) cardiology and neurology clinics and inpatients (wards 3F, 3M) with CHC/Ds and their caregivers completed identical self-administered computerized questionnaires separately. Adolescents without a caregiver present were included and caregivers of adolescents with cognitive delay were included. Non-English speakers were excluded. Data was inputted directly into RedCAP for analysis. The questions asked what health services the youth/caregivers accessed for certain medical issues and the perceived FP’s role in managing the youth’s health.

**Results:** Fifty-one youth and 49 caregivers were surveyed. When asked if youth see their FP without their caregiver, 67% of youth reported never, 24% sometimes and 2% always. Therefore, in this abstract we report the caregiver data, as representing the primary managers of the youth’s care. Ninety eight percent of youth have a FP, while 43% of youth have a paediatrician. Of those who have a FP, 39% have seen their FP 2 times or less in the past 2 years and 14% have not seen their FP in the last year. While youth/caregivers accessed a FP up to 84% of the time for basic health issues, surprisingly they accessed BCCH specialists frequently (49% prescription refill, 35% mental health, 14% sexual health, 8% immunizations.)

**Conclusion:** The results demonstrate that most youth with CHC/Ds have a FP but they do not see them often. Additionally, patients are seeking care from specialists for primary care management issues. How attachment is defined and perceived needs to be more deeply explored.
Intrauterine device acceptance and prior contraceptive satisfaction among women at second trimester abortion

Author(s): Norman WV, Brooks M, Brant R, Soon JA, Kaczorowski J

Abstract: Objective: This report details enrollment findings related to a Canadian randomized controlled trial comparing immediate to delayed intrauterine contraception (IUC), placement after a second trimester abortion. We report acceptance of IUC, prior contraceptive satisfaction, adherence to the CONSORT criteria and challenges faced in the recruitment process.

Study Design: Women seeking second trimester abortion (12 to 24 weeks gestation) and selecting either of two IUC as their preferred contraception method were enrolled and randomized to insertion timing either immediately or four weeks post abortion. Enrolled participants completed a Contraception Satisfaction Questionnaire (CSQ) detailing prior contraceptive satisfaction. The trial is registered at controlledtrials.com and received full ethics approval.

Results: Among 1813 women assessed, 1500 (83%) met eligibility criteria and IUC was chosen for post-abortion contraception by over half (792/1500, 53%). These women were then invited to participate in the study and 59.8% (474/792) chose to enrol. Participants had an average age of 26.0 (SD 6.8) years, and average gestational age of 16.1 (SD 3.1) weeks. Almost half (48.4%) had a prior abortion and 46.9% a prior birth. Two-thirds were using a contraception method at conception, but almost a third of these were using methods in the lower tiers of effectiveness. There was a weak correlation between prior contraceptive compliance and education level.

Conclusion: More than half of eligible women seeking a second-trimester abortion chose an IUC for post-abortion contraception when counselling and cost-free methods were offered. Offering comprehensive information on the range of contraceptive methods along with cost-free IUCs is an effective strategy to increase uptake of intrauterine contraception among Canadian women who wish to prevent further unintended pregnancy.
Student: Christopher Koo

Board #: 52

Session #: 5

Supervisor: Edmond Chan

Title: Recruitment to a new registry for children with eosinophilic esophagitis

Author(s): Christopher Koo, Vishal Avinashi, Preeti Vekaria, Edmond S Chan

Abstract:

**Background:** Eosinophilic esophagitis (EoE) is a disease resulting from chronic inflammation of the esophagus, predominantly with eosinophils. This leads to narrowing of the esophagus, which impedes passage of food from the mouth to the stomach. Thus, patients have difficulty swallowing, difficulty eating, poor nutrition, poor growth, and poor quality of life. Children can experience these problems from early infancy, and symptoms usually persist into adulthood. Although the pathophysiology remains largely unknown, there is a strong link with allergic disease. Most EoE patients also present with food allergy, asthma, allergic rhinitis, and/or atopic dermatitis.

As EoE has mainly been studied in the past decade, relatively few practicing physicians are aware of it. Patients suffer an average of 4.5 years before diagnosis, which requires an endoscopy and biopsy. Furthermore, there is no known cure, and evidence for treatment is relatively sparse. In July 2012, the first Canadian EoE clinic of its kind was established at BC Children’s Hospital, where a gastroenterologist, allergist, and dietician see EoE patients concurrently. This environment presents an opportunity to conduct research and shed light on this mysterious disease.

Our objective is to create a registry for EoE patients seen at BC Children’s Hospital and gather longitudinal information on patient characteristics. Our initial research goal is to see how many families will consent to participate in the registry. A secondary purpose of the registry is to allow targeted recruitment of patients into future studies based on specific patient criteria.

**Methods:** Following ethics approval and registry design in REDCap software, we have begun recruitment for patients seen previously in the EoE clinic. We are conducting chart review to collect variables including ethnicity, medical history, symptoms, EoE triggers, treatment, allergic background, diet, and growth. We will continue recruiting at future clinics prospectively and compare the rate of consent to retrospective recruitment.
Student: Vincent Ng
Board #: 53
Session #: 5
Supervisor: Simon Dobson & Julie Bettinger
Title: Optimizing management of patients with adverse events following immunization: A retrospective chart review
Author(s): Vincent Ng, Simon Dobson, Julie Bettinger

Abstract:
Context: As the prevalence and incidence of vaccine-preventable diseases have decreased, concerns about vaccine safety have risen. Vaccines are much safer and more cost-effective than therapeutic or hospital intervention; however, adverse events following immunization (AEFIs) have been observed. Despite their infrequency, such episodes are much less tolerated by the public than adverse drug reactions. Active and passive surveillance for AEFIs within Canada capture information on the acute occurrence of AEFIs, but do not provide insight to the outcome of revaccination in patients with previous AEFIs. Therefore, the appropriate management of patients with proven AEFIs is not known. To address this, a Special Immunization Clinic (SIC) was set up in British Columbia with a further proposal of implementing a national SIC network that extends standardized clinical care of such patients across the country.

Objective: Our study aims to pilot test and provide improvements for the SIC Data Collection Form that will be used to systematically capture information about the investigation, diagnosis, and management of patients with AEFIs. Ultimately, the data from the SIC will be used to determine best practice for revaccination of patients with AEFIs.

Methods: This study is a retrospective chart review involving patients referred to the BC Children’s Hospital SIC from 1 January 2008 to 22 May 2013. We will review the medical charts of those who had an AEFI or a contraindication to vaccination. We selected 56 patients using comprehensive inclusion criteria, including:

- Fever ≥40.5 °C
- Local reaction, e.g. cellulitis, abscess, or nodule
- Arthus reaction
- Persistent crying
- Convulsions
- Hypotonic-hyporesponsive episode
- Arthralgia/arthritis
- Allergic-like symptoms

Results: Chart details from the 56 patients will be abstracted onto the SIC Data Collection Form. We will establish the form’s validity in providing an accurate patient “portrait” and identify any deficiencies in the form, including missing or insufficient information that hinders its use.

Future impact: We will standardize clinical assessments and management of patients with AEFIs across Canada and thereby improving health care provider confidence, patient management, and vaccine uptake.
Abstract:
Background: Unwanted, intrusive thoughts of harm coming to one’s infant (accidental harm thoughts) are experienced universally by new mothers, and close to half of all new mothers experience unwanted, intrusive thoughts of harming their infant (intentional harm thoughts). Both accidental and intentional harm thoughts can be extremely upsetting to the women who experience them. Perinatal caregivers often worry that these thoughts may be harbingers of child abuse. However, evidence suggests that the occurrence of these thoughts is normal, and is much more likely to lead to the onset and/or exacerbation of obsessive-compulsive disorder (OCD), a potentially debilitating anxiety disorder, than to child harm. Responding appropriately to women who report these kinds of thoughts is critical.

Key Objectives:
1. Document the prevalence of maternal postpartum thoughts of accidental and intentional infant-related harm
2. Determine if maternal thoughts of intentional harm predict infant harming behaviours
3. Determine if maternal thoughts of harm predict postpartum OCD among vulnerable women
4. Determine if there is an increase in the prevalence of OCD from pregnancy to the early postpartum

Participants: Participants will be 800 pregnant women living in British Columbia.

Procedures: Questionnaires and interviews are administered at 33-weeks gestation, and at 1-month and 3-months postpartum. Prenatal questionnaires will assess demographic information and reproductive history, parenting attitudes, beliefs about thoughts, OCD symptoms and mood. Postpartum questionnaires will also include assessment of birth information and infant temperament. Postpartum interviews will assess postpartum thoughts of harm, OCD and depression. All assessments will include questionnaires assessing social support and sleep. The final assessment will also include anonymous questions about infant harm.

Outcomes: Recruitment is scheduled to begin in August 2013. This research will determine (a) the prevalence of postpartum thoughts of harm and ppOCD, and (b) whether or not there is an association between postpartum intrusive thoughts of infant-related harm, and i) postpartum OCD and ii) maternal harming behaviours. This knowledge will be integral to the development of interventions for pregnant and postpartum women and educational material for maternity care providers, pertaining to these frequently distressing, and all too often debilitating, thoughts.
Student: Sarah Wang
Board #: 55
Session #: 5
Supervisor: Jean-Pierre Chanoine
Title: Preliminary data: PEP measurement of sympathetic nervous system function at rest and under stress in childhood obesity
Author(s): Sarah Wang, Brenden Hursh, Mir Fazeli, Jean-Paul Collet, Jean-Pierre Chanoine

Abstract:
Background: The rate of childhood obesity is increasing in Canada and current weight management approaches have had limited success. Obesity is associated with autonomic nervous system (ANS) dysfunction, an imbalance characterized by decreased parasympathetic and increased sympathetic function. In adults, the presence of ANS dysfunction has been shown to correlate with increased morbidity and mortality. Additionally, ANS dysfunction may contribute to a pro-inflammatory state. There are few studies investigating the function of the autonomic nervous system in childhood obesity. Pertinent to this report, the pre-ejection period (PEP) is a systolic time interval that inversely correlates with the sympathetic nervous system function. In this preliminary investigation, as part of the “Autonomic Dysfunction and Inflammation in Childhood Obesity” study, we aim to describe the function of the ANS using PEP in children with obesity (OB) as compared to children with normal weight (NW).

Methods: 8 NW (BMI 15-85th percentile) and 8 OB (BMI > 95th percentile) children and adolescents aged 12-18 were recruited. Participants were asked to fast for 10 hours. Information on the participant’s age, gender, ethnicity, puberty stage, weight, height, BMI, waist circumference, and presence of acanthosis was obtained from a brief medical history and physical exam. A glucometer was used to determine fasting glucose levels. Impedance Cardiography was used to measure PEP at rest, during perceived stress (participants trace a star while looking into a mirror), and during physical stress (participants perform a sustained grip test). Concurrently, heart rate (HR) and blood pressure (BP) were also measured.

Results: Descriptive statistics will be provided for each of the two sample populations. Using the Wilcoxon Rank Sum Test, the PEP, HR, and BP at baseline and the percent change during perceived and physical stress tests will be compared between OB and NW groups. The study will provide exploratory data on ANS function in childhood obesity.
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<td>Participants:</td>
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<td>Ka Hong (Casey) Chan</td>
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<td>Sharlyn Khan</td>
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<td>Vivian Kwan</td>
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Coarctation of the aorta (CoA) is a congenital narrowing of the aorta accounting for 5 – 8% of congenital heart lesions. This narrowing impedes blood flow and increases blood pressure that may result in impaired heart function. Surgical repair of coarctation has classically been the primary therapy. In the last two decades, transcatheter approaches through balloon angioplasty and stenting have been gaining wide acceptance. Recent studies have shown that stenting is a safe and effective procedure in reducing systemic blood pressure as well as the gradient across the area of coarctation. Moreover, mid-term follow up data from the Congenital Cardiovascular Interventional Study Consortium, the largest study to date, suggests that stenting is a superior therapy compared to balloon angioplasty and surgery due to lower complication rates and blood pressure gradients. Despite successful repairs, it has been shown that hypertension may persist in up to 50% of adolescents and 90% of patients over 50. Patients that appear normotensive at rest may also exhibit an abnormal blood pressure during exercise, with a study showing that this may be associated with progression to resting hypertension. However, the reasons behind the persistent hypertension still remain unclear. This makes optimum treatment for CoA difficult to elucidate, especially considering that hypertension is a well-established risk factor for other cardiovascular diseases. Numerous factors have been implicated including increased abnormal blood pressure regulatory systems, arteriopathy and arterial stiffness. Yet, there is little literature on the long-term impact and management of hypertension following stenting. Potential changes of the biophysical properties of the aorta as well as the flow pattern through it are also inadequately understood. Our group has developed a non-invasive technique to assess elastic properties of the aorta in children. The purpose of our study is to determine the rates of hypertension and long-term effects of coarctation stenting on aortic flow properties at rest and during exercise in children and adolescents.
Student: Michelle Chiu
Board #: 57
Session #: 6
Supervisor: Mark Ansermino
Title: The RRate Mobile App: Developing a robust measure of respiratory rate for use in low-resource settings
Author(s): Michelle Chiu, Dustin Dunsmuir, Mark Ansermino

Abstract:
Background: Every year, pneumonia kills 2 million children under five and is the leading cause of death in that age group. The World Health Organization’s guidelines use high respiratory rate, defined as >50 in children 2-12 months and >40 in children 1-5 years, as the primary sign for diagnosing pneumonia in low-resource settings. The main method of measuring respiratory rate, the ARI Timer, is time consuming. There is an urgent need for a robust alternative that can help community health workers in developing countries to more quickly and accurately diagnose childhood pneumonia.

Objectives: The RRate mobile app requires users to tap the phone’s touchscreen each time the child inhales, and calculates the child’s respiratory rate based on the length of the tap intervals. Our study objective was to evaluate and improve RRate’s algorithm to achieve an average error of <4%.

Methods: We developed a gold standard set of 10 video recordings with a respiratory rate range of 17 to 59 breaths/minute and an age range of 0 to 5 years. We recruited 22 adults with varying degrees of medical experience to watch the videos in random order, and measure the respiratory rate in each video using RRate and the ARI timer.

Results: We analyzed 44 algorithms for their accuracy and efficiency. Algorithms with high accuracy had low efficiency, so we calculated the trade-off ratio for each algorithm that had an average error of <4%. The algorithm with the best trade-off ratio used the median of four tap intervals and a consistency threshold of 15%. The average error of this algorithm was 1.17 breaths/minute (95% CI: -4.02 to +4.17) and the normalized error was 3.47%. The average number of taps needed was 5.7, which equals 8.6 seconds for a respiratory rate of 40 and 6.8 seconds for a respiratory rate of 50. The ARI timer’s average error was 0.47 (normalized: 1.51%), but required 60 seconds for each measurement.

Conclusion: RRate successfully measured respiratory rate with an accuracy of <4% error, and had much better efficiency than the ARI timer. Our next step is to evaluate RRate in realistic clinical settings.
Abstract:

Background: Social media has become the target for marketing adolescents with over 47% of adolescents owning smart phones. The Vaccine Evaluation Center (VEC) is conducting a study (QUEST) in adolescent girls to evaluate whether 2 doses is non-inferior to 3 doses of the quadrivalent HPV vaccine in the prevention of persistent infections of HPV 16, 18, 6 or 11. A greater understanding of how to promote the study to adolescent females through social media could help with study recruitment and sustain retention over a 10 year study.

Objective: The objective is to evaluate the role of social media in enhancement of recruitment and retention strategies of adolescent females.

Methods: A literature review of current social media trends with adolescents using medical, business and marketing database is conducted. Interviews with recruiters working on the Quest study were carried out in 7/9 staff.

Results: The results from the literature review indicate there is a growing trend to adapt social media sites and applications (APPS) that parents and older generations are not active members. Facebook is no longer a plausible means of promoting to adolescent as most are migrating to Twitter, Instagram, and Tumblr. To advertise and promote the project recruiter felt that social media is essential but personal contacts such as school presentations are equally important.

Conclusions: Appropriate mass social media advertising is necessary to attract adolescents girls and evolving with social media trends for long term retention. 7 recruiters agree social media is necessary for recruitment along with onsite visits.

Future Direction: The study will need to begin diversifying the social media outlets to target adolescent girls. To invest in sites such as Twitter, Instagram, Pinterest and Tumblr while choosing one means of social media as a hub. To develop contests and manage how to get “likes”, “repsins” and “retweets” to spread the message (peer recruiting) of the HPV study and allow promotion to be lead by the adolescents rather than the researchers.
Abstract:
Charting the Territory is a longitudinal study with 258 participating families whose children have progressive, life-threatening metabolic disorders, neurodegenerative disorders and chromosomal disorders. These children experience health issues that challenge the family over time, making it important to assess the families’ health and functioning.

The Family Adaptability and Cohesion Scales (FACES) is a standardized questionnaire measuring the adaptability and cohesion of an “ideal family” – a family with parents and children old enough to communicate and actively participate in decisions. While the FACES is validated and commonly used, the authors did not address atypical families, e.g. where a couple has a non-verbal child with life threatening condition.

Does the FACES questionnaire provide valid data regarding families whose composition is atypical?

Preliminary results show that families can be grouped into three categories: the first and second categories are atypical whose only child is the index case and/or siblings who may not be active members of the family. The third category consists of typical families for the FACES with one sick child and one or more siblings who participate actively in the family.

Overall mean and standard deviation for each subscale – adaptability and cohesion – will be reported and compared to normative samples. ANOVA will be conducted to evaluate statistical differences between means for each family category.

Using family categories to analyze the FACES will inform future research focusing on rare childhood population as to whether the FACES is a valid tool to conduct analysis.
Student:            Vivian Kwan  
Board #:            60  
Session #:          6  
Supervisor:    Kenneth Poskitt  
Title:          Seizures are independently associated with altered brain development in infants with congenital heart disease  
Author(s):      Vivian Kwan, Vann Chau, Jill Zwicker, Elavazhagan Chakkarapani, Kenneth J Poskitt, Steven P Miller  

Abstract:  
Background: Although seizures occur commonly in term newborns with congenital heart disease (CHD), it is unknown whether seizures affect brain development in this population.  
Objective: To determine if seizures are associated with altered brain development in term newborns with CHD.  
Design/Methods: Between May 2006 and February 2013, term neonates with CHD were recruited at BC Children’s Hospital. Participants underwent MRI (standard MRI, diffusion tensor imaging (DTI), and MR spectroscopy) on median day of life 4 (IQR 3-8). Standard MRI scans were evaluated using validated scales for white matter injury, intraventricular hemorrhage, and cerebellar hemorrhage. Fractional anisotropy (FA) on DTI, which provides a measure for micro-structural development, was recorded bilaterally in 12 regions of interest in the gray and white matter, and averaged bilaterally. N-acetylaspartate/choline ratios on MR spectroscopic imaging, which reflects brain metabolism, were collected in 8 regions and averaged bilaterally. During and immediately after surgery, amplitude-integrated EEG was collected to detect seizures. Clinical data were recorded from detailed chart reviews.  
Results: The cohort comprised of 45 term neonates with CHD (36 transposition of great vessels, 5 single ventricle physiology and 4 others): median gestational age at birth was 38.9 weeks (IQR 38-40) and median birth weight was 3240 grams (IQR 2930-3690). Of these, 35 infants were classified as “2 ventricles without arch obstruction”, 6 as “single ventricle without arch obstruction”, and 4 as “single ventricle with arch obstruction”. The clinical characteristics did not differ between the newborns with or without seizures. Seizures occurred in 6 of the 45 newborns (13%); 5 of the seizures occurred in newborns with TGA (83%) and 1 with other (17%). In a multivariate regression model adjusting for risk factors (gestational age at scan, stroke, white matter injury, and cardiac anatomic classifications), newborns with seizures had lower N-acetylaspartate/choline ratios (p<0.001). In addition, the presence of seizures trended towards lower FA (p=0.077).  
Conclusions: In this prospective cohort of term newborns with CHD, seizures were independently associated with altered brain metabolic development and a trend toward abnormal brain micro-structural development. Prevention and improved management of seizures may offer potential to improve brain development in infants with CHD.
**Abstract:**

**Background:** Imbalances in the vaginal microbiota can lead to negative reproductive health outcomes for women, including increased risk of acquiring HIV, sexually transmitted infections and preterm birth. The study’s objective was to use cpn60 (60kDa chaperonin) metagenomic profiling to explore differences between the vaginal microbiome of HIV-positive and negative reproductive aged women.

**Methods:** 54 HIV-positive women were recruited from the Oak Tree Clinic and 91 HIV-negative women were recruited through passive advertisement. Demographics, clinical information and vaginal samples for gram stains and genomic analyses were collected during study visits. Nugent’s scores were used to assess gram stains. Total DNA was extracted from vaginal swabs and PCR amplified with cpn60 universal primers. Cpn60-sequence libraries were generated using 454-GS-FLX Titanium; which used cpn60 reference database (cpndb_nr_vag20121004) to match with known bacterial organisms. Multiple regression analyses were used to find associations between cohort and taxa groups. LEfse algorithm was used to compare bacterial abundance and diversity.

**Results:** HIV-positive - Mean age: 36.5y (range: 22-49y); ethnicity: 18 Caucasian, 9 Black, 12 Aboriginal, 4 Asians, 4 South Asians, 6 mixed ethnicity; mean CD4 count: 288cells/mm3 (range: 90-930 cells/mm3); mean viral load: 13,392copies/mL (range: <40-355,245copies/mL); 62% of women had suppressed viral loads; 85% of women were on antiretroviral therapy. Using clinical gram stain assessment, 30%(16/53) of women had bacterial vaginosis and 9%(5/53) had intermediate scores.

HIV-negative - Mean age: 29.9y (range: 18- 48y); ethnicity: 58 Caucasian, 4 Black, 1 Aboriginal, 19 Asian, 6 South Asian, 2 mixed ethnicity. Using clinical gram stain assessment, 4%(4/91) had bacterial vaginosis and 10%(9/91) had intermediate scores.

231 phylotypes were identified. Results suggest that on average, taxa including Gardnerella vaginalis (except GroupB), Megasphaera, Clostridia, Prevotella timonensis and amnii, Bifidobacteria breve and Dialister micraerophilus were more frequently seen in HIV-positive women. Lactobacillus jensenii, Lactobacillus criputus and Garnerella vaginalis GroupB were more commonly observed in HIV-negative women. Further statistical analyses of relationships between behavioral demographics and vaginal microbiome are underway.

**Conclusion:** Culture-independent methods exhibited substantial variation in vaginal microbiome among HIV-positive and negative women. Exploration of these trends using a larger sample size, and relationships of such shifts within other cohort groups is warranted.
Nutritional status of pre-operative surgical patients: Prevalence of malnutrition and impact on surgical outcomes

Author(s): Roseanne Lim, Erik Skarsgard

Abstract:
The detrimental effects of malnutrition in childhood are well established. These include poor growth, impaired educational performance and social achievement, and nutrition-related disease burdens as adults. However, limited information is available on the prevalence of malnutrition in pediatric hospital patients and the impact of malnutrition on surgical outcomes. The goal of this study is to conduct a retrospective chart review to estimate the prevalence of malnutrition in patients who underwent orthopedic hip or spine surgeries at BC Children’s Hospital. We hypothesize that malnutrition is prevalent in this population, and confirmation of our hypothesis will justify our next study, which will be to validate a malnutrition screening tool for use in pediatric surgical patients. The medical records of 70 orthopedic patients (20 hip and 50 spine), who have undergone surgery between January 2012 and December 2012, will be reviewed for age, gender, height and weight measurements, and discharge diagnoses. The existence of malnutrition will be estimated by two methods: Body Mass Index (BMI) percentile ranking and by calculated Z-scores. The weight and height measurements will be used to calculate patient BMI and the obtained BMI values are plotted for percentile ranking. The BMI values of children ages 0-2 years are plotted according to the World Health Organization (WHO) Growth Standards and Centers for Disease Control (CDC) growth charts are used for children ages 2-18 years. Children and teens will be categorized as underweight (less than the 5th percentile), healthy weight (5th to 84th percentile), overweight (85th to 94th percentile), or obese (equal to or greater than the 95th percentile). BMI Z-scores will be calculated by subtracting the mean BMI of the reference population from the patient’s BMI and dividing it by the standard deviation of the reference population. Malnutrition will be defined as having a BMI standard deviation of greater than 2 or less than -2. Our study is still ongoing with pending results and conclusion. The first part of the study is expected to be completed by the end of August 2013.
Abstract:
Current clinical measures of stereo vision for children include only small disparities that can be fused for single vision (fine stereopsis). However, previous research from our lab demonstrated that children with amblyopia and poor fine stereopsis performed just as well as controls when the disparities were large and gave rise to double vision (coarse stereopsis). Yet the poor visual acuity that is the hallmark of amblyopia is a potential confound in interpreting these results. We subsequently explored whether coarse stereopsis might be spared in children with strabismus without amblyopia, another condition that is characterized by poor fine stereopsis, and whether this might serve as a useful predictor of stable eye alignment following strabismus surgery.

First, we recruited children aged 4-12 from the BCCH Ophthalmology Clinic who had strabismus with no other visual or general health problems. Participants were included if they had good visual acuity and poor stereoaucity on clinical tests. To measure fine and coarse stereoscopic depth perception, we used a Pokemon-themed videogame that presented stimuli over a large range of disparities from fused (fine) to double (coarse) using liquid crystal shutter glasses synchronized to a computer. We assessed the accuracy with which participants judged whether the cartoon characters were nearer or farther away than a zero-disparity reference frame. Our preliminary results confirmed that patients with strabismus and poor fine stereopsis show residual coarse stereopsis.

Next, we conducted a chart review of all children (with or without amblyopia) who had undergone strabismus surgery and for whom we had existing measurements on the previously described task. We extracted information about the type of strabismus, number of surgeries, and eye alignment measures pre- and post-surgery (at several time points). Data analysis is in progress, and we predict that residual coarse stereopsis will be positively correlated with stable eye alignment after strabismus surgery.

The next step will be to conduct a prospective study to determine the extent to which the presence of coarse stereopsis before strabismus surgery predicts surgical outcomes. Ultimately, this research could provide clinicians with more evidence on which to base their treatment decisions for pediatric strabismus patients.
Abstract:

Background: Tonsillar enlargement can be related to several health conditions in the pediatric population, most notably, Obstructive Sleep Apnea (OSA). Reliable monitoring and documentation of tonsil size is necessary in clinical and research settings. Tonsil grading systems or scales allow clinicians to precisely record and communicate changes in tonsil size. There is however, significant variability associated with the use of tonsil grading systems which may potentially make tonsil grading unreliable. Different medical settings employ the use of multiple tonsil grading systems and the reliability of these systems in a ‘real-life’ setting has never been formally studied. Therefore, there is a need to compare commonly used existing tonsillar grading scales and to assess their reliability/reproducibility in clinical settings.

Objectives: We aimed to investigate the intra-observer reliability and inter-observer reliability of 3 different tonsil grading scales: Brodsky Grading Scale, Friedman Grading Scale, and a Modified-3-grade scale, which was designed in Vancouver. We hypothesized that a 3-grade scale may provide a greater intra-observer and inter-observer reliability when compared to the other tonsil grading scales.

Methods: This study aimed to recruit a minimum of 80 children between ages 3-18 with no major craniofacial abnormalities, who were attending the Pediatric Otolaryngology outpatient clinic at BC Children’s Hospital. For each child, 2 separate tonsil assessments (with at least a 5 minute interval in between) were conducted by 4 independent observers with different clinical backgrounds. These included: 2 Otolaryngologists, 1 Fellow/Resident, and 1 Medical Student. Each observer assessed and graded tonsil sizes using 3 different scales.

Results: We are currently in the data collection phase of this study. To assess the reliability of the tonsil grading systems, we will derive the Intra-Class Correlation Coefficient (ICC) and Cronbach a. An ICC cut-off value of 0.75 will be used to indicate an acceptable reliability level.

Conclusions: As this study is still in progress, it is difficult to derive any conclusions at this point. We expect, however, that the results of this study will help improve tonsil size assessment in kids and will lead to better medical care for the pediatric population.