



Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

Commons West, Easel 21

11:00 AM to 1:00 PM

Using RNA Sequencing to Identify Abnormal Splice Junctions in Undiagnosed Patients with Developmental and Epileptic Encephalopathies

Samra Yemane Gebrehiwot, Sophomore, Pre-Sciences

Mentor: Alison Muir, Pediatrics

Mentor: Heather Mefford, Pediatrics

Developmental epileptic encephalopathies (DEEs) are a group of severe neurological disorders that present with seizures in early infancy and developmental delays. A genetic cause can be identified in up to 50% of affected individuals and is most commonly a *de novo* genetic change. However, for some patients a genetic change is not evident through DNA sequencing. In this research project, we are using RNA sequencing to search for and identify abnormal splicing events that may be due to splice-altering variants in the genome of previously undiagnosed patients. Splicing occurs naturally in unaffected and affected individuals; it is the process of introns being removed from mRNA transcripts and exons being joined. The process of RNA sequencing allows us to discover abnormal splicing errors by looking at splice junctions, which are sites on the intron and exon border where splicing normally occurs. We search for abnormal splicing defects using RNA sequencing by comparing the splice-junctions from our patients to a reference database of healthy controls (Genotype-Tissue Expression Project), filtering out common splice junctions, allowing us to identify unique and abnormal splice junctions. If we identify abnormal splicing, we will also investigate the genome of the patient to determine whether there is an underlying DNA change. Using RNA sequencing, we hope to provide a genetic diagnosis for a subset of our undiagnosed patients and for more individuals affected by DEE in the future.

SESSION 1P

MCNAIR SESSION - BIOLOGICAL MANIPULATIONS TO DEVELOP MEDICAL AND ENVIRONMENTAL INTERVENTIONS

Session Moderator: Barbara Juarez, Psychiatry

MGH 295

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Functional Analysis of an *ARPC4* Variant Associated with Microcephaly

Dianne Laboy, Senior, Biology (Molecular, Cellular & Developmental)

Louis Stokes Alliance for Minority Participation, Mary Gates Scholar, McNair Scholar

Mentor: Heather Mefford, Pediatrics

Mentor: Alison Muir, Pediatrics

Many genes are associated with microcephaly, a condition characterized by a small head size. Through exome-sequencing, we identified *de novo* missense mutations in an actin polymerization gene, *ARPC4*, in four individuals with microcephaly, mild developmental delay, and mild intellectual disability. *ARPC4* is involved in actin filament formation. Actin is an essential component of the cell's cytoskeleton that gives the cell its structure and aids in cell movement and division. The goal of this research project is to understand the molecular mechanisms that lead to the disease phenotype observed in these patients. I began by using CADD, a measurement used to predict the deleteriousness of single nucleotide variants. The mutations identified in the patients were located in highly conserved loci, indicating they might be pathogenic. To provide further evidence of the pathogenicity of the *ARPC4* variants, we were interested in determining the functional effect these variants have on actin polymerization – specifically, the role of *ARPC4* in this mechanism. I established fibroblast cell lines from two patients with the same *ARPC4* mutation. I performed immunofluorescence staining for actin to quantify the amount of actin present in the patients and control cell lines. Preliminary data from this experiment suggests that there is greater abundance of actin filaments in the control sample compared to the patients' cells. To ob-

serve the effect of decreased actin abundance on cell migration, I executed a scratch migration assay. The results from this study elucidate the impact of *ARPC4* in actin polymerization, and establish actin deficiency as a clinically recognizable cause of microcephaly.

SESSION 1Q

BIOLOGICAL STRUCTURE AND FUNCTION

Session Moderator: Matt Kaerberlein, Pathology
JHN 022

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Investigating Nuclear Localization Patterns in NUP188 Patients using Live Cell Fluorescence

Natalie J. Weed, Senior, Economics, Neurobiology

Mary Gates Scholar, UW Honors Program

Mentor: Heather Mefford, Pediatrics

Mentor: Alison Muir, Pediatrics

The nucleus is the information center of the cell, acting as a hub for DNA storage, regulation, and replication. Therefore, transport of molecules into and out of the nucleus is vital for proper biological functioning. This transport is tightly regulated via the nuclear membrane, and a collection of proteins called nuclear pore proteins that interact with molecular signals. These signals allow molecules that cannot passively diffuse through the nuclear membrane (typically greater than 60 kD) to be shuttled into or out of the nucleus via a variety of pathways. Like all biological pathways, this process can be disrupted and lead to phenotypic abnormalities. Our lab identified two siblings with biallelic variants in NUP188, a known nuclear pore protein; we identified four additional cases in three families through collaborators. Clinical features include brain abnormalities with thin corpus callosum, progressive microcephaly, severely delayed myelination; congenital cataracts; mild dysmorphic features; and hypoventilation leading to death in infancy. In order to better understand our patients' phenotype, we are investigating nuclear import pathways as a potential mechanism of disease. Using green fluorescent protein (GFP), we can directly visualize protein localization in living cells, without the use of additional stains such as immunohistochemistry. Based on size restriction of the nuclear pore, we used a vector construct with three repeats of GFP and our nuclear localization signal (NLS). Specifically, we are investigating four main NLS pathways: Importin $\alpha+\beta$ (SV40 NLS), Kap $\beta 2$ (hnRNP NLS), Importin β (CREB NLS), and no NLS. We have been able to successfully create a viral vector for each NLS. Next steps include optimizing expression in patient cell lines. This approach will allow us to quantify and visualize discrepancies between patient and

control localization patterns, leading to better understanding of causes of patient phenotype and potential novel therapies.

SESSION 1T

BRAIN FUNCTION, DYSFUNCTION AND REPAIR

Session Moderator: Kathleen Millen, Pediatrics

JHN 175

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Investigating Splice Site Variants Associated with Epilepsy

Apoorva Chowdhary, Senior, Biochemistry

Mary Gates Scholar

Mentor: Heather Mefford, Pediatrics

Mentor: Alison Muir, Pediatrics

Developmental and epileptic encephalopathies (DEEs) are an early-onset form of epilepsy characterized by intractable seizures and severe cognitive and developmental impairment. While most genetic variants that cause DEE reside in the coding regions of genes, splice-site variants can also be pathogenic. Splice-site variants are changes in the DNA close to, or on, the exon-intron boundary, which can cause aberrant splicing, resulting in exon exclusion or intron inclusion within spliced mRNA, generating a protein that is non-functional, partially functional, or aberrantly expressed. Aberrant splicing can have pathogenic consequences, but predicting which variants near splice-sites will have an effect on splicing is difficult. I am studying three potential splice-site variants in three genes associated with DEE: SYNGAP1, SCN1B, and WWOX. I used RNA extracted from fibroblasts from three DEE patients, each with one of these variants, to confirm whether the splice-site variants cause aberrant splicing and what the predicted consequences of this aberrant splicing is on the protein. I have been able to discover the effects of the variants on splicing in WWOX and SYNGAP1. In SYNGAP1, a synonymous variant at the exon-intron junction caused an exon 4 deletion, resulting in a severely truncated protein (SYNGAP1:p.Glu120A1afs*20). In WWOX, a duplication which included exon 5 resulted in a transcript with the inclusion of two copies of exon 5, leading to a frameshift mutation and predicted truncated protein (WWOX:p.His173Glyfs*13). We are still investigating the effects of the intronic variant in SCN1B, which appears to decrease expression of the gene. We are using nonsense-mediated decay inhibitors in order to better understand the mechanism through which this decreased expression occurs. This research could potentially improve the care of these patients by providing genetic evidence of the causes of DEE, which can be the basis for advancing better treatments.

POSTER SESSION 2

Commons West, Easel 35

1:00 PM to 2:30 PM

Low Temperature Apatite (U-Th)/He Thermochronology Analysis Along the Seattle Fault Zone, Washington

*Monica R. Hill, Senior, Earth and Space Sciences: Geology
UW Honors Program*

Mentor: Alison Duvall, Earth and Space Sciences

The Seattle Fault Zone (SFZ) is a region of complex, east-west striking thrust faults in the Puget Sound area in Washington. Radiocarbon dating of landslides has determined that the SFZ most recently ruptured ~1100 years ago. At this time, non-glacial sedimentary rocks from the Blakeley Formation (26-37 Mya) were uplifted up to 7 meters along the hanging wall of the fault. In this study, we use low temperature (U-Th)/He thermochronology to constrain the timing of slip on the main thrust of the Seattle Fault Zone. The dating technique is used on apatite crystals obtained from samples of the Blakeley sandstone to develop a time-temperature cooling history as the rocks were uplifted and subsequently exhumed. The apatite (U-Th)/He thermochronometer records the date that the mineral passed through the approximately 70C closure temperature. Based on a 25C/km geothermal gradient, this closure temperature represents the upper 2-3 km of the crust. The samples were collected from a transect along the hanging wall of the fault, from Alki Point, Seattle in the west to East Lake Sammamish, Issaquah in the east. All samples were collected between 0.5 and 1 m from the surface, and at similar elevations in order to account for different exhumation rates. The rate of exhumation along the fault will be determined from plots of the apatite time-temperature cooling history, where younger dates indicate more rapid exhumation rates. From this data, we will back out erosion rates in order to constrain the total fault uplift.

POSTER SESSION 3

Commons West, Easel 23

2:30 PM to 4:00 PM

Map of Marine Terraces on Haida Gwaii

*Gabrielle Therese Bugayong (Gabby) Alampay, Senior,
Earth and Space Sciences: Geology*

Mentor: Philip Schoettle-Greene

Mentor: Alison Duvall, Earth and Space Sciences

Marine terraces are geomorphic features created by wave erosion of the land, which is modulated by crustal uplift or past sea level changes. In this study, I consider possible driving mechanisms that could have generated the marine terraces on the archipelago of Haida Gwaii, Canada. Southwest of the island, there is a young subduction zone that was only initiated

~6 Ma. This subduction has generated a M_w 7.8 earthquake in 2012. From this study, we can find out more information about the plate boundary, including uplift and deformation histories. The island has also experienced ice coverage during the last glacial maximum. Since then, the ice has melted. As a result, the island may have uplifted in response to the ice melting during the Holocene. To obtain the data needed, I am using newly released LIDAR data which contain high resolution elevation topography to map the landforms digitally. The purpose of this project is to provide a resource for locations of the terraces and also to find patterns in distribution and in elevation of marine terraces in Haida Gwaii.

POSTER SESSION 4

Commons East, Easel 56

4:00 PM to 6:00 PM

Testing the Relationship between Earthquakes and Coseismic Landslides: A Case Study from the Loma Prieta Earthquake, CA

*Natalie Elizabeth Wisdom, Senior, Earth & Space Sciences
(Environmental)*

UW Honors Program

Mentor: Alison Duvall, Earth and Space Sciences

My research is a case study of coseismic landslides triggered by the M6.9 earthquake in Loma Prieta, California on 17 October 1989. Coseismic landslides bring deadly consequences, often resulting in more casualties and infrastructure damage than from the earthquake directly. In addition, coseismic landslides are understudied geomorphic features and are difficult to predict, resulting in a high safety hazard. My study area, located in the Forest of Nisene Marks State Park, California, a regional park in the Santa Cruz Mountains where the earthquake shaking was strongest, includes over a hundred coseismic landslides triggered from the 1989 earthquake. I am using geographic information systems (GIS) software to map and analyze the landslides on a high-resolution LiDAR Digital Elevation Model (DEM) and using data from the United States Geological Survey to create an inventory of the Loma Prieta coseismic landslides. This dataset will be compared against seismic shaking strength estimates and local lithology to look for spatial patterns in sliding. I am also using this dataset, an event of known age, to test a recently developed landslides Surface Roughness - Age model. I expect to find that the zones with greatest seismic shaking produced the most or biggest landslides, certain lithologies are prone to greatest failure, and that the 1989 coseismic landslides currently have approximately equal surface roughness. Learning how certain lithologies respond to seismic shaking will help predict future coseismic landslides, and evaluating the current surface roughness will help validate, or invalidate, the Surface Roughness - Age model.