Analysis of Differential mRNA and miRNA Expression in Alzheimer's Disease Mouse Model.

Project Summary:

Our project will evaluate genome-wide RNA expression patterns from brain and blood in an Alzheimer's Disease (AD) mouse model. This analysis will provide insight regarding the mechanisms of AD pathology as well as determine a possible diagnostic tool utilizing RNA expression patterns found in the blood as biomarkers for AD.

Objectives:

[1] Evaluate the role of miRNA in AD pathology. This portion of the project will compare differences in miRNA expression in the hippocampus of AD and normal mice by using qPCR to amplify miRNA with demonstrated changes in methylation status. Changes in expression will then be analyzed to determine the interaction between miRNA expression and AD development.

[2] Comparative analysis of RNA expression in normal and AD hippocampi. In this portion of the project, differences in RNA expression will be analyzed using RNA-Sequencing. Expression changes shown by sequencing will be examined to identify AD-significant RNA expression patterns.

[3] Comparative analysis of RNA expression in normal and AD blood. This portion of the project will focus on determining differential RNA expression between blood samples found in AD and normal mice.

[4] Comparison of RNA expression patterns in brain and blood. Differential expression data for hippocampus will be compared to data for blood to determine whether characteristic patterns of expression in AD are tissue-specific.

Rationale:

Rationale for Objective 1: Previous work by our group has demonstrated a correlation between AD pathology and changes in epigenetic markers including cytosine methylation of gene promoter regions (*1*). Several genes determined to have AD-related changes in methylation code for miRNA, which is known to affect gene expression. Research suggests that miRNA play a key role in AD development by alteration of gene expression, particularly in that of amyloid-ß (Aß) production and apoptosis of postmitotic neurons (*2, 3*). Thus differences in miRNA

expression in the hippocampus of AD mice would be expected and may give insight into the role miRNA have in Aß plaque formation.

Rationale for Objectives 2-3: The AD brain displays altered gene expression compared to that of a normal brain. Similarly, other tissues including blood also exhibit these changes in expression (*4*). Analysis of specific genes changing expression in AD will give a better understanding of the unique mechanisms involved in AD development.

Rationale for Objective 4: A comparative analysis of differential RNA expression in the hippocampus and blood of AD mice could show itself valuable in the development of an inexpensive diagnostic test for AD using AD-specific biomarkers found in the blood.

Experimental Design and Methods:

Genomic DNA from mouse hippocampi and blood (n=3) will be isolated, reverse transcribed to cDNA, and sequenced using an Illumina MiSeq sequencer to determine differential mRNA expression in AD. Gene-by-gene qPCR confirmations will be conducted to verify the genome-wide analysis. To accomplish objective 1, a miScript PCR assay (Qiagen) will be used in which mature miRNA is polyadenylated and reverse transcribed into cDNA using oligo-dT primers followed by a qPCR analysis of mir gene fold changes (n=3). Once completed, expression data will be overlaid with epigenetic changes and transcription factor binding sites.

Significance:

Previous research has established that mRNA and miRNA expression play a critical role in AD development (*2, 4*) In addition, our research shows that many genes coding for both mRNA and miRNA display AD-related changes in methylation status. Thus, a specific analysis of these genes could shed new light on the role of epigenetic modification in AD development. Examination of genome-wide changes in expression could also give new insight regarding the specific mechanisms involved in AD pathology. Finally, specific RNA expression patterns in AD hippocampi and blood samples could provide a diagnostic tool to effectively and efficiently diagnose AD from biomarkers present in the blood.

References:

1. **N. Taher *et al.*, Amyloid-beta alters the DNA methylation status of cell-fate genes in an Alzheimer's disease model. *Journal of Alzheimer's disease : JAD* 38, 831 (2014).**
2. **S. Absalon, D. M. Kochanek, V. Raghavan, A. M. Krichevsky, MiR-26b, upregulated in Alzheimer's disease, activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33, 14645 (Sep 11, 2013).**
3. **C. Delay, F. Calon, P. Mathews, S. S. Hebert, Alzheimer-specific variants in the 3'UTR of Amyloid precursor protein affect microRNA function. *Molecular neurodegeneration* 6, 70 (2011).**
4. **B. J. Naughton *et al.*, Blood Genome-Wide Transcriptional Profiles Reflect Broad Molecular Impairments and Strong Blood-Brain Links in Alzheimer's Disease. *Journal of Alzheimer's disease : JAD*, (Jul 30, 2014).**