Title: Stable Isotope Tracing of Mitochondrial Proteins and mtDNA Dynamics in Alzheimer's Tauopathy.

Principal Investigator: Takhar Kasumov, Ph.D.
Associate Professor, Department of Pharmaceutical Sciences
College of Pharmacy, NEOMED
E-mail: tkasumov@neomed.edu

Alzheimer's disease (AD), the leading cause of dementia and a major global health challenge, is a progressive neurodegenerative disorder characterized by cognitive decline and memory loss [1-3]. Pathologically, AD is marked by extracellular β-amyloid accumulation and intracellular neurofibrillary tangles formed from tau aggregation [4]. Mitochondrial dysfunction is a central contributor to neurodegeneration in AD. Mitochondrial DNA (mtDNA), due to its limited repair capacity and proximity to reactive oxygen species production sites, is particularly susceptible to oxidative damage [5]. Several forms of pathogenic tau impair neuronal bioenergetics and may lead to disrupted mitochondrial dynamics (fusion and fission balance), and block mitophagy, preventing clearance of defective mitochondria [6]. Using our mass spectrometry platform, we demonstrated that differentially abundant proteins in tauopathy mouse brain are predominantly associated with tau aggregation and autophagy regulation, accompanied by impaired mitochondrial integrity and function.

We hypothesize that tauopathy-related disruptions in mitochondrial dynamics alter the synthesis and turnover rates of mitochondrial proteins, with a particular impact on mtDNA-encoded subunits of the respiratory chain in the brain.

We will develop and apply a novel ^2H_2O-based stable isotope method to quantify mitochondrial proteins and mtDNA synthesis in the AD brain. Oral ^2H_2O administration establishes a steady-state enrichment, enabling turnover measurements of proteins [7, 8] and DNA [9] using high-resolution LC-MS/MS. We have validated this approach across multiple tissues [10-12] and, in preliminary brain studies, showed similar synthesis rates for nuclear- and mtDNA-encoded proteins. Using this approach, we will define how tauopathy disrupts mitochondrial dynamics, mtDNA integrity, and proteome turnover in the brains of *htau* mice (expressing all six human tau isoforms). This project will provide the direct *in vivo* measurements of mtDNA and mitochondrial proteome turnover in tauopathy, revealing mechanisms by which tau-induced mitochondrial defects drive neurodegeneration.

Impact: This project will deliver the novel isotope-based method to quantify mtDNA and mitochondrial protein synthesis in the prefrontal cortex and hippocampus, regions critically affected in AD. By linking tauopathy-induced defects in mitophagy, fusion–fission dynamics, and mtDNA integrity to impaired proteome turnover, these studies will define mechanisms of mitochondrial failure that drive synaptic dysfunction and identify therapeutic targets for AD.

Background and Significance: The brain is one of the most metabolically active organs, consuming roughly 20% of the body's total energy, primarily in the form of ATP produced in healthy and functional mitochondria. Brain aging and tauopathy disrupt the mitochondrial respiratory complexes (ETC), imbalancing mitochondrial dynamics and biogenesis, ultimately amplifying neuronal stress and degeneration. However, it remains unknown how disrupted mitochondrial dynamics affect protein turnover with tauopathy progression.

Goals and Objectives: Our objective is to define how progressive and advanced-diseases stage tauopathy affects mitochondrial dysfunction and global mitochondrial proteome turnover in AD brain. To test this, we will use pre-symptomatic, progressive-disease-stage, and advanced-disease-stage symptomatic *htau* mice as a tauopathy model. We will use age and sex-matched *C57BL/6* wild-type mice as healthy controls.

Aim 1. Determine whether tauopathy preferentially decreases synthesis of mtDNA-encoded proteins. *Hypothesis: Tauopathy-associated mtDNA defects reduce synthesis of mtDNA-encoded proteins, driving mitochondrial dysfunction.* We will evaluate the respiratory functions of hippocampal and PFC mitochondria, and measure mtDNA and mitochondrial proteome dynamics by $^{2}H_2O$ labeling and LC-MS/MS.

Aim 2. Define how disrupted mitochondrial dynamics affect protein turnover in tauopathy. *Hypothesis: Tauopathy and aging impair mitochondrial fusion and fission, causing dysregulated protein turnover*. We will measure mitochondrial dynamics and turnover to determine their interaction in AD.

Innovation: Our approach to mitochondrial protein DNA dynamics is novel and represents a major advance in flux studies. Using $^{2}H_2O$ metabolic labeling of mtDNA, proteins, and their acetylated forms, we developed a high-resolution MS method that quantifies low isotopic enrichment of peptide fragment ions. Unlike conventional tracers, this approach enables simultaneous measurement of protein, DNA, and biomolecule turnover; assessment of PTMs on protein stability and aggregation; and evaluation of the interplay between mtDNA synthesis, mitochondrial dynamics, and proteome turnover. It employs a safe, inexpensive isotope delivered in drinking water, avoiding costly high-dose i.v. ^{13}C tracer infusions that disrupt metabolic homeostasis. This cost-effective method enables direct in vivo measurement of mtDNA and mitochondrial proteome dynamics.

Student Involvement: Students will gain hands-on training in proteomics sample preparation, LC-MS/MS, mass spectrometry software and data analysis, bioinformatics, and Western blot–based tauopathy-induced mitochondrial dysfunction characterization.

Methods and Data Analysis: We will administer $^{2}H_2O$ to *htau* and wild-type mice and collect the cortical and hippocampal brain regions at different time points, i.e., 1, 3, 7, 14, 21 and 30 days. We will prepare the proteomics samples for LC-MS/MS analysis. Raw MS data will be processed using a SwissProt mouse database, 1% FDR, tryptic peptides with up to two missed cleavages, 6-ppm precursor and 20-ppm fragment tolerances. We will also assess mitochondrial function and enzymatic activity in hippocampal and cortical tissues. Cell culture models, i.e., N2A cells and SHY5Y cells, will be used to explore downstream mechanistic pathways and how altered mitochondrial dysfunction disrupts proteostasis.

Significance of Expected Findings: This study will define mechanisms of mitochondrial failure that drive synaptic and brain proteome dysfunction and neurodegeneration, and identify dynamic biomarkers and therapeutic targets for AD.

References

1. Chandrashekar, D.V., et al., *Alcohol as a Modifiable Risk Factor for Alzheimer's Disease-Evidence from Experimental Studies*. Int J Mol Sci, 2023. **24**(11).
2. Sullivan, E.V. and A. Pfefferbaum, *Alcohol use disorder: Neuroimaging evidence for accelerated aging of brain morphology and hypothesized contribution to age-related dementia*. Alcohol, 2023. **107**: p. 44-55.
3. Bostrand, S.M.K., et al., *Associations between alcohol use and accelerated biological ageing*. Addict Biol, 2022. **27**(1): p. e13100.
4. Polanco, J.C., et al., *Amyloid-beta and tau complexity - towards improved biomarkers and targeted therapies*. Nat Rev Neurol, 2018. **14**(1): p. 22-39.
5. Combs, B., et al., *Frontotemporal Lobar Dementia Mutant Tau Impairs Axonal Transport through a Protein Phosphatase 1gamma-Dependent Mechanism*. J Neurosci, 2021. **41**(45): p. 9431-9451.
6. Rawat, P., et al., *Phosphorylated Tau in Alzheimer's Disease and Other Tauopathies*. Int J Mol Sci, 2022. **23**(21).
7. Kasumov, T., et al., *Measuring protein synthesis using metabolic (2)H labeling, high-resolution mass spectrometry, and an algorithm*. Anal Biochem, 2011. **412**(1): p. 47-55.
8. McCullough, A., et al., *HDL flux is higher in patients with nonalcoholic fatty liver disease*. Am J Physiol Endocrinol Metab, 2019. **317**(5): p. E852-E862.
9. Macallan, D.C., et al., *Measurement of cell proliferation by labeling of DNA with stable isotope-labeled glucose: studies in vitro, in animals, and in humans*. Proc Natl Acad Sci U S A, 1998. **95**(2): p. 708-13.
10. Li, L., et al., *Proteome Dynamics Reveals Pro-Inflammatory Remodeling of Plasma Proteome in a Mouse Model of NAFLD*. J Proteome Res, 2016. **15**(9): p. 3388-404.
11. Lee, K., et al., *Hepatic Mitochondrial Defects in a Nonalcoholic Fatty Liver Disease Mouse Model Are Associated with Increased Degradation of Oxidative Phosphorylation Subunits*. Mol Cell Proteomics, 2018. **17**(12): p. 2371-2386.
12. Aghayev, M., et al., *Chronic alcohol consumption reprograms hepatic metabolism through organelle-specific acetylation in mice*. Mol Cell Proteomics, 2025: p. 100990.

Student Fellow Training/Mentoring Plan: The mentoring program aims to equip student with skills in mass spectrometry and bioinformatics. Guided by the National Academies' recommendations, the program offers structured mentoring, career planning, and training in scientific presentation and writing. Key topics include coworker interactions, work habits, and thorough documentation of research. Students will be trained by senior lab members and graduate students. They will join weekly journal clubs to critique articles and learn paper submission. Professional practices instruction will cover the scientific method, hypothesis formulation, research design, and timeline creation. Progress will be monitored through interviews, weekly meetings, and a final poster presentation of summer research findings.