UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

Online Proceedings

Poster Session 1

MGH 258, Easel 182

11:00 AM to 1:00 PM

Specific c-Jun N-terminal Kinase (JNK) Activities in a Chronic Epilepsy Rat Model

Marium Narejo Khan, Senior, Neurobiology
Mentor: Nicholas Poolos, Neurology
Mentor: Francis Concepcion, Neurology

c-Jun N-terminal kinases (JNKs) are members of the mitogen-activated protein kinases (MAPKs) family that are derived from three genes: *Jnk1*, *Jnk2*, and *Jnk3*. JNKs have been implicated in several cellular responses to homeostatic insults, including inflammation and apoptosis. We previously reported in a chronic epilepsy rat model significant elevated levels of phosphorylated JNKs (pJNKs), which indicate increased JNK activities. Additionally, we demonstrated that pharmacological manipulations of JNK proportionally affected seizure frequency. In this set of experiments, we attempted to identify which of the JNK isoforms (JNK1, JNK2, JNK3) contribute to the overall increased pJNK levels in our animal model of epilepsy. This would provide us insights as to the role(s) of JNKs in this disease. We measured the phosphorylation levels of the individual isoforms after pJNK enrichment from the CA1 hippocampal tissue of chronic epileptic rats and their age controls. The amount of protein was normalized by pJNK levels between experimental and control samples. We found a significant increase in activation levels of JNK2 in chronic epilepsy at 130 $\pm$ 9% (n=6, p=0.018) when compared to naïve, nonepileptic controls but insignificant changes in activation levels of JNK1 (97 $\pm$ 14%, n=5, p=0.83) and JNK3 (98 $\pm$ 17%, n=6, p=0.92). Previously, we had found in rats that JNK1 predominantly exists in the 46kDa size; JNK3 predominantly exists in the 54 kDa; and JNK2 exists in both sizes equally. We further analyze which of the JNK bands (46 kDa and 54 kDa or both) contribute to the elevated phosphorylated JNK levels. Given the previous pharmacological observation that JNK manipulation does influence seizure frequency in epilepsy, this investigation is imperative as it will allow us to narrow our focus to a specific JNK isoform to study further.

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.

Elucidating the Kinetics of STAT1 Phosphorylation in Response to TLR4 and IFNAR Agonists in Microglia

Rachel Anne Arnold, Senior, Neurobiology
UW Honors Program
Mentor: Jonathan Weinstein, Neurology

Ischemic preconditioning (IPC) is a robust, neuroprotective phenomenon in which a brief ischemic exposure confers resistance to injury from subsequent prolonged ischemia. Characterizing IPC may provide insight into better treatment options for those at high risk of ischemic stroke. Microglia, the immune cells of the brain, play an important role in the immune response to IPC. Previously, our laboratory found that the type 1 interferon signaling pathway in microglia is important in IPC-mediated neuroprotection. This signaling pathway is dependent upon activation of Toll-like receptor 4 (TLR4) and type 1 interferon receptor (IFNAR1). We hypothesize that in this pathway, damage-induced molecular patterns (DAMPs), which are released by brain tissues under ischemic conditions, activate TLR4 resulting in a signal cascade that activates IFNAR1, leading to phosphorylation of signal transducer and activator of transcription 1 (STAT1). Phosphorylated STAT1 (pSTAT1) then forms a complex with other proteins and induces transcription of multiple interferon-stimulated genes (ISGs). ISG expression alters the microglial phenotype, leading to neuronal and axonal protection against subsequent ischemia-related brain injury. The kinetics of type 1 interferon signaling in microglia are not yet fully understood. We aimed to further characterize this pathway by culturing primary microglia from wild-type mice, exposing them to TLR4 agonists or type 1 interferons directly, and quantifying pSTAT1 levels using flow cytometry at multiple time points. A time course of STAT1 phosphorylation in response to innate immune stimuli will provide a clearer picture of the kinetics of microglial type 1 interferon signaling in the setting of ischemia. These findings

will enable us to optimize experimental timing for future experiments involving more complex and physiologic stimuli. Optimization of the kinetics of the pSTAT1 assay will also allow us to investigate how genetic ablation of specific innate immune signaling pathways (like TLR4 or IFNAR1) might modulate the microglial response to ischemia.

SESSION 1T

BRAIN FUNCTION, DYSFUNCTION AND REPAIR

Session Moderator: Kathleen Millen, Pediatrics
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Evaluating the Presence of Microglia Progenitor Cells in the Adult Mouse Brain
Chloe Netania Winston, Sophomore, Pre-Sciences
Mentor: Gwenn Garden, Neurology
Mentor: Katherine Prater, Neurology

Microglia, the immune cells of the central nervous system, are long lived. In mice, microglia have an average lifespan of 15.5 months. When microglia are experimentally depleted from the mouse brain, microglia populations quickly return to steady state levels. The mechanisms of this observed repopulation are unclear. More importantly, the mechanisms of microglia replenishment in the healthy brain are not well understood. The literature supports two competing hypotheses. One is that microglia proliferate simply by dividing. Another possibility is that pools of microglia progenitor cells within the central nervous system divide and differentiate into microglia. I hypothesize that microglia proliferate primarily through the differentiation of progenitor cells. Available data to date suggest CD133 as a potential marker for microglia progenitor cells. In order to study these putative progenitor populations, I used a genetic reporter mouse line in which administration of tamoxifen induces TdTomato expression specifically in CD133-expressing cells. TdTomato, a red fluorescent protein, allows these cells to be visualized under a fluorescence microscope. Importantly, all the progeny of these cells also express TdTomato, allowing us to determine whether CD133 cells generate new microglia over time. After tamoxifen treatment at the age of 10 weeks, mice were sacrificed at three and nine months of age. Brains were fixed, sectioned, and labeled with antibodies to a microglia specific protein and to TdTomato. Daughter microglia that differentiated from CD133-expressing cells express both markers. Using a fluorescence microscope, I identified several microglia daughter cells of CD133-expressing cells. This suggests that microglia populations replenish in the healthy brain at least in part through the division of CD133-expressing cells. We can apply this new knowledge about how new microglia are generated in the healthy mouse brain to further our understanding of how microglia population dynamics are affected in both health and disease.

POSTER SESSION 2

MGH 258, Easel 179
1:00 PM to 2:30 PM

Investigating Neurotoxicity and Endothelial Activation after Immunotherapy with CAR T-Cell Cancer Treatment
Katie Kaur Mand, Senior, Neurobiology
Levinson Emerging Scholar, Mary Gates Scholar
Mentor: Juliane Gust, Neurology

Chimeric antigen receptor (CAR) T-cell therapy is the latest treatment option available for those suffering from certain forms of cancer such as lymphoma and leukemia. These engineered cells are able to recognize specific proteins found in tumors, and subsequently induce CAR-T cell proliferation, cytokine secretion, and lysis of the cancerous cells. Despite its promise, a percentage of patients who receive this treatment develop a range of neurotoxic symptoms. My research project tests the hypothesis that endothelial activation of vascular tissue in the brain, which would allow for increased permeability of immune cells through the blood-brain barrier, is contributing to the development of these clinical symptoms. Using a technique called immunohistochemistry, I used the antibodies claudin-5 and cd31 to fluorescently label tight-junction proteins and adhesion molecules of endothelial cells from brain tissue harvested from a developed mouse model. This mouse model received CAR-T cell injections and underwent behavioral testing to confirm the presence of neurotoxicity symptoms. I then used microscopy skills to visualize the labeling of the endothelial cells and proteins. If my hypothesis is correct, I expect to see a quantifiable decrease in the number of cerebral tight-junction proteins connecting endothelial cells along the blood-brain barrier, as compared to negative control tissue that received no CAR-T cell injections. In order to make these comparisons, I will use a software program such as Image-Pro Premier software (Media Cybernetics) to help me quantify the positive fluorescence labeling of endothelial cell proteins and adhesion molecules in both the control and experimental tissue. Tissue with less tight-junction proteins and adhesion molecules would permit the influx of foreign particles into the CNS. Understanding the cause of CAR T-cell related neurotoxicity will be first step in promoting prevention and increasing the effectiveness of this new cancer immunotherapy.

POSTER SESSION 2

MGH 258, Easel 181

1:00 PM to 2:30 PM

Optimization of Canine Flow Cytometry Panel

Griffen Tyler Girvan, Senior, Biology (Molecular, Cellular & Developmental)
Tommy Henry (Tommy) Taslim, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Julie Crudele, Neurology

Flow Cytometry is a quantitative data collection method which utilizes a laser and optics system to measure forward and side-scattering light from single cells in a heterogeneous solution, which when analyzed describe the structure and internal complexity of the cells in solution. Additionally, varying wavelengths of light emitted from the cytometer excite particular fluorescent dyes that can be conjugated to known antibodies, so that when cells contain the known antibody's antigen, the dye color will be present. This allows for cell identification and/or protein expression to be determined and quantified within a heterogeneous mixture of cells. With the given technology, we optimized a flow cytometry panel for the use of analyzing immune responses to gene therapy treatments in canines. We stained extracellular and intracellular protein markers on canine peripheral blood mononuclear cells (PBMCs) with fluorescent dye conjugated antibodies thought to recognize canine antigens. This entailed staining the extracellular markers with conjugated antibodies, fixing and permeabilizing the cell, and doing the same to intracellular markers. Once this had been carried out, the cells were run through a flow cytometer to excite the dyes with varying wavelengths of light to highlight separate dye colors. Analysis of multicolored dye presence in cells post-excitement allowed for identification and quantification of cell types. We identified antibodies that recognize canine antigens and developed a multicolor panel identifying T helper cells, cytotoxic T cells, T regulatory cells, and B cells in canine samples. Once optimized, we used this panel to characterize immune responses in dogs following gene therapy. With a reliable canine cytometry panel, future canine immune responses, both broadly and in isolated muscular tissues, can be characterized.

POSTER SESSION 2

MGH 258, Easel 180

1:00 PM to 2:30 PM

Identification of Optimal Peptide Sequences for a Canine IFNγ ELISpot Positive Control

Ai Che, Senior, Biochemistry
Leeseok (Lee) Song, Junior, Biochemistry
Mentor: Julie Crudele, Neurology

Interferon-gamma Enzyme-Linked Immunosorbent Spot Assay (IFNγ ELISpot) is a laboratory technique that quantifies the number of cells producing interferon gamma (IFNγ) by utilizing antibodies that selectively bind to IFNγ molecules, resulting in spot formation corresponding to individual IFNγ-producing cells. Since cytotoxic T cells (CD8 T cells) and their helper Th1 cells (CD4 T cells) produce IFNγ to activate macrophages and inflammatory responses, quantifying IFNγ-producing cells allows for characterization of host immune responses. Our lab utilized a canine IFNγ ELISpot to test for immune responses against novel proteins expressed following gene therapy in dogs. Currently, we used a routinely given vaccine for canine flu, distemper, adenovirus, and parvovirus as a biological positive control. However, we tested our experimental proteins with peptides, making a whole-protein positive control, which required internal processing, flawed. Our goal was to optimize this ELISpot by identifying peptides from the vaccine that stimulates an IFNγ immune response in peripheral blood mononuclear cells (PBMCs) and splenocytes. Utilizing a commercially available canine IFNγ ELISpot, we stimulated PBMCs and splenocytes. These cells included lymphocytes (T cells) and macrophages, which acted as antigen presenting cells. We compared stimulation with the entire vaccine and various vaccine peptides in order to identify peptides that can be used as a biological positive control. These were compared to traditionally used mitogens that indiscriminately activated all lymphocytes. This optimization allows for greater confidence in the results obtained from our canine IFNγ ELISpot. The improved technique serves as a powerful tool to assist in preclinical trials of vaccine production and gene therapy. It is utilized in our lab to test for CD8 T cell-mediated immune responses against novel dystrophins following gene therapy in Duchenne muscular dystrophy dogs.

POSTER SESSION 2

MGH 241, Easel 149

1:00 PM to 2:30 PM

***LRP10* Mutations in Familial Parkinson's Disease**

Tarun Singh Gandhi, Senior, Biochemistry
UW Honors Program
Mentor: Cyrus Zabetian, Neurology
Mentor: Dora Yearout, GRECC, VAPSHCS

Parkinson's Disease (PD) is a neurodegenerative movement disorder characterized by muscular rigidity, slow movement of the limbs and resting tremor. The onset and progression of PD is attributed to the combined effect of environmental and genetic risk factors, with several specific disease-causing genes having been identified. PD onset is determined by the loss of more than 80% of dopamine-synthesizing neurons from the substantia nigra as well as formation of α-synuclein protein aggregates, called Lewy Bodies. Recent research has

identified several potential pathogenic variants in the *LRP10* gene on chromosome 14, suggesting that *LRP10* may be a novel PD causative gene. It has been shown that the *LRP10* protein is implicated in vesicular transport and that it may regulate α-synuclein aggregation, intracellular trafficking, and cell-to-cell transmission. The goal of my project was to analyze the association between *LRP10* and PD by sequencing the regions on *LRP10* that harbor these variants described in other families and check for co-segregation in the relatives of mutation carriers. DNA samples from 188 PD familial patients were sequenced. *LRP10* regions of interest that were examined included exons 1-7 (protein coding regions) and intron 5 (a non-protein coding region). These *LRP10* regions in patient DNA were amplified by polymerase chain reaction and sequenced subsequently using Sanger Sequencing. Relatives of *LRP10* mutation carriers were then analyzed for co-segregation between the variant and PD. It was expected that certain *LRP10* variants would be found to segregate with the disease in inheritance or increase the likelihood of its onset. The molecular function of both the normal and mutated *LRP10* protein are still largely unknown and future research into their mechanisms will provide valuable insight into their specific role in Lewy Body formation and dopaminergic neuronal loss.

POSTER SESSION 3

Commons East, Easel 52

2:30 PM to 4:00 PM

Identifying Effects of Deep Brain Stimulation on Parkinson's Disease using Voice Samples

Huy Phi, Senior, Neurobiology
Mentor: Reza Hosseini Ghomi, Psychiatry and Behavioral Sciences

Parkinson's Disease (PD) is a neurological disease that affects motor function. Symptoms include muscle rigidity, tremors, slowed movements, and altered voice. Deep Brain Stimulation (DBS) is a therapeutic intervention addressing PD symptoms, by implanting an electrode into the brain, delivering electrical impulses to areas affected by PD. DBS patients, however, must come into clinic every few months for multihour reprogramming sessions to adjust DBS settings according to the progressions of their symptoms, making DBS treatment an arduous and expensive process. This significantly limits the accessibility of DBS, because only limited locations/providers who can offer this service. Our lab has developed a computer algorithm that can derive digital biomarkers, indicators for the severity/presence of a disease, for PD from voice samples. We aim to investigate the correlation between patient voice and physical symptoms, with manipulation of stimulation in the on/off states. Our findings could aid clinicians in their monitoring, management and adjustment of DBS protocol, without the need for patients to come into clinic. We tested this by obtaining motor scores via the Unified Parkinson's Disease Rating Scale (UPDRS), and gathering voice samples with DBS turned on and off, during patients' visit at UW clinics. Voice samples were analyzed for specific biomarkers, using our machine learning algorithm. We expected to observe different voice-associated biomarkers present with DBS turned on/off, to demonstrate a correlation between DBS and voice/motor symptoms. Once the relationship between DBS and voice is established, the ultimate goal is to develop a closed looped DBS device which can auto tune itself based on patient voice alone. In an age where audio collection devices, such as smartphones are so accessible, the use of voice data has exciting potential as a clinical tool, optimizing DBS therapy protocol and efficiency for administering clinicians and patients with PD.

POSTER SESSION 3

MGH 258, Easel 179

2:30 PM to 4:00 PM

Identification of a Causal Gene for a Novel Form of Hereditary Spastic Paraplegia

Olga Sarby Cherepakhin, Senior, Biology (Molecular, Cellular & Developmental), Anthropology: Medical Anth & Global Hlth
Mary Gates Scholar, UW Honors Program
Mentor: Dong-Hui Chen, Neurology

Hereditary Spastic Paraplegia (HSP) is a classification for a group of neurogenetic diseases that cause affected individuals to have severe contractions and stiffness in the lower limb muscles. This group includes a genetically diverse range of disorders that vary in age of onset, rate of progression, and severity. The purpose of my project is to identify the causal gene involved in this novel form of HSP. My lab acquired DNA samples from affected and unaffected members of a family with an unassigned autosomal dominant HSP. At first, we obtained whole exome sequencing on DNA from three affected family members. In a file containing all the variants (differences from a reference DNA exome) detected in any of these three subjects, we looked for previously identified causal variants to ensure that the family did not have a known HSP subtype. Then we identified potential variants by filtering for those that were present in one copy (heterozygous) in all three family members, since this form is autosomal dominant. We further filtered them for low prevalence in the Genome Aggregation Database, since HSP is not a common disorder. Candidate variants for testing were selected based on their predicted change in the protein, relevance of gene function, and predicted impacts from CADD and GERP models. We are currently in the process of analyzing the candidates. We are amplifying and sequencing them using DNA from all the family members to determine whether these variants are present in all those who are affected and absent from

those who are not affected, since that is how the causal variant would present. My project will contribute to our understanding of the pathogenesis of and improve clinical diagnostics for HSP. The implications of my research could also extend to other genetic disorders if a novel genetic mechanism is elucidated.

POSTER SESSION 3

MGH 258, Easel 180

2:30 PM to 4:00 PM

Variants in STUB1 Causes Autosomal-Dominant Late-Onset Spinocerebellar Ataxia

Elyana Lux Heigham, Senior, Neurobiology
UW Honors Program
Mentor: Dong-Hui Chen, Neurology

Heritable spinocerebellar ataxias (SCAs) are rare genetic neurological disorders that affect the cerebellum and sometimes the spinal cord. As a result, those with SCA often have problems with movement and coordination. In my experiment, I analyzed a pedigree where several family members had a dominant, late-onset spinocerebellar ataxia. The family tested negative on the tests for all known variants causing dominant spinocerebellar ataxias. Thus, my goal was to find what genetic variant was responsible for the SCA in this pedigree. I began by obtaining exome sequences for two of the affected family members. The exome sequences listed all of the variants present in each individual's exome. My approach to variant filtering steps were selecting shared heterozygous variant, an assessment of population frequency, functional significance, and evolutionary conservation, and then prioritizing the remaining variants based on candidate gene function and expression, animal models and relevance to neurologic disease. The process resulted in a list of candidate variants. I then created primers for my candidate variants and ran PCRs with DNA samples of both affected and unaffected family member. The DNA fragments generated from the PCR were then sequenced. The only candidate that cosegregated was a variant c.158T>C, p.Ile53Thr in STUB1 a gene known to be associated to a recessive SCA. The pathology study to review the abnormality in the patient autopsy brain was performed by our collaborators. Our finding confirmed that STUB1 can cause autosomal dominant hereditary cerebellar ataxia in addition to recessive form of this disease. It is increasingly apparent that variants once associated only with one form of inheritance are in fact capable of causing both recessive and dominant forms.

POSTER SESSION 3

Balcony, Easel 108

2:30 PM to 4:00 PM

Deletion of Inflammatory MicroRNA Regulates Behavioral Phenotypes in a Mouse Model of Alzheimer's Disease

Rachael Annie Hu, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, Undergraduate Research Conference Travel Awardee
Mentor: Gwenn Garden, Neurology
Mentor: Macarena Aloi, Pathology

Microglia are innate immune cells in the CNS that exhibit a sustained pro-inflammatory response in the Alzheimer's disease (AD) brain. Sustained pro-inflammatory responses by microglia can promote excessive synaptic pruning and neuronal death, exacerbating neurodegeneration. MicroRNAs can regulate microglia inflammatory behaviors by modifying gene expression at the post-transcriptional level by suppressing expression of target genes. MiR-155 is a microRNA that targets suppressors of inflammation and is dysregulated in neurodegenerative disorders. Additionally, miR-155 deletion has been reported to be neuroprotective in several models of neural injury and degeneration. The impact of microglia specific miR-155 regulation on the neuroinflammatory response or behavioral outcomes of AD models has yet to be elucidated. We hypothesize that miR-155 deletion in microglia decreases neuroinflammatory response to AD, thus improving memory impairments typically observed in AD. We use a mouse model expressing a transgene of associated mutant forms of human amyloid precursor protein and presenilin 1 (APP/PS1). We crossed APP/PS1 mice with a tamoxifen-inducible Cre model or a constitutive Cre model to conditionally or constitutively delete miR-155 in microglia. We use open field chambers and T-maze to assess general behavior and spatial memory at 6, 9, and 12 months. When miR-155 was deleted specifically in microglia, no difference was seen in the spatial memory as measured through T-maze tests, compared to APP/PS1 mice. However, increased locomotor activity was seen in open field tests at 6 and 9 months. Similarly, when miR-155 was deleted in microglia and peripheral myeloid cells, there were no significant differences in spatial memory, though increases in locomotor activity at 6 and 9 months and potential decreases in anxiety at 6 months were also seen in open field. These results suggest that miR-155 may play a more complex role in the regulatory response of neuroinflammation during AD.