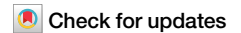


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Mitochondrial bioenergetic signatures differentiate asymptomatic from symptomatic Alzheimer's disease



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Asymptomatic Alzheimer's disease (AsymAD) refers to individuals who, despite exhibiting amyloid- β plaques and tau pathology comparable to Alzheimer's disease (AD), maintain cognitive performance similar to cognitively normal individuals. The resilience mechanism in AsymAD individuals remains understudied. We performed a systematic analysis comparing AsymAD and AD across multiple cohorts (ROSMAP, Banner and Mount Sinai), brain regions (BA6, BA9, BA36 and BA37) and neuronal and glial cell types using proteomics and transcriptomics data. AsymAD brains exhibited preserved mitochondrial bioenergetics, characterized by enhanced oxidative phosphorylation, electron transport chain activity, fatty acid and lipid metabolism, and branched-chain amino acid utilization. Pathways regulating mitochondrial complex biogenesis and calcium homeostasis were also upregulated. Key mitochondrial proteins such as MRPL47, CPT2, BCAT2, and IDH2, were consistently upregulated in AsymAD. At the cellular level, excitatory neurons, including superficial, mid-layer, and deep-layer subtypes, exhibited the most preserved mitochondrial function, whereas vulnerable inhibitory subtypes, including PVAlb and SST neurons, showed increased cellular abundance and bioenergetic activity. In contrast, microglia and oligodendrocytes proportions were reduced in AsymAD relative to AD. Our findings identify preserved mitochondrial bioenergetics in AsymAD and suggest that enhancing NADH metabolism via NAD⁺ precursor-based interventions may potentially help in maintaining cognitive function despite amyloid and tau pathology.

Alzheimer's disease (AD) is characterized by the accumulation of amyloid- β plaques and tau tangles^{1,2}, which historically drove therapeutic strategies aimed at reducing these hallmark pathologies^{3–7}. Despite decades of effort, most clinical trials of amyloid-targeted drugs, such as solanezumab (Eli Lilly)⁸, and aducanumab (Biogen)^{9,10}, have failed, whereas for Lecanemab^{10,11} (Eisai/Biogen), the number of side effects is worse than the benefits offered, highlighting the need to explore alternative mechanisms that contribute to AD pathogenesis. Analysis of postmortem brain samples, positron emission tomography (PET) amyloid imaging studies and neuropsychological and memory binding assessments have revealed a distinct group of individuals termed the asymptomatic AD (AsymAD) cohort who exhibit plaque and

tangle burdens comparable to those of AD patients but who maintain cognitive performance similar to that of the control/resistant cohort^{12–14}. Unlike resistant individuals who resist pathology, these resilient individuals tolerate amyloid and tau accumulation without developing dementia. The mechanisms underlying this resilience remain poorly understood. Large-scale proteomic analysis using weighted gene co-expression network analysis (WGCNA) for brain and cerebrospinal fluid (CSF) samples revealed mitochondria as key molecular modules that are disrupted in AD compared with AsymAD, while transcriptomic analyses from separate studies have highlighted the downregulation of specific mitochondrial genes, such as *MT-CO1*, *MT-CO3*, *MT1G*, and *MT2A*, in AD compared with resilient

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AD^{14–16}. However, the mechanisms by which mitochondria contribute to resilience in AsymAD individuals remain largely unexplored^{14–16}.

Mitochondria are central to brain function and provide the energy required to sustain neuronal activity^{17,18}. Each neuron can harbor hundreds to millions of mitochondria, reflecting the high metabolic demands of the brain^{19,20}. Mitochondrial dysfunction impairs energy production, leading to neuronal death or compensatory degradation of brain tissue, which could contribute to the cortical atrophy observed in AD^{21–23}. In contrast, asymptomatic individuals have preserved brain volume despite having equivalent pathology^{24,25}. Importantly, recent evidence suggests that mitochondrial dysfunction is not merely a consequence of AD but may act as a causal driver of neurodegeneration. Activation of mitochondrial G proteins has been shown to compensate for bioenergetic failure, leading to improved memory and reduced neurodegeneration in an AD mouse model²⁶. In humans, glucose hypometabolism precedes amyloid and tau deposition and may serve as a more sensitive diagnostic marker than classical pathology does^{27–30}. Other findings, such as improved acylcarnitine profiles in resilient individuals³¹ and enhanced branched-chain amino acid (BCAA) utilization in APOE2 carriers relative to APOE4 carriers, further support the role of bioenergetic resilience in mitigating AD risk³².

Although several studies have independently identified mitochondria as a critical component in AD pathology, none have specifically focused on AsymAD individuals to investigate how mitochondrial features are preserved compared to AD across cohorts, brain regions, and cell types. To address this gap, we systematically analyzed proteomic and transcriptomic data to examine how mitochondrial regulatory and metabolic functions are maintained in AsymAD. We aimed to elucidate the mechanisms of mitochondrial resilience in AsymAD by identifying conserved mitochondrial

features that may contribute to protection against neurodegeneration. Using proteomic data from the dorsolateral prefrontal cortex (BA9) across two cohorts (ROSMAP and Banner), we identified mitochondrial pathways that are maintained in asymptomatic individuals. We also applied machine learning classifiers to extract a minimal set of mitochondrial proteins and trained a logistic regression model to distinguish AD from AsymAD, which validated their expression across four brain regions (BA6, BA9, BA36, and BA37) and three cohorts (Banner, ROSMAP, and Mount Sinai). To understand the cell type-specific differences between AD and AsymAD, we analyzed single-nucleus RNA sequencing data and examined six major brain cell types: excitatory neurons, inhibitory neurons, astrocytes, microglia, oligodendrocyte precursor cells (OPC), and oligodendrocytes, which were resolved into 14 subtypes. These results position mitochondrial bioenergetic preservation as a core feature of AsymAD, suggesting new strategies for maintaining brain function despite amyloid and tau pathology.

Results

Study design for categorizing AD, AsymAD, and control samples

We included three groups in our study: Alzheimer's disease (AD), asymptomatic AD (AsymAD), and control groups. We applied an integrated approach combining proteomics, machine learning, and single-nucleus RNA sequencing to identify mitochondrial genes/proteins that were differentially expressed between AD and AsymAD across cell types, brain regions, and cohorts (ROSMAP, Banner, and Mount Sinai) (Fig. 1a). In our classification, AsymAD cases were defined as individuals with CERAD scores of 1–3 and Braak stages of 3–6 but without dementia at their last evaluation, whereas AD cases

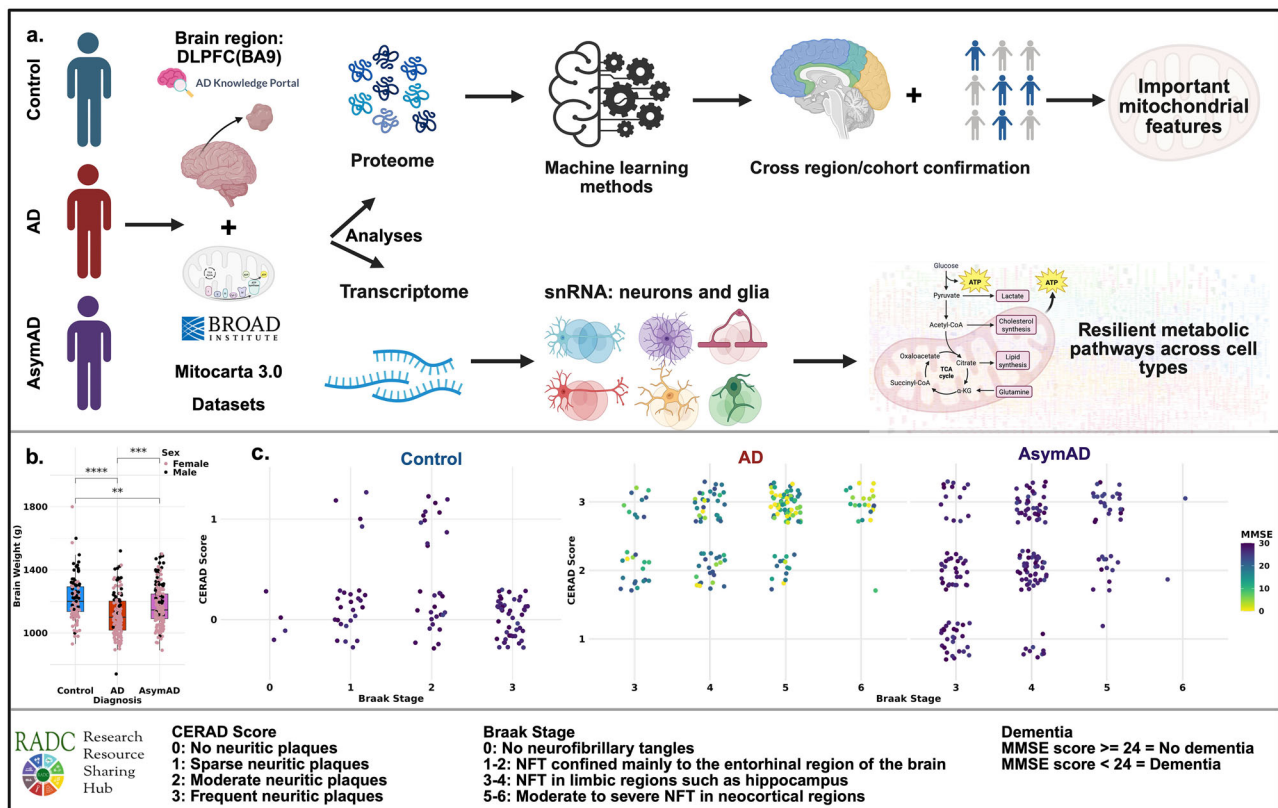


Fig. 1 | Asymptomatic AD (AsymAD) has a similar pathology to that of AD but a similar cognition to that of controls. **a** Schematic for the overall theme of the paper, highlighting the 3 approaches used—proteomics, transcriptomics, and machine learning—to identify key mitochondrial proteins and pathways. **b** Box plot showing the brain weight (in grams) for ROSMAP individuals (** = $p < 0.01$; *** = $p < 0.001$;

**** = $p < 0.0001$). **c** Scatter plot showing the Braak stages vs. the CERAD score for controls, AD patients, and AsymAD patients, colored according to the MMSE score per individual. (Created in BioRender. Mandal, P. (2026) <https://BioRender.com/s92lftm>).

had CERAD scores of 2–3 and Braak stages of 3–6 with dementia at the last evaluation. Although AsymAD patients exhibited a pathological burden similar to that of AD patients, they had greater brain weight (Fig. 1b) and maintained their cognitive performance within the normal range, which was comparable to that of controls, as reflected by MMSE scores ≥ 24 (Fig. 1c).

AsymAD patients exhibit increased mitochondrial resilience compared to AD

To identify differentially expressed proteins (DEPs) between AsymAD vs. AD, we first calculated the bias due to cell type abundance, given that AsymAD brains retain cognition and show minimal brain atrophy despite the presence of neuropathological hallmarks (Fig. 1b). Using established marker proteins for neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells reported by Johnson et al.¹⁴, we observed that compared with AD brains, AsymAD brains exhibited higher neuronal abundance but lower numbers of microglia, oligodendrocytes, and endothelial cells (Supplementary Fig. 1a, b).

To control for these differences and capture true protein-level changes, we applied the limma workflow, incorporating cell type marker scores as covariates in the design matrix. We further validated the correction using Fisher's exact test to assess cell type enrichment before and after adjustment. We observed that the initial enrichment of neuronal, microglial, and endothelial proteins in the uncorrected data was removed post correction, confirming that the final set of DEPs reflects protein levels independent of cell-type composition (Supplementary Fig. 1c–iii).

To explore the biological processes associated with the 1757 DEPs obtained after cell type correction, we performed pathway enrichment analysis using the KEGG, Reactome, and WikiPathways databases. With respect to the pathways associated with upregulated DEPs in AsymAD vs. AD, we observed enrichment for metabolism, translation, the TCA cycle, and ETC, suggesting that AsymAD individuals retain metabolic, mitochondrial and translational activity better than AD individuals do (Supplementary Fig. 1d). In contrast, pathways associated with downregulated DEPs in AsymAD vs. AD were enriched for AUF1-mediated RNA destabilization, translational inhibition, and components of the innate immune system, indicating a potential shift toward enhanced protein synthesis and reduced inflammatory response in AsymAD (Supplementary Fig. 1e). Among the 1757 DEPs, 258 mapped to the MitoCarta 3.0 database, indicating significant alterations in mitochondrial protein expression in AD patients compared with AsymAD patients. To further validate the robustness of our cell type-corrected pipeline, we used ANOVA with Tukey's HSD and limma without cell-type correction to identify mitochondrial DEPs across diagnoses. A total of 221 DEPs were commonly identified across all three approaches in AsymAD vs. AD. In terms of AD vs. control, we identified 198 overlapping DEPs, confirming that our strategy yielded a reliable and conservative set of mitochondrial proteins (Supplementary Fig. 1f–h).

Among the 258 overlapping DEPs between the cell type-corrected data and MitoCarta 3.0, 229 DEPs were upregulated and 29 were downregulated in AsymAD vs. AD (Fig. 2a, Supplementary Data 1). Pathway enrichment analysis of these 229 upregulated proteins revealed the involvement of mitochondrial regulatory and metabolic pathways. The top enriched metabolic pathways included the ETC, OXPHOS, the TCA cycle, and BCAA, fatty acid and propanoate metabolism. With respect to regulatory pathways, the top enriched pathways included “Biogenesis of Complexes I, III, and IV”, “Mitochondrial translation”, “Protein import and degradation”, “Fusion and fission regulation”, and “Mitochondrial calcium ion transport” (Fig. 2b and c, Supplementary Data 2).

Since OXPHOS was the most enriched pathway in AsymAD, we examined the overlap of our dataset with proteins reported in the ETC. In total, 57 proteins overlapped with ETC complexes (Fig. 2d and Supplementary Data 3). We found the following % overlap with the complexes: 53.1% for Complex I, 12.5% for Complex II, 62.5% for Complex III, and 23.5% for Complex IV. We observed the highest coverage, accounting for more than half of the proteins in Complexes I and III (Fig. 2e and

Supplementary Data 3). We compared the protein expression of mitochondrial complexes across AD, AsymAD, and control groups and observed that AsymAD and control groups shared similar expression profiles across all four complexes, whereas AD was consistently downregulated (Fig. 2f and Supplementary Data 4). These findings support our observation that OXPHOS is the most conserved pathway in AsymAD. The heatmap displays median protein expression in Complexes I–IV after correcting for cell type abundance, highlighting true protein abundance changes rather than shifts driven by cellular composition. Among the 13 mitochondrial DNA-encoded proteins, 3 (MT-ND3, MT-ND4, and MT-CO3) were significantly upregulated in AsymAD compared with AD, which could be attributed to their small size compared with nuclear-encoded mitochondrial proteins (Fig. 2f and Supplementary Data 4).

In addition to OXPHOS, we also examined key metabolic and regulatory proteins that are essential for energy generation. Proteins involved in fatty acid oxidation (CPT2; Carnitine Palmitoyltransferase 2, ACADSB; Acyl-CoA Dehydrogenase, Short/Branched Chain), BCAA metabolism (ECHS1; Enoyl-CoA Hydratase, Short Chain 1, BCKDHB; Branched-Chain Ketoacid Dehydrogenase E1 Subunit Beta), and the TCA cycle (SUCLA2; Succinate-CoA Ligase ADP-Forming Beta Subunit and IDH2; Isocitrate Dehydrogenase 2) were significantly upregulated in AsymAD vs. AD (Fig. 2g and Supplementary Data 5). In addition, calcium regulatory proteins (MCU; Mitochondrial Calcium Uniporter, VDAC2; Voltage-Dependent Anion Channel 2), mitochondrial translation factors (MRPL37; Mitochondrial Ribosomal Protein L37, TOMM40; Translocase of Outer Mitochondrial Membrane 40), and structural components (PDHB; Pyruvate Dehydrogenase E1 Beta Subunit, FIS1; Fission 1 Homolog) were also upregulated in AsymAD, with expression levels resembling those of Controls (Fig. 2h and Supplementary Data 5). All up- and downregulated mitochondrial proteins are provided in Supplementary Data 1.

Mitochondrial features associated with fatty acid, the TCA cycle, and amino acid metabolism distinguish AsymAD from AD

Using the 258 DEPs for AsymAD vs. AD in the BA9 region, we developed a machine learning model to identify the most informative features that distinguish AsymAD individuals from AD individuals. Protein expression data were imputed using k-nearest neighbors (KNN), and proteins with >15% missing values were plotted. KNN imputation did not introduce major shifts in mean or variance and preserved the overall distribution of protein expression (Supplementary Fig. 2a, b). Following imputation, we applied four classifiers, namely, random forest, gradient boosting, recursive feature elimination (RFE), and least absolute shrinkage and selection operator-cross validation (LASSO-CV), and the features selected by each were used to train a logistic regression model with 10-fold cross-validation (Fig. 3a). In addition, we tested the combined intersection of features identified by all four classifiers ($n = 16$; Fig. 3b, black outline, Supplementary Data 6). Among the classifiers, RFE selected 43 features (Supplementary Fig. 2c, d) and achieved the best performance (training AUC = 0.92, validation AUC = 0.88, accuracy = 0.81) (Fig. 3c and Supplementary Data 6). To assess biological relevance, we compared the 43 RFE-selected proteins against previously nominated AD-associated proteins from AGORA³³. Six proteins overlapped (Fig. 3b, pink outline, Supplementary Data 7), of which four proteins, ALDH4A1, DBT, DAP3, and IDH2, were present in the top 20 SHAP features (Fig. 3d and Supplementary Data 7). SHAP analysis revealed that higher expression of MRPL47, FTH1, BDH1, and CPT2 was predictive of AsymAD, whereas greater expression of MACROD1, ALDH9A1, DAP3, and FASN was associated with AD. Notably, several proteins involved in BCAA and fatty acid metabolism were enriched among the predictors of AsymAD (Supplementary Fig. 2e).

Finally, to test the robustness of these features, we examined their expression across additional cohorts and brain regions. IDH2 (*isocitrate dehydrogenase 2*) and DBT (*dihydrolipoamide branched chain transacylase E2*), which are involved in the TCA cycle and BCAA metabolism, were consistently upregulated in AsymAD compared with AD across BA6, BA9, BA36, and BA37 (Fig. 3e and Supplementary Data 8), whereas MACROD1

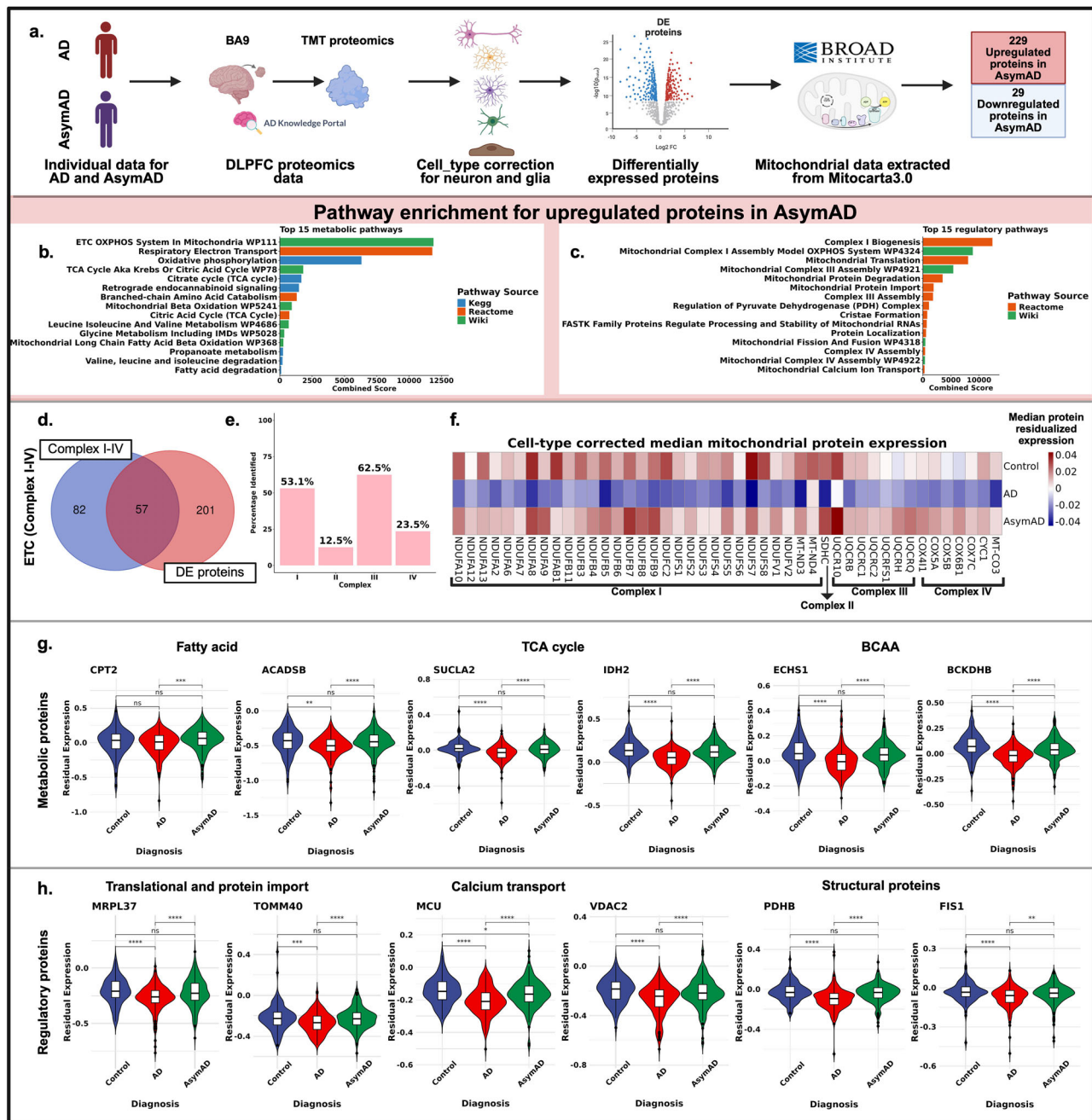


Fig. 2 | Compared with AD, AsymAD maintains mitochondrial bioenergetics (ETC, the TCA cycle, BCAA and fatty acid). **a** Schematic diagram representing 229 upregulated and 29 downregulated mitochondrial proteins between AsymAD and AD using BA9 proteomics data after correcting for cell type bias. **b, c** Pathway enrichment analysis shows upregulation of metabolic and regulatory mitochondrial pathways in AsymAD vs. AD. **d** Venn diagram showing the number of mitochondrial proteins overlapping with mitochondrial OXPHOS Complexes I–IV. **e** Percentage bar plot of overlap for the DEPs across mitochondrial complexes. **f** Residualized median expression of the protein heatmap of mitochondrial

complexes across control, AD, and AsymAD individuals after cell type, PMI, age of death, and sex correction. **g** (i–vi) Violin plot of key proteins involved in fatty acids, the TCA cycle, and BCAAs across the control, AD, and AsymAD groups using *t* tests. **h** (i–vi) Violin plot of key proteins involved in the regulation of mitochondrial functions, namely, translation, calcium transport, and structural proteins, across the control, AD, and AsymAD groups using *t* tests. (ns = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$). (Created in BioRender. Mandal, P. (2026) <https://BioRender.com/14j5mzj>).

(mono-ADP-ribosylhydrolase 1; a post-translational modification involved in DNA repair, transcription, and stress response) was consistently downregulated in AsymAD across the same regions, and BCAT2 (branched-chain aminotransferase 2) was upregulated in BA9 and BA37 (Fig. 3f and Supplementary Data 8). Among the 43 features selected by the RFE, several solute carrier mitochondrial family (SLC25) proteins were upregulated in AsymAD, including SLC25A46 (mitochondrial dynamics and lipid transfer) and SLC25A27 (uncouples oxidative phosphorylation). We also

observed upregulation of DAP3 (Death-Associated Protein 3 (Mitochondrial Ribosomal Protein S29)) and FIS1 (Fission 1 Homolog), which are essential for mitochondrial maintenance (Supplementary Fig. 2f–g).

Mitochondrial bioenergetic resilience across brain cell types in AsymAD

snRNA-seq data from the BA9 brain region were analyzed, with individuals classified as control, AD, or AsymAD, on the basis of previously established

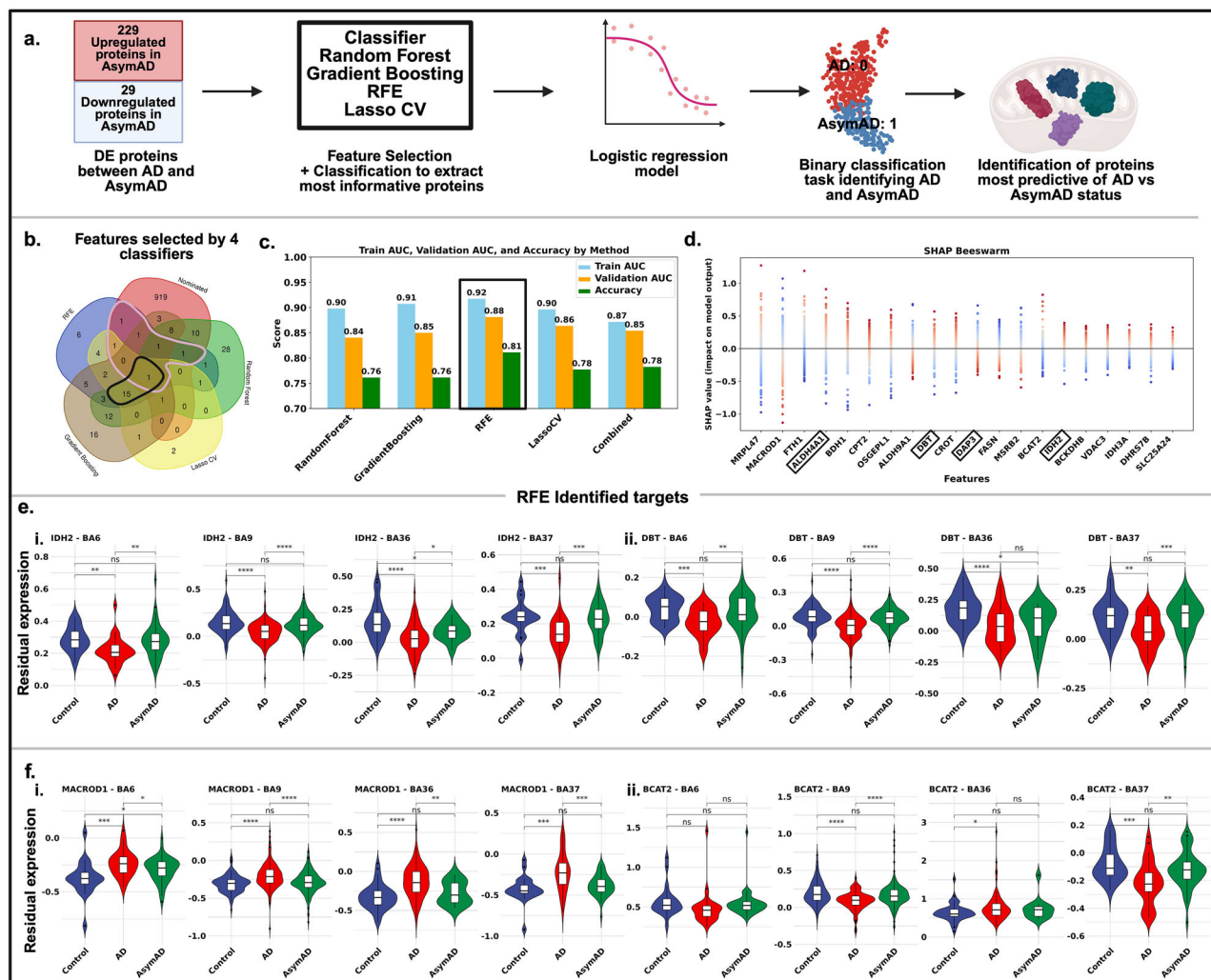


Fig. 3 | Identification of the top 20 mitochondrial features that distinguish AsymAD from AD. **a** Schematic of different classifiers used to identify key mitochondrial features from the 229 upregulated and 29 downregulated proteins between AsymAD and AD identified using BA9 proteomics data. **b** Venn diagram of common proteins identified by each classifier and nominated proteins (pink = common nominated proteins with RFE; black = common protein). **c** Bar plot reporting the training AUC, validation AUC, and accuracy for each classifier model, including the common proteins from all classifiers. **d** SHAP plot of the top 20 proteins used by the

RFE-logistic model to distinguish AsymAD from AD. **e** Violin plot of nominated targets + identified by RFE, IDH2, and DBT proteins across 4 brain regions (statistical significance was calculated using *t* test). **f** Violin plot of proteins identified by RFE, MACROD1, and BCAT2 across 4 brain regions (statistical significance was calculated using *t* test) (ns = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$). (Created in BioRender. Mandal, P. (2026) <https://BioRender.com/6jdsktz>).

criteria published by Johnson et al.¹⁴ (Supplementary Fig. 3a). Across 1.9 million cells, six major cell types were considered: excitatory neurons, inhibitory neurons, astrocytes, microglia, oligodendrocyte precursor cells (OPCs), and oligodendrocytes. The original snRNA-seq dataset contained a large number of subtypes, including 14 excitatory neuronal subtypes, 25 inhibitory neuronal subtypes, 3 astrocytic subtypes, and 5 immune populations. To reduce complexity while retaining biological meaning, these were consolidated into broader categories (refer to the methods section: Clubbing of cell types in single-nucleus RNA sequencing data). Specifically, excitatory neurons were grouped into superficial, mid-layer, and deep-layer excitatory neurons, whereas inhibitory neurons were grouped into the following groups: the PVALB, VIP, SST, and LAMP5 subtypes (Supplementary Fig. 3b). Other populations, including astrocytes, microglia, OPCs, and oligodendrocytes, were kept as originally defined by Mathys et al.³⁴.

We observed comparable proportions of male and female samples, as well as different diagnosis groups (Control, AsymAD, and AD individuals) (Fig. 4a and Supplementary Data 9). To assess whether shifts in cell type composition occurred across disease progression, we calculated the

proportion of each cell type subclass within each group. As expected, the inhibitory neuronal subtypes Inh SST and Inh PVALB were reduced in AD, which is consistent with their known vulnerability, but were retained in AsymAD (Fig. 4b and Supplementary Data 9).

We observed an interesting pattern in which the number of microglia and oligodendrocytes progressively increased from the control group to the AsymAD group to the AD group, which is consistent with previous reports of microglial expansion and oligodendrocyte enrichment during AD progression^{34–36}. Similar results were observed for oligodendrocytes from the cell type-resolved proteomics data analysis performed earlier (Supplementary Fig. 1b). The remaining cell types did not significantly differ (Supplementary Fig. 3c).

To investigate cell type-specific changes between AsymAD and AD, we performed MAST differential expression analysis (Fig. 4c and Supplementary Data 10). The greatest number of mitochondrial DEGs was observed in excitatory neurons, the majority of which were upregulated in AsymAD compared with AD, followed by astrocytes and inhibitory neurons. In contrast, oligodendrocytes uniquely exhibited a greater proportion

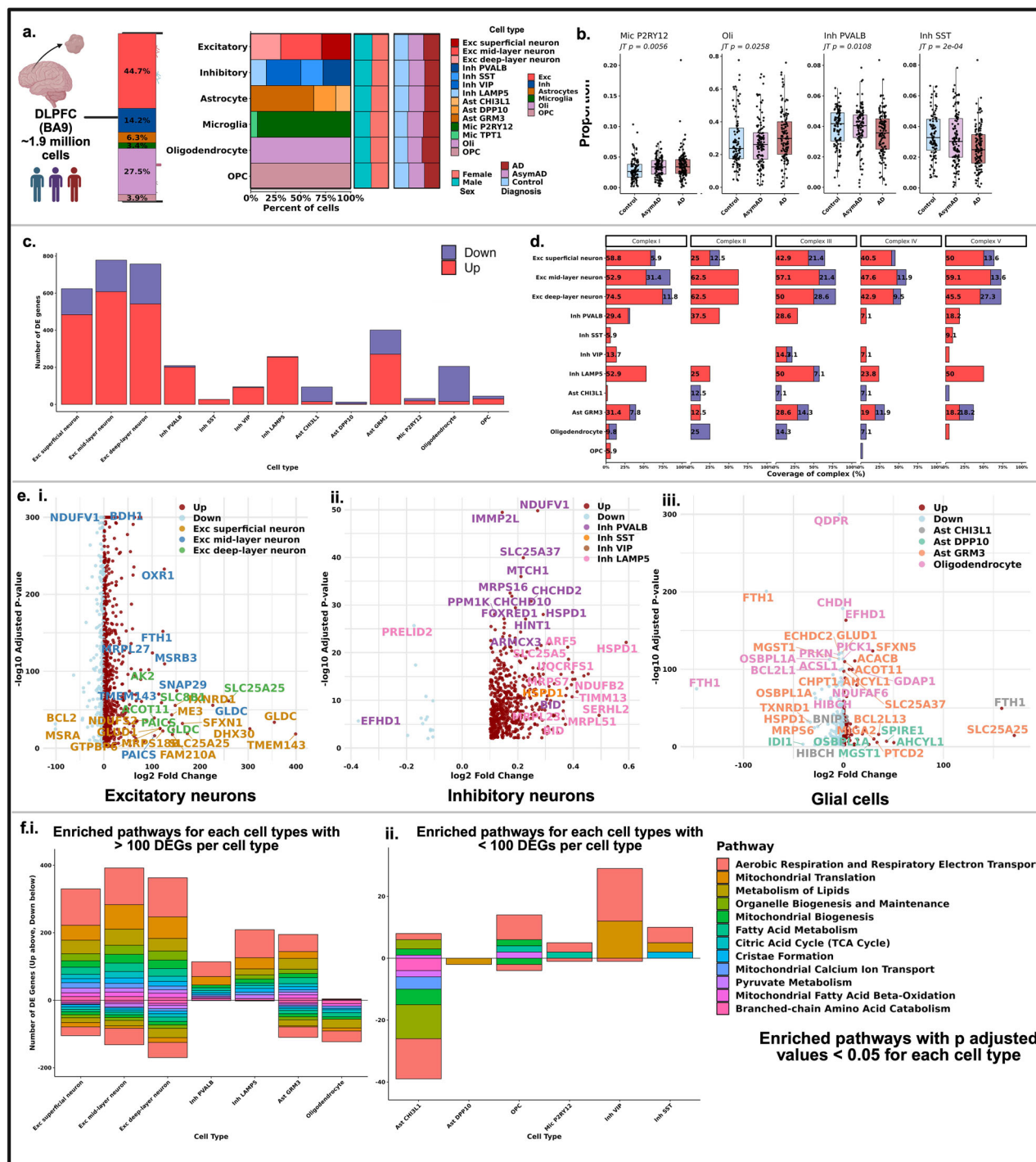


Fig. 4 | Cell type-specific mitochondrial changes in AsymAD vs. AD. **a** BA9 DLPFC from the control, AsymAD, and AD groups were used for analysis, with equal proportions of male and female samples clubbed into 6 main types and 14 subtypes. **b** Box plots of the proportion of each cell type per diagnosis to identify a trend compared with the control AsymAD AD using Jonckheere's trend test. **c** Bar plot of up- and downregulated DEGs between AsymAD vs. AD obtained using MAST analysis per cell subtype. **d** Bar plot reflecting the coverage of nuclear-encoded genes of the mitochondrial complex present per cell subtype. **e** Volcano plot

showing the top DEGs across cells color coded by up and downregulation and subtype; (i) DEGs for excitatory neuronal subtypes (superficial, mid-layer, and deep-layer neurons); (ii) DEGs for inhibitory neuronal subtypes (PVALB, VIP, SST, and LAMP5); and (iii) DEGs for glial cell subtypes (Ast CH13L1, Ast DPP10, Ast GRM3, and oligodendrocytes). **f.i** Bar plot showing the top enriched pathways associated with more than 100 DEGs per cell subtype; **f.ii** bar plot showing the top enriched pathways associated with less than 100 DEGs per cell subtype. (Created in BioRender. Mandal, P. (2026) <https://BioRender.com/0hy8j9w>).

of downregulated genes, differing from the upregulation trends observed in other cell types (Fig. 4c and Supplementary Data 10). Given that OXPHOS was the most preserved pathway in AsymAD (Fig. 2b), we next assessed the coverage of mitochondrial Complexes I-V for DEGs (both up- and down-regulated in AsymAD vs. AD) across cell types (Fig. 4d and Supplementary

Data 11). Excitatory neurons showed the highest coverage, especially the upregulated genes in mid-layer and deep-layer neuronal subtypes when they were mapped to mitochondrial complexes. Among inhibitory neuron subtypes, genes whose expression was predominantly upregulated in PVALB and LAMP5 neurons, followed by VIP and SST, were mapped to

mitochondrial complexes. In astrocytes, the homeostatic population (Ast GRM3³⁷) exhibited the strongest coverage of Complexes I & III, whereas activated astrocytes (Ast CHI3L1³⁸) exhibited greater coverage of Complex II, driven largely by downregulated genes, indicating reduced activity compared with homeostatic astrocytes. Oligodendrocytes showed more coverage of Complex II for genes whose expression was mostly downregulated, while OPCs demonstrated upregulation of Complex I genes. These results indicate broad preservation of mitochondrial bioenergetics across cell types in AsymAD, with activated or inflammatory astrocytes and oligodendrocytes showing reduced activity, likely reflecting lower metabolic demands within their environment.

To investigate the most significantly differentially expressed genes (DEGs) across cell subtypes, we generated volcano plots for the three major cell classes: excitatory neurons, inhibitory neurons, and glial cells (Fig. 4e.i–iii and Supplementary Data 10). In excitatory neurons, *NDUFV1*, *BDH1*, *BCL2*, *TREM143*, *GLDC*, and *SLC25A25* emerged as the top DEGs between AsymAD vs. AD. In inhibitory neurons, prominent DEGs included *NDUFV1*, *EFHD1*, *PRELID2*, *SLC25A37*, and *IMMP2L*. With respect to glial cells, *QDPR*, *FTH1*, and *SLC25A25* were the genes whose expression was the most significantly altered. Several of these genes overlapped with the protein features identified by RFE, suggesting consistency between the transcriptional and proteomic signatures of mitochondrial resilience. To determine which pathways were associated with the DEGs, we performed Reactome pathway enrichment analysis for each subclass of cells (Fig. 4f.i–ii and Supplementary Data 12). Across most cell types, we observed the upregulation of metabolic pathways associated with the upregulated DEGs, including those related to the ETC, lipid and fatty acid metabolism, and the TCA cycle, as well as regulatory pathways such as mitochondrial translation, calcium ion transport, and cristae formation. Oligodendrocytes were the only cells that predominantly exhibited downregulated pathways. Similarly, activated astrocytes marked as Ast CHI3L1 displayed marked downregulation of metabolic pathways compared to the homeostatic astrocytic population (Ast GRM3).

To investigate why oligodendrocytes uniquely exhibited downregulation in AsymAD, we compared this cell type across all three conditions: AD vs. control, AsymAD vs. AD, and AsymAD vs. control (Supplementary Fig. 3d). We observed a distinct pattern, especially for mitochondrial metabolic pathways, whose expression was upregulated in the AD vs. control group and in the AsymAD vs. control group, indicating higher mitochondrial activity in the presence of pathology compared to no pathology. However, in AsymAD vs. AD, these pathways were downregulated, suggesting that oligodendrocyte mitochondrial activity is selectively lower in the AsymAD brain environment relative to AD.

Identifying small-molecule compounds associated with bioenergetic balance in AsymAD

Using the 258 differentially expressed proteins identified in Fig. 1, we queried DrugBank to identify small-molecule compounds associated with these proteins. NetworkAnalyst analysis revealed 45 druggable proteins corresponding to 16 candidate drugs or small molecules. To assess the biological and pathological relevance of these interactions, we integrated data from the AD Atlas³⁹ (<https://adatlas.org/>) to compile protein-metabolite associations, their relationships to AD-related phenotypes (including cognition, tau pathology, and global pathology burden), and small-molecule interactions into a unified network to identify hub nodes. Among these proteins, the *BDH1*, *IDH3A*, and *ALDH9A1* proteins, identified from convergence across machine learning, snRNA-seq, and proteomics analyses (highlighted in bold and represented in the Venn diagram in Fig. 5 and Supplementary Data 13), were found to interact with dihydroorotate and hydroxylysine. Many proteins in this network were linked to NAD⁺/NADH-dependent redox reactions, highlighting the centrality of mitochondrial redox balance in AsymAD bioenergetic resilience. While NADH itself does not cross the blood-brain barrier, these findings emphasize that modulating brain NAD⁺ metabolism could restore redox equilibrium and support mitochondrial function. These results highlight a

convergent network of proteins, metabolites, pathologies, and druggable targets that collectively point to mitochondrial NAD⁺/NADH metabolism as an important feature of bioenergetic stability in AsymAD (Fig. 5 and Supplementary Data 13).

We further characterized how each of the 16 small molecules interacts with broader protein networks using DrugBank via BioGRID and annotated their pathways through Reactome. NADH exhibited the greatest number of interacting protein partners (144) (Supplementary Fig. 4a) and was enriched primarily in pathways related to the electron transport chain (ETC), complex I biogenesis, and mitochondrial protein degradation (Supplementary Fig. 4b). L-glutamic acid, followed by 69 interacting partners (Supplementary Fig. 4a), is involved in neurotransmission and synaptic signaling. Among all the identified compounds, “metabolism of amino acids and derivatives” emerged as the most significantly targeted pathway, highlighting the importance of metabolic regulation in the context of AsymAD (Supplementary Fig. 4b). Comprehensive pathway and protein-compound interaction details are provided in Supplementary Data 1. This convergence of protein, metabolite, and small-molecule interactions highlights redox modulation as a potential metabolic strategy to sustain neuronal function and counteract Alzheimer’s disease progression.

Discussion

In this systematic study, we leveraged proteomics and transcriptomics from post-mortem brain samples and developed machine learning models to identify important metabolic features that distinguish asymptomatic (AsymAD) individuals from symptomatic AD patients. The main findings of the study are as follows: (i) Proteomics revealed that AsymAD individuals maintain enhanced mitochondrial metabolic functions, including BCAA metabolism (*ECHS1*, *BCKDHB*), the TCA cycle (*IDH2*, *SUCLA2*), fatty acid oxidation (*CPT2*, *ACADSB*), and regulatory functions such as mitochondrial translation (*MRPL37*, *TOMM40*), and structural protein formation (*PDHB*, *FIS1*), and calcium ion transport (*MCU*, *VDAC2*), compared with AD; (ii) the results of mitochondrial OXPHOS complex analysis revealed that most ETC complexes I–IV required for OXPHOS and ATP generation were preserved in AsymAD and Controls but were markedly reduced in AD; (iii) the results of machine learning models identified *MRPL47*, *IDH2*, *CPT2*, *DBT*, *MACROD1*, and *BCAT2* as among the top distinguishing mitochondrial features between AsymAD and AD, with a cross-cohort and brain region statistical analysis revealing the expression of these proteins in AsymAD to be consistent with that in Controls but dysregulated in AD; (iv) single-nucleus RNA-seq analysis across neurons and glial cell types demonstrated enhanced expression of mitochondrial genes related to bioenergetics in AsymAD compared to AD, with key genes such as *NDUFV1*, *BDH1*, *SLC25A37*, *SLC25A5*, *SLC25A25*, and *FTH1* enriched across multiple cell types; and (v) protein–small molecule interaction analysis position mitochondrial NAD⁺/NADH-dependent redox pathways as core determinants of bioenergetic stability in AsymAD.

The mitochondrial oxidation of glucose, fatty acids, and amino acids provides metabolic flexibility, which is essential for maintaining homeostasis³¹. While AsymAD and AD brains exhibit similar levels of amyloid plaques and tau tangles, the absence of cognitive decline and brain atrophy in AsymAD suggests the presence of additional protective mechanisms. Our findings point to mitochondrial protein preservation in AsymAD as a potential driver of this degree of cognitive resilience. While glucose has traditionally been viewed as the brain’s primary energy source, emerging evidence challenges this notion. Recent studies have shown that neurons, particularly at synapses, preferentially utilize fatty acids, supporting increased metabolic and enzymatic activity⁴⁰. These findings underscore the importance of studying mitochondrial adaptability and fuel switching, which may enable the brain to meet changing energy demands and protect against neurodegeneration. In our cross-cohort, multiregional analyses, the TCA cycle and the ETC consistently emerged as the most preserved mitochondrial pathways in AsymAD compared with AD. The TCA cycle is a central metabolic hub that integrates and links lipid metabolism, BCAA

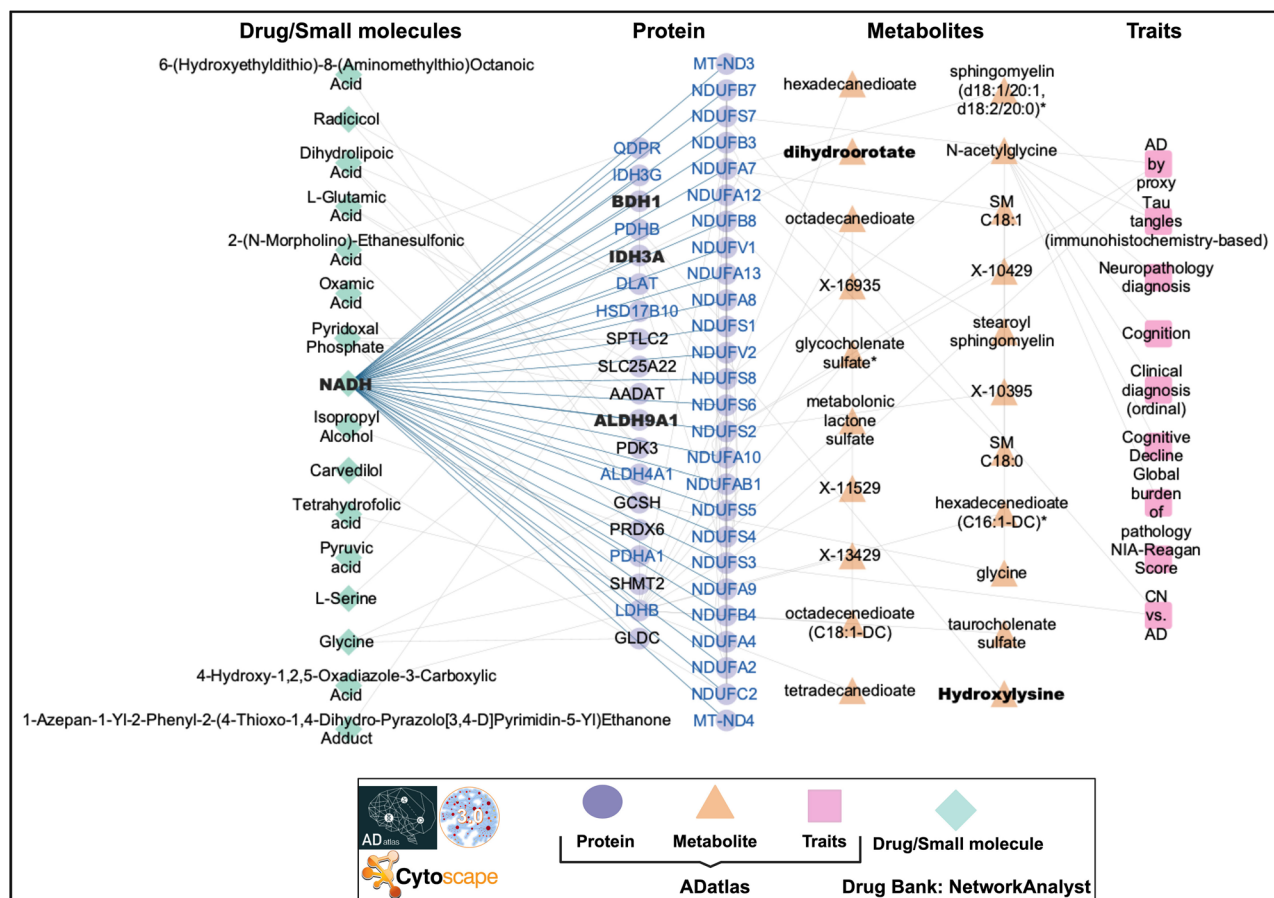


Fig. 5 | Integrated network of proteins, metabolites, AD pathological features, and linked drugs/small molecules. (Created in BioRender. Mandal, P. (2026) <https://BioRender.com/02mccgq>).

metabolism, fatty acid oxidation, and ETC activity, all of which are essential for maintaining mitochondrial membrane integrity and sustaining ATP production (Fig. 6).

Key proteins that are consistently enhanced in AsymAD compared with AD include SUCLA2 (-succinate-CoA Ligase-ADP-Forming Subunit Beta) and IDH2 (isocitrate dehydrogenase 2), which are involved in the TCA cycle; CPT2 (carnitine palmitoyltransferase 2) and ACADSB (acyl-CoA dehydrogenase, short/branched chain), which regulate fatty acid oxidation; and ECHS1 (Enoyl-CoA hydratase, short chain 1), along with BCKDHB (branched-chain ketoacid dehydrogenase E1 subunit beta), both of which are central to BCAA metabolism. Additionally, solute carrier family transporters from the SLC25 family, including SLC25A46 (involved in mitochondrial lipid homeostasis and dynamics) and SLC25A27/UCP4 (essential for neuronal energy homeostasis), were maintained at levels comparable to those of the controls, supporting their role in sustaining mitochondrial function in AsymAD.

In addition to metabolic enzymes, we also observed the preservation of mitochondrial regulatory proteins involved in structural integrity, protein assembly, calcium handling, and translation. These include PDHB (a component of the pyruvate dehydrogenase complex), FIS1 (a fission regulator), and calcium transporters such as MCU (mitochondrial calcium uniporter) and VDAC2 (voltage-dependent anion channel 2). Notably, members of the mitochondrial ribosomal large subunit (MRPL) family, including MRPL37 and MRPL47, were upregulated in AsymAD. These proteins are essential for mitochondrial translation and critical for maintaining OXPHOS-dependent energy production⁴¹. Our study further highlights the importance of investigating mitochondrial biology from a cell type-specific perspective. The majority of mitochondrial functions are governed by nuclear-encoded genes, with more than 1100 mitochondrial genes regulating key aspects of

metabolism⁴². Leveraging single-nucleus RNA sequencing (snRNA-seq), we captured the expression of these nuclear-encoded mitochondrial genes across diverse brain cell types, allowing us to dissect their roles in maintaining ETC activity, OXPHOS, the TCA cycle, lipid metabolism, fatty acid oxidation, and BCAA metabolism in A5mAD vs. AD.

We observed a consistent trend of upregulated mitochondrial energy pathways across most cell types in AsymAD, apart from oligodendrocytes and activated astrocytes (Ast CHI3L1). Among all cell types, ETC function, mitochondrial translation, and lipid metabolism were the most robustly preserved pathways. Some key genes, such as *NDUFV1* (NADH:Ubiquinone Oxidoreductase Core Subunit V1), *BDH1* (3-Hydroxybutyrate Dehydrogenase, Type 1), *FTH1* (Ferritin Heavy Chain 1) and solute carrier family SLC25 genes such as *SLC25A37* (iron homeostasis), *SLC25A5* (involved in OXPHOS by coupling ATP production), and *SLC25A25* (which regulates ATP levels), were enriched across neuronal and glial cell types.

In terms of cell type abundance, AsymADs maintain vulnerable neuronal populations, such as those of inhibitory PVAlB and SST neurons, which are typically reduced in AD³⁴. In contrast, microglia and oligodendrocytes are more abundant in AD. The increased mitochondrial activity and expansion of the oligodendrocyte population in AD may reflect a compensatory response to heightened myelination demand in a disease environment characterized by hypomyelination.

We leveraged the AD Atlas and DrugBank to identify the pathological associations and small molecules linked to the proteins differentially expressed between AsymAD and AD. NADH emerged as the most highly connected small molecule across 16 candidate protein targets. Early clinical trials using NADH in small human cohorts have shown mixed cognitive outcomes^{43–45}, which could be attributed to its poor stability and inability to cross the blood-brain barrier^{46,47}. Current strategies have focused on NAD⁺

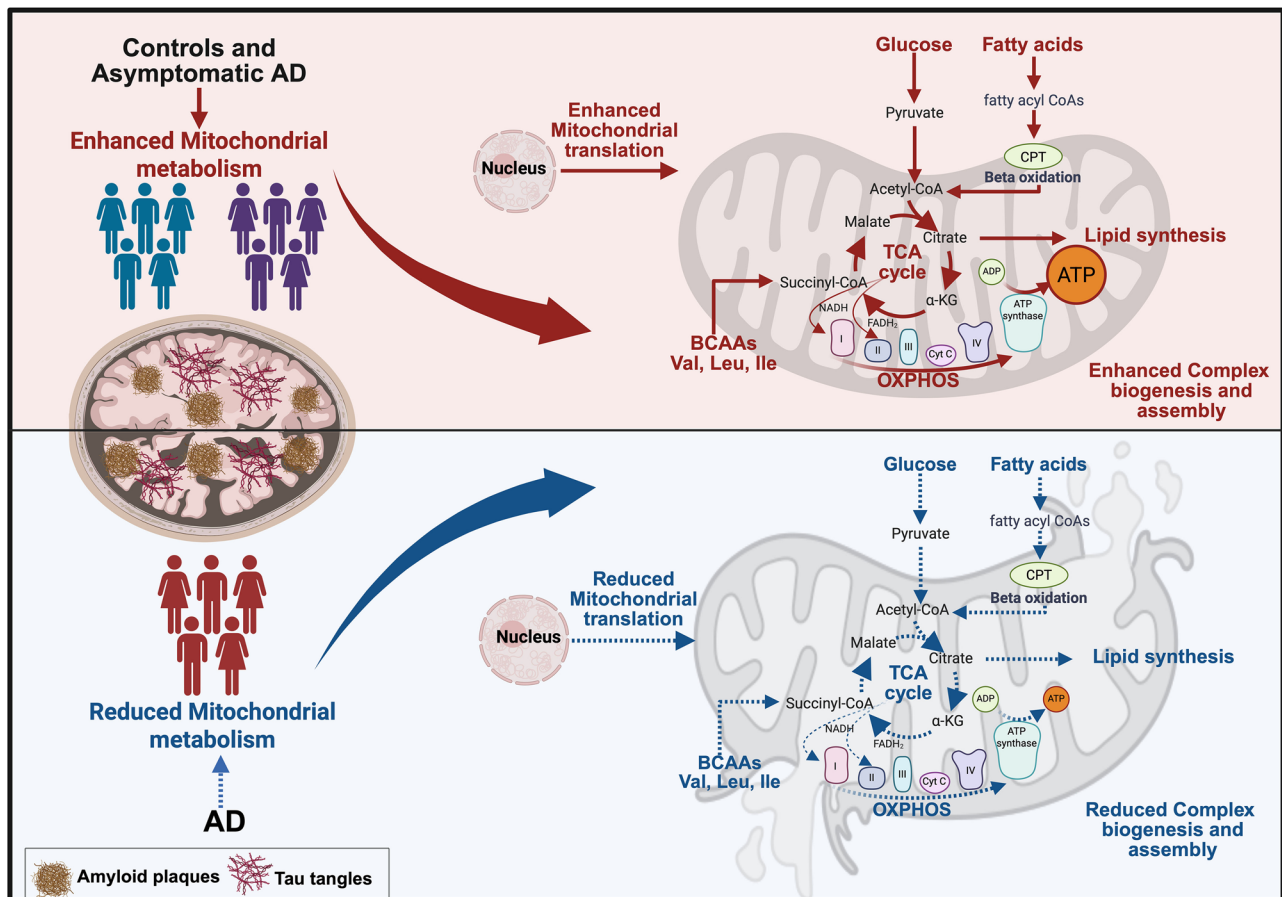


Fig. 6 | Overview of upregulated mitochondrial regulatory and metabolic pathways in AsymAD compared with AD. Red bold arrows highlight enhanced mitochondrial bioenergetic activity across glucose, fatty acid, and branched-chain amino

acid (BCAA) pathways, whereas Alzheimer's disease (AD) is characterized by massive downregulation across all mitochondrial bioenergetic pathways. (Created in BioRender. Mandal, P. (2026) <https://BioRender.com/i1ukvp3>).

(the oxidized form of NADH) precursors, such as NR (nicotinamide riboside) and NMN (nicotinamide mononucleotide), which have been shown to increase NAD⁺ levels, restore mitochondrial function, and attenuate A β /tau pathology in multiple AD mouse and *C. elegans* models⁴⁸. An ongoing clinical trial is now determining the optimal NR dose for human AD patients (ClinicalTrials.gov ID: NCT05617508). Given that the NAD⁺ balance regulates key metabolic and neuroprotective pathways⁴⁹, our finding that NADH-linked targets are central in AsymAD individuals suggests that early therapeutic intervention to sustain mitochondrial bioenergetics via NAD⁺ metabolism may help prevent progression to symptomatic AD, offering a mechanistically distinct alternative to anti-amyloid therapies, which are often associated with adverse effects 8–12. These results highlight the need for more advanced NAD⁺-boosting therapeutics to move toward clinical translation.

Mitochondria are the only organelles in a cell whose own genome can encode unique small peptides known as mitochondria-derived micro peptides, including SHLP2, MOTS-c, and SHMOOSE, that have been implicated in protection against neurodegenerative diseases (AD/PD)^{50–53}. Among centenarians living beyond 100 years, those carrying APOE4 and who also possessed the mitochondria-derived peptide humanin P3S performed better on cognitive tests than those lacking this variant did. In mouse models of AD with humanized APOE4, treatment with P3S led to a marked reduction in amyloid- β deposition and an increase in the expression of OXPHOS genes⁵⁴.

In this study, mild cognitive impairment (MCI) is defined by Mini-Mental State Examination (MMSE) scores <24, whereas both the control and AsymAD groups have MMSE scores $\geq$ 24 and therefore are unaffected by this stratification. Because our primary objective was to identify biological

features distinguishing cognitively resilient AsymAD individuals from symptomatic AD patients overall, we did not further subdivide AD patients by disease stage; however, future studies stratifying AD patients into MCI, moderate, and severe stages will be important to determine how mitochondrial biology evolves throughout disease progression.

This study has a few limitations. First, analyses were performed on post-mortem brain tissue, which, despite the corrected post-mortem interval, could show variability in mitochondrial protein stability and activity at death. Second, we used a cross-sectional study design, which allowed us to use samples from multiple cohorts and integrate them for analysis. Therefore, we cannot pinpoint whether the observed mitochondrial adaptations drive cognitive resilience in AsymAD or represent compensatory responses to disease pathology. Because our analyses are based on cross-sectional postmortem data representing a single end-stage time point, this study was not designed to establish causal relationships. In future studies incorporating longitudinal data, experimental data from functional assays, or patient-derived cell lines, we will be able to confirm whether the identified proteins and pathways enhance mitochondrial functions, leading to resilience. Together, these findings, along with those of prior work, highlight mitochondria as critical organelles capable of influencing neuronal vulnerability and suggest that individuals with AD pathology remain asymptomatic or progress to clinical disease.

Methods

Data acquisition

Proteomics and snRNA sequencing data were obtained from the AD Knowledge portal of the Synapse database (<https://www.synapse.org/>). Data from four brain regions, namely, the dorsolateral prefrontal cortex (DLPFC;

BA9), were obtained from a consensus from 2 cohorts: ROSMAP (Religious Orders Study (ROS) involving older Catholic nuns, priests, and brothers across the United States; Memory and Aging Project (MAP) involving older people across the Chicago metropolitan area) and Banner (Banner Sun Health Research Institute, Arizona); the parahippocampal gyrus (PHG; BA36), which was obtained from Mt Sinai (Mount Sinai School of Medicine Brain Bank, New York); and the premotor cortex (BA6) and temporal cortex (BA37), which were obtained from ROSMAP. For the snRNA-seq analysis, we leveraged postmortem brain data from the ROSMAP cohort for all 6 cell types: excitatory, inhibitory neurons, astrocytes, microglia, OPCs, and oligodendrocytes. Synapse IDs are provided in the data availability section.

Individual identification

We grouped the samples into control, AD, and AsymAD classes using the following rubric published by Johnson et al.¹⁴. Samples with a CERAD score of 0–1 and a Braak score of 0–3 without dementia at the last evaluation were defined as controls (if the Braak score was equal to 3, then the CERAD score was equal to 0); cases with a CERAD score of 1–3 and a Braak score of 3–6 without dementia at the last evaluation were defined as AsymAD; and cases with a CERAD score of 2–3 and a Braak score of 3–6 with dementia at the last evaluation were defined as AD. Individuals with dementia were defined on the basis of their MMSE scores, specifically MMSE scores < 24 or CDR scores ≥ 1. The proteomics data consisted of a total of 488 samples from BA9 (106 control, 182 AD, and 200 AsymAD), BA36 (45 control, 92 AD, and 13 AsymAD), BA6 (25 control, 33 AD, and 53 AsymAD), and BA37 (25 control, 34 AD, and 52 AsymAD). For the snRNA-seq analysis, we extracted a total of 347 samples, consisting of 100 controls, 126 AD patients, and 121 AsymAD patients.

Mitocarta3 dataset

We extracted information from the MitoCarta3.0 dataset⁵⁵, a comprehensive, curated resource that catalogs genes associated with mitochondrial structure, function, and biogenesis across various tissues and species. It includes more than 1100 mitochondria-associated genes identified through integrative approaches such as proteomics, mitochondrial targeting sequence prediction, and experimental validation. MitoCarta3.0 also provides tissue-specific expression profiles, providing a more in-depth understanding of mitochondrial dynamics in different physiological and pathological contexts.

Differentially expressed protein analysis

To identify the differentially expressed proteins in the dataset, we employed two approaches:

(i) Without cell type correction:

Differential expression was assessed across three comparisons, AsymAD vs. AD, AD vs. Control, and AsymAD vs. Control, using two approaches. Proteomic data from the BA9 DLPFC were preprocessed and corrected for the postmortem interval (PMI), age at death, and sex, with outliers removed and values log-normalized using TAMPOR¹⁴. First, ANOVA followed by Tukey's HSD test was applied to identify differentially expressed proteins. Second, the limma package in R (4.4.1) was used with Benjamini–Hochberg (BH) correction. In both approaches, proteins with an adjusted *p* value < 0.05 were considered significant in our analysis. Mitochondrial proteins were identified using the MitoCarta 3.0 database.

(ii) With cell type correction:

To account for cellular composition, cell type marker proteins for neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells were obtained from a published reference dataset¹⁴. The expression matrix was aggregated by protein name to generate a protein × individual matrix of log-normalized expression values. For each sample, the mean expression of marker proteins for each cell type was calculated, yielding a matrix of cell type marker scores (rows = individuals, columns = cell types). These marker scores were then included as covariates in the limma design matrix prior to

model fitting and BH correction.

$$\text{design} = \text{model.matrix}(\sim \text{diagnosis} + \text{Astrocytes} + \text{Microglia} + \text{Neuron} + \text{Oligodendrocytes} + \text{Endothelia}, \text{data} = \text{cell_type_marker_scores})$$

Proteins with an adjusted *p* value < 0.05 were considered significantly differentially expressed. Mitochondrial proteins were identified using the MitoCarta 3.0 database, which is consistent with prior analyses.

Identifying features using machine learning models

Significant differentially expressed (DE) proteins identified from the proteomic analysis of the BA9 region were used as inputs for machine learning. Among the 258 DE proteins that distinguished AsymAD from AD, genes whose expression data were missing were imputed using k-nearest neighbor (KNN) imputation. Four classifiers, namely, random forest⁵⁶, gradient boosting⁵⁷, recursive feature elimination (RFE)⁵⁸, and least absolute shrinkage and selection (LASSO-CV)⁵⁹, were applied to extract the most informative protein features. Proteins prioritized by these classifiers were subsequently used to train a logistic regression model to classify individuals as having AD or AsymAD on the basis of protein expression. For RFE, the optimal number of features was determined by using the RFECV function and tested by plotting the mean cross-validated area under the curve (AUC) against the number of features. The best performance was achieved with 43 features, which were subsequently used to train the model. To avoid overfitting, 10-fold cross-validation was performed across all the classifier models. Finally, SHAP (SHapley Additive exPlanations) analysis was conducted to interpret model predictions and identify the top 20 proteins most strongly contributing to discrimination between AD and AsymAD.

Clubbing of cell types in single-nucleus RNA sequencing data

All eight cell-type objects were normalized and preprocessed by Mathys et al.³⁴. The original cell-type annotations from the published manuscript³⁴ were retained and reformatted into more concise groupings in the table below, following the conventions outlined in the subsequent publication⁶⁰. Mic MKI67, T cells, and CAMs were excluded from further analysis because the number of cells precluding meaningful DEGs/pathway information was limited. Table 1 provides information on the cell clusters used for analysis.

Differentially expressed gene analysis

Differential gene expression analysis was performed using the MAST package⁶¹ in R (4.4.1). A log fold-change threshold of 0.1 was applied, with the minimum expression cutoff set at *min.pct* = 0.1 (i.e., genes expressed in at least 10% of cells in either group). Postmortem interval (PMI), age at death, and sex were included as latent variables to account for potential confounding effects. Genes with a false discovery rate (FDR)-adjusted *p* value < 0.01 were considered significantly differentially expressed. Mitochondrial genes were identified using the MitoCarta 3.0 database, which is consistent with previous analyses.

Mapping of mitochondrial complexes

Mitochondrial complexes I–V were mapped using the MitoCarta 3.0 database⁵⁵. For the proteomics data, both nuclear- and mitochondrial DNA-encoded (MT) genes were retained for all the complexes. For the snRNA-seq data, only nuclear-encoded mitochondrial complex genes were included, as nuclear genes were exclusively analyzed.

Pathway enrichment analysis

We performed pathway enrichment analysis using the differentially expressed genes and proteins identified from our analysis as inputs and the Enrichr database (<https://maayanlab.cloud/Enrichr/>). We used KEGG Human 2021, Reactome Pathways 2024, and WikiPathways Human 2024 for all analyses. Significantly enriched pathways were identified on the basis of adjusted *p* values (<0.05). The top pathways were ranked using the combined score, which integrates both statistical significance and the magnitude of enrichment, allowing comparison across multiple databases.

Table 1 | Cell cluster information used for study design and analysis

Cell type objects	Original names (Matthys et al.)	New clustered names
Excitatory set 1	Exc L2-3 CBLN2 LINC02306	Exc superficial neuron
Excitatory set 2	Exc L3-4 RORB CUX2 Exc L4-5 RORB GABRG1 Exc L4-5 RORB IL1RAPL2 Exc L3-5 RORB PLCH1	Exc mid-layer neurons
Excitatory set 3	Exc NRGN Exc RELN CHD7 Exc L5-6 RORB LINC02196 Exc L6 THEMIS NFIA Exc L6b Exc L6 CT Exc L5/6 IT Car3 Exc L5 ET Exc L5/6 NP	Exc deep-layer neurons
Inhibitory	Inh PVALB CA8 (Chandelier) Inh PVALB HTR4 Inh GPC5 RIT2 Inh PVALB SULF1 Inh L5-6 PVALB STON2	Inh PVALB
	Inh RYR3 TSHZ2 Inh VIP CLSTN2 Inh VIP ABI3BP Inh ALCAM TRPM3 Inh PTPRK FAM19A1 Inh VIP TSHZ2 Inh SORCS1 TTN Inh VIP THSD7B Inh SGCD PDE3A	Inh VIP
	Inh CUX2 MSR1 Inh L3-5 SST MAFB Inh ENOX2 SPHKAP Inh L5-6 SST TH Inh L6 SST NPY Inh FBN2 EPB41L4A	Inh SST
	Inh LAMP5 RELN Inh LAMP5 NRG1 (Rosehip) Inh L1-6 LAMP5	Inh LAMP5
	Inh L1 PAX6 CA4 Inh L1-2 PAX6 SCGN	Inh PAX6
Astrocytes	Ast CHI3L1 Ast DPP10 Ast GRM3	Ast CHI3L1 Ast DPP10 Ast GRM3
Microglia	Mic P2RY12 Mic TPT1 Mic MKI67	Mic P2RY12 Mic TPT1 Mic MKI67
OPC	OPC	OPC
Oligodendrocytes	Oligodendrocytes	Oligodendrocytes

Protein-small molecule interaction analysis

To identify small molecules and drugs linked to the differentially expressed proteins between AsymAD and AD, we queried the DrugBank database using NetworkAnalyst to construct a protein-drug interaction network. To further explore the metabolite and pathological associations associated with the proteins, we leveraged the AD Atlas platform, which integrates metabolite and AD pathological trait data. A comprehensive network merging proteins, drugs, metabolites, and AD-related traits was visualized using Cytoscape. For downstream pathway analysis, we used BioGRID to identify protein interactors of the identified drugs/small molecules, followed by Reactome pathway enrichment analysis using R (version 4.4.1) to highlight key pathways associated with each small molecule.

Statistics and reproducibility

Fisher’s exact test was applied to evaluate cell type abundance bias in the differentially expressed protein sets, both before and after cell type correction. Significant proteins were defined as those with an adjusted *p* value < 0.05, while the background set included all the proteins. For each cell type, a

2 × 2 contingency matrix was constructed consisting of (i) marker proteins in the significant set, (ii) marker proteins not in the significant set, (iii) non-marker proteins in the significant set, and (iv) non-marker proteins not in the significant set. Fisher’s test *p* values were generated for each cell type across all three comparisons (AsymAD vs. AD, AD vs. Control, AsymAD vs. Control) and subsequently corrected for multiple hypothesis testing using the Benjamini–Hochberg (BH) procedure. Violin plots were generated for all the brain regions (BA9, BA6, BA36, and BA37) to compare protein expression across the control, AD, and AsymAD groups using *t* tests. The results were visualized using ggpubr() and ggplot2() functions in R (4.4.1).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The proteomic and snRNA sequencing data used in the study can be obtained from the AD Knowledge portal of the Synapse database (<https://www.synapse.org/Home:x>). For proteomics data, 4 brain regions were utilized from proteomics, namely, the dorsolateral prefrontal cortex (DLPFC; BA9) data and metadata, which were obtained from a consensus from 2 cohorts: ROSMAP (Religious Orders Study (ROS) involving older Catholic nuns, priests, and brothers across the United States; Memory and Aging Project (MAP) involving older people across the Chicago metropolitan area) and Banner (Banner Sun Health Research Institute, Arizona) (syn25006659 and syn25006658), and the para gyrus hippocampus (PHG; BA36), which was obtained from Mt Sinai (Mount Sinai School of Medicine Brain Bank, New York; syn25006652 and syn25006648), and the premotor cortex (BA6) and temporal cortex (BA37), which were obtained from ROSMAP (syn25006866 and syn25006867; syn25006835, respectively). For snRNA-seq analysis, we leveraged postmortem brain data from the ROSMAP cohort for all 6 cell types-excitatory, inhibitory neurons, astrocytes, microglia, OPCs and oligodendrocytes (syn52293433 and syn3157322). Numerical source data for all graphs in the manuscript can be found in Supplementary Data file.

Code availability

Codes used for analysis are available in the following GitHub repository: https://github.com/BaloniLab/Asymptomatic_vs_symptomatic_Alzheimers_disease (Nov 2025). Codes can also be accessed at Mandal & Baloni. Mitochondrial bioenergetic signatures differentiate asymptomatic from symptomatic Alzheimer’s disease. Zenodo. <https://doi.org/10.5281/zenodo.19633113>⁶².

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Author contributions

P.M.: Data Curation, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing. P.B.: Resources, Methodology, Conceptualization, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Supervision. E.T.: Resources, Investigation, Writing - Review & Editing. M.A.: Investigation, Writing - Review & Editing. R.K.D.: Resources, Investigation, Writing - Review & Editing.

Competing interests

E.T. is a co-inventor of four U.S. Patents, 11,161,814; 10,336,700; 10,774,045; and 12,017,992, which are relevant to the development of mitochondria-targeted therapeutics. She and the Mayo Clinic own the IP on this technology. R.K.D. is an inventor on a series of patents on the use of metabolomics for the diagnosis and treatment of CNS diseases and holds equity in Metabolon Inc., Chymia, and Metabosensor. M.A. is a co-inventor (through Duke University/Helmholtz Zentrum München) on patents on applications of metabolomics in diseases of the central nervous system; M.A. holds equity in Chymia LLC and IP in PsyProtix and Atai that are exploring the potential for therapeutic applications targeting mitochondrial metabolism in depression. The remaining authors declare that they have no competing interests.

Additional information

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