Epilepsy, Asthma and Diabetes

Session Moderator: Ian Sweet, Medicine
Mary Gates Hall Room 251
1:00 PM to 2:30 PM

* Note: Titles in order of presentation.

UV-Mediated Toxicity of a Known Longevity Compound
Devon Brian (Devon) Chandler Brown, Senior, Biology (General), Biochemistry
Mary Gates Scholar
Mentor: Matt Kaeberlein, Pathology
Mentor: Lara Shamieh, Biology, Regis University

Ethosuximide is a T-type calcium channel inhibitor whose anticonvulsant properties are used clinically to treat epileptic seizures. Recently this compound was also found to have positive effects on lifespan in an invertebrate model organism, the nematode *C. elegans*, which may translate to similar effects in humans. While the therapeutic benefits of ethosuximide are promising, we report a light-mediated reaction that produces a previously unreported by-product. Mortality assays using *C. elegans* show that acute, high dosage exposure and long-term, low dosage exposure have much greater toxicity than unreacted ethosuximide. This property is unique, as several structurally related compounds do not exhibit similar biological properties. Using chromatographic separation and a variety of spectroscopic techniques, we seek to identify the unknown product of the reaction. Once the product is identified, it will be possible to use genetic analysis in *C. elegans* to elucidate the biological basis of the toxicity. Ethosuximide is known to cause severe skin irritation in the presence of sunlight, and consequently finding the mechanism of UV induced toxicity will allow mitigation of this effect. Given ethosuximide’s current use as a clinical anti-convulsant and its potential as an anti-aging therapeutic, it is important to limit the potential side-effects of the drug.

Copy Number Variation in Brain Development and Epilepsy
Cynthia Hsu, Senior, Biology (Molecular, Cellular & Developmental), Biochemistry
Howard Hughes Scholar, Mary Gates Scholar, NASA Space Grant Scholar, Washington Research Foundation Fellow
Mentor: Heather Mefford, Pediatrics

Epilepsy is a disorder of electrical signals inside the brain causing recurring seizures that range from blank stares to full-fledged convulsions. 200,000 new cases of epilepsy are diagnosed each year and the prevalence of active epilepsy is estimated at almost 3 million in the United States (Epilepsy Foundation). There are many different types of epilepsy, ranging from mild, treatable forms that eventually resolve to severe debilitating forms. We are investigating the genetic causes of epileptic encephalopathies, a group of severe epilepsy syndromes characterized by severe cognitive and behavioral disturbances resulting from epileptic seizures in infancy and early childhood. While mutations in some genes, such as PDHA1 and Sox6, have been found to be associated to epileptic encephalopathies, much is still unknown about the genetic causes of epileptic encephalopathies. We hypothesize that some of these patients will have large deletions or duplications of their genome in regions critical to brain development and function. To identify these critical genes, we are performing array comparative genomic hybridization (CGH) on 400 patient DNA samples. Our preliminary data on more than 250 samples suggest that up to 10% of patients carry a deletion or duplication that may be the cause of their epilepsy. One example is a homozygous deletion of part of the CNTNAP2 gene seen in two siblings. The CNTNAP2 gene is known to be important for brain development and has been implicated in specific language disorder, autism, mental retardation, and schizophrenia. Further analysis revealed that the parents were both heterozygous carriers of the mutation. We sequenced the breakpoint and identified a microduplication that is the likely cause of the deletion. Array CGH studies are ongoing and will lead to the identification of additional new candidate genes for epilepsy.
Mechanisms leading to airway remodeling in asthmatic children are poorly understood. Periostin, an integrin ligand and extracellular matrix protein, has been proposed as a gene involved in asthmatic airway remodeling. Periostin expression by bronchial epithelial cells (BEC) from asthmatic adults was recently shown to be greater than from healthy adults. Our aims are to determine if periostin expression by BEC’s from asthmatic children is greater than from healthy children, unstimulated and in response to IL4 and IL13 stimulation or rhinovirus-16 (RV-16) infection. We carefully phenotyped atopic asthmatic (n=6) and healthy children (n=6) ages 6-16 yrs. who underwent anesthesia for an elective surgical procedure. Bronchial brushings were performed to obtain BECs. BECs were grown and differentiated in transwells at an air-liquid interface. Differentiated cells were then stimulated with IL4 and IL13 (50ng/mL each), infected with RV16 at multiplicity of infection of 0.1, or maintained unstimulated. RNA was isolated and gene expression measured using real-time quantitative PCR. Periostin expression was normalized using GAPDH as a housekeeping gene. We found that periostin expression by asthmatic BEC’s was 4.5-fold greater than by healthy BEC’s. Following IL-4 & IL-13 exposure periostin expression by asthmatic BEC’s was 1.1-fold greater than by healthy BEC’s. Following RV-16 infection periostin expression by asthmatic BEC’s was 2-fold greater than by healthy BEC’s. To our knowledge, these data are the first from pediatric human BEC’s demonstrating greater periostin expression by cells from asthmatic children than from healthy children. These results suggest that airway epithelial periostin expression is unique to cells from asthmatics. Identification of a unique airway epithelial gene expression pattern from asthmatic children may be useful as a biomarker to aid in the diagnosis of asthma in young children, and will assist in the design of future mechanistic studies to assess the role of periostin expression in asthmatic airway remodeling.

The Role of Obesity-Associated Inflammation in Insulin Resistance
Harpreet Singh (Harpreet) Dhaliwal, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Mario Kratz, Epidemiology
Mentor: Brian Van Yserloo, Medicine

With increasingly sedentary lifestyles, obesity and metabolic diseases have become growing concerns. Obesity triggers the release of inflammatory proteins. These proteins interfere with the functions of hormones secreted from adipocytes (fat cells), leading to insulin resistance, and associated metabolic diseases such as type II diabetes. Previous research, using mouse models, has suggested that obesity-associated low-grade chronic inflammation is an important factor in the development of insulin resistance. A key focus of our research is to evaluate to what extent these findings apply to humans. We believe adipose inflammation comes from macrophages somehow infiltrating adipose tissue and becoming activated. If this is true, then we may be able to measure correlations between insulin resistance and obesity-associated inflammation. As part of our study, we have recruited subjects of varying adiposity and insulin sensitivity, and; we are specifically testing the hypothesis that obesity-associated inflammation is an important factor in the development of insulin resistance. Blood and fat samples from study participants have been assayed to identify potential correlations between inflammation, adiposity, insulin resistance, and insulin sensitizing hormones in humans. In our analyses, we have found increased expression of inflammatory cytokines and chemokines in subcutaneous adipose tissue of obese, insulin resistant subjects (relative to leaner, more insulin sensitive subjects). Furthermore, we have found the expression of these pro-inflammatory genes to negatively correlate with the adipose tissue expression and plasma concentrations of adiponectin, a key insulin-sensitizing hormone. We conclude that insulin resistance in humans is associated with adipose tissue inflammation. Our findings are one step toward further understanding the etiology of insulin resistance and type 2 diabetes in humans.

Cytochrome C Reduction Measurement by Absorbance in Pancreatic Islets of Langherhans as a Method to Clarify the Role of Cytochrome C in the Control of Insulin Secretion
Kelli Marie Mc Entee, Senior, Spanish
Mary Gates Scholar
Mentor: Ian Sweet, Medicine

In diabetes research, much work is done to elucidate the factors leading to insulin deficiency on a cellular level. The beta cells in the pancreas are the site of insulin production, and it has been shown that the survival of these cells is dependent upon their metabolism and the rate of electron transport. Cells undergoing dysfunction or apoptosis exhibit marked declines in both of these areas. The rate of electron transport is directly linked to the reduction of cytochrome c, an electron carrier in the electron transport chain. Our lab has previously shown that the number of electrons available to cytochrome c can be measured by its absorbance at 550nm. Because of this property, we can measure real-time reduction of cytochrome c by measuring the absorbance of light transmitted across a bed of cells. Our current data is collected by measuring absorbance across a bed of 1000 islets, however, it would be more time and cost effective to heighten the sensitivity of the system to reduce the number of cells necessary. In order to do this, we need to increase the signal produced by our spectrophotome-
Evidence for a High-Energy Process that Couples Calcium Influx and Cytochrome C Reduction to Insulin Secretion in the INS-1 Cell Line

Mark James (Mark) Lisowski, Senior, Biology (Molecular, Cellular & Developmental), Biochemistry
Mary Gates Scholar
Mentor: Ian Sweet, Medicine

Dysfunction of pancreatic β-cells leading to inadequate insulin secretion is a major determinant in the development of diabetes. Therefore, a detailed understanding of the intracellular processes that mediate insulin secretion rate (ISR) is critical to advancing diabetes research. Previous studies in our laboratory have shown that both an increase in calcium influx and the reduction of a specific protein in the electron transport chain (cytochrome c) are essential for stimulation of ISR. These results have lead to the hypothesis that together these two signals lead to an increase in energy generation needed for the activation of processes regulating ISR.

In this study, we tested our hypothesis by comparing the relationship between calcium influx, cytochrome c reduction, energy turnover and ISR in a cell line model, INS-1 cells, characterized by normal glucose-ISR dose response dependency, but low absolute ISR. Based on our hypothesis, we predicted that the ability of calcium influx and cytochrome c reduction to stimulate energy production (as reflected by oxygen consumption rate (OCR)) would diminish in proportion to ISR. Initial studies showed that stimulation of INS-1 cells with glucose increased cytochrome c reduction similarly to normal islets, whereas ISR was only about 10% of what islets normally secrete. To test whether the calcium-sensitive component of OCR was low, the effects of calcium channel blockade was assessed. The change in OCR in INS-1 cells was barely measurable (0.01) compared to 0.15 nmol/min/10^6 islet cells). However, the low rate of calcium-stimulated energy generation was greatly increased by pharmacologic activation of calcium channels, which concomitantly increased ISR to levels seen in islets. In summary, this data supports the scenario where energy generation stimulated by adequate calcium influx and increased cytochrome c reduction regulates rates of insulin secretion. Dysfunction of this regulatory mechanism represents a possible cause for diabetes.