**Name of Institution:** University of Pittsburgh

**Project Title:** Defining the role of glucocerebrosidase and TFEB in Parkinson’s disease

**Investigators:**
Dr. Emily Rocha (Postdoctoral Researcher and Grant Awardee)
Dr. Greenamyre (Postdoctoral Supervisor)

**Biography:** My main postdoctoral project focused on the role of glucocerebrosidase and autophagy-lysosomal degradation in Parkinson’s disease (PD). This work was based largely on the recent clinical observation that a mutation in GBA1, which encodes for the lysosomal hydrolase glucocerebrosidase, is now considered one of the most prevalent genetic risk factors for PD. My research demonstrated that overexpression of GBA1 in vivo is neuroprotective and is able to prevent accumulation of soluble and insoluble α-synuclein and blocking glucocerebrosidase promoted the accumulation of α-synuclein in rodents. The most impactful finding that I made was an age-dependent reduction in glucocerebrosidase in the brains of healthy subjects beginning after the 6th decade of life. Collectively, these data illustrate that there is a link between glucocerebrosidase activity levels and α-synuclein aggregation and reductions in glucocerebrosidase activity levels may contribute to the onset of PD.

**Objective:** (1) Determine whether glucocerebrosidase requires transcription factor EB (TFEB)-mediated upregulation of CLEAR-network proteins to prevent accumulation of intracellular α-synuclein and (2) Assess the therapeutic potential of glucocerebrosidase and TFEB by determining whether they are capable of removing high levels of intracellular α-synuclein.

**Background:** Glucocerebrosidase is a lysosomal lipid hydrolase that cleaves glucosylceramide (GlcCer) and glucosylsphingosine (GluSph) into glucose and ceramide or sphingosine, respectively. Mutations in GBA1 that result in a 30-50% reduction in lysosomal glucocerebrosidase activity have emerged in the past few years as a major genetic risk factor for PD and has been shown to exacerbate the progression of the disease. PD patients who are heterozygous carriers of a GBA1 mutation (GBA1-PD) usually develop clinical symptoms at an earlier age; have more severe cognitive symptoms and increased α-synuclein accumulation relative to PD patients who are not GBA1 mutation (nonGBA1-PD) carriers. Therefore, it is not surprising that 6-7% of early-onset PD patients are GBA1 mutation carriers. We hypothesize that reductions in glucocerebrosidase may be causative and accelerate the degenerative processes in PD.

The only known regulator of glucocerebrosidase is the transcription factor EB (TFEB), which also regulates lysosomal biogenesis and autophagy-lysosomal degradation pathways by upregulating Coordinated Lysosomal Expression and Regulation (CLEAR)-network proteins. Deficits in autophagy-lysosomal degradation are implicated in the pathophysiology of PD, and overexpression of TFEB or GBA1 can prevent degeneration in the substantia nigra and reduce α-synucleinopathy. We and others have demonstrated that this neuroprotection is associated with changes in CLEAR-network proteins involved in autophagy-lysosomal degradation. Moreover, recent histological examination of substantia nigral neurons in PD-patients revealed cytoplasmic retention of TFEB that co-localized with α-synuclein in the Lewy bodies. We hypothesize that high levels of cytoplasmic α-synuclein sequesters TFEB in the cytoplasm, preventing up-regulation of CLEAR-network proteins – including GCase and other autophagy-related proteins, which disrupts lysosomal degradation and likely promotes accumulation of undegraded macromolecules. Furthermore, the neuroprotective effects observed by overexpressing glucocerebrosidase and TFEB may be mediated by enhanced autophagy-lysosomal function, which promotes degradation of unwanted material.
Methods/Design: I intend to use primary midbrain neurons isolated from embryonic day 17 rats to study how high levels of α-synuclein affect glucocerebrosidase and TFEB activity and contribute to the pathogenesis of PD. We intend to increase intracellular α-synuclein levels using two approaches (1) exposure to a low sub-lethal concentration of rotenone and (2) exposure to exogenous oligomeric α-synuclein. These culture conditions will allow us to measure how changes in α-synuclein levels contribute to deficits in autophagy-lysosomal degradation pathways and evaluate the role of TFEB in glucocerebrosidase-mediated removal of α-synuclein. To achieve our objectives, glucocerebrosidase and TFEB will be overexpressed in primary midbrain neurons using lentiviruses. Upon confirmation that optimal vector transduction has been achieved, cultures will be exposed to either rotenone, purified α-synuclein oligomers or vehicle. We hypothesize that rotenone or α-synuclein treatment may exacerbate both α-synuclein accumulation and autophagy deficits, causing glycolipid accumulation, mimicking animal models of PD associated with glucocerebrosidase reductions. Therefore, we will measure changes in α-synuclein, glycolipids (GluCer and GluSph) and autophagy deficits at several time points following rotenone and α-synuclein exposures.

In my second set of experiments, primary midbrain neuronal cultures will first be treated with either rotenone or oligomeric α-synuclein. Once maximal α-synuclein accumulation is achieved, neuronal cultures will then be transduced with the same human GBA1 or TFEB lenti-viruses described in my first set of experiments. A subset of these cultures will be co-transduced with human GBA1 while simultaneously silencing TFEB using RNAi to determine whether TFEB-mediated upregulation of autophagy-related CLEAR-network proteins are required to facilitate removal of α-synuclein. Aim 2 will assess whether (1) GCase can reverse intracellular α-synuclein and glycolipid accumulation and (2) glucocerebrosidase requires TFEB-mediated induction of autophagy-related proteins for neuroprotection.

We intend to use a variety of techniques to determine whether glucocerebrosidase and TFEB can prevent rotenone and α-synuclein induced accumulation of intracellular α-synuclein and deficits in autophagy-lysosomal degradation. I intend to use immunofluorescence coupled with live cell imaging to assess TFEB translocation and also determine whether glucocerebrosidase can induce activation of TFEB. This will be coupled with quantification of CLEAR-network proteins involved in autophagy as well as measuring changes in autophagic flux. Immunofluorescence will confirm (1) accumulation of α-synuclein caused by exposure to either rotenone or oligomeric α-synuclein and (2) identify the subcellular location of α-synuclein accumulation by co-labeling with lysosomal specific markers and a nuclear specific marker. I intend to also use Tandem mass spectrometry to measure changes in the glycolipid substrates of glucocerebrosidase. Changes in glycolipid levels will be correlated with α-syn accumulation.

Relevance to Diagnosis/Treatment of Parkinson’s disease: My research will contribute to a better understanding the role glucocerebrosidase and TFEB play in the pathogenesis of PD. Elucidating the role of these proteins in the disease pathology will provide novel mechanistic insights regarding α-synuclein toxicity and may lead to novel therapeutics interventions designed to reduce the α-synuclein accumulation.