**Characterization of the promoter for amyloid precursor protein and its affect on transcription**

Alzheimer’s disease is a neurodegenerative disorder associated with loss of memory and cognitive function. One of the hallmarks of Alzheimer’s is the extracellular accumulation of amyloid precursor protein (APP) into large insoluble amyloid-β plaques. APP and amyloid plaques are normally cleared by microglial cells, but when they accumulate in the extra cellular environment neuron degradation occurs. Neurodegeneration may be caused by the over expression of the APP gene however little is known about the regulation of the upstream promoter region of the APP gene under normal conditions. Genomic studies reveal quantitative phenotypic effects in APP protein products.

My **primary goal** is to better understand the molecular mechanisms involved in APP protein regulation that leads to amyloid plaque accumulation. Characterizing the promoter region of APP will uncover how it is normally regulated in neurons and improve the understanding of how it leads to Alzheimer’s.

By using novel genomic and proteomic techniques, I aim to understand how the APP promoter is regulated normally. I propose to test the **central hypothesis** that mutations in the upstream promoter region will increase accumulation of amyloid plaques, with the following specific aims:

**Aim 1:** To determine conserved base pairs in APP that are necessary for normal APP gene expression levels in the neuronal cells.

**Hypothesis:** Specific and conserved base pairs are necessary for normal APP gene expression levels in neuronal cells.

**Approach:** Use CRISPR/Cas9 technology on 5 proposed upstream promoter sequences found in previous research [1, 2, 3] for *APP* to uncover the promoter’s effect on APP activation to understand amyloid plaque accumulation in the brain.

1. Design a vector for zebrafish containing the APP promoter, coding regions, and a histidine tag.
2. Use CRISPR/Cas9 in the 5’ promoter region at sites -369, -479, -534, -1023, and -3830.
3. Quantify the amount of APP transcribed with protein spectrometry, gel electrophoresis, and a zebrafish behavioral maze test.

**Aim 2:** To determine if mutagenesis performed in Aim 1 affects the solubility of the resulting amyloid plaques.

**Hypothesis:** Mutagenesis will have no impact on plaque solubility.

**Approach:** Test the solubility of the promoter mutant amyloid plaques with the Alzheimer’s drugs Florabetaben, Methionine Sulfoxide, and Flutemetamol. These drugs treat Alzheimer’s by solubilizing amyloid plaques in the brain.

1. Use the zebrafish model created in Aim 1 to plate neurons
2. Treat each group with one of the Alzheimer’s drugs
3. Quantify the amount of remaining amyloid plaque with protein spectrometry, gel electrophoresis, and a zebrafish behavioral maze test.

**Aim 3:** To determine the phosphorylation levels of mutant APP promoter protein product in the brain tissue.

**Hypothesis:** There will be a increase in phosphorylation levels in the mutant APP promoter protein product that is proportional to APP expression because of the higher abundance of APP cleavage sites.

**Approach:** Use SILAC-based mass spectrometry to quantify the phosphorylation present in mutant APP promoter protein products. Mutant proteins will be compared relative to the WT promoter protein product.

1. Feed the zebrafish model created in Aim 1 with food containing a heavy isotope of Carbon.
2. Quantify the phosphorylation levels with mass spectrometry.
3. Compare the phosphorylation levels to the level of APP transcription

Characterizing the upstream promoter region for *APP* will improve our understanding of how APP regulation leads to the accumulation of amyloid plaques in Alzheimer’s disease. Knowing which sites in the promoter are responsible for this regulation will lead to new ways to prevent the accumulation of Aβ in patients with Alzheimer’s and patients who are at risk for the disease.

References

[1] Guyant-Maréchal, L. et al. 2007. Variations in the APP gene promoter region and risk of Alzheimer disease. Neurology. 68: 684-687.

[2] Theuns, J. et al. 2006. Promoter Mutations that Increase Amyloid Precursor-Protein Expression are Associated with Alzheimer Disease. The American Journal of Human Genetics. 78: 936-946.

[3] Bailey, J. et al. 2011. Functional activity in of the novel Alzheimer’s amyloid β-peptide interacting domain (AβID) in the *APP* and *BACE1* promoter sequences and implications in activating apoptotic genes in amyloidogenesis. Gene. **488**: 1-2: 13-22.