Title: ACSS2 and Acetylation Remodeling in Alcohol-Induced Tauopathy.

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Alzheimer's disease (AD), the sixth leading cause of death in the United States, increases markedly with age. Chronic alcohol consumption accelerates brain aging and heightens AD risk by disrupting pathways essential for neuronal homeostasis[1]. Alcohol impairs brain energy metabolism and perturbs histone acetylation, critical regulators of proteostasis, synaptic plasticity, and memory formation[2, 3]. Alcohol use is also associated with increased tauopathy, yet the mechanistic link between alcohol-driven metabolic dysfunction and AD progression remains poorly defined.

This proposal centers on lysine acetylation, a post-translational modification dysregulated by both aging and alcohol exposure. Aberrant tau acetylation promotes the accumulation of neurofibrillary tau aggregates, a defining feature of AD-related tauopathy. In parallel, alcohol-induced reductions in specific histone acetylation marks impair autophagy-mediated tau clearance, collectively contributing to pathological tau accumulation.

Glucose is the primary ACLY-dependent source of acetyl-CoA for histone acetylation, whereas nuclear ACSS2 activates acetate derived from exogenous sources or released from local histone "reservoir" lysines[4]. Alcohol impairs brain glucose uptake, increases reliance on acetate metabolism, and provides alcohol-derived acetate that competes with glucose for energy production. Using our stable-isotope–based mass spectrometry platform, we showed that alcohol not only provides carbon for histone acetylation but also reprograms acetylation dynamics by facilitating direct acetate flux from metabolic reservoir sites to transcriptionally active lysine residues[5].

Here, we will define how alcohol-disrupted acetylation dynamics drive alcohol-accelerated tauopathy.

We hypothesize that alcohol-induced metabolic and oxidative stress enhances ACSS2-dependent mobilization of acetate from reservoir lysines to activating lysines, increasing chromatin accessibility and inducing aberrant transcriptional programs that impair memory and promote pathological tau accumulation.

We will quantify site-specific acetylation stoichiometry and determine acetyl-CoA sources using ethanol-d_6, [U-^{13}C]glucose, and unlabeled histone-derived acetate. These tracers will resolve alcohol-derived, glucose-derived, and inter-lysine acetate transfer. Finally, ChIP-based analyses using site-specific acetyl-lysine antibodies will characterize functional epigenetic consequences in mouse hippocampus and cortex.

Impact: This work will provide new mechanistic insight into how alcohol-induced histone acetylation remodeling disrupts proteostasis and memory, establishing a foundation for future studies targeting acetylation-driven neurodegeneration.

Background and Significance: Lysine acetylation links cellular metabolism to gene regulation and proteostasis through acetyl-CoA–dependent modification of histones, transcription factors, and tau. Nuclear acetyl-CoA pools are shaped by HAT/HDAC activity and by metabolic enzymes, including PDH, ACLY, and ACSS2. ACSS2 converts acetate to acetyl-CoA for histone acetylation during metabolic

stress and facilitates direct intra-histone acetate transfer from reservoir to activating lysines, as shown in yeast quiescence exit.

Brain glucose metabolism is impaired in AD due to reduced glucose uptake and utilization. NAD^+-dependent sirtuins (SIRT1 and SIRT6) oppose acetylation by deacetylating histones, transcription factors, and tau (e.g., K174ac). Alcohol further imposes metabolic stress by inhibiting glucose metabolism, generating acetate, inducing oxidative stress, depleting NAD^+, and reducing sirtuin-dependent deacetylation. However, it remains unknown whether alcohol-induced acetate redistribution, via ACSS2-dependent mobilization, drives dysregulated histone acetylation and contributes to AD-related pathology.

Goals and Objectives: Our objective is to define how *alcohol-induced metabolic stress alters histone acetylation and accelerates brain aging and tauopathy.* We hypothesize that alcohol-induced metabolic and oxidative stress enhances *ACSS2-dependent acetate mobilization* from reservoir lysines to transcriptionally activating lysines, leading to increased chromatin accessibility, aberrant gene expression, memory impairment, and pathological tau accumulation. To test this, we will use $ACSS2^{+/+}$htau and $ACSS2^{-/-}$htau mice as a tauopathy model.

Aim 1: Determine the role of ACSS2 in alcohol-disrupted histone acetylation. We hypothesize that ethanol impairs systemic and brain metabolism by altering mitochondrial function, reducing glucose oxidation, and increasing reliance on ACSS2-dependent acetate utilization. In $ACSS2^{+}/^{+}$htau and $ACSS2^{-}/^{-}$htau mice, we will quantify cortical and hippocampal glucose metabolism and acetyl-CoA flux. Stable isotope MS will distinguish ACLY- vs. ACSS2-derived acetylation using glucose-, ethanol-, and histone-reservoir–derived acetate.

Aim 2: Assess the epigenetic consequences of alcohol-disrupted histone acetylation. We will determine how alcohol-induced changes in H4K16ac (proteostasis/autophagy) and H4K12ac (learning/memory) affect chromatin regulation. Using H4K16ac- and H4K12ac-specific antibodies and ChIP-qPCR/ChIP-seq, we will define transcriptional changes associated with tau accumulation and metabolic stress in hippocampus and cortex.

Student Involvement: Students will gain hands-on training in proteomics sample preparation, LC-MS/MS, isotope-tracer data analysis, ChIP methods, and Western blot–based tauopathy characterization.

Methods and Data Analysis: Female $ACSS2^{-}/^{-}$, htau, and $ACSS2^{-}/^{-}$;htau mice will receive ethanol-d_6 and [U-^{13}C]glucose. Nuclear histones will be extracted and analyzed by LC-MS/MS. 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 assess mitochondrial function, glucose oxidation, and enzymatic activity in hippocampal and cortical tissues. Complementary cell culture models will evaluate how altered acetylation disrupts proteostasis.

Significance of Expected Findings: This study will clarify how alcohol-disrupted histone acetylation rewires brain metabolism and contributes to cognitive impairment and tauopathy. The integration of metabolic tracing, epigenetics, and proteostasis analyses will provide mechanistic insights into alcohol-accelerated AD progression and support future development of therapeutic strategies targeting HATs, HDACs, ACSS2, or acetyl-CoA metabolism.

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 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.

References

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