

RESEARCH

Open Access



Cell type-specific potential pathogenic genes and functional pathways in Alzheimer's Disease

Xiao-Lan Wang^{1,2*} and Lianjian Li^{3,4}

Abstract

Background: Alzheimer's disease (AD) is a pervasive age-related and highly heritable neurodegenerative disorder but has no effective therapy. The complex cellular microenvironment in the AD brain impedes our understanding of pathogenesis. Thus, a comprehensive investigation of cell type-specific responses in AD is crucial to provide precise molecular and cellular targets for therapeutic development.

Methods: Here, we integrated analyzed 4,441 differentially expressed genes (DEGs) that were identified from 263,370 single-cells in cortex samples by single-nucleus RNA sequencing (snRNA-seq) between 42 AD-pathology subjects and 39 normal controls within 3 studies. DEGs were analyzed in microglia, astrocytes, oligodendrocytes, excitatory neurons, inhibitory neurons, and endothelial cells, respectively. In each cell type, we identified both common DEGs which were observed in all 3 studies, and overlapping DEGs which have been seen in at least 2 studies. Firstly, we showed the common DEGs expression and explained the biological functions by comparing with existing literature or multil-omics signaling pathways knowledgebase. We then determined the significant modules and hub genes, and explored the biological processes using the overlapping DEGs. Finally, we identified the common and distinct dysregulated pathways using overall DEGs and overlapping DEGs in a cell type-specific manner.

Results: Up-regulated LINGO1 has been seen in both oligodendrocytes and excitatory neurons across 3 studies. Interestingly, genes enriched in the mitochondrial module were up-regulated across all cell types, which indicates mitochondrial dysfunction in the AD brain. The estrogen signaling pathway seems to be the most common pathway that is disrupted in AD.

Conclusion: Together, these analyses provide detailed information of cell type-specific and overall transcriptional changes and pathways underlying the human AD-pathology. These findings may provide important insights for drug development to tackle this disease.

Keywords: Alzheimer's disease, cell type-specific, transcriptomic, mitochondrial dysfunction, estrogen signaling pathway

*Correspondence: xiao-lan.wang@etu.unistra.fr

¹ Department of Nephrology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
Full list of author information is available at the end of the article



© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Background

Alzheimer's disease (AD) is the most common form of dementia characterized by the accumulation of extracellular amyloid- β (A β) and neurofibrillary tangles, and progressive synaptic and neuronal dysfunction and degeneration [1–3]. However, AD pathogenesis is not restricted to neurons and increasing evidence suggests that multiple cell type interactions in the brain promote AD development [4–8]. Immune response-induced chronic neuroinflammation has been considered as a critical component in the progression of AD, but anti-inflammatory drug candidates still failed in clinical trials [9–11]. The complex interplay of cells in the brain may limit our understanding of the pathological mechanisms of AD. Thus, it's important to clearly understand how different cell types contribute to AD progression and outcome.

Microglia serve as the brain's resident macrophages with immune-modulating and phagocytic capability. Compelling evidence revealed an extended microglial gene network in AD [12–14] and has firmly linked microglia to A β deposition and synaptic loss [15, 16]. Microglia in AD, a double-edged sword, protect against the initiation of AD, and their activation-induced inflammation also leads to AD progression [8, 17, 18]. Astrocytes provide trophic and metabolic support to neurons in the brain, however, microglia secreted inflammatory cytokines activate the neurotoxic A1 astrocytes, which cooperate with microglia to mediate complement-dependent neuronal loss [6, 19]. Activated astrocytes have been found from both postmortem AD patients and animal models [4, 20]. Moreover, a recent *in vivo* study showed that astrocytes are also involved in the engulfment of apoptotic neurons to maintain brain homeostasis [21], which may play an important role in the AD brain.

Genome-wide associated studies have indicated that most of the risk factors for AD are expressed by microglia (such as APOE and TREM2) and astrocytes (such as CLU), which are associated with immune response, as well as oligodendrocytes (such as BIN1) [13, 20, 22]. Oligodendrocytes produce the myelin that ensheathes axons, provide trophic and metabolic support to neurons, and regulate neuronal connectivity. Reduced oligodendrocytes and myelin have been observed consistently in AD, which may be caused by A β toxicity and increased inflammation and oxidative stress in the brain [5, 23, 24]. Moreover, in most AD patients, A β deposition has been seen around perivascular, which leads to endothelial cell dysfunction and death, increases the permeability of the blood-brain barrier (BBB) and neuroinflammation, and further contributes to AD progression [7, 25, 26].

Therefore, it's critical to comprehensively analyze the transcriptomic changes in different cell types in the AD brain. Here, we integrated analyzed the differentially expressed genes (DEGs) identified from cortex samples of AD individuals and normal controls in 3 single-nucleus RNA sequencing (snRNA-seq) studies (Mathys et al, Grubman et al, and Lau et al) [27–29]. Our comprehensive molecular profiling of the human AD brain may figure out the cell type-specific and overall pathogenic genes and disease-associated signaling pathways, which may provide therapeutic targets for AD.

Methods

Data Source and identification of overlapping DEGs

Transcriptome results were from age and sex-matched AD-pathology individuals and normal controls. In the study of Mathys et al, 24 prefrontal cortex samples were used in each group and statistical enrichment for sets of marker genes [30, 31] was used for the identification of cell types. 1,031 DEGs were identified from 70,634 single-cells transcriptomes in 6 cell types (FDR-corrected $P < 0.01$ in two-sided Wilcoxon-rank-sum test, absolute log fold change > 0.25 , and FDR-corrected $P < 0.05$ in Poisson mixed model). Gene expression results of Mathys et al were obtained from Supplementary Material 4. To be noticed, mitochondrial genes have been removed in the study of Mathys et al. In the study of Lau et al, 21 prefrontal cortex samples (AD = 12, normal controls = 9) were included for transcriptome analysis, and Seurat was used to identify the cell types. 2,190 DEGs were identified from 169,496 nuclei in 6 cell types (adjusted $P < 0.1$, absolute log₂ fold change ≥ 0.1). DEGs results were obtained from Supplementary file 4. In the study of Grubman et al, BRETIGEA [32] was used to identify the cell types. Gene expression results were downloaded from <http://adsn.ddnetbio.com>, and FDR < 0.01 , absolute log fold change > 0.5 were considered as statistically significant differences between AD and normal controls ($n = 6$ entorhinal cortex samples per group). 1,726 DEGs were defined from 13,214 nuclei in 8 classified cell types. DEGs that were used in this study have been added in the [Supplemental Files](#).

Venn Diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was used to calculate and draw a venn map for each cell type, and overlapping DEGs were retained for further analysis. The log₂ fold change of overlapping DEGs was used to generate Heatmap using R software (version 3.4.210).

Construction of PPI network and module analysis

Overlapping DEGs was used to construct protein-protein interaction (PPI) network [33, 34] using STRING analysis

(<http://string-db.org> Version:11.0) and was further analyzed in Cytoscape software (3.7.2 version) by both cytoHubba and Molecular Complex Detection (MCODE) plugins in Cytoscape to select significant hub genes and modules from the PPI network [35, 36] in each cell type. Hub genes were ranked by MCC method in cytoHubba and presented by nodes with different colors in figures (red to yellow means ranking from high essential to essential). Edges mean interactions, reactions, or regulations among nodes in the network. Modules were identified by MCODE and presented with a circle layout. The selection criteria of MCODE: degree cutoff = 2, node score cutoff = 0.2, and k-score = 2, max. Depth = 100.

Signaling pathways analysis

The Signaling Pathway Project (<https://www.signalingpathways.org/index.jsf>), a multi-omics knowledgebase for cellular signaling pathway [37, 38], was used to evaluate the cellular signaling pathways that single differentially expressed gene involved in. Human transcriptomics datasets were chosen, and FDR significant cut-off = 1E-02.

GO biological process and KEGG pathways enrichment analysis

The annotation function of GO biological process of overlapping DEGs was carried out using the online DAVID Bioinformatics database 6.8 [39], which is a database resource for understanding high-level functions and utilities of the genes. KEGG pathway enrichment analysis of all DEGs and overlapping DEGs [40] was performed in the online DAVID Bioinformatics database 6.8. *P*-value < 0.05 was considered as significant differences for both GO analysis and KEGG pathway enrichment analysis. *P*-value, fold enrichment, and gene counts in each term were used to create a Bubble chart in R software (version 3.4.210).

Results

Disrupted immune response, energy supply, and oxidative stress, as well as reduced protein degradation in microglia

Human genetic studies pointed out a key role of microglia in the pathogenesis of AD [13]. To identify the potential pathogenic genes and cellular processes in microglia, we integrated analyzed the DEGs in 3 studies. We

observed 5 common DEGs, and only 2 genes showed the concordant change in microglial cells (Fig. 1A and B, and Table 1). Concordant up-regulated PTPRG and MYO1E, and discordant SPP1, VSIG4, and RNF149 are involved in inflammation [41–45]. The further investigation of these common genes in multi-omics signaling pathways knowledgebase showed that except RNF149, the other genes are also involved in the estrogen signaling pathway in human tissue [46, 47]. These findings indicate that the dysregulated immune response and the involvement of the estrogen signaling pathway may be the common characteristic of microglia in AD.

There were another 61 overlapping DEGs among 3 studies, including up-regulated APOE and APOC1 (Fig. 1A and B), the risk factors for developing AD [48, 49], suggesting the importance of these DEGs in AD-pathology progression. We then identified the important modules and hub genes among the total 66 overlapping DEGs. The top-ranked genes (up-regulated MT-ND1–4, MT-CO2, MT-CO3, MT-ATP6, and MT-CYB) in the PPI network were enriched in the mitochondrial module (8 nodes and 28 edges) from both Grubman et al and Lau et al studies (Fig. 1B and C). The changed sugar metabolism and mitochondrial function have also been observed in a large-scale proteomic analysis of AD brain, especially in glial cells [50]. GO enrichment analysis showed that the genes-RPS19, RPS28, RPL35, RPS27A, RPLP1, and RPS6 that involved in the second module (8 nodes and 27 edges) were enriched in SRP-dependent cotranslational protein targeting to membrane, mRNA catabolic process, translational initiation, and ribosomal processes (Fig. 1B–D). While hub genes-UBC and RPS27A, which involved in protein ubiquitination to eliminate the toxic protein aggregation, including ribosomes, were down-regulated in microglia (Fig. 1B and C) [51–54]. Moreover, UBC and RPS27A were also involved in negative regulation of apoptotic and transcription processes, TGF beta signaling pathway, inflammatory signaling pathways, and activation of MAPK activity in AD (Fig. 1D). DIRC3, a lncRNA, was up-regulated, which may interact with peroxisome proliferator-activated receptors (PPARs) according to the multi-omics signaling pathways knowledgebase (Fig. 1B and C).

Microglial cells in AD were also enriched for genes involved in the regulation of neuron death,

(See figure on next page.)

Fig. 1 Microglia. Overlapping differentially expressed genes (DEGs) and pathways in microglia from 3 studies. **A** Venn diagram showing overlapping DEGs detected in microglia. **B** List of overlapping DEGs at least in 2 studies. Heatmap colored by gene expression in each study (red: up-regulated, blue: down-regulated). **C** PPI network of overlapping DEGs. Significant modules are indicated in circle layout. Significance of hub genes is indicated by color; red to yellow means significance from high to low. **D** Top 20 significant gene ontology (GO) terms for overlapping DEGs. **E** Significant KEGG pathways of DEGs in microglia. Red circles show pathways generated using overlapping DEGs. In bubble charts, significance is indicated by color, and the gene number of DEGs is indicated by the size of a dot

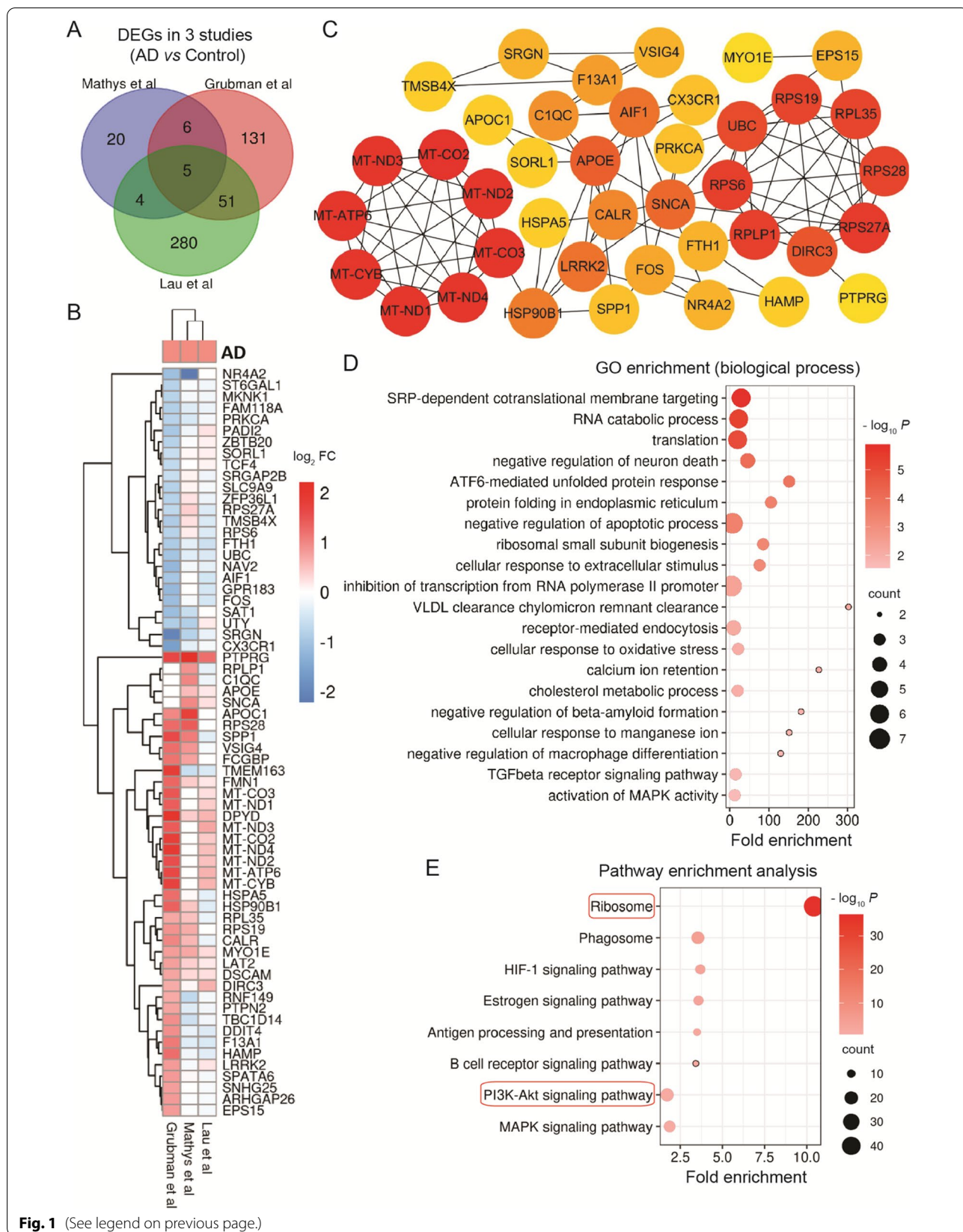


Table 1 Common DEGs in 3 studies (AD versus Control)

Studies Genes	Mathys et al	Grubman et al	Lau et al	Studies Genes	Mathys et al	Grubman et al	Lau et al
Microglia (5)				Oligodendrocytes (10)			
<i>PTPRG</i>	↑	↑	↑	<i>LINGO1</i>	↑	↑	↑
<i>MYO1E</i>	↑	↑	↑	<i>MID1IP1</i>	↑	↑	↑
<i>SPP1</i>	↑	↑	↓	<i>SLC38A2</i>	↑	↑	↑
<i>RNF149</i>	↓	↑	↓	<i>CCP110</i>	↑	↑	↑
<i>VSIG4</i>	↑	↑	↓	<i>KCNH8</i>	↓	↓	↓
Excitatory neurons (6)				<i>CARNS1</i>	↓	↓	↓
<i>DPP7</i>	↑	↑	↑	<i>LDLRAD4</i>	↓	↓	↓
<i>LINGO1</i>	↑	↑	↑	<i>GPM6A</i>	↓	↓	↓
<i>CHD5</i>	↑	↑	↑	<i>SLC5A11</i>	↓	↑	↑
<i>PER1</i>	↑	↑	↑	<i>RPS27</i>	↑	↑	↓
<i>TSPAN7</i>	↓	↓	↓	Inhibitory neurons (0) ↓			
<i>SLC26A3</i>	↑	↑	↓	Endothelial cells (0)			
Astrocytes (20)							
<i>PLXNB1</i>	↑	↑	↑	<i>ITGB8</i>	↓	↓	↓
<i>AHNAK</i>	↑	↑	↑	<i>PREX2</i>	↓	↓	↓
<i>CSRP1</i>	↑	↑	↑	<i>SLCO1C1</i>	↓	↓	↓
<i>SLC38A2</i>	↑	↑	↑	<i>GRM3</i>	↓	↓	↓
<i>VCAN</i>	↑	↑	↑	<i>DIO2</i>	↓	↓	↓
<i>RHPN1</i>	↑	↑	↑	<i>CIRBP</i>	↓	↓	↓
<i>MRAS</i>	↑	↑	↑	<i>CABLES1</i>	↓	↓	↓
<i>PLEC</i>	↑	↑	↑	<i>GRIA2</i>	↓	↓	↓
<i>PLEKHA5</i>	↑	↑	↑	<i>RALGAPA2</i>	↓	↓	↓
<i>SYNM</i>	↑	↑	↑	<i>ITGB4</i>	↑	↑	↓

Red color means concordant up-regulated genes and green color means concordant down-regulated genes in 3 studies

lipid-related clearance, response to oxidative stress (such as up-regulated LRRK2, APOE, and SNCA), response to extracellular stimulus, microglia activation (such as down-regulated AIF1), unfolded protein response, protein folding in endoplasmic reticulum (ER), endocytosis, and calcium ion retention (such as

HSP90B1 and CALR) (Fig.1B-D). The KEGG pathway enrichment analysis of the DEGs showed that ribosome, phagosome, antigen processing and presentation, HIF-1 signaling pathway, estrogen signaling pathway, B cell receptor signaling pathway, PI3K-Akt signaling pathway, and MAPK signaling pathway

were enriched in microglial cells (Fig.1E). Especially the ribosome and PI3K-Akt signaling pathway were enriched in at least 2 studies (Fig.1E).

Disturbed cellular homeostasis in astrocytes and deficit of neuronal support

Astrocytes support neuronal functions, including recycling of neural transmitters, modulation of synaptic transmission, stimulation of synaptogenesis, regulation of ion concentration in the extracellular space, and maintenance of BBB. Here, we observed 20 common DEGs in 3 studies, and 19 genes (11 up-regulated and 8 down-regulated) showed concerted changes in astrocytes (Fig.2A and B, and Table 1). These genes were enriched for glutamate receptors (down-regulated GRIA2 and GRM3), glutamate secretion (up-regulated SLC38A2), thyroid hormone perturbation (down-regulated SLCO1C1 and DIO2) [55, 56], extracellular matrix organization (ITGB8, ITGB4, and VCAN), circadian clock regulation (down-regulated CIRBP) [57], permeability of BBB (up-regulated PLEKHA5) [58], and cell proliferation (up-regulated RHPN1) [59], indicating the dysfunction of astrocytes and neurons in AD. While we also observed the altered PLXNB1, MRAS, CSRP1, AHNAK, SYNM, PREX2, RALGAPA2, CABLES1, and PLEC expression, which has been described in previous human AD studies (Fig.2B) [60–67].

Overlapping DEGs among 3 studies showed high concerted gene expression in astrocytes (Fig.2B). In overlapping DEGs constructed PPI network, the most significantly changed genes were enriched in mitochondrial module, as we observed in microglia. GO enrichment showed that the hub genes (SLC6A1, SLC1A2, SLC1A3, NTRK2, GRIA2, GRM3, PLP1, SPARCL1, GPM6A, and NRXN1), which were mostly involved in the second module, were enriched for processes related to learning, glutamate secretion, signal transduction, chemical synaptic transmission, neuron cell-cell adhesion, neurotransmitter secretion, synaptic assembly (Fig.2C and D). These alterations indicate the disrupted neuronal signal transduction in AD. In the third module, genes-DNAJB1, HSP90AA1, HSPA1B, PTGES3, FKBP4, HSPA1A, HSPB1, and CST3, were enriched for negative regulation of inclusion body assembly, cellular response to heat, regulation of protein ubiquitination, protein refolding, unfolded protein, and negative regulation of cell death (Fig.2C and D). In addition, some hub genes were enriched in extracellular matrix organization (VCAN and GFAP), activation of insulin secretion and glutamate catabolic process (GLUL), and negative regulation of neuron differentiation (SOX2) (Fig.2C and D). To be noticed that APOE which involved in retinoid metabolic process, triglyceride homeostasis, and NMDA

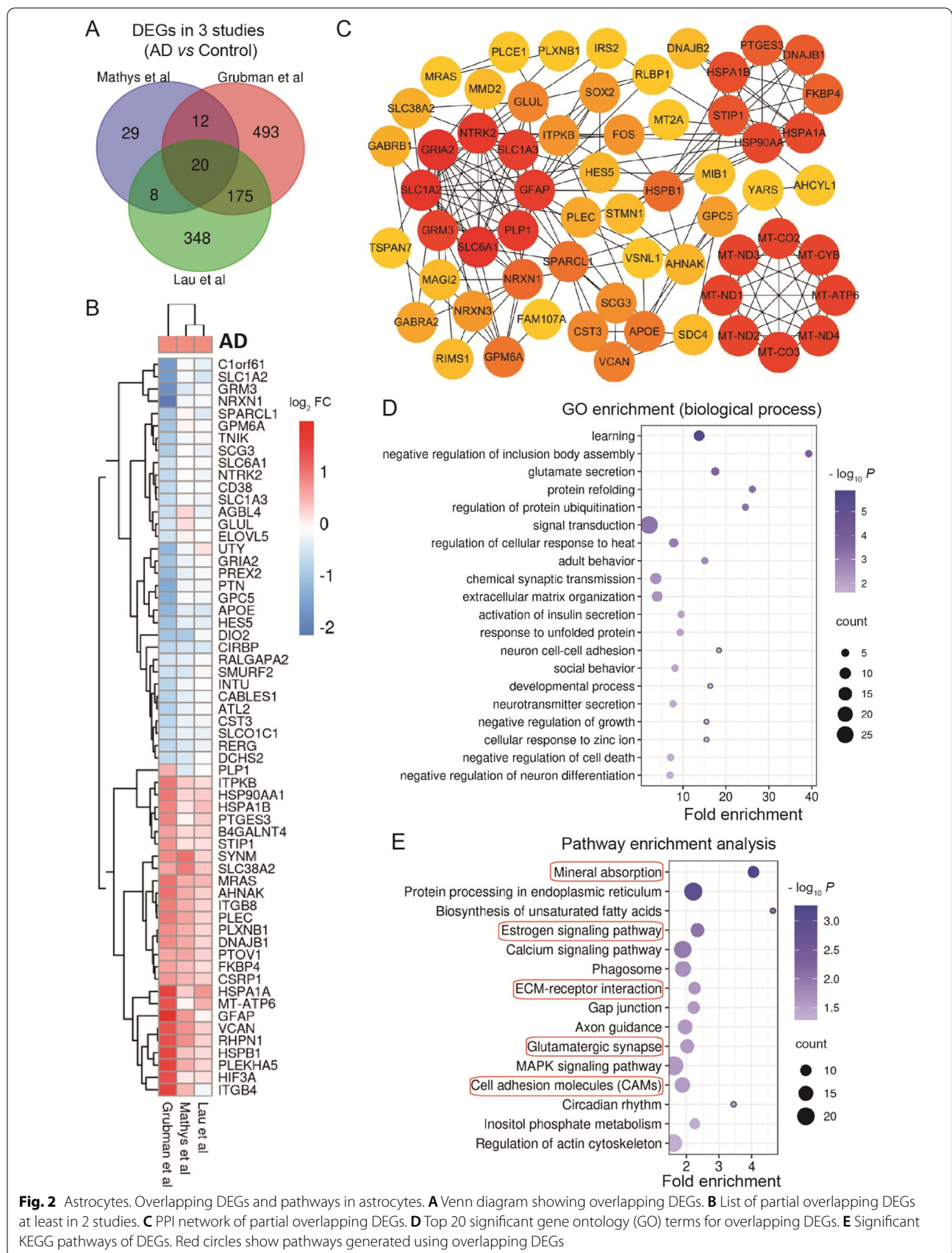
glutamate receptor clustering was down-regulated in astrocytes (Fig.2B and D).

Further KEGG pathway enrichment showed that the overlapping DEGs were mainly enriched for pathways, such as mineral absorption, cell adhesion molecules, glutamatergic synapse, GABAergic synapse, extracellular matrix (ECM)-receptor interaction, and estrogen signaling pathway (Fig.2E). Moreover, overall DEGs in astrocytes were also involved in protein processing in ER, biosynthesis of unsaturated fatty acids, calcium signaling pathway, phagosome, gap junction, axon guidance, MAPK signaling pathway, circadian rhythm, inositol phosphate metabolism, and regulation of actin cytoskeleton (Fig.2E).

Increased oxidative stress in oligodendrocytes

Oligodendrocytes dysfunction has been associated with neurodegenerative disease and neuroimaging studies showed that the myelin loss happened in the preclinical phase of AD [68, 69]. Here, we observed 10 common DEGs in 3 studies (Fig.3A). Up-regulated LINGO1 was involved in axonogenesis and signal transduction, while MID1IP1 and SLC38A2 were related to AD pathology, which has been reported in previous studies [70, 71]. Multil-omics signaling pathways knowledgebase showed that CCP110, KCNH8, CARNIS1, LDLRAD4, and GPM6A might be involved in the cell cycle, immune response, and estrogen receptor pathway [42].

In overlapping DEGs constructed PPI network, the top-ranked module (9 nodes and 36 edges) was enriched for up-regulated mitochondria related-genes (MT-ND1–4, MT-CO2, MT-CO3, MT-ATP6, MT-CYB, and NDUFS2) (Fig.3B and C). Genes in the second module (dysregulated RPS27, RPS35, RPS6, UBC, and RPLP1) and together with the hub genes (down-regulated S100B and CTNNA2) were enriched in SRP-dependent cotranslational protein targeting to membrane, mRNA catabolic process, and regulation of apoptotic process (Fig.3B-D). Down-regulated S100B, CTNNA2, SNAP25, SLC1A2, CNTNAP2, and NRXN3 were involved in axonogenesis, glutamate secretion, long-term synaptic potentiation, and adult behavior (Fig.3B-D). HSPA1A and MT3 were involved in cellular response to oxidative stress and hypoxia. Besides, down-regulated DEGs were also enriched in glutamate receptor (GRID2), stabilizing the myelin sheath (MOBP), and promoting oligodendrocyte terminal differentiation (OPALIN) (Fig.3B-D). The up-regulated PLP1 and ITPKB have been considered as risk factors of AD in previous studies [72, 73]. These altered hub genes and related biological processes indicate the increased cellular oxidative stress and injury of oligodendrocytes, which further affect neural transmission.



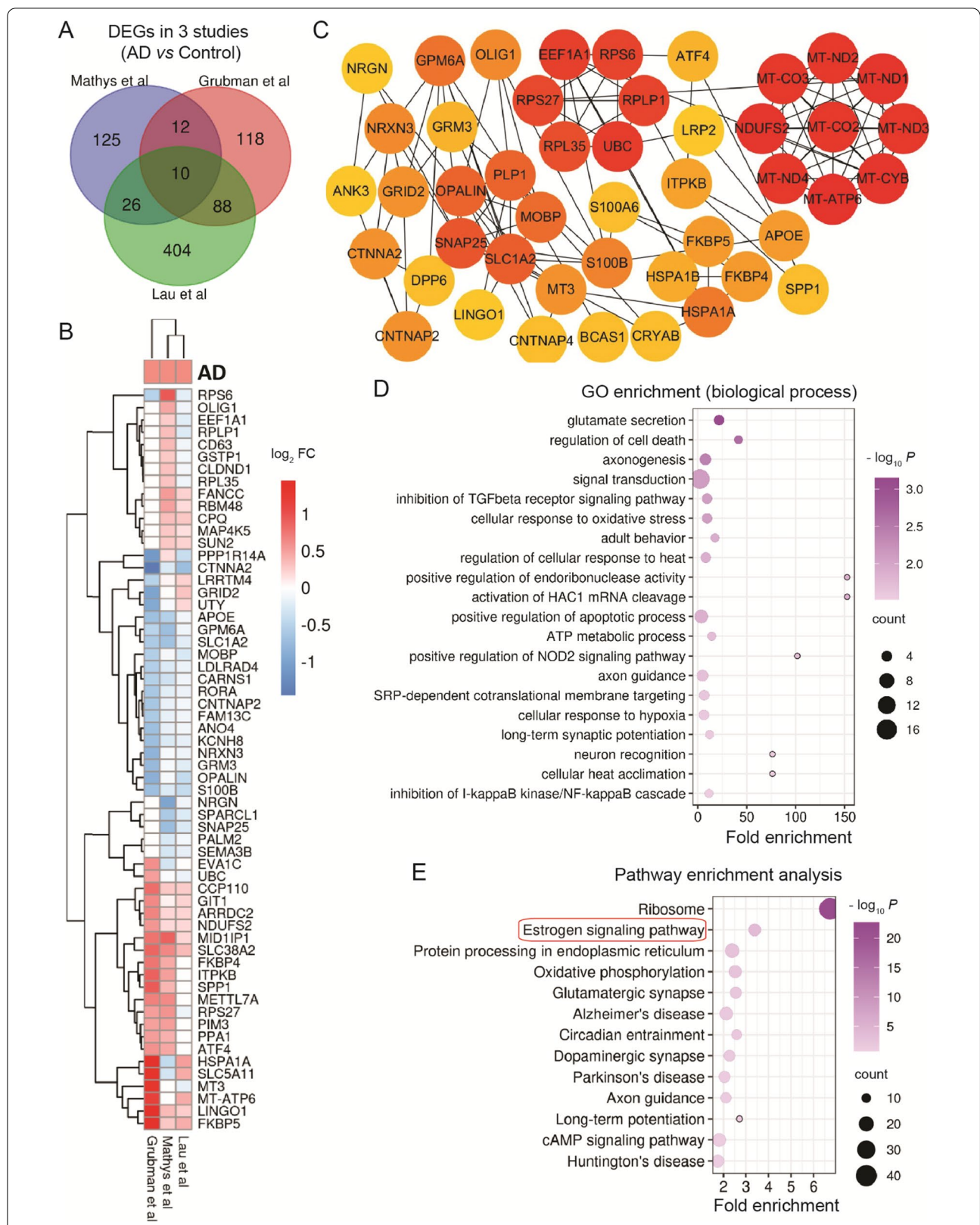


Fig. 3 Oligodendrocytes. Overlapping DEGs and pathways in oligodendrocytes. **A** Venn diagram showing DEGs. **B** List of partial overlapping DEGs at least in 2 studies. **C** PPI network of partial overlapping DEGs. **D** Top 20 significant gene ontology (GO) terms for overlapping DEGs. **E** Significant KEGG pathways of DEGs. Red circles show pathways generated using overlapping DEGs

Using the DEGs, we explored the KEGG pathway enrichment in oligodendrocytes, including ribosome, estrogen signaling pathway, protein processing in ER, oxidative phosphorylation, glutamatergic and dopaminergic synapse, circadian entrainment, axon guidance, long-term potentiation, cAMP signaling pathway, AD, Parkinson's disease, and Huntington's disease (Fig. 3E). It seems that these DEGs include the transcriptional changes of other neurodegenerative diseases.

Dysfunction of cellular metabolism and protein degradation in excitatory neurons

Synaptic dysfunction and neuronal loss are the main characteristics of AD. In excitatory neurons, we observed 6 common DEGs in 3 studies (Fig. 4A). These overlapping DEGs were enriched in axonogenesis (up-regulated LINGO1), histone H4 acetylation (up-regulated CHD5 and PER1) [74], and entrainment of circadian clock (PER1) (Fig. 4B–D). TSPAN7, regulation of spine maturation and AMPA receptor trafficking, was down-regulated in excitatory neurons [75]. While SLC26A3 was enriched in regulation of membrane potential, membrane hyperpolarization, oxalate transport, and sulfate transmembrane transport. Multilomics signaling pathways knowledgebase showed that the up-regulated DPP7 was involved in innate immune response (Fig. 4B–D).

Moreover, in the PPI network of overlapping DEGs, the top-ranked genes (up-regulated MT-ND1–4, MT-ND4L, MT-CO2, MT-CO3, MT-ATP6, MT-CYB, and CYCS) were enriched in mitochondrial module (with 10 nodes and 45 edges) (Fig. 4B–C). While the genes-NDUFA8, NDUFS5, and COX6C that related to mitochondrial electron transport chain were down-regulated (Fig. 4B–C). These changes in mitochondria indicate the disorder of oxidative phosphorylation. Among the hub gene, down-regulated SNAP25 has been considered as a potential biomarker in AD [76]. Up-regulated GRIN1, a glutamate receptor, was enriched in the regulation of membrane potential. Up-regulated PPFIA3 together with the genes (SYN1 and RAB3A) from the second module were enriched in neurotransmitter secretion. Down-regulated RAB3A and CCK were enriched in axonogenesis and regulation of synaptic vesicle fusion to presynaptic membrane. RIMS1, associated with synaptic transmission, and LRRC4B, related to cell adhesion, were disturbed (Fig. 4B–D). Multilomics signaling pathways knowledgebase showed that down-regulated NSF was related to inflammation. Up-regulated SYN1, down-regulated RAB3A, RPH3A, SST, and CCK were closely associated with AD development [77–81] (Fig. 4B–C). Together, these changed gene expressions indicate the deficit of synaptic function in excitatory neurons.

The KEGG pathway enrichment analysis showed that the overlapping DEGs were enriched in AD, Parkinson's disease, Huntington's disease, and endocytosis (Fig. 4E), suggesting these DEGs may be the common gene set involved in neurodegenerative diseases. Overall DEGs were also involved in oxidative phosphorylation, synaptic vesicle cycle, MAPK signaling pathway, glutamatergic synapse, GnRH signaling pathway, ribosome, gap junction, long-term depression, lysosome, one carbon pool by folate, purine metabolism, long-term potentiation, proteasome, glycerophospholipid metabolism, endocrine and other factor-regulated calcium reabsorption, and neurotrophin signaling pathway (Fig. 4E).

Mitochondrial dysfunction in inhibitory neurons

In inhibitory neurons, there were no common DEGs in 3 studies (Fig. 5A). The 35 overlapping DEGs among 3 studies were used to construct the PPI network. The significant module included up-regulated mitochondrial genes and down-regulated mitochondrial respiratory chain-related genes (NDUFA4, NDUFA12, NDUFS3, and COX4I1) (Fig. 5B and C). These DEGs together with down-regulated ATP1F1, ATP5J, and MRPS16 indicate the mitochondrial dysfunction in inhibitory neurons. SOD1, which is involved in response to reactive oxygen species, was down-regulated (Fig. 5B–D). In addition, the overlapping DEGs were also enriched in neurotransmitter secretion, axonogenesis (up-regulated LINGO1), exocytosis, NMDA receptor activity, MAPK cascade, and cell adhesion (Fig. 5B–D). Overlapping DEGs were enriched in pathways including AD, Huntington's disease, Parkinson's disease, and long-term potentiation. Overall DEGs were also involved in Rap1 signaling pathway, cAMP signaling pathway, and estrogen signaling pathway (Fig. 5E).

Increased energy metabolism and immune response in endothelial cells

Endothelial cells are a central element to form the BBB and regulate molecular transport into the brain. BBB impairments have been seen in the preclinical stages of the AD brains [82]. Here, only 2 studies classified the endothelial cells and we have 24 overlapping DEGs (Fig. 6A). Except for MALAT1, the overlapping DEGs showed high concordant up-regulation in endothelial cells (Fig. 6B). Interestingly, mitochondria and nutrient transporter related genes, such as glucose transporter-SLC2A1, intracellular calcium and amino acid transporter-SLC3A2, transporter activity regulator-SLCO4A1, and the physical barrier-CLDN5, were significantly increased, suggesting the increased uptake of nutrients and energy production in AD brain [83–85] (Fig. 6B).

GO analysis showed that DEGs were also enriched in response to unfolded protein (HSP90AA1, SERPINH1,

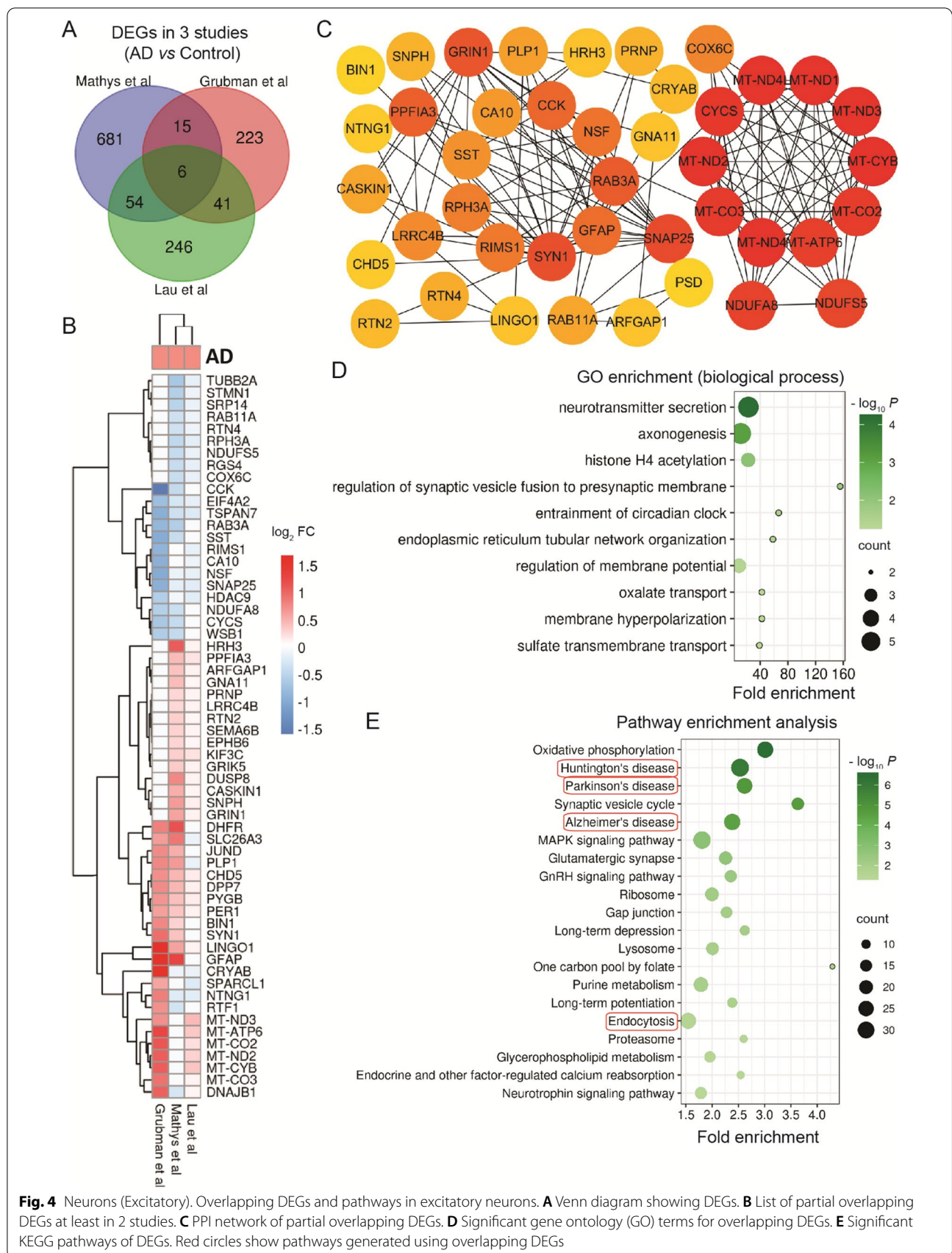


Fig. 4 Neurons (Excitatory). Overlapping DEGs and pathways in excitatory neurons. **A** Venn diagram showing DEGs. **B** List of partial overlapping DEGs at least in 2 studies. **C** PPI network of partial overlapping DEGs. **D** Significant gene ontology (GO) terms for overlapping DEGs. **E** Significant KEGG pathways of DEGs. Red circles show pathways generated using overlapping DEGs

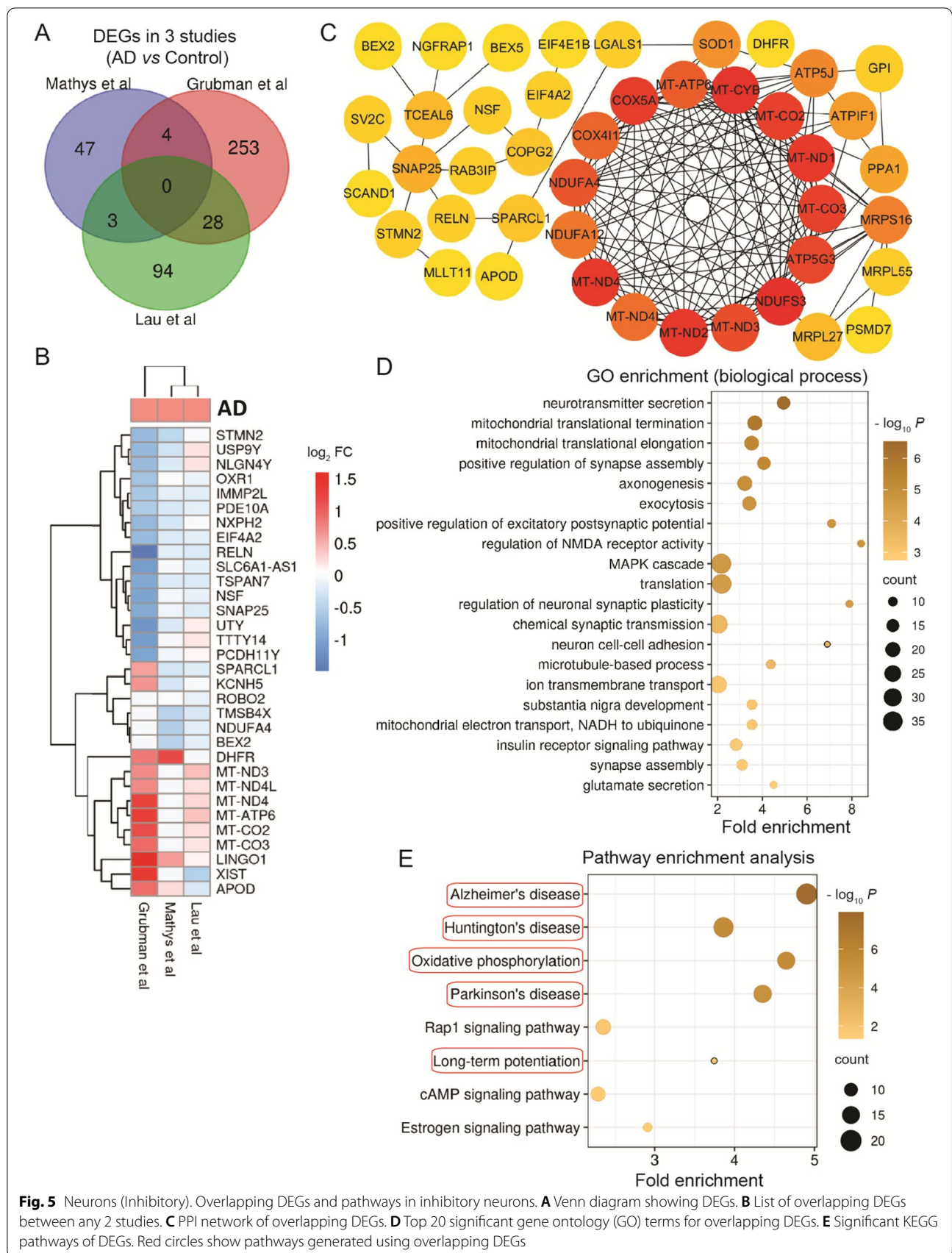


Fig. 5 Neurons (Inhibitory). Overlapping DEGs and pathways in inhibitory neurons. **A** Venn diagram showing DEGs. **B** List of overlapping DEGs between any 2 studies. **C** PPI network of overlapping DEGs. **D** Top 20 significant gene ontology (GO) terms for overlapping DEGs. **E** Significant KEGG pathways of DEGs. Red circles show pathways generated using overlapping DEGs

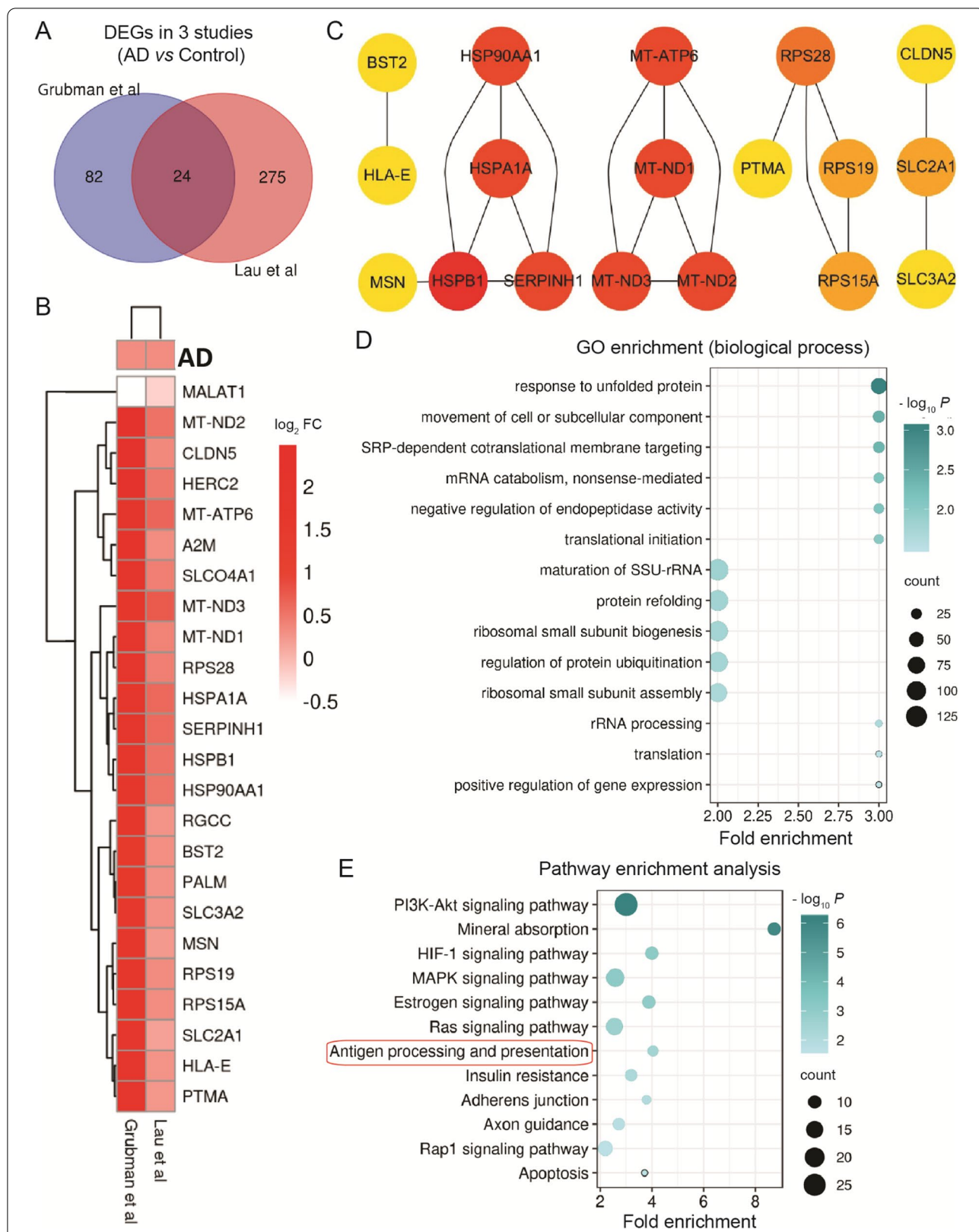


Fig. 6 Endothelial cells. Overlapping DEGs and pathways in endothelial cells. **A** Venn diagram showing DEGs. **B** List of overlapping DEGs between 2 studies. **C** PPI network of overlapping DEGs. **D** Significant gene ontology (GO) terms for overlapping DEGs. **E** Significant KEGG pathways of DEGs. Red circles show pathways generated using overlapping DEGs

and HSPB1), movement of cell or subcellular component (HSPB1, MSN, and PALM), SRP-dependent cotranslational protein targeting to membrane, mRNA catabolism, nonsense-mediated, translational initiation, rRNA processing and translation (RPS28, RPS15A, and RPS19), maturation of SSU-rRNA, ribosomal small subunit processing (RPS28 and RPS19), protein refolding, regulation of protein ubiquitination (HSP90AA1 and HSPA1A), negative regulation of endopeptidase activity (BST2, SERPINH1, and A2M), and positive regulation of gene expression (RGCC, MSN, and HSPA1A) (Fig. 6C and D). Also, the overlapping DEGs were enriched in immune response (PTMA, HLA-E, and MALAT1) and ubiquitin-protein ligase binding (HERC2) [86, 87]. These changes indicate the cellular stress and protein degradation in endothelial cells.

The common KEGG pathway between the 2 studies was antigen processing and presentation (Fig. 6E). The overall DEGs were also involved in PI3K-Akt signaling pathway, mineral absorption, HIF-1 signaling pathway, MAPK signaling pathway, estrogen signaling pathway, ras signaling pathway, insulin resistance, adherens junction, axon guidance, rap1 signaling pathway, and apoptosis (Fig. 6E).

Discussion

In recent decades, it has been reported that numerous risk factors, different cell types, and complex signaling pathways are involved in AD pathogenesis. However, there is currently no effective therapy to cure the disease. Here, we integrated analyzed the cell type-specific transcriptomic and functional changes to understand the common and distinct molecule changes and networks across different cell types in AD.

Common molecule and pathways changes in AD

Up-regulated LINGO1, a negative regulator of neuronal processes, was the only differential gene observed in multiple cell types across all studies, which has been predicted to be a potential target of AD therapy [29, 88]. Moreover, we found the coordinated up-regulation of mitochondrial genes across all cell types in the studies of Lau et al and Grubman et al. Increasing evidence indicates the mitochondrial disorder in AD brain [7, 89–91]. Mitochondria, as the center of cellular metabolism, not only provide enough energy supply to maintain the essential cellular processes but may cause mitochondrial-related oxidative damage. Mitochondrial dysfunction could impair astrocyte's neuroprotective effect and synaptic activity [92]. Besides, altered energy metabolism also implicates mitochondrial dysfunction, which has been seen in microglia, astrocytes, and oligodendrocytes

[5, 50]. Metabolic processes affect the immune response and oxidative stress and vice versa [93–96]; the unbalance of this complex interplay aggravates the oxidative stress, dysfunction of cellular metabolism, and immune response as we have observed in AD brain, especially in microglia, oligodendrocytes, and endothelial cells. Overall, the mitochondrial transcriptome alterations are the most significant and consistent changes that cross all cell types, while the mitochondrial dysfunction has also been approved in a recent proteomic study in the human AD brain [90].

The overall transcriptomic changes in each cell type revealed more possible impaired pathways involved in AD-pathology. Common KEGG pathways enriched in different cell types were shown in Table 2. The dysregulated MAPK signaling pathway has been observed in microglia, astrocytes, excitatory neurons, and endothelial cells. Inhibition of the p38 MAPK signaling pathway to treat AD has been suggested since decades ago [97, 98]. The antigen processing and presentation, HIF-1 signaling pathway, and PI3K-Akt signaling pathway were enriched in microglia and Endothelial cells. These pathways together with mitochondrial and estrogen signaling pathways form a complex network and play an extensive and important role in AD pathology [99]. Phagosome was enriched in microglia and astrocytes, but the

Table 2 Common KEGG pathways in different cell types of AD brain

Pathways	Cell types
Estrogen signaling pathway	Mic, Astro, Oligo, Neuron (In), Endo
MAPK signaling pathway	Mic, Astro, Neuron (Ex), Endo
Ribosome	Mic, Oligo, Neuron (Ex)
Glutamatergic synapse	Astro, Oligo, Neuron (Ex)
Axon guidance	Astro, Oligo, Endo
Oxidative phosphorylation	Oligo, Neuron (Ex), Neuron (In)
Long-term potentiation	Oligo, Neuron (Ex), Neuron (In)
Alzheimer's disease	Oligo, Neuron (Ex), Neuron (In)
Parkinson's disease	Oligo, Neuron (Ex), Neuron (In)
Huntington's disease	Oligo, Neuron (Ex), Neuron (In)
Phagosome	Mic, Astro
HIF-1 signaling pathway	Mic, Endo
PI3K-Akt signaling pathway	Mic, Endo
Antigen processing and presentation	Mic, Endo
Mineral absorption	Astro, Endo
cAMP signaling pathway	Oligo, Neuron (In)
Protein processing in ER	Astro, Oligo
Gap junction	Astro, Neuron (Ex)
Rap1 signaling pathway	Neuron (In), Endo

Mic: Microglia; Astro: Astrocytes; Oligo: Oligodendrocytes; Neuron (Ex): Excitatory neurons; Neuron (In): Inhibitory neurons; Endo: Endothelial cells

phagocytosis of astrocytes could be compensation for microglial dysfunction [100]. The estrogen signaling pathway seems to be the most common pathway that is disrupted in AD.

Distinct molecule and pathways changes in AD

Except for the DEGs that cross different cell types, we also identified the cell type-specific common genes and hub genes in 3 studies and explored their related biological processes and pathways that involve in AD-pathology. Besides the immunometabolism and oxidative stress-related genes, the ribosomal genes, reduced polyubiquitin-related genes-UBC and RPS27A, and genetic risk genes-APOE and APOC1 have also been seen in microglia. The most possible impaired pathways in microglia were the ribosome and PI3K-Akt signaling pathways. Ribosome dysfunction has been regarded as an early event of AD, which may be caused by oxidative damage [101]. Reduced PI3K-Akt signaling pathway has been reported in postmortem AD brain and is closely related to microglia inflammation [102, 103]. These pathways further indicate the microglial oxidation and inflammation in AD. Surprisingly, the endothelial cells were enriched for genes related to immune response and protein ubiquitination and were also involved in antigen processing and presentation. It has been seen the amyloid deposition around cerebral vessels in the preclinical stage of AD, which could impair the BBB integrity and further lead to T cell infiltration and activation in the brain [25, 28, 50, 104, 105]. Thus, the microglia and endothelial cells are the main roles that contributed to the inflammation in AD brain.

While in astrocytes, the most significant transcriptional changes were associated with neuronal signal transduction and extracellular matrix organization. The main impaired pathways were glutamatergic and GABAergic synapses, cell adhesion molecules, ECM-receptor interaction, and mineral absorption in astrocytes. Extracellular matrix promotes the formation of neural networks [106]. Therefore, the astrocytes lost the function to support neuronal activity and maintain brain homeostasis in AD, which has been seen in oligodendrocytes as well [27, 28, 107]. Moreover, the estrogen signaling pathway was impaired in both astrocytes and oligodendrocytes. Reduced expression of estrogen receptors has been reported in hippocampal neurons of AD patients [108]. Restore the estrogen-related signaling could be an effective therapy for the treatment of AD [99]. To be noticed, the transcriptomic alterations in oligodendrocytes, excitatory neurons, and inhibitory neurons could be not specific to AD, but partially overlap with gene sets related to neurodegenerative diseases, such as Parkinson's disease

(PD) and Huntington's disease (HD). Aberrant myelination and alteration in oligodendrocytes have been identified as common pathophysiological features of AD, PD, and HD [109–112]. Additionally, it's well known that progressive deficit of structure or dysfunction of neurons and neuronal loss are the main characteristics of neurodegeneration.

We also identified the dysregulated pathways in specific cell types across studies, for instance, ribosome and PI3K-Akt signaling pathway in microglia, cell adhesion molecules, glutamatergic synapse, ECM-receptor interaction, and estrogen signaling pathway in astrocytes, estrogen signaling pathway in oligodendrocytes, endocytosis in excitatory neurons, oxidative phosphorylation in inhibitory neurons, and antigen processing and presentation in endothelial cells.

Conclusion

In summary, except for the consensus alteration of mitochondrial genes, there are no DEGs that can cross all cell types. Our comprehensive study provides the precise cellular changes, points out the complexity of the transcriptional network in AD pathology, and further highlights the value of cell type-specific transcriptomic analysis. The identified pathogenic genes and functional pathways in AD brain may provide a helpful resource for future investigations and serve as therapeutic targets and biomarkers for the disease.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12883-021-02407-1>.

Additional file 1: Supplementary File 1. DEGs are identified and used in microglial cells. **Supplementary File 2.** DEGs are identified and used in astrocytes. **Supplementary File 3.** DEGs are identified and used in oligodendrocytes. **Supplementary File 4.** DEGs are identified and used in excitatory neurons. **Supplementary File 5.** DEGs are identified and used in inhibitory neurons. **Supplementary File 6.** DEGs are identified and used in endothelial cells.

Acknowledgements

Not applicable.

Patient consent statement

Not applicable.

Clinical trial registration

Not applicable.

Authors' contributions

X-LW and LJJ conceived the work, analyzed the data, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

No funding supported this work.

Availability of data and materials

The snRNA-seq data were obtained from the Supplementary files of 2 articles (Mathys et al., [29]; Lau et al., [28]) and single-cell atlas (<http://adsn.ddnetbio.com>). All data generated or analysed during this study are included in this published article.

Declarations**Ethics approval and consent to participate**

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Author details

¹Department of Nephrology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. ²Université de Strasbourg, Strasbourg, France. ³Department of Surgery, Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan 430061, China. ⁴Hubei Province Academy of Traditional Chinese Medicine, Wuhan 430076, China.

Received: 12 June 2021 Accepted: 28 August 2021

Published online: 02 October 2021

References

- Katzman R, Saitoh T. Advances in Alzheimer's disease. *FASEB J*. 1991;5:278.
- Mattson MP. Pathways towards and away from Alzheimer's disease (vol 430, pg 631, 2004). *Nature*. 2004;431:107. <https://doi.org/10.1038/nature02940>.
- Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol*. 1997;41:17. <https://doi.org/10.1002/ana.410410106>.
- Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol*. 2015;14:388. [https://doi.org/10.1016/S1474-4422\(15\)70016-5](https://doi.org/10.1016/S1474-4422(15)70016-5)
- Zhang XW, Wang RH, Hu D, Sun XY, Fujioka H, Lundberg K, et al. Oligodendroglial glycolytic stress triggers inflammasome activation and neuropathology in Alzheimer's disease. *Sci Adv*. 2020 6:ARTN eabb8680. <https://doi.org/10.1126/sciadv.abb8680>
- Liddel SA, Guttenplan KA, Larke LEC, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017;541:481. <https://doi.org/10.1038/nature21029>.
- Parodi-Rullan R, Sone JY, Fossati S. Endothelial Mitochondrial Dysfunction in Cerebral Amyloid Angiopathy and Alzheimer's Disease. *J Alzheimers Dis*. 2019;72:1019. <https://doi.org/10.3233/Jad-190357>.
- Sarlus H, Heneka MT. Microglia in Alzheimer's disease. *J Clin Invest*. 2017;127:3240. <https://doi.org/10.1172/JCI90606>.
- Ising C, Heneka MT. Functional and structural damage of neurons by innate immune mechanisms during neurodegeneration. *Cell Death Disease*. 2018;9:Art120. <https://doi.org/10.1038/S41419-017-0153-X>
- Laurent C, Dorothee G, Hunot S, Martin E, Monnet Y, Duchamp M, et al. Hippocampal T cell infiltration promotes neuroinflammation and cognitive decline in a mouse model of tauopathy. *Brain*. 2017;140:184. <https://doi.org/10.1093/brain/aww270>.
- Fu WY, Wang XJ, Ip NY. Targeting Neuroinflammation as a Therapeutic Strategy for Alzheimer's Disease: Mechanisms, Drug Candidates, and New Opportunities. *ACS Chem Neurosci*. 2019;10:872. <https://doi.org/10.1021/acscchemneuro.8b00402>.
- Nott A, Holtman IR, Coufal NG, Schlachetzki JCM, Yu M, Hu R, et al. Brain cell type-specific enhancer-promoter interactome maps and disease-risk association. *Science*. 2019;366:1134. <https://doi.org/10.1126/science.aay0793>.
- Jansen IE, Savage JE, Watanabe K, Bryois J, Williams DM, Steinberg S, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51:404. <https://doi.org/10.1038/s41588-018-0311-9>.
- Schwabe T, Srinivasan K, Rhinn H. Shifting paradigms: The central role of microglia in Alzheimer's disease. *Neurobiol Disease*. 2020;143:Art104962. <https://doi.org/10.1016/J.Nbd.2020.104962>
- Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA, Klunk WE, et al. Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. *J Neurosci*. 2008;28:4283. <https://doi.org/10.1523/Jneurosci.4814-07.2008>
- Cai ZY, Hussain MD, Yan LJ. Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer's disease. *Int J Neurosci*. 2014;124:307. <https://doi.org/10.3109/00207454.2013.833510>.
- Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. *J Cell Biol*. 2018;217:459. <https://doi.org/10.1083/jcb.201709069>.
- Felsky D, Roostaei T, Nho K, Risacher SL, Bradshaw EM, Petyuk V, et al. Neuropathological correlates and genetic architecture of microglial activation in elderly human brain. *Nature Communications*. 2019;10:ARTN 409. <https://doi.org/10.1038/s41467-018-08279-3>
- Fakhoury M. Microglia and Astrocytes in Alzheimer's Disease: Implications for Therapy. *Curr Neuropharmacol*. 2018;16:508. <https://doi.org/10.2174/1570159x15666170720095240>.
- Arranz AM, De Strooper B. The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. *Lancet Neurol*. 2019;18:406. [https://doi.org/10.1016/S1474-4422\(18\)30490-3](https://doi.org/10.1016/S1474-4422(18)30490-3).
- Damisah EC, Hill RA, Rai A, Chen FY, Rothlin CV, Ghosh S, et al. Astrocytes and microglia play orchestrated roles and respect phagocytic territories during neuronal corpse removal in vivo. *Sci Adv*. 2020;6:ARTN eaba3239. <https://doi.org/10.1126/sciadv.aba3239>
- Karch CM, Goate AM. Alzheimer's Disease Risk Genes and Mechanisms of Disease Pathogenesis. *Biol Psychiatry*. 2015;77:43. <https://doi.org/10.1016/j.biopsych.2014.05.006>.
- Nasraby SE, Rizvi B, Goldman JE, Brickman AM. White matter changes in Alzheimer's disease: a focus on myelin and oligodendrocytes. *Acta Neuropathol Com*. 2018;6:ARTN 22. <https://doi.org/10.1186/s40478-018-0515-3>
- Xu J, Chen SW, Ahmed SH, Chen H, Ku G, Goldberg MP, et al. Amyloid-beta peptides are cytotoxic to oligodendrocytes. *J Neurosci*. 2001;21. <https://doi.org/10.1523/Jneurosci.21-01-J0001.2001>
- Ghiso J, Fossati S, Rostagno A. Amyloidosis Associated with Cerebral Amyloid Angiopathy: Cell Signaling Pathways Elicited in Cerebral Endothelial Cells. *J Alzheimers Dis*. 2014;42:S167. <https://doi.org/10.3233/Jad-140027>.
- Keable A, Fenna K, Yuen HM, Johnston DA, Smyth NR, Smith C, et al. Deposition of amyloid beta in the walls of human leptomeningeal arteries in relation to perivascular drainage pathways in cerebral amyloid angiopathy. *BBA-Mol Basis Dis*. 2016;1862:1037. <https://doi.org/10.1016/j.bbadis.2015.08.024>.
- Grubman A, Chew G, Ouyang JF, Sun GZ, Choo XY, McLean C, et al. A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nat Neurosci*. 2019;22:2087. <https://doi.org/10.1038/s41593-019-0539-4>.
- Lau SF, Cao H, Fu AKY, Ip NY. Single-nucleus transcriptome analysis reveals dysregulation of angiogenic endothelial cells and neuroprotective glia in Alzheimer's disease. *P Natl Acad Sci USA*. 2020;117:25800. <https://doi.org/10.1073/pnas.2008762117>.
- Mathys H, Davila-Velderrain J, Peng ZY, Gao F, Mohammadi S, Young JZ, et al. Single-cell transcriptomic analysis of Alzheimer's disease. *Nature*. 2019;570:332. <https://doi.org/10.1038/s41586-019-1195-2>.
- Lake BB, Chen S, Sos BC, Fan J, Kaeser GE, Yung YC, et al. Integrative single-cell analysis of transcriptional and epigenetic states in the human adult brain. *Nat Biotechnol*. 2018;36:70. <https://doi.org/10.1038/nbt.4038nbt.4038>.
- He Z, Han D, Efimova O, Guijarro P, Yu Q, Oleksiak A, et al. Comprehensive transcriptome analysis of neocortical layers in humans, chimpanzees and macaques. *Nat Neurosci*. 2017;20:886. <https://doi.org/10.1038/nn.4548nn.4548>.

32. McKenzie AT, Wang M, Hauberg ME, Fullard JF, Kozlenkov A, Keenan A, et al. Brain Cell Type Specific Gene Expression and Co-expression Network Architectures. *Sci Rep*. 2018;8:8868. <https://doi.org/10.1038/s41598-018-27293-5>.
33. Xia JG, Benner MJ, Hancock REW. NetworkAnalyst - integrative approaches for protein-protein interaction network analysis and visual exploration. *Nucleic Acids Res*. 2014;42:W167. <https://doi.org/10.1093/nar/gku443>.
34. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics*. 2003;4:ArtN 2. <https://doi.org/10.1186/1471-2105-4-2>
35. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. *Bmc Syst Biol*. 2014;8:ArtN S11. <https://doi.org/10.1186/1752-0509-8-S4-S11>
36. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13:2498. <https://doi.org/10.1101/gr.1239303>.
37. Becnel LB, Ochsner SA, Darlington YF, McOwiti A, Kankanamge WH, Dehart M, et al. Discovering relationships between nuclear receptor signaling pathways, genes, and tissues in Transcriptome. *Science Signaling*. 2017;10:ARTN eaah6275. <https://doi.org/10.1126/scisignal.aah6275>
38. Ochsner SA, Abraham D, Martin K, Ding W, McOwiti A, Kankanamge W, et al. The Signaling Pathways Project, an integrated 'omics knowledge-base for mammalian cellular signaling pathways. *Sci Data*. 2019;6:ArtN 252. doi: <https://doi.org/10.1038/S41597-019-0193-4>
39. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4:44. <https://doi.org/10.1038/nprot.2008.211>.
40. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res*. 2012;40:D109. <https://doi.org/10.1093/nar/gkr988>.
41. Igarashi Y, Nakatsu N, Yamashita T, Ono A, Ohno Y, Urushidani T, et al. Open TG-GATes: a large-scale toxicogenomics database. *Nucleic Acids Res*. 2015;43:D921. <https://doi.org/10.1093/nar/gku955>.
42. Realegeno S, Kelly-Scumpia KM, Dang AT, Lu J, Teles R, Liu PT, et al. S100A12 Is Part of the Antimicrobial Network against Mycobacterium leprae in Human Macrophages. *Plos Pathog*. 2016;12:ARTN e1005705. <https://doi.org/10.1371/journal.ppat.1005705>
43. Lorenzetto E, Moratti E, Vezzalini M, Harroch S, Sorio C, Buffelli M. Distribution of different isoforms of receptor protein tyrosine phosphatase gamma (Ptpg-RPTP gamma) in adult mouse brain: upregulation during neuroinflammation. *Brain Struct Funct*. 2014;219:875. <https://doi.org/10.1007/s00429-013-0541-7>.
44. Herold C, Hooli BV, Mullin K, Liu T, Roehr JT, Mattheisen M, et al. Family-based association analyses of imputed genotypes reveal genome-wide significant association of Alzheimer's disease with OSBP16, PTPRG, and PDCL3. *Mol Psychiatry*. 2016;21:1608. <https://doi.org/10.1038/mp.2015.218>.
45. Rangaraju S, Dammer EB, Raza SA, Rathakrishnan P, Xiao HL, Gao TW, et al. Identification and therapeutic modulation of a pro-inflammatory subset of disease-associated-microglia in Alzheimer's disease. *Mol Neurodegener*. 2018;13:ARTN 24. <https://doi.org/10.1186/s13024-018-0254-8>
46. Naciff JM, Khambatta ZS, Reichling TD, Carr GJ, Tiesman JP, Singleton DW, et al. The genomic response of Ishikawa cells to bisphenol A exposure is dose- and time-dependent. *Toxicology*. 2010;270:137. <https://doi.org/10.1016/j.tox.2010.02.008>.
47. Gonit M, Zhang JA, Salazar MD, Cui HJ, Shatnawi A, Trumbly R, et al. Hormone Depletion-Insensitivity of Prostate Cancer Cells Is Supported by the AR Without Binding to Classical Response Elements. *Mol Endocrinol*. 2011;25:621. <https://doi.org/10.1210/me.2010-0409>.
48. Zhou Q, Zhao F, Lv ZP, Zheng CG, Zheng WD, Sun L, et al. Association between APOC1 Polymorphism and Alzheimer's Disease: A Case-Control Study and Meta-Analysis. *PLoS One*. 2014;9:ARTN e87017. doi: <https://doi.org/10.1371/journal.pone.0087017>
49. Zhou XP, Chen Y, Mok KY, Kwok TCY, Mok VCT, Guo QH, et al. Non-coding variability at the APOE locus Communications. 2019; 10:ArtN 3310. doi: [10.38/S41467-019-10945-Z](https://doi.org/10.38/S41467-019-10945-Z)
50. Johnson ECB, Dammer EB, Duong DM, Ping LY, Zhou MT, Yin LM, et al. Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. *Nat Med*. 2020;26:769. <https://doi.org/10.1038/s41591-020-0815-6>.
51. Tramutola A, Di Domenico F, Barone E, Perluigi M, Butterfield DA. It Is All about (U)biqutin: Role of Altered Ubiquitin-Proteasome System and UCHL1 in Alzheimer Disease. *Oxid Med Cell Longev*. 2016;2016:ArtN 2756068. <https://doi.org/10.1155/2016/2756068>
52. Hegde AN, Smith SG, Duke LM, Pourquoi A, Vaz S. Perturbations of Ubiquitin-Proteasome-Mediated Proteolysis in Aging and Alzheimer's Disease. *Front Aging Neurosci*. 2019; 11:ArtN 324. <https://doi.org/10.3389/Fnagi.2019.00324>
53. An H, Harper JW. Ribosome Abundance Control Via the Ubiquitin-Proteasome System and Autophagy. *J Mol Biol*. 2020;432:170. <https://doi.org/10.1016/j.jmb.2019.06.001>.
54. Sung MK, Porras-Yakushi TR, Reitsma JM, Huber FM, Sweredoski MJ, Hoelz A, et al. A conserved quality-control pathway that mediates degradation of unassembled ribosomal proteins. *Elife*. 2016; 5:ARTN e19105. <https://doi.org/10.7554/eLife.19105>
55. Nelson PT, Katsumata Y, Nho K, Artiushin SC, Jicha GA, Wang WX, et al. Genomics and CSF analyses implicate thyroid hormone in hippocampal sclerosis of aging. *Acta Neuropathol*. 2016;132:841. <https://doi.org/10.1007/s00401-016-1641-2>.
56. McAninch EA, Rajan KB, Evans DA, Jo S, Chaker L, Peeters RP, et al. A Common DIO2 Polymorphism and Alzheimer Disease Dementia in African and European Americans. *J Clin Endocrinol Metab*. 2018;103:1818. <https://doi.org/10.1210/jc.2017-01196>.
57. Hoekstra MMB, Emmenegger Y, Hubbard J, Franken P. Cold-inducible RNA-binding protein (CIRBP) adjusts clock-gene expression and REM-sleep recovery following sleep deprivation. *Elife*. 2019;8:ARTN e43400. <https://doi.org/10.7554/eLife.43400>
58. Eisele SC, Gill CM, Shankar GM, Brastianos PK. PLEKHA5: A Key to Unlock the Blood-Brain Barrier? *Clin Cancer Res*. 2015;21:1978. <https://doi.org/10.1158/1078-0432.CCR-14-2604>.
59. Cui P, Su JC, Li QM, Xu GM, Zhu NX. LncRNA RHPN1-AS1 Targeting miR-625/REG3A Promotes Cell Proliferation And Invasion Of Glioma Cells. *Oncotargets Ther*. 2019;12:7911. <https://doi.org/10.2147/Ott.S209563>.
60. Yu L, Petyuk VA, Gaiteri C, Mostafavi S, Young-Pearse T, Shah RC, et al. Targeted brain proteomics uncover multiple pathways to Alzheimer's dementia. *Ann Neurol*. 2018;84:78. <https://doi.org/10.1002/ana.25266>.
61. Pereira AC, Gray JD, Kogan JF, Davidson RL, Rubin TG, Okamoto M, et al. Age and Alzheimer's disease gene expression profiles reversed by the glutamate modulator riluzole. *Mol Psychiatry*. 2017;22:296. <https://doi.org/10.1038/mp.2016.33>.
62. Bossers K, Wirz KTS, Meerhoff GF, Essing AHW, van Dongen JW, Houba P, et al. Concerted changes in transcripts in the prefrontal cortex precede neuropathology in Alzheimer's disease. *Brain*. 2010;133:3699. <https://doi.org/10.1093/brain/awq258>.
63. Manavalan A, Mishra M, Feng L, Sze SK, Akatsu H, Heese K. Brain site-specific proteome changes in aging-related dementia. *Experimental Mol Med*. 2013;45:ARTN e39. <https://doi.org/10.1038/emm.2013.76>
64. Kim JH, Franck J, Kang T, Heinsen H, Ravid R, Ferrer J, et al. Proteome-wide characterization of signalling interactions in the hippocampal CA4/DG subfield of patients with Alzheimer's disease. *Scientific Reports*. 2015;5:ArtN 11138. <https://doi.org/10.1038/Srep11138>
65. Silva ART, Grinberg LT, Farfel JM, Diniz BS, Lima LA, Silva PJS, et al. Transcriptional Alterations Related to Neuropathology and Clinical Manifestation of Alzheimer's Disease. *PLoS One*. 2012;7:ARTN e48751. <https://doi.org/10.1371/journal.pone.0048751>
66. Shigemizu D, Akiyama S, Asanomi Y, Boroevich KA, Sharma A, Tsunoda T, et al. Risk prediction models for dementia constructed by supervised principal component analysis using miRNA expression data. *Communications Biology*. 2019;2:ARTN 77. <https://doi.org/10.1038/s42003-019-0324-7>
67. Kuhn A, Kumar A, Beilina A, Dillman A, Cookson MR, Singleton AB. Cell population-specific expression analysis of human cerebellum. *BMC Genomics*. 2012;13:ArtN 610. <https://doi.org/10.1186/1471-2164-13-610>

68. Fischer FU, Wolf D, Scheurich A, Fellgiebel A, Neuroimaging AD. Altered whole-brain white matter networks in preclinical Alzheimer's disease. *Neuroimage-Clin*. 2015;8:660. <https://doi.org/10.1016/j.nicl.2015.06.007>.
69. Ettl B, Schlachetzki JCM, Winkler J. Oligodendroglia and Myelin in Neurodegenerative Diseases: More Than Just Bystanders? *Mol Neurobiol*. 2016;53:3046. <https://doi.org/10.1007/s12035-015-9205-3>.
70. Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J, Podtelezchnikov AA, et al. Integrated Systems Approach Identifies Genetic Nodes and Networks in Late-Onset Alzheimer's Disease. *Cell*. 2013;153:707. <https://doi.org/10.1016/j.cell.2013.03.030>.
71. Patel H, Hodges AK, Curtis C, Lee SH, Troakes C, Dobson RJB, et al. Transcriptomic analysis of probable asymptomatic and symptomatic Alzheimer brains. *Brain Behav Immun*. 2019;80:644. <https://doi.org/10.1016/j.bbi.2019.05.009>.
72. McKenzie AT, Moyon S, Wang MH, Katsyly I, Song WM, Zhou XX, et al. Multiscale network modeling of oligodendrocytes reveals molecular components of myelin dysregulation in Alzheimer's disease. *Mol Neurodegener*. 2017;12:ARTN 82. <https://doi.org/10.1186/s13024-017-0219-3>.
73. Stygelbout V, Leroy K, Pouillon V, Ando K, D'Amico E, Jia YH, et al. Inositol trisphosphate 3-kinase B is increased in human Alzheimer brain and exacerbates mouse Alzheimer pathology. *Brain*. 2014;137:537. <https://doi.org/10.1093/brain/awt344>.
74. Esposito M, Sherr GL. Epigenetic Modifications in Alzheimer's Neuropathology and Therapeutics. *Front Neurosci-Switz*. 2019;13:ArtN 476. <https://doi.org/10.3389/Fnins.2019.00476>.
75. Bassani S, Cingolani LA, Valnegri P, Folci A, Zapata J, Gianfelice A, et al. The X-Linked Intellectual Disability Protein TSPAN7 Regulates Excitatory Synapse Development and AMPAR Trafficking. *Neuron*. 2012;73:1143. <https://doi.org/10.1016/j.neuron.2012.01.021>.
76. Ohrfelt A, Brinkmalm A, Dumurgier J, Zetterberg H, Bouaziz-Amar E, Hugon J, et al. A Novel ELISA for the Measurement of Cerebrospinal Fluid SNAP-25 in Patients with Alzheimer's Disease. *Neuroscience*. 2019;420:136. <https://doi.org/10.1016/j.neuroscience.2018.11.038>.
77. Maesako M, Zoltowska KM, Berezovska O. Synapsin 1 promotes A beta generation via BACE1 modulation. *PLoS One*. 2019;14:ARTN e0226368. <https://doi.org/10.1371/journal.pone.0226368>.
78. Blennow K, Bogdanovic N, Alafuzoff I, Ekman R, Davidsson P. Synaptic pathology in Alzheimer's disease: Relation to severity of dementia, but not to senile plaques, neurofibrillary tangles, or the ApoE4 allele. *J Neural Transmission*. 1996;103:603. <https://doi.org/10.1007/Bf01273157>.
79. Plagman A, Hoscheidt S, McLimans KE, Klinedinst B, Pappas C, Anantharam V, et al. Cholecystokinin and Alzheimer's disease: a biomarker of metabolic function, neural integrity, and cognitive performance. *Neurobiol Aging*. 2019;76:201. <https://doi.org/10.1016/j.neurobiolaging.2019.01.002>.
80. Tan MGK, Lee C, Lee JH, Francis PT, Williams RJ, Ramirez MJ, et al. Decreased rabphilin 3A immunoreactivity in Alzheimer's disease is associated with A beta burden. *Neurochem Int*. 2014;64:29. <https://doi.org/10.1016/j.neuint.2013.10.013>.
81. Solarski M, Wang HS, Wille H, Schmitt-Ulms G. Somatostatin in Alzheimer's disease: A new Role for an Old Player. *Prion*. 2018;12:1. <https://doi.org/10.1080/19336896.2017.1405207>.
82. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and Dysfunction of the Blood-Brain Barrier. *Cell*. 2015;163:1064. <https://doi.org/10.1016/j.cell.2015.10.067>.
83. Winkler EA, Nishida Y, Sagare AP, Rege SV, Bell RD, Perlmutter D, et al. GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nat Neurosci*. 2015;18:521. <https://doi.org/10.1038/nn.3966>.
84. Meier S, Bell M, Lyons DN, Ingram A, Chen J, Gensel JC, et al. Identification of Novel Tau Interactions with Endoplasmic Reticulum Proteins in Alzheimer's Disease Brain. *J Alzheimers Dis*. 2015;48:687. <https://doi.org/10.3233/Jad-150298>.
85. Oikari LE, Pandit R, Stewart R, Cuni-Lopez C, Quek H, Sutharsan R, et al. Altered Brain Endothelial Cell Phenotype from a Familial Alzheimer Mutation and Its Potential Implications for Amyloid Clearance and Drug Delivery. *Stem Cell Rep*. 2020;14:924. <https://doi.org/10.1016/j.stemcr.2020.03.011>.
86. Zheng QY, Huang T, Zhang LS, Zhou Y, Luo H, Xu HX, et al. Dysregulation of Ubiquitin-Proteasome System in Neurodegenerative Diseases. *Front Aging Neurosci*. 2016;8:ArtN 303. <https://doi.org/10.3389/Fnagi.2016.00303>.
87. Ma PZ, Li YL, Zhang W, Fang FQ, Sun J, Liu MZ, et al. Long Non-coding RNA MALAT1 Inhibits Neuron Apoptosis and Neuroinflammation While Stimulates Neurite Outgrowth and Its Correlation With MiR-125b Mediates PTGS2, CDK5 and FOXQ1 in Alzheimer's Disease. *Curr Alzheimer Res*. 2019;16:596. <https://doi.org/10.2174/1567205016666190725130134>.
88. Fernandez-Enright F, Andrews JL. Lingo-1: a novel target in therapy for Alzheimer's disease? *Neural Regeneration Research*. (2016) 11:88. <https://doi.org/10.4103/1673-5374.175048>.
89. Wang WZ, Zhao FP, Ma XP, Perry G, Zhu XW. Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: recent advances. *Mol Neurodegener*. 2020;15:ArtN 30. <https://doi.org/10.1186/S13024-020-00376-6>.
90. Wang H, Dey KK, Chen PC, Li YX, Niu MM, Cho JH, et al. Integrated analysis of ultra-deep proteomes in cortex, cerebrospinal fluid and serum reveals a mitochondrial signature in Alzheimer's disease. *Mol Neurodegener*. 2020;15:ArtN 43. <https://doi.org/10.1186/S13024-020-00384-6>.
91. Swerdlow RH. Mitochondria and Mitochondrial Cascades in Alzheimer's Disease. *J Alzheimers Dis*. 2018;62:1403. <https://doi.org/10.3233/Jad-170585>.
92. Gollihue JL, Norris CM. Astrocyte mitochondria: Central players and potential therapeutic targets for neurodegenerative diseases and injury. *Ageing Res Rev*. 2020;59:ArtN 101039. <https://doi.org/10.1016/J.Arr.2020.101039>.
93. O'Neill LAJ, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol*. 2016;16:553. <https://doi.org/10.1038/nri.2016.70>.
94. Afridi R, Lee WH, Suk K. Microglia Gone Awry: Linking Immunometabolism to Neurodegeneration. *Front Cell Neurosci* 2020;14:ArtN 246. <https://doi.org/10.3389/Fncel.2020.00246>.
95. Frisard M, Ravussin E. Energy metabolism and oxidative stress - Impact on the metabolic syndrome and the aging process. *Endocrine*. 2006;29:27. doi: Doi 10.1385/Endo:29:1:27
96. Buck MD, O'Sullivan D, Geltink RIK, Curtis JD, Chang CH, Sanin DE, et al. Mitochondrial Dynamics Controls T Cell Fate through Metabolic Programming. *Cell*. 2016;166:63. <https://doi.org/10.1016/j.cell.2016.05.035>.
97. Lee JK, Kim NJ. Recent Advances in the Inhibition of p38 MAPK as a Potential Strategy for the Treatment of Alzheimer's Disease. *Molecules*. 2017;22:ArtN 1287. <https://doi.org/10.3390/Molecules22081287>.
98. Munoz L, Ammit AJ. Targeting p38 MAPK pathway for the treatment of Alzheimer's disease. *Neuropharmacology*. 2010;58:561. <https://doi.org/10.1016/j.neuropharm.2009.11.010>.
99. Uddin MS, Rahman MM, Jakaria M, Rahman MS, Hossain MS, Islam A, et al. Estrogen Signaling in Alzheimer's Disease: Molecular Insights and Therapeutic Targets for Alzheimer's Dementia. *Mol Neurobiol*. 2020;57:2654. <https://doi.org/10.1007/s12035-020-01911-8>.
100. Konishi H, Okamoto T, Hara Y, Komine O, Tamada H, Maeda M, et al. Astrocytic phagocytosis is a compensatory mechanism for microglial dysfunction. *Embo J*. (2020) 39. doi:
101. Ding QX, Markesbery WR, Chen QH, Li F, Keller JN. Ribosome dysfunction is an early event in Alzheimer's disease. *J Neurosci*. 2005;25:9171. <https://doi.org/10.1523/Jneurosci.3040-05.2005>.
102. Gabbouj S, Ryhanen S, Marttinen M, Wittrahm R, Takalo M, Kemppainen S, et al. Altered Insulin Signaling in Alzheimer's Disease Brain - Special Emphasis on PI3K-Akt Pathway. *Front Neurosci-Switz*. (2019) 13. doi: ArtN 62910.3389/Fnins.2019.00629
103. Cianciulli A, Porro C, Calvella R, Trotta T, Lofrumento DD, Panaro MA. Microglia Mediated Neuroinflammation: Focus on PI3K Modulation. *Biomolecules*. (2020) 10. doi: ArtN 13710.3390/Biom10010137
104. Attems J, Yamaguchi H, Saido TC, Thal DR. Capillary CAA and perivascular A beta-deposition: Two distinct features of Alzheimer's disease pathology. *J Neurol Sci*. 2010;299:155. <https://doi.org/10.1016/j.jns.2010.08.030>.
105. Snyder HM, Corriveau RA, Craft S, Faber JE, Greenberg SM, Knopman D, et al. Vascular contributions to cognitive impairment and dementia including Alzheimer's disease. *Alzheimers Dement*. 2015;11:710. <https://doi.org/10.1016/j.jalz.2014.10.008>.

106. Lam D, Enright HA, Cadena J, Peters SKG, Sales AP, Osburn JJ, et al. Tissue-specific extracellular matrix accelerates the formation of neural networks and communities in a neuron-glia co-culture on a multi-electrode array. *Scientific Reports*. 2019;9:Artn 4159. <https://doi.org/10.1038/S41598-019-40128-1>
107. Hillen AEJ, Burbach JPH, Hol EM. Cell adhesion and matricellular support by astrocytes of the tripartite synapse. *Prog Neurobiol*. 2018;165:66. <https://doi.org/10.1016/j.pneurobio.2018.02.002>.
108. Zhao LQ, Woody SK, Chhibber A. Estrogen receptor beta in Alzheimer's disease: From mechanisms to therapeutics. *Ageing Res Rev*. 2015;24:178. <https://doi.org/10.1016/j.arr.2015.08.001>.
109. Nasrabad SE, Rizvi B, Goldman JE, Brickman AM. White matter changes in Alzheimer's disease: a focus on myelin and oligodendrocytes. *Acta Neuropathol Commun*. 2018;6:22. <https://doi.org/10.1186/s40478-018-0515-3>.
110. Butt AM, De La Rocha IC, Rivera A. Oligodendroglial Cells in Alzheimer's Disease. *Adv Exp Med Biol*. 2019;1175:325. https://doi.org/10.1007/978-981-13-9913-8_12.
111. Errea O, Rodriguez-Oroz MC. Oligodendrocytes, a New Player in the Etiology of Parkinson's Disease. *Mov Disord*. 2021;36:83. <https://doi.org/10.1002/mds.28393>.
112. Casella C, Lipp I, Rosser A, Jones DK, Metzler-Baddeley C. A Critical Review of White Matter Changes in Huntington's Disease. *Mov Disord*. 2020;35:1302. <https://doi.org/10.1002/mds.28109>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

