1. Title: Gene-Environment Interactions in Parkinson's Disease
PI: Sheila Fleming, Ph.D. Assistant Professor in the Department of Pharmaceutical Sciences
Location: Research and Graduate Education Building, RGE-400

2. Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by the loss of dopaminergic neurons in the substantia nigra and the development of lewy bodies and lewy neurites in the brain and periphery. While the cause of the majority of cases is unknown, it is generally considered that gene-environment interactions underlie most cases of PD. Therefore, the identification of gene-environment interactions associated with PD-like pathology and neurodegeneration is an important goal in the field. ATP13A2 is a P_5-ATPase of the P-type ion transport ATPase superfamily and loss of function mutations cause the neurodegenerative condition Kufor-Rakeb Syndrome, an autosomal recessive form of PD. The function of ATP12A2 is unclear but *in vitro* studies suggest it may be involved in the lysosomal degradation of proteins, polyamine and heavy metal transport (manganese and/or zinc), and mitochondrial function, all mechanisms that can overlap with PD. An important next step is to determine how loss of function of ATP13A2 *in vivo* interacts with environmental factors such as heavy metals and toxicants that interfere with cellular transport, protein degradation, and mitochondrial function. It is hypothesized the loss of ATP13A2 function causes an increased vulnerability to the toxic effects of certain heavy metals and pesticides associated with PD. This hypothesis will be tested using Atp13a2-deficient mice that have been shown to develop age-dependent motor impairments, enhanced accumulation of lysosomal storage material, and increased accumulation of the PD protein alpha-synuclein. Wildtype and Atp13a2-deficient mice will be exposed to different metals and toxicants associated with PD (ex. manganese). Sensorimotor function will be measured and in the brain accumulation of the PD protein alpha-synuclein and neurodegeneration will be determined. A combination of behavioral, cellular, and molecular techniques will be employed.

3. Background and Rationale: PD belongs to a group of diseases known as synucleinopathies, where the presynaptic protein alpha-synuclein abnormally accumulates in the brain and periphery. Alpha-synuclein is a major component of lewy bodies, the pathological hallmark of synucleinopathies and a key protein in the study of PD. Inherited forms of PD show that mutated or increased alpha-synuclein can lead to the development of PD. Thus, the identification of genetic and environmental factors that can increase alpha-synuclein accumulation and toxicity could have a major impact on the development of therapeutics for the disease. P-type ATPases are a large family of proteins involved in the transport of cations and other substrates across cell membranes through the utilization of energy from ATP hydrolysis (Schultheis et al., 2004; van Veen et al., 2014). Functionally, they are involved in essential cellular processes including vesicular transport and excitability. ATP13A2 is most abundant in the brain and loss of function mutations in humans causes Kufor-Rakeb Syndrome, an autosomal recessive form of PD. More recently, ATP13A2 polymorphisms have been linked to an enhancement of the neurotoxic effects of manganese in an elderly population. Loss of

ATP13A2 function in mice causes age-related sensorimotor impairments, gliosis, enhanced lysosomal storage material, and increased alpha-synuclein accumulation (Schultheis et al., 2013; Kett et al., 2015). This suggests ATP13A2 could be an important factor in gene-environment interactions associated with PD.

4. Goals and Objectives: The goal of these studies is to understand the role of ATP13A2 in cellular dysfunction and neurodegeneration. The objective is to characterize ATP13A2 x environmental exposure interactions and determine the mechanisms by which they contribute to behavioral dysfunction and neurodegeneration *in vivo*.

5. Investigative Methods: A combination of behavioral, cellular, molecular, and genetic methods will be employed to determine the effect of different environmental exposures in Atp13a2-deficient mice.

Environmental Exposures. Separate cohorts of wildtype and Atp13a2-deficient mice will be exposed to manganese. Mice will then be behaviorally tested to determine the effect of the exposures on sensorimotor function and cognition. In the brain alpha-synuclein accumulation, mitochondrial bioenergetics, and neurodegeneration of the nigrostriatal dopaminergic system will be determined.

Behavioral methods. Sensorimotor function will be assessed using a battery of tests shown to be sensitive in genetic mouse models of PD (Schallert et al., 1978; Fleming et al., 2004; Schultheis et al., 2013). Cognitive function will be determined using tests that measure aspects of attention, memory, and executive function.

Alpha-Synuclein Accumulation (brain). Soluble and insoluble alpha-synuclein protein will be measured using both immunoblot and immunohistochemistry techniques.

Mitochondrial Bioenergetics. Mitochondrial bioenergetics will be measured in multiple brain regions using Seahorse analysis.

Neurodegeneration (brain). Neuron counts will be measured using immunohistochemistry in the substantia nigra. Dopamine neurons in substantia nigra pars compacta and dopamine terminals in the striatum will be identified utilizing tyrosine hydroxylase immunohistochemistry protocols routinely used in the lab.

6. Proposed Method of Data Analysis: A combination of parametric and non-parametric statistics will be used to analyze the behavior and tissue data. For parametric statistics, 2X2 randomized ANOVA will be used to analyze genotype (wildtype and Atp13a2-deficient) and treatment (vehicle and manganese). Post hoc comparisons will use the Bonferroni corrected factor when multiple comparisons are being made. For scores that do not meet the assumptions of ANOVA nonparametric statistics will be used to compare genotypes and treatment.

7. Significance of Anticipated Findings: It is anticipated that Atp13a2-deficient mice will be more sensitive to the toxic effects of environmental exposures compared to wildtype mice. It is anticipated that exposed Atp13a2-deficient mice will show more severe alterations in behavior than exposed wildtype mice and vehicle-treated Atp13a2-deficient mice. In the brain it is expected that exposed Atp13a2-deficient mice will have increased alpha-synuclein accumulation, impaired mitochondrial function, and nigrostriatal cell loss compared to exposed wildtype mice and vehicle-treated Atp13a2-deficient mice. These findings will be significant because they will reveal a novel gene-environment interaction that could lead to neurodegeneration in humans. This would also identify ATP13A2 as a potential target for neuroprotection or therapeutic intervention.

8. Appendix:

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Kett LR, Stiller B, Bernath MM, Tasset I, Blesa J, Jackson-Lewis V, Chan RB, Zhou B, Di Paolo G, Przedborski S, Cuervo AM, Dauer WT. 2015. α-Synuclein-Independent Histopathological and Motor Deficits in Mice Lacking the Endolysosomal Parkinsonism Protein Atp13a2. J Neurosci. 2015; 5(14): 5724-542.

Schallert T, Whishaw IQ, Ramirez VD, Teitelbaum P. 1978. 6-hydroxydopamine and anticholinergic drugs. Science. 1978; 202(4373): 1216-1217.

Schultheis PJ, Fleming SM, Clippinger AK, Lewis J, Tsunemi T, Giasson B, Dickson DW, Mazzulli JR, Bardgett ME, Haik KL, Ekhator O, Chava AK, Howard J, Gannon M, Hoffman E, Chen Y, Prasad V, Linn SC, Tamargo RJ, Westbroek W, Sidransky E, Krainc D, Shull GE. Atp13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited α-synuclein accumulation and age-dependent sensorimotor deficits. Hum Mol Genet. 2013; 22(10): 2067-2082.

Schultheis PJ, Hagen TT, O'Toole KK, Tachibana A, Burke CR, McGill DL, Okunade GW, Shull GE. Characterization of the P5 subfamily of P-type transport ATPases in mice. Biochem Biophys Res Commun. 2004; 323(3): 731-8.

van Veen S, Sørensen DM, Holemans T, Holen HW, Palmgren MG, Vangheluwe P. Cellular function and pathological role of ATP13A2 and related P-type transport ATPases in Parkinson's disease and other neurological disorders. Front Mol Neurosci. 2014; 7:48.

Student Fellow Training/Mentoring Plan:

Plan. This is a large project that is ongoing in the lab. The PI will work with the student to determine what aspect of the project best suits his/her interests, abilities, and goals. The student would have the option to work mainly on one aspect of the project (such as behavioral testing and analysis or tissue processing and immunohistochemistry) or multiple aspects of the study. The student will meet with the PI on a weekly basis to discuss project progress and literature in the field. In addition to individual meetings the

student will attend regular lab meetings where each person in the lab discusses the project they are working on and the progress or setbacks they have encountered. Short PowerPoint presentations are encouraged during these meetings as they will keep the student on track for the final poster session at the end of the summer.

*Resource*s. The lab has all resources necessary for the student to complete a summer project. Mutant mice are available and behavioral testing protocols are already established. Supplies and space for tissue processing are also available.

Location. The experiments will be conducted primarily in the laboratory area in RGE-200. There is behavioral testing space in C-129 where motor and cognitive testing will take place. The student will have a desk and access to a computer in the write-up area for data analysis and presentation.