Linking ER–Mitochondria Calcium Flux to Oxidative Stress and Protein Clearance Deficits in Alzheimer's Disease

Swetha Dammalapati*, Anusha Natraj†

Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by mitochondrial dysfunction, impaired protein clearance, and accumulation of pathological aggregates. In this study, we analyzed a multidimensional dataset comprising control, mild cognitive impairment (MCI), early AD, and late AD human brain samples. We investigated mitochondrial reactive oxygen species (ROS), autophagic flux, lipid peroxidation (4-HNE), mitochondrial dynamics (Mitofusin 2), lysosomal abundance, and amyloid peptide ratios (Aβ42/Aβ40), along with gene expression profiles for predictive modeling. Our results reveal a strong positive correlation between AD progression and both mitochondrial ROS and Aβ42/Aβ40 ratios, highlighting increased oxidative stress and amyloidogenic burden with disease severity. Conversely, autophagic flux showed a robust decline, while 4-HNE levels rose significantly in late AD, indicating impaired clearance mechanisms and elevated oxidative damage. Lysosome abundance increased in late-stage AD, potentially reflecting compensatory or dysfunctional remodeling. Mitofusin 2 expression exhibited moderate upregulation with progression, consistent with altered mitochondrial–ER tethering. Finally, machine learning models based on gene expression achieved modest classification accuracy ($\sim$40%), suggesting that transcriptional changes alone are insufficient for robust stage discrimination. Collectively, these findings provide an integrated view of mitochondrial stress, protein aggregation, and clearance pathway dysfunction across AD stages, and highlight the need for multimodal approaches to improve disease staging and mechanistic understanding.

Introduction

Alzheimer's disease is a growing global health concern that impairs mental and cognitive development, and it also disrupts neural function. Alzheimer's disease is a form of dementia, which can cause major dependence for daily activities, disability, and even mortality. Estimates approximate that 50 million people live with Alzheimer's worldwide [7], impacting millions who deal with cognitive decline of their loved ones.

Memory loss can be caused by a variety of problems in the brain and cellular pathways. Calcium ions are brought into the mitochondria through mitochondrial-associated membranes (MAMs). MAMs have been associated with mitochondrial dysfunction, calcium influx, and Aβ deposition [1]. The influx of calcium ions causes reactive oxygen species (ROS) to form through triggering mitochondrial responses. ROS are highly reactive molecules that are unstable and can damage cellular components and DNA, but can also be critical as signaling molecules regulating essential processes [5]. ROS can also damage the mitochondria and lysosomes. Lysosomes break down internal structures that are malfunctioning or misfolded. Damage of lysosomes causes their deacidification and subsequent cell dysfunction [10]. Misfolded proteins are unable to be degraded due to lysosomal dysfunction. These misfolded proteins develop into neurofibrillary tangles [8] and amyloid plaques [6]. The accumulation of misfolded proteins leads to inflammation. Inflammation of the cell causes the recruitment of immune cells to stop or prevent further damage. However, this

*Milpitas High School
†Dougherty Valley High School

ultimately leads to apoptosis by the death of the immune cells. The cells in the region therefore die, and synaptic connections are lost. Synaptic connections store memories, and hypoxic conditions can cause impaired neural communication, as inadequate oxygen supply to the synapse disrupts function.

Apart from this, genetics can also cause metabolic dysfunction in neurons, particularly in the Krebs cycle. Misfolded protein accumulation can occur, as discussed previously. Additionally, apolipoprotein E (APOE) genes can increase the risk of developing Alzheimer's disease. APOE genes provide instructions for making proteins involved in fat and cholesterol transport. Most people have APOE gene 3, which is the most common. However, APOE gene 2 can decrease the chance of Alzheimer's by threefold. Meanwhile, having APOE gene 4 increases the risk of Alzheimer's by four times due to the amyloid-β plaques [9].

Methods

Dataset Description

We analyzed a dataset comprising human subject samples stratified by clinical diagnosis into four groups: Control, Mild Cognitive Impairment (MCI), Early Alzheimer's disease (Early AD), and Late Alzheimer's disease (Late AD). Each entry is annotated with demographic variables including age, sex, and brain region of measurement (hippocampus, temporal cortex, parietal cortex, or prefrontal cortex).

The dataset integrates molecular, cellular, and physiological variables relevant to Alzheimer's pathology. These include indices of ER–mitochondria interactions (e.g., MAM contact index, ER–mitochondria distance, and calcium flux), mitochondrial function (ROS levels, ATP concentration), lysosomal properties (pH, V-ATPase and cathepsin D activity, autophagic flux), and markers of protein aggregation and stress (Aβ42/40 ratio, Aβ oligomer concentration, phospho-tau (pTau181), ubiquitinated proteins, and lipid peroxidation products). In addition, expression levels of key genes and proteins implicated in neurodegeneration were measured, including MFN2, VAPB, PTPIP51, IP3R, VDAC1, MCU, APP, and LAMP1, along with a binary indicator for presenilin-1 mutation (PSEN1).

Together, the dataset captures a multidimensional view of mitochondrial–ER crosstalk, lysosomal biology, and proteinopathy, enabling quantitative assessment of cellular processes linked to Alzheimer's disease progression.

Machine Learning Models

We trained three classifiers on the gene expression subset: support vector machines (SVM, radial basis kernel) [3], XGBoost [2], and a multi-layer perceptron (MLP) [4]. Data were split 80/20 into training and test sets, with 5-fold cross-validation on the training partition. Hyperparameters were optimized via grid search, and model performance was assessed using accuracy, precision, recall, F1-score, and confusion matrices. All models were implemented in the `scikit-learn` framework [11] and executed with standardized preprocessing pipelines to ensure reproducibility.

Results

We first examined whether mitochondrial oxidative stress increases with Alzheimer's disease (AD) progression. A significant positive correlation was observed between disease severity and mitochondrial reactive oxygen species (ROS) levels ($r = 0.867$), indicating a progressive increase in oxidative stress (Figure 1, left). Similarly, the ratio of Aβ42 to Aβ40 peptides, a known hallmark of amyloid pathology, also increased with disease severity ($r = 0.988$, $p = 4.89 \times 10^{-41}$), demonstrating a nearly linear relationship (Figure 1, right). These findings support the hypothesis that mitochondrial dysfunction and amyloid accumulation intensify with advancing AD.

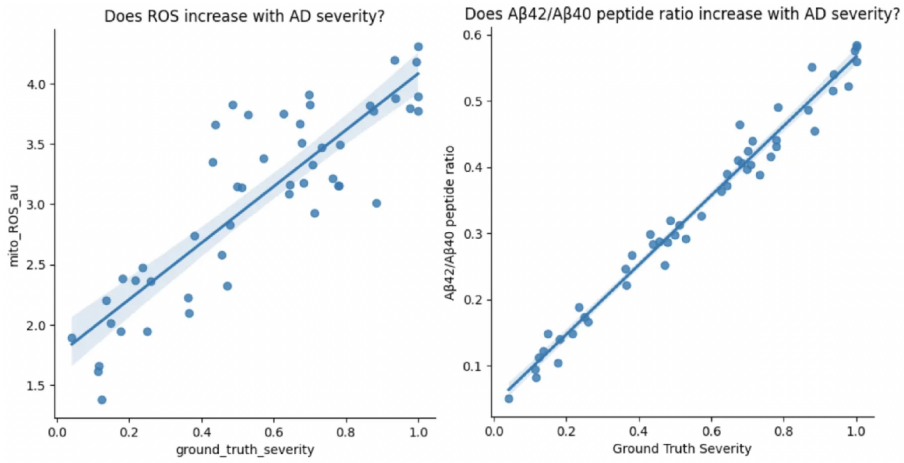

Figure 1: Mitochondrial ROS and Aβ42/Aβ40 peptide ratio increase with AD progression. (Left) Correlation of ROS with ground truth severity ($r = 0.867$). (Right) Correlation of Aβ42/Aβ40 ratio with AD severity ($r = 0.988$, $p < 10^{-40}$).

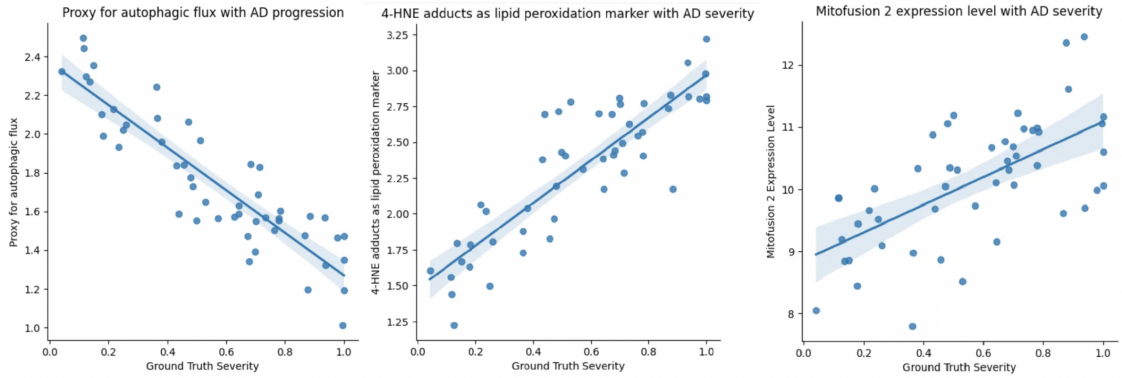

Figure 2: Cellular clearance and oxidative stress markers across AD progression. (Left) Decline in autophagic flux with severity ($r = -0.896$). (Middle) Increased 4-HNE adducts with severity ($r = 0.87$). (Right) Upregulation of Mitofusin 2 expression ($r = 0.634$).

Autophagic Flux, Lipid Peroxidation, and ER–Mitochondria Calcium Flux

We next investigated autophagic flux as a measure of cellular clearance capacity. A strong negative correlation was observed between disease severity and autophagic flux ($r = -0.896$, $p = 1.66 \times 10^{-18}$), suggesting that autophagy becomes progressively impaired with AD progression (Figure 2, left). In parallel, we assessed 4-HNE adduct levels as a proxy for lipid peroxidation. While values remained relatively low in controls, mild cognitive impairment (MCI), and early AD cases, late AD samples showed substantially elevated levels. Quantitatively, 4-HNE correlated positively with disease severity ($r = 0.87$, $p = 2.50 \times 10^{-16}$) (Figure 2, middle).

In addition to these markers, analysis of ER–mitochondria Ca^{2+} flux revealed a pronounced positive association with AD severity (Figure 3). Control subjects exhibited relatively low Ca^{2+} transfer, whereas MCI, early AD, and late AD groups showed progressively elevated flux values. This stepwise increase suggests that dysregulated Ca^{2+} shuttling from the ER to mitochondria emerges early and persists across disease stages. Importantly, the steep rise in MCI and early AD cohorts indicates that mitochondrial calcium overload may precede overt neuronal loss, thereby driving oxidative stress and energy deficits. Together with impaired autophagy and enhanced lipid

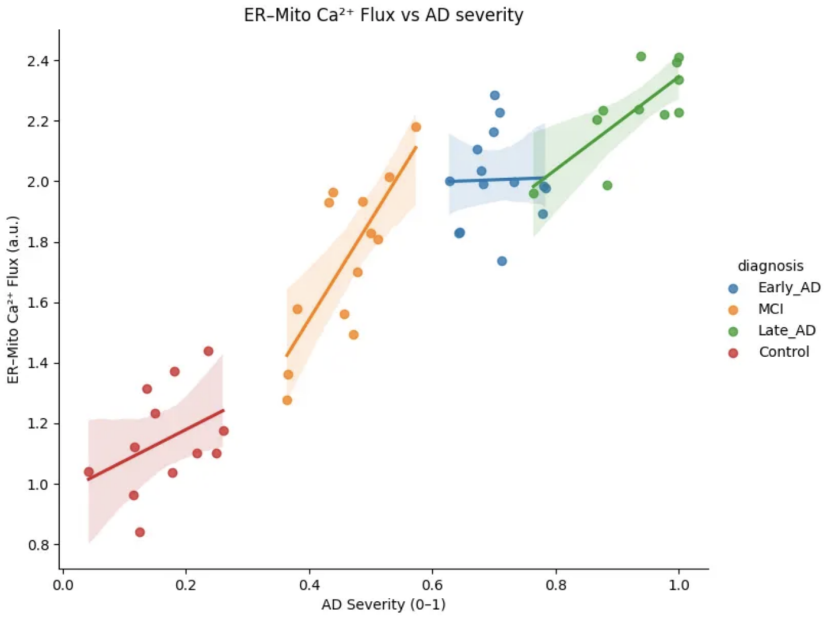

Figure 3: ER–mitochondria Ca^{2+} flux increases progressively with AD severity. Control samples show minimal flux, whereas MCI, early AD, and late AD groups display stepwise elevations, highlighting dysregulated Ca^{2+} handling across disease stages.

peroxidation, these findings underscore the convergence of disrupted mitochondrial–ER communication and defective clearance pathways in Alzheimer's progression.

Mitochondrial Dynamics

Mitofusin 2 (MFN2), a regulator of mitochondrial fusion and ER–mitochondria tethering, showed a moderate but significant increase with disease severity ($r = 0.634$, $p = 7.58 \times 10^{-7}$) (Figure 2, right). This suggests an adaptive or maladaptive remodeling of mitochondrial networks in AD, possibly reflecting cellular stress responses.

Lysosomal Alterations in Early vs. Late AD

To further probe cellular clearance mechanisms, we compared lysosome abundance between early- and late-stage AD groups. Boxplot analysis revealed that lysosome numbers per cell were significantly higher in late AD compared to early AD (median increase from ~ 93 to ~ 105 lysosomes per cell, with a broader distribution extending to ~ 130 in late AD) (Figure 4). These results indicate an accumulation of lysosomes with advancing disease, potentially reflecting compensatory upregulation or impaired lysosomal clearance.

Predictive Modeling of AD Stages from Gene Expression

Finally, we evaluated whether gene expression profiles could discriminate disease stages. Classifiers including SVM (RBF kernel), XGBoost, and MLP were trained on the dataset. Predictive performance was modest, with SVM and XGBoost achieving $\sim$40% accuracy (4 true positives, $\sim$6 true negatives on average), while the MLP underperformed at $\sim$10% accuracy (Figure 5). These findings suggest that while transcriptional alterations are present, they may not be sufficient on their own for robust stage classification without additional molecular or imaging features.

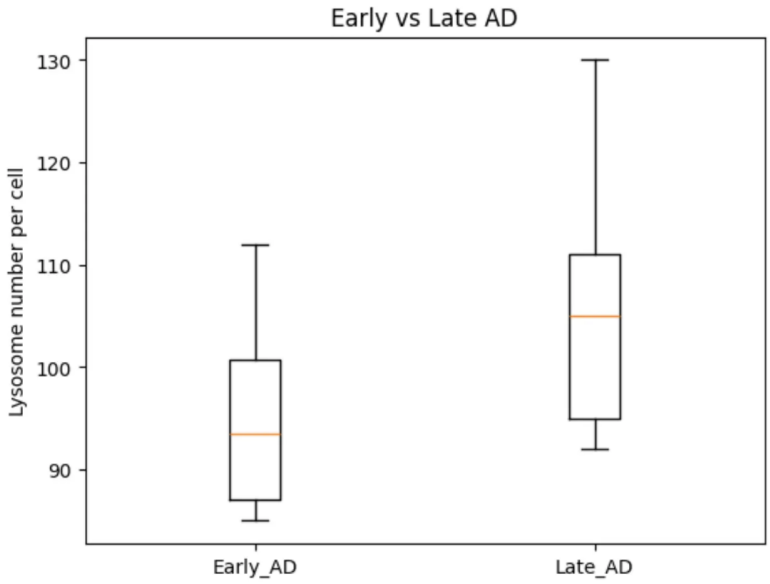

Figure 4: Comparison of lysosome number per cell between early- and late-stage AD. Late AD samples showed significantly higher lysosome abundance, with a wider distribution reaching ∼130 per cell.

Model	Accuracy	True Positives	True Negatives (avg)
SVM (RBF)	0.4	4	6.00
XGBoost	0.4	4	6.00
MLP	0.1	1	5.25

Figure 5: Classification performance of machine learning models for predicting AD stage from gene expression. Both SVM (RBF) and XGBoost achieved ∼40% accuracy, while MLP performed poorly (∼10%).

Conclusion

We provide an integrated view of mitochondrial dysfunction, protein aggregation, and clearance pathway impairments across the continuum of Alzheimer's disease progression. Our results demonstrate that mitochondrial oxidative stress and amyloidogenic burden (Aβ42/Aβ40 ratio) increase steadily with severity, while autophagic flux declines and lipid peroxidation becomes pronounced in late stages. We further show that ER–mitochondria Ca^{2+} flux rises progressively from mild cognitive impairment through late AD, suggesting that dysregulated calcium handling is an early and sustained pathological driver. Together with the observed upregulation of Mitofusin 2 and increased lysosomal abundance, these findings point to maladaptive remodeling of mitochondrial networks and compensatory yet inefficient clearance responses.

Importantly, predictive modeling based solely on gene expression achieved limited accuracy, underscoring that transcriptional changes alone are insufficient for reliable staging and highlighting the need for multimodal approaches that combine molecular, cellular, and imaging features. By linking mitochondrial–ER communication, oxidative stress, and protein clearance deficits, our work emphasizes convergent pathways that underlie disease progression. Future efforts aimed at therapeutic intervention should focus on restoring calcium homeostasis, mitochondrial resilience, and autophagic efficiency to slow or halt the trajectory of Alzheimer's pathology.

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