1. Project title: Role of alcohol-induced acetylation in Alzheimer's disease (AD).

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2. Abstract: Alcohol (EtOH) consumption, prevalent in the United States, is strongly associated with an elevated risk of late-onset Alzheimer's disease (AD), a leading cause of dementia. Excessive alcohol intake increases the likelihood of AD by a staggering 300%, underscoring the urgent need to investigate the link between Alcohol Use Disorder (AUD) and increased AD risk.

A possible AUD-AD connection may stem from disrupted brain protein homeostasis due to EtOH metabolism. Post-translational acetylation at lysine side chain of proteins by acetyl-CoA (AcCoA) has emerged as an essential regulatory mechanism in protein stability, intermediary metabolism, and epigenetics. EtOH detoxification produces AcCoA and depletes NAD^+, key factors involved in acetylation. Tau acetylation is implicated in tauopathy, accumulation of hyperphosphorylated microtubule-associated protein tau (p-tau), in AD. Yet, how alcohol metabolism is linked to altered acetylation of tau in AD remains unknown.

Site-specific tau acetylation dynamic in tauopathy is poorly understood, and the influence of alcohol on acetylation-dependent tauopathy remains entirely uncharted. EtOH metabolism-induced NAD^+ deficiency may hinder brain deacetylation, potentially disrupting tau turnover and increasing p-tau accumulation. As EtOH-derived acetate contributes to mouse brain histone acetylation, it may also induce epigenetic alterations linked to tauopathy. Hence, the EtOH-induced shift in site-specific acetylation dynamics, rather than mere changes in acetylation levels, can influence brain function through epigenetic mechanisms and p-tau aggregation.

Our group developed a mass spectrometry (MS)-based method to examine acetylome dynamics in vivo. Here, we aim to employ this method to establish a connection between AUD and AD. *The central hypothesis is that alcohol-induced altered brain acetylation dynamics contribute to the accumulation of toxic acetylated tau*.

We will measure site-specific acetylation turnover of histones and tau in the hippocampus and cortex of alcoholic *htau* mouse model of tauopathy to determine whether the altered acetylation results from impaired acetylation or deacetylation. Utilizing ChiP-Seq, we will identify histone acetylation-regulated transcriptional changes to uncover modified signaling pathways.

The impact. This study will also establish the feasibility of the acetylome dynamics method, which also can be used to investigate the selectivity and specificity of deacetylase and acetyltransferase inhibitors or activators *in vivo* and motivate the development of new AD therapies.

3. Background and Significance: EtOH consumption in the United States is strongly linked to a higher risk of late-onset Alzheimer's disease (AD), a major cause of dementia. Among the 14 million Americans with AUD, 9% have concurrent brain disorders, including increased AD risk[1, 2]. A possible AUD-AD connection may stem from disrupted brain protein metabolism and EtOH-induced epigenetic changes. Post-translational acetylation at lysine side chain of proteins by AcCoA, a central metabolite, plays a pivotal role in protein stability and epigenetics. While the liver primarily metabolizes EtOH, acetate produced enters the brain, competing with glucose metabolism[3]. Acyl-CoA synthetase 2 (ACSS2) converts acetate to AcCoA, affecting histone acetylation in the brain, crucial for gene expression control in neural activity[4]. AD-related tauopathy, characterized by tau protein accumulation, is associated with altered acetylation[5]. However, the link between EtOH metabolism and dysregulated acetylation in AD remains underexplored. Acetylation is a dynamic process impacting protein behavior through temporal changes in acetylation, deacetylation, and protein decay[6, 7]. Reversible acetyl transfer allows cells to adapt changes through transcriptional and enzymatic regulations. In general, histone acetylation activates transcription, while deacetylation silences it[8]. Importantly, an active acetylated histone marks have faster turnover than

silent marks[9, 10]. Acetylation also regulates enzyme activity by influencing catalytic function and substrate accessibility[11]. Additionally, acetylation may enhance protein accumulation by influencing turnover. The dynamics of site-specific tau acetylation in tauopathy are poorly understood, and the impact of EtOH-dependent acetylation on tauopathy is entirely unexplored. EtOH-derived acetate, contributing to brain histone acetylation[4], may induce epigenetic alterations linked to tauopathy. Therefore, the EtOH-induced shift in site-specific acetylation dynamics, rather than mere changes in acetylation levels, can influence brain function through epigenetic mechanisms and tau aggregation. We have devised a mass spectrometry (MS)-based method to examine acetylome dynamics *in vivo*. Here, we will employ this method to establish a connection between AUD and AD.

4. Goals and Objectives: Our *objective* is to evaluate the link between EtOH-induced protein acetylation and AD in AUD. *Our central hypothesis posits that* EtOH *-induced changes in acetylation dynamics contribute to the accumulation of toxic acetylated tau.* We will this hypothesis through studies in the following Specific Aims:

Aim 1: Investigate the role of alcohol-regulated site-specific acetylation on brain protein stability *in vivo*. We will employ our in vivo $^{2}H_2O$-labeling method to examine acetylome dynamics in the hippocampus and frontal cortex of normal and alcoholic htau mice. Additionally, we will evaluate the development of AD characteristics, such as p-tau accumulation and neurofibrillary tangle formation to understand the role of AD in tauopathy.

Aim 2: Examine the effect of chronic alcohol consumption on brain protein acetylation turnover *in vivo*. We will measure site-specific acetylation turnover of histones and tau, to determine whether the altered acetylation in the mice from Aim 1 results from impaired acetylation or deacetylation. Through ChiP-Seq, we'll identify histone acetylation-regulated transcriptional changes to uncover modified signaling pathways in tauopathy.

5. A student will be involved in the analysis of collected brain samples. Specifically, a student will gain experience in proteomics sample preparation, MS acquisition, and data analysis using bioinformatic tools and statistical methods. In addition, a student will learn how to characterize tauopathy using Western blots.

6. Investigative Methods and Data Analysis: We'll conduct kinetic studies on the *htau* mice to investigate alcohol-dependent tauopathy using our lab's established EtOH-feeding paradigm. Age-, and weight-matched female *htau* mice will be randomized into control pair-fed (**PF**) and EtOH-fed (**EF**) groups and fed their respective diets for 25 days. To assess the impact of acetylation, mice will be treated with $^{2}H_2O$ will start during the EtOH feeding experiment. To assess alcohol-related changes in acetylation stoichiometry, we will isolate nuclear cytosolic, and mitochondria fractions. Histones will be acid extracet from nuclear fraction. Cytosolic and mitochondrial acetylated peptides will be enriched using PTMScan kit for ^{Ac}K motif and analyzed by LC-MS/MS. We will use commercially available anti-tauK174ac and anti-p-tau (ser202 and ser416) to assess the role of site-specific tau acetylation in tau phosphorylation and tauopathy.

Raw MS data files including peptide ion masses and fragment spectra obtained from the Q-Exactive Plus mass spectrometry will be processed using MaxQuant against SwissProt mouse protein database. Trypsin-digested peptide sequences will be searched at up to a maximum of two missed cleavages. Database searching was performed with 6 ppm mass tolerance for precursor ions and 20 ppm for fragment ions. The false discovery rate (FDR) will be set to a maximum of 1% false identifications from a reversed sequence database.

7. **Significance of anticipated findings:** We aim to uncover the influence of alcohol on brain proteome and acetylome dynamics in a mouse model of AD, offering essential insights into the mechanisms underpinning AUD-related AD. The acetylome dynamics method could be used to evaluate the efficacy of HDAC and KAT inhibitors and activators in vivo and catalyzing the development of innovative treatments for individuals with alcoholic AD.

References

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Student Fellow Training/Mentoring Plan:

The goal of the mentoring program is to provide skills, knowledge and experience to prepare a student fellow to excel in mass spectrometry and bioinformatics technology. To accomplish this goal, the mentoring plan will follow the guidance of the National Academies of Science and Engineering on how to enhance the research experience, by providing a structured mentoring plan, career planning assistance, and opportunities to learn a number of career skills such as developing scientific presentation and writing skill.

Mutual expectations will be discussed and agreed upon in advance. The plan topics will include (a) interaction with coworkers, (b) work habits, and (c) documentation of research methodologies and experimental details so that work can be continued by other researchers and colleagues. Participation in the journal clubs in which graduate students and postdocs meet weekly, along with a faculty facilitator, to discuss and critique recent journal articles and to discuss how to write and submit papers.

Instruction in Professional Practices will be provided on a regular bases in the context of the research work and will include fundamentals of the scientific method in the design of research question, formulation of a hypothesis and description of defined approaches to test the hypothesis. She/he will learn to identify research questions, definition of objectives, description of approach and rationale and construction of a work plan and timeline. Success of this mentoring will be assessed by tracking the student fellow's progress through interviews to assess satisfaction with the mentoring program, and tracking of progress through weekly discussions in group meeting. At the end of the internship, the results of the summer research will be presented in a poster session.