UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

Poster Session 1

Commons East, Easel 61

11:00 AM to 12:30 PM

The Ontology of Craniofacial Development and Malformation (OCDM): Standardizing the Classification of Orofacial Clefting

Kathie H (Kathie) Wang, Senior, Neurobiology, Biochemistry
Mentor: Carrie Heike, Pediatrics, Seattle Children's Hospital
Mentor: Timothy Cox, Pediatrics (Craniofacial Medicine)

Cleft lip with or without cleft palate is among the most common congenital birth defects, but its phenotypic presentation is highly variable. This variability not only impacts the clinical management of patients, but also poses many challenges for recurrence risk counseling. Population studies indicate a diversity of causative factors, including genetic and non-genetic factors. Although some genetic causes have been identified, further progress in this area will require more precise classification of subphenotypes. Numerous cleft lip/palate classification systems have been developed over the years, but there is a lack of consistency among these models. These discrepancies lead to problems regarding precision and interpretation when documenting clefts for research or treatment purposes. The Ontology of Craniofacial Development and Malformation (OCDM) project, part of the National Institute of Dental and Craniofacial Research's FaceBase Program, aims to develop a framework of anatomical terminology to facilitate integration of diverse sources of research and clinical data, including genetic, embryological, and phenotypic information. The overarching goal is to maximize sharing of research data and promote collaboration to drive research into the causes and treatment of craniofacial disorders. To this end, we have comprehensively reviewed existing classification systems with respect to the methods of categorization of cleft types, including placement, severity, and other distinguishing features. Along with wide variation in terminology, there was also wide variation in the extent of content among systems. Each system categorized clefting differently, but none encompassed all other systems' classifications, nor all cleft types observed in the clinic. It is predicted that integrating these systems through a common anatomical ontology will serve to facilitate standardization of cleft classifications and terminology. In addition, this foundation should enable further subclassification to ultimately benefit studies exploring relationships between causes and manifestations of orofacial clefting, as well as underpin advances in interventional therapies and surgical treatments.

Session 1D

Medical Therapeutics and Endocrinology

Session Moderator: Ian Sweet, Medicine

231 MGH

1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Expression of Costimulatory Molecules in Juvenile Idiopathic Arthritis versus Severe Gingivitis

Megan Christine Yuasa, Senior, Biology (Physiology)
Mentor: Anne Stevens, Pediatrics

The most common rheumatological disorder found in children is juvenile idiopathic arthritis (JIA), a disabling disease of unknown etiology and with no cure to date. Previous studies have suggested autoantibodies of adult rheumatoid arthritis (RA) cross-react with oral pathogens, suggesting that oral infection could trigger arthritis. Severe gingivitis, an inflammatory disease of the gums, is present in 50-100% of adolescents, and may exhibit this same interaction with JIA. Costimulatory molecules on antigen presenting cells are induced during an inflammatory response to regulate T lymphocytes. Specifically, programmed death ligand-1 (PD-L1) known to be expressed during infection, is also highly upregulated in JIA. To test the hypothesis that PD-L1 expression is induced by oral pathogens associated with gingivitis, peripheral blood cells were isolated from JIA patients and healthy children. Gingivitis was scored by oral examination. PD-L1 expression was assayed on myeloid DCs and monocytes by flow cytometry. Preliminary data on a subset of subjects (n=7) showed a higher percentage of monocytes with PD-L1 in JIA patients compared to controls; however JIA patients had a lower density of PD-L1 per cell. There was no association between extent of gingivitis and PD-L1 expression. The results of this study could contribute to a new field of JIA therapy targeted at costimulatory molecules and oral hygiene.

SESSION 1T

MOLECULAR AND CELLULAR BIOLOGY

Session Moderator: Hannele Ruohola-Baker, Biochemistry
111 JHN
1:15 PM to 2:45 PM
* Note: Titles in order of presentation.

Sequencing DHODH in Persons with Miller Syndrome: Expanding the Spectrum of Mutations
Naomi Tweyo (Naomi) Nkinsi, Freshman, Pre-Sciences
Mentor: Heidi Gildersleeve, Department of Medicine Pediatrics Genetics

Miller syndrome is a rare autosomal recessive disorder resulting from mutations in the DHODH gene. DHODH encodes the enzyme dihydroorotate dehydrogenase, which is involved in de novo pyrimidine biosynthesis. Clinical characteristics of Miller syndrome include craniofacial abnormalities, coloboma of the eyelids, cleft palate, and missing postaxial digits on the hands and feet. To broaden the spectrum of causal mutations, DNA samples obtained from two unrelated affected probands were Sanger sequenced to screen for mutations in DHOHD. In family 1, the affected individual was a compound heterozygote for a mutation in one of the canonical splice bases of intron 6-7 (c.820+1G>A), and a missense mutation in exon 8 (c.C1036T, p.R346W). DNA samples obtained from both parents of the affected proband were screened using Sanger sequencing and each parent was found to be heterozygous for one of the DHODH mutations. The c.C1036T mutation has been reported now in three families with Miller syndrome and functional studies have demonstrated a reduction of DHODH activity by 40%-70% in yeast with this mutation. No pathogenic mutations in DHODH were found in family 2. The next steps we will take include whole genome sequencing of the proband and parents in family 2 to determine if there are mutations in other genes that cause Miller syndrome. Our results mark the first report of a splice site mutation in a patient with Miller syndrome and further expand the spectrum of causative mutations in DHODH.

POSTER SESSION 2

Commons East, Easel 52
12:45 PM to 2:15 PM

Exploring the Genetics of Seizures in Individuals with Fragile X Syndrome
Adiba Khan, Junior, Biochemistry, Communication (Journalism)
Mary Gates Scholar
Mentor: Heather Mefford, Pediatrics

Those who have studied genetics have a keen understanding of the fact that incredibly small changes can have tremendous, and often tragic, consequences. In patients with Fragile X Syndrome (FXS), a repeating sequence of genetic information (CGG) in an X chromosomal region is repeated more often than in the general population. This results in a spectrum of conditions including intellectual disability, anxiety, and physical features that deviate from the norm. About one fifth of people who have FXS also have seizures; the underlying cause of seizures in this fraction of FXS individuals is unknown. We hypothesize that FXS individuals who have seizures will have specific genetic variants –duplications and deletions of genetic information –that are not seen in FXS individuals without seizures. The experimental group is composed of DNA samples of FXS patients with seizures, while the control group will be composed of DNA samples of FXS patients without seizures. In order to find genetic variants, I utilize a genomic technique called array-comparative genomic hybridization (array-CGH). In array-CGH, DNA samples are tagged with fluorescent dyes to contrast patient DNA (tagged with pink dye) from control DNA (tagged with blue dye). The ultimate product of this technique is a quantitative representation of any differences between patient and control DNA, which can be processed and analyzed by computer software. By comparing the genomes of these patients, we will determine if there are common genetic variants across the experimental group that are distinct from the control group. If such variants are found, the next step is to associate specific genes with the subset. Establishing an association between a gene and the FXS and seizure phenotype is essential because it deepens our understanding of the condition and provides a foundation for future therapeutic treatments.

POSTER SESSION 2

Commons East, Easel 54
12:45 PM to 2:15 PM

The Design and Implementation of a Novel Method to Study the Dependence of Microbubble Cavitation and Collapse on Ultrasound Frequency
Alexander (Alex) Esibov, Senior, Bioengineering
Mary Gates Scholar
Mentor: Carol H. Miao, Pediatrics
Mentor: Misty Noble, Seattle Children's Research Institute

Ultrasound (US) and microbubbles (MBs), as tools for gene transfection, have promising implications for gene therapy. Through permeation, US facilitates the diffusion of a gene or drug from the capillaries into the cell. Binding of the gene or drug to the MBs can further enhance the delivery efficiency to the cell, and when exposed to US, the MBs cavitate, allowing the gene to diffuse into its target destination. Optimal US parameters (intensity, exposure time, central fre-

quency) and MB characteristics (concentration, size, distribution, stability) are interdependent with regards to transfection and cavitation optimization, and thus the optimization of each individual parameter is important. Preliminary data has indicated that the optimal intensity and exposure time for US is 2 W/cm^2 for 3 minutes. However, optimizing US frequency has not been widely explored. The goal of this project is to investigate the effect of US frequency on MB cavitation, with both pure MBs and DNA-bound MBs . In order to do this, the following areas have been completed or are in the process of being completed. 1. A method has been designed to isolate home-made MBs by size; 2. A novel set-up to record the interaction of the US with the MBs with a hydrophone has been created and tested. The signals must be collected and processed with a written MATLAB program; 3. The results must be applied to *in vitro* US transfection with MBs of human embryonic kidney 293 (HEK 293) cells to demonstrate the effect of frequency on transfection results. By expanding research in US-mediated gene therapy with MBs, it is the hope that one day this method will be advanced towards clinical application as a means of non-invasive, localized, and efficient gene delivery.

SESSION 2J

INFECTIOUS DISEASES

Session Moderator: James Mullins, Microbiology

254 MGH

3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

In Vitro Expansion of Factor VIII-Specific T Regulatory Cells

Bryn Smith, Senior, Microbiology
Washington Research Foundation Fellow
Mentor: Carol H. Miao, Pediatrics
Mentor: Chao Lien Liu, Immunology and Immunotherapies, Seattle Childrens Research Institute

Hemophilia A is characterized by a deficiency of factor VIII, a protein necessary for the formation of blood clots. Treatment involves infusions of replacement clotting factor but about one-third of patients develop inhibitors to the clotting factor, resulting in reduced efficacy of the infusion and increased cost of treatment. Immunosuppressant drugs have been found to reduce an inhibitor response, but their off-target effects can complicate treatment further. Alternatively, T regulatory cells have a naturally suppressive function and their proliferation in vivo in response to treatment with IL-2 has been studied previously in the Hemophilia A mouse model. This project aims to expand a population of factor-VIII specific Treg cells in vitro by co-stimulation with anti-CD3 and anti-Crry, in addition to IL-2 treatment. Treg cells are harvested from the spleen, and then isolated by magnetic separation and FACS Aria sorting for CD25high cells. Analysis by flow cytometry has indicated that the expanded cells maintain Foxp3 expression – an intracellular marker for Treg cells – and further experiments are being done to analyze the suppressive functionality of the expanded cells compared to fresh Treg cells. Future expansions will be focused on factor VIII-specific Tregs using the Hemophilia A mouse model and the techniques mentioned previously. The goal is to generate an expanded population of these suppressive cells that show specificity to only factor VIII antigen. This method of expansion has translational potential for suppressing the inhibitory immune response to treatment in human Hemophilia patients. Treating patients with their own expanded cells can reduce possible rejection of the treatment and provide promising long-term results.

POSTER SESSION 3

MGH 241, Easel 172

2:30 PM to 4:00 PM

Interim Report of Pilot Study Assessing the Association of Demographic and Clinical Variables on Quality of Care in Caregivers of Youth with Type 1 Diabetes

Neil Anthony (Neil) Panlasigui, Junior, Comparative History of Ideas
Marissa Amurao Tabile, Recent Graduate,
Mentor: Joyce Yi-Frazier, Seattle Children's Research Institute

Proper patient-provider communication (PPC) regarding choices available to patients and management of diabetes-specific care can lead to better patient outcomes. Our pilot study describes the specific demographic and clinical characteristics associated with PPC in families with children who have type 1 diabetes (T1D). Thirty-four caregivers of youth with T1D ages 2-18 were recruited for a study describing quality of care (QOC) measures, specifically PPC and quality of diabetes-specific education. Parent-report of patient quality of life (QOL), via a standardized assessment, was also collected. Clinical measures including insulin regimen and current glycosylated hemoglobin (A1c) was recorded from the medical record. Descriptive statistics, including Pearson and Spearman correlation coefficients, chi-squared tests and one-way analyses of variance tests were used to explore differences between demographic and clinical variables with QOC. Thus far, 34 caregivers have completed enrollment. On a scale of 4-16, with higher scores reflecting better PPC, the mean was 13.8 +/- 2.6 (range 8-16), indicating relatively high PPC overall. When asked to indicate whether 7 diabetes-specific topics (i.e., what to do for a low or high blood sugar) have been addressed, 23/34 (67.6%) reported all 7 as being discussed (mean = 6.6 +/- 0.7; range 5-7). Among clinical variables (QOL, A1c, and insulin regimen), QOL associated

with PPC (r=.42, p=.01). For quality of diabetes education, income was the only demographic variable associated (families with higher income were more likely to report maximum education scores; chi-squared = 9.48, p=.05). No clinical variables were associated with diabetes education scores. Initial results from this pilot study indicate high levels of quality of care in this population. Certain demographic indicators, such as income, did affect quality of care indicators. Quality of life was also an important correlate with PPC, which suggests adequate communication plays a role in patient outcomes.

POSTER SESSION 3

Commons East, Easel 83

2:30 PM to 4:00 PM

Do Physiological Levels of Human Hepatic Lipase Reduce the Development of Atherosclerosis?

Dean Ricks (Dean) Spencer, Senior, Biochemistry
Mary Gates Scholar
Dongyang Chen, Senior, Applied Music (String Instruments)
Mentor: Helen Dichek, Pediatrics
Mentor: Jennifer Lam, Pediatric Endocrinology

Atherosclerotic cardiovascular disease is the leading cause of death in North America. Hepatic lipase (HL) a liver enzyme that hydrolyzes lipoprotein triglyceride, plays a critical role in lipid metabolism and may influence the development of atherosclerosis. Hepatic lipase may serve dual roles in the development of atherosclerosis: 1) HL may be pro-atherogenic by converting low-density lipoprotein (LDL) to small-dense LDL, causing build up of cholesterol in arteries. 2) HL may be anti-atherogenic by increasing the cholesterol-poor form of high-density lipoprotein (HDL) that removes cholesterol from the arteries, decreasing build up of cholesterol in arteries. Previous studies have reported that elevated levels of human hepatic lipase (hHL) reduce the development of atherosclerosis. However, those studies were done in transgenic mouse models with supra-physiologic levels of hHL and may not accurately reflect the role of physiologic levels of HL on atherosclerosis. Therefore, we created a physiologic model of hHL expression to analyze the role of hHL in the development of atherosclerosis. This "humanized" mouse model expresses hHL at physiologic levels in the liver. We hypothesize that physiologic levels of HL will reduce the development of atherosclerosis compared to mice lacking HL. To test this hypothesis we bred the transgenic hHL mice onto a low-density lipoprotein receptor knock out background (which is an established model for atherosclerosis). Littermates with and without the hHL transgene were fed a high-fat, cholesterol enriched ("Western") diet to accelerate atherosclerosis development, after which the aortas were harvested, dissected longitudinally, and pinned to expose the inner aortic walls for visualization. Cholesterol buildup was stained and the ratios of atherosclerotic accumulation to total aortic surface area were quantitatively measured via Adobe Photoshop. Results from this study will clarify the contribution of hHL to atherosclerosis development. Ultimately, our results will guide the development of new treatments to prevent and treat atherosclerosis.

POSTER SESSION 3

Commons East, Easel 81

2:30 PM to 4:00 PM

GPSM2 Mutations Cause the Brain Malformations and Hearing Loss in Chudley-McCullough Syndrome

Abbey D (Abhay) Knickerbocker, Senior, Biochemistry, Biology (Molecular, Cellular & Developmental)
Mentor: Dan Doherty, Pediatrics

GPSM2 is required for orienting the mitotic spindle during asymmetric cell division during development. The hearing loss and brain malformations in Chudley-McCullough Syndrome (CMS) suggest that this autosomal recessive condition is due to mutations in GPSM2. The clinical phenotype of CMS includes partial agenesis of the corpus callosum, arachnoid cyst, cerebellar dysplasia, gray matter heterotopia, and frontal polymicrogyria. This combination of brain malformations is highly distinctive and not seen in any other genetic syndrome. Homozygosity mapping and sequencing of affected individuals revealed eight molecular variations in the G protein-signaling modulator 2 gene, GPSM2, that underlie CMS. To identify additional GPSM2 mutations, I utilized a molecular inversion probe (MIP) method enabling minimal DNA input, high output performance, and low-cost sequencing for less than $1 per gene per sample. Each MIP is a common 30 bp linker flanked by an extension arm and a ligation arm giving a total MIP length always equal to 70 bp. The unique arms of each MIP target a specific 112 bp genomic region for a gap-fill and circularization. Post-capture PCR amplification with primers corresponding to the common linker was used to adjoin sample-specific barcodes. Samples were then PCR amplified, pooled together, and ran on Illumina MiSeq. Currently, I am sequencing six confirmed CMS patients in addition to eight samples we obtained from patients presenting only with agenesis of the corpus collosum and hearing loss. These results are pending. By sequencing patients who present with classic CMS features, as well as those who present with limited CMS features, I hope to identify a genotype-phenotype correlation in Chudley-McCullough patients.