PROJECT SUMMARY (See instructions):

Aspects of Alzheimer's disease (AD) pathobiology are often clinically silent for years prior to the onset of dementia. New biomarkers that are sensitive to central nervous system (CNS) system changes early and throughout the disease course are needed. Here, we will show that a new proteomics procedure created in The Patrie Lab is amenable to routine biomarker discovery investigations in rodent models of AD. We highlight the procedure in a proof of concept study intended to establish if in a streptozotocin (Stz) rat model protein glycosylation in cerebrospinal (CSF) changes over time. Stz rats exhibit clinical molecular and vascular traits over time that may emulate pathogenesis of AD, such as: brain insulin resistance (BIR), neuroinflammation, and Aβ deposition. We will monitor CSF glycosylation in rats over time and compare changes to metabolism and vascular markers associated with BIR, neuroinflammation, and Aβ deposition in brain and biofluids. These pilot studies may be generally categorized as 1) development of procedures for creation of rat models of AD, as well as, evaluate underlying pathologies; 2) the creation of a "top-down" proteome screening procedure that can rapidly monitor intact protein microheterogeneity changes in the context of tissue or biofluid samples deriving from the animal (or humans); and 3) creation of a computational and statistical framework to support such proteoform-level analysis. Our long-term vision for this study is to establish a team and procedures that can routinely be applied in collaborations with diverse AD investigators across the UT System, where our team will help to create animal models for tissue and biofluid sampling for follow-on proteomics screens.

Our work will create innovative procedures related to application of off-gel isoelectric focusing, superficially porous liquid chromatography, and Fourier transform mass spectrometry (IEF-SPLC-FTMS) in a routine screening environment. In our previous work 100 man-hours were required to process data from a single CSF proteome with IEF-SPLC-MS, which prevents regular application of this approach in discovery screens. To address this bottleneck, we propose a computational model: glycoproteoform differential network analysis (GDNA) that will rapidly assign observed glycoproteoforms to network maps that will subsequently support: 1) efficient annotation of MS spectra, 2) Poisson-based scoring during glycoproteoform identification from MS/MS datasets, and 3) glycoproteoform differential expression in longitudinal studies in rodents. Expected outcomes associated with this project include the development of strong working relationship between investigators with diverse pedigrees from across the UT Southwestern. Our team will have created standardized operational procedures for AD model generation including collection 50-200 µL of CSF, as well as, have established microscopy and immunohistochemistry procedures for evaluation of brain metabolism and vascular alterations in the rodents over time. We also expect to have obtained substantial data on a new proteomics screening procedure, akin to traditional 2D gel electrophoresis, but with superior throughput, up to 40× improved resolving power, and detection limits as low as 100 attomole. Similarly, upon completion of our unique proteoform-level automation procedures we expect the timeframe for compilation of IEF-SPLC-FTMS datasets, assignment of proteoforms, and differential expression analysis between datasets will be reduced to <30 min.

RELEVANCE (See instructions):

This proposal will create and apply new and innovative proteome screening procedures, whole animal models sampling procedures, and computational/statistical analysis procedures that may be broadly applied to study aspects of AD pathobiology through biomarkers created through the course of the disease. Our proposal meets the 2015 UT BRAIN seed program agenda by forging collaborations from across UT Southwestern campus. Upon completion of the first year The Patrie Lab will have established a strong working relationship with NeuroModels Center, Cell/Tissue Imaging Facility, and the Clinical Statistics departments; developing procedures that will not only permit biomarker discovery at a proteoform-level on Stz treated rats, but will also be applicable to prospective screens on other systems, such as, models that seek to establish prognostic markers that may be useful in trails on novel therapeutics. We will also be able to further refine hypothesis regarding the metabolic and vascular biomarkers observed in Stz rats and verify them in screens on AD patients.