UT SOUTHWESTERN
MEDICAL CENTER

Steven M. Patrie, Ph.D.
Endowed Scholar & Assistant Professor
Department of Pathology

Monday, August 26th, 2015

The Darrell K Royal Research Fund for Alzheimer's disease
PO Box 5839
Austin, TX 78763
866-946-3606

Dear Mrs. Edith Royal, the DKR Fund Board of Directors, and Scientific Review Committee:

It gives me great pleasure to provide this annual report (vide infra) for the scientific research project entitled: *Molecular Proteotyping*.

By 2050, approximately 80 million people are expected to suffer from Alzheimer's disease (AD) worldwide. The U.S. National Alzheimer's Plan (USNAP) reaffirms the government's commitment to conquering AD and related dementias, which includes finding ways to diagnose, prevent and treat the disease by 2025. There is evidence implicating O- and N-glycosylation changes in AD patients. Plus gene, metabolic, and inflammatory disturbances shown in AD may compound to impact the secretory pathway and protein glycosylation. An accurate understanding of long evolving, systemic dysfunction in AD spectrum disorders may be possible with glycosylation based-markers from biofluids. Cerebrospinal fluid (CSF) represents an accessible source of biomarkers from the brain, and many proteins in CSF are known to be glycosylated. With continued support my work will show that top-down mass spectrometry procedures are amenable to informative glycoprotein-based biomarker screens across the CSF proteome, setting the stage for first of its kind population-based research in human subjects.

With the warmest regards,

Steven M. Patrie

5323 Harry Hines Blvd., ND6.214, Dallas, TX, 75390
Telephone 217-649-1870
Steven.Patrie@UTSouthwestern.edu

In this first year of funding, Dr. Patrie has begun the creation of new computational tools to support the automated processing of glycoprotein variants, called glycoproteoforms, in cerebrospinal fluid of Alzheimer's disease (AD) patients. The work was recently presented at the annual conference of the American Society of Mass Spectrometry and is being written up in separate manuscripts that are anticipated to be published late 2015 or early 2016. To briefly summarize, in humans a single glycoprotein can occur in 1000s of variants simultaneously. Such heterogeneity occurs because of the complex nature of carbohydrate biosynthesis and the complexity of how they attach to a protein. Despite the availability of high fidelity bioanalytical tools for observance of upwards of 10,000s of glycoproteoforms in patient CSF, current informatics resources can not interpret them. This means that for screens on patients the bulk of the observed data is not used. To address this limitation, the Patrie Lab has created a unique algorithm that enumerates hundreds of glycoproteoform variants from theoretical biosynthesis paths involved in protein glycosylation. They can show that an individual glycoproteoform can be accurately assigned in mixtures of 1000s of glycoproteoforms based upon the highly accurate physiochemical property measurements (mass, pI, and hydrophobicity) obtained by a novel proteomics workflow developed in the Patrie Lab. The assignments include unique chemical identifiers that derive from the core sugar components for all carbohydrates attached to a glycoprotein. Based on the intensity of the different glycoproteoforms observed the new informatics procedure opens the door to rapid examination of general classes of enzymes in the carbohydrate biosynthesis network that are expected to be modulated by neurodegeneration and neuroinflammation in AD patients. In our second year, we will continue to refine these procedures as well as begin to apply them in exploratory studies on a small subset of AD and control patients. A key aspects of this work will refine our algorithms for high-throughput screens on individual patients which will include the creation of new of data visualization tools that are intended to aid the clinicians interrogation of 1000s of CSF glycoproteoforms from patient samples.