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Systemic alterations of tricarboxylic acid cycle enzymes in Alzheimer's disease

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Mitochondrial dysfunction, especially tricarboxylic acid (TCA) cycle arrest, is strongly associated with Alzheimer's disease (AD), however, its systemic alterations in the central and peripheral of AD patients are not well defined. Here, we performed an integrated analysis of AD brain and peripheral blood cells transcriptomics to reveal the expression levels of nine TCA cycle enzymes involving 35 genes. The results showed that TCA cycle related genes were consistently down-regulated in the AD brain, whereas 11 genes were increased and 16 genes were decreased in the peripheral system. Pearson analysis of the TCA cycle genes with A β , Tau and mini-mental state examination (MMSE) revealed several significant correlated genes, including pyruvate dehydrogenase complex subunit (PDHB), isocitrate dehydrogenase subunits (IDH3B, IDH3G), 2-oxoglutarate dehydrogenase complex subunit (DLD), succinyl-CoA synthetase subunit (SUCLA2), malate dehydrogenase subunit (MDH1). In addition, SUCLA2, MDH1, and PDHB were also uniformly down-regulated in peripheral blood cells, suggesting that they may be candidate biomarkers for the early diagnosis of AD. Taken together, TCA cycle enzymes were systemically altered in AD progression, PDHB, SUCLA2, and MDH1 may be potential diagnostic and therapeutic targets.

KEYWORDS

Alzheimer's disease, tricarboxylic acid (TCA) cycle, transcriptomics, brain, peripheral blood cells

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease, and its main clinical manifestations are cognitive impairment and progressive decline of memory ability (GBD 2016 Neurology Collaborators, 2019). The main pathology of AD includes amyloid (A β) plaque deposition and neurofibrillary tangles (NFTs) formed by hyperphosphorylation of Tau (Hodson, 2018; Scheltens et al., 2021). With the acceleration of population aging, the prevalence of AD has increased dramatically. In 2018, Alzheimer's Disease International (ADI) estimated that there were about 50 million people with dementia worldwide, and it is expected that the global prevalence will increase by 2 times by 2050 (Scheltens et al., 2021), but the clinical treatment of AD is rarely effective. Therefore, there is an urgent need to find new regulatory molecules and the mechanisms for AD. There has been clear evidence that brain glucose metabolism is significantly reduced before cognitive decline (Cunnane et al., 2011; Ding et al., 2013), with positron emission tomography (PET) imaging showing a 20–30% reduction in brain glucose utilization of AD patients (de Leon et al., 1983).

Neurons have a high energy demand and are mainly operated by mitochondria, whose defects lead to synaptic dysfunction, neuroinflammation and neuronal death (Shoshan-Barmatz et al., 2018). Electron transport chain (ETC) and tricarboxylic acid (TCA) cycle are the most important metabolic pathways in mitochondria. The TCA cycle can completely oxidize acetyl-CoA and provide electron carriers for the oxidative phosphorylation pathway (Butterfield and Halliwell, 2019). Therefore, the dysregulation of TCA cycle metabolic pathways may be closely related to the insufficient energy supply in AD brain. Previous studies on the expression levels of TCA cycle enzymes were mostly limited to brain tissue samples, and the sample size was small, making the validity of the data insufficient (Bubber et al., 2005; Sang et al., 2022).

In order to systematically elucidate the relationship between TCA cycle and energy metabolism in AD and find the key molecules involved, we integrated the transcriptome data of brain tissue (Xu et al., 2018) (Alzdata; AD = 269, Ctrl = 271)¹ and peripheral blood cells (Sood et al., 2015) (GSE63060: Ctrl = 104, MCI = 80, AD = 145; GSE63061: Ctrl = 134, MCI = 109, AD = 139), and systematically analyzed the changes of TCA cycle enzymes in the central and peripheral tissues. By Pearson analysis, we identified genes correlated with A β and Tau pathology, as well as mini-mental state examination (MMSE), while PDHB, SUCLA2, and MDH1 may be potential diagnostic and therapeutic targets.

Materials and methods

Data acquisition

All datasets used to explore the expression of TCA cycle enzymes in the brain and periphery of AD patients are from the National Biotechnology Information Center (NCBI) Gene Expression Comprehensive Database (GEO) (Barrett et al., 2013). Specifically, brain data were obtained from 20 GSE series dataset, which have been integrated in the Alzdata database (see text footnote 1) (Xu et al., 2018), for a total of 269 AD patients and 271 normal aging subjects, including four brain regions [entorhinal cortex (EC): 39 vs. 39, hippocampus (HP): 74 vs. 65, temporal cortex (TC): 52 vs. 39, and frontal cortex (FC): 104 vs. 128]. Alzdata database has integrated and normalized multiple datasets across platforms, providing a reliable data resource for the exploration of pathological mechanisms and drug development related to AD. Specifically, individual expression datasets for each brain region were normalized, log₂ transformed, and then merged by the compact algorithm in the ComBat R package to eliminate batch effects (Xu et al., 2018). Peripheral blood cells transcriptomes were obtained from GSE63060 (Ctrl = 104, MCI = 80, AD = 145) and GSE63061 (Ctrl = 134, MCI = 109, AD = 139), which were two large European blood cells RNA expression datasets, and contributing to find reliable peripheral biomarkers for AD diagnosis. Differentially expressed genes (DEGs) were identified using limma package in R, which included linear regression analysis adjusted for age and gender. The mRNA expression profile and A β /Tau pathological scores in AD model mice were obtained from Mouse Dementia

Network (Matarin et al., 2015).² The data required for MMSE correlation analysis were obtained from GSE1297.

Correlation of potential genes with A β , Tau and MMSE

To confirm the association between brain biomarkers and A β /Tau pathology, we performed correlation analysis between the mRNA expression of related genes and A β /Tau pathology scores in AD mouse brain. Specifically, immunofluorescence was applied to perform A β ₄₀ and phosphorylated Tau semi-quantitative analysis, of which 56 brain tissue samples with A β pathological grading, and 14 brain tissue samples with Tau pathological grading. In addition, to confirm the association between brain biomarkers and cognitive decline, we performed correlation analysis between mRNA expression of related genes and MMSE scores in AD patients (GSE1297: Ctrl = 9, MCI = 22). Graphpad prism software (Graphpad software, La Jolla, CA, USA) was used for Pearson correlation analysis between gene mRNA expression and A β /Tau pathology, MMSE scores.

Statistical analysis

Differentially expressed genes (DEGs) were identified using limma package in R,³ which included linear regression analysis adjusted for age and gender, and *P*-values < 0.05 was considered to be significant.

Results

The mRNA expression levels of TCA cycle genes in brain tissues from 269 AD patients and 271 controls

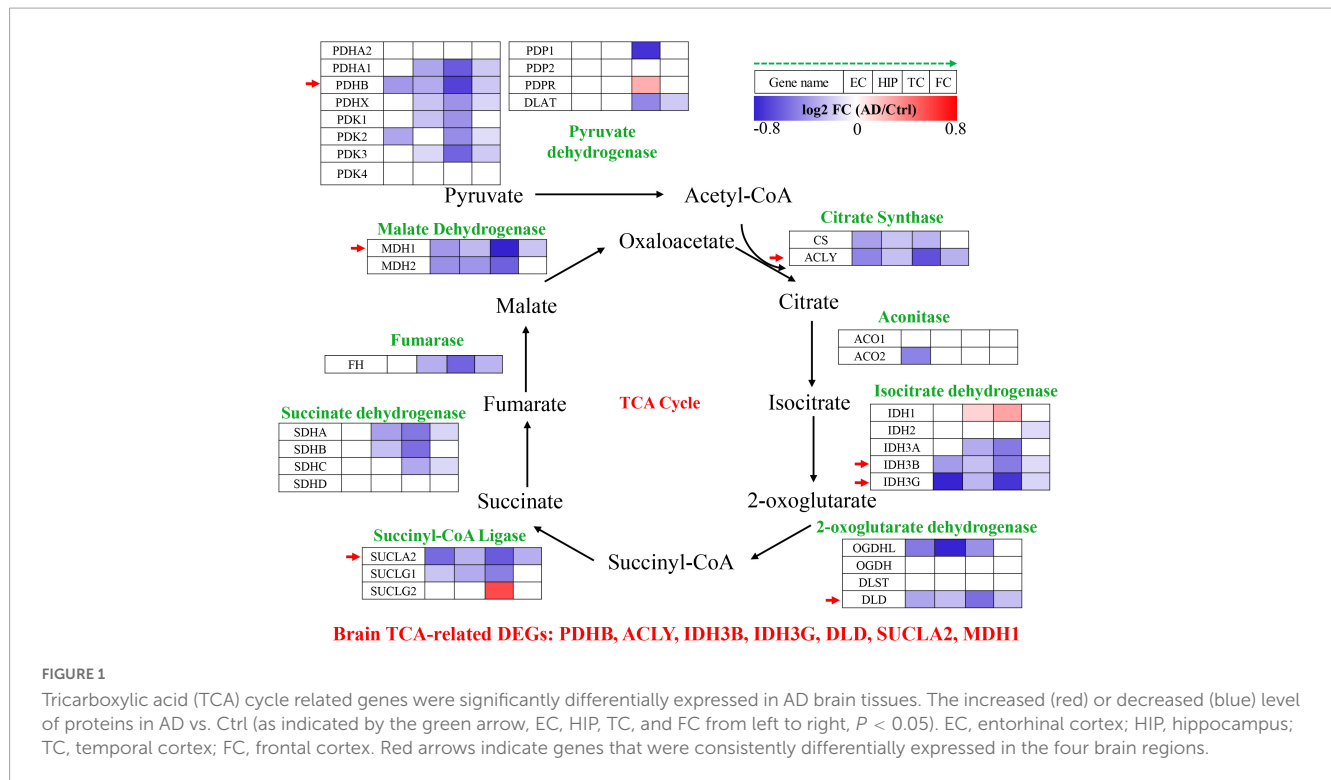
The TCA cycle consists 35 genes, forming 9 key enzyme complexes that collectively drive the conversion of pyruvate to acetyl-CoA and complete oxidation. Brain data were obtained from the Alzdata database (see text footnote 1) (Xu et al., 2018), for a total of 269 AD patients and 271 normal aging subjects, including four brain regions (entorhinal cortex, hippocampus, temporal cortex, and frontal cortex). The mRNA expression levels of TCA cycle genes in brain tissues were shown in **Figure 1** and **Table 1**.

Pyruvate dehydrogenase complex (PDHC) mainly promotes the conversion of pyruvate to acetyl-CoA, which then enters the tricarboxylic acid cycle (Seifert et al., 2007). Pyruvate dehydrogenase E1 component subunit α and β (PDHA1, PDHB), pyruvate dehydrogenase protein X component (PDHX), [pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme (PDK1, PDK2, PDK3), and dihydrolipoyllysine S-Acetyltransferase (DLAT) were significantly decreased in multiple brain regions of AD. PDHB showed the greatest perturbation, which was significantly decreased

¹ <http://www.alzdata.org/>

² www.mouseac.org

³ <https://www.bioconductor.org/packages/release/bioc/html/limma.html>



in EC, HIP, TC and FC. Citrate synthase [CS, ATP-citrate synthase (ACLY)] acts synergistically to regulate the conversion balance of citrate to oxaloacetate and acetyl-CoA (Marin-Garcia et al., 1998; Potapova et al., 2000), which were also significantly reduced in multiple brain regions of AD. Aconitase (ACO1, ACO2) promoted citrate to isocitrate conversion, which were not changed significantly in AD brain, only ACO2 was decreased significantly in EC region. Isocitrate dehydrogenase mainly including IDH1, IDH2, IDH3A, IDH3B, IDH3G were significantly altered in AD brain. IDH1 was significantly elevated in HIP and TC brain regions, and IDH2 was significantly decreased only in FC brain region. Notably, both IDH3B and IDH3G were significantly decreased in the four brain regions EC, HIP, TC and FC. Moreover, 2-oxoglutarate dehydrogenase (OGDHL, DLD), succinyl-CoA ligase (SUCLA2, SUCLG1), succinate dehydrogenase [succinate dehydrogenase [ubiquinone] flavoprotein subunit (SDHA), succinate dehydrogenase [ubiquinone] iron-sulfur subunit (SDHB), SDHC], fumarase (FH), and malate dehydrogenase (MDH1, MDH2) were significantly decreased in multiple brain regions of AD.

Altogether, TCA cycle related genes were consistently down-regulated in the AD brain, PDHB, ACLY, IDH3B, IDH3G, DLD, SUCLA2, and MDH1 were significantly decreased in the four brain regions EC, HIP, TC and FC, which may be potential brain biomarkers (Figure 1).

Correlation of brain biomarkers with Aβ and Tau pathology in AD mouse models

To determine the association between brain biomarkers and AD progression, we analyzed the expression of brain biomarkers in AD mice and correlated them with Aβ and Tau pathology (Figure 2A). Pearson correlation analysis found that the IDH3B,

IDH3G, SUCLA2, MDH1, PDHB showed moderate negative correlations with Aβ pathology ($|r| = 0.189-0.439, p < 0.05$; Figure 2B), and strong negative correlations with Tau pathology ($|r| = 0.653-0.847, p < 0.05$; Figure 2C), implying that their reduction significantly exacerbates AD pathology.

Correlation of brain TCA-related DEGs with MMSE scores in AD patients

To confirm the association between brain biomarkers and cognitive decline, we performed correlation analysis between mRNA expression of related genes and MMSE scores in AD patients (GSE1297: Ctrl = 9, MCI = 22; Figure 3A). Pearson correlation analysis found that the IDH3G, DLD, SUCLA2, MDH1 showed moderate positive correlations with MMSE scores ($r = 0.365-0.441, p < 0.05$; Figure 3B), suggesting that their expression can effectively track the progression of cognitive decline.

The mRNA expression levels of TCA cycle genes in peripheral blood of patients with MCI and AD and Ctrl

Peripheral blood cells transcriptomes were obtained from GSE63060 (Ctrl = 104, MCI = 80, AD = 145) and GSE63061 (Ctrl = 134, MCI = 109, AD = 139), which were two large European blood cells RNA expression datasets. The expression of TCA cycle genes in the peripheral blood of AD patients were shown in Figure 4 and Table 2, with 16 genes significantly reduced and 11 genes significantly increased, which may be affected by the complex microenvironment of the peripheral system. Specifically, citrate synthase (CS), aconitase (ACO1, ACO2) and isocitrate

TABLE 1 The mRNA expression levels of TCA cycle genes in brain tissues from 269 AD patients and 271 controls.

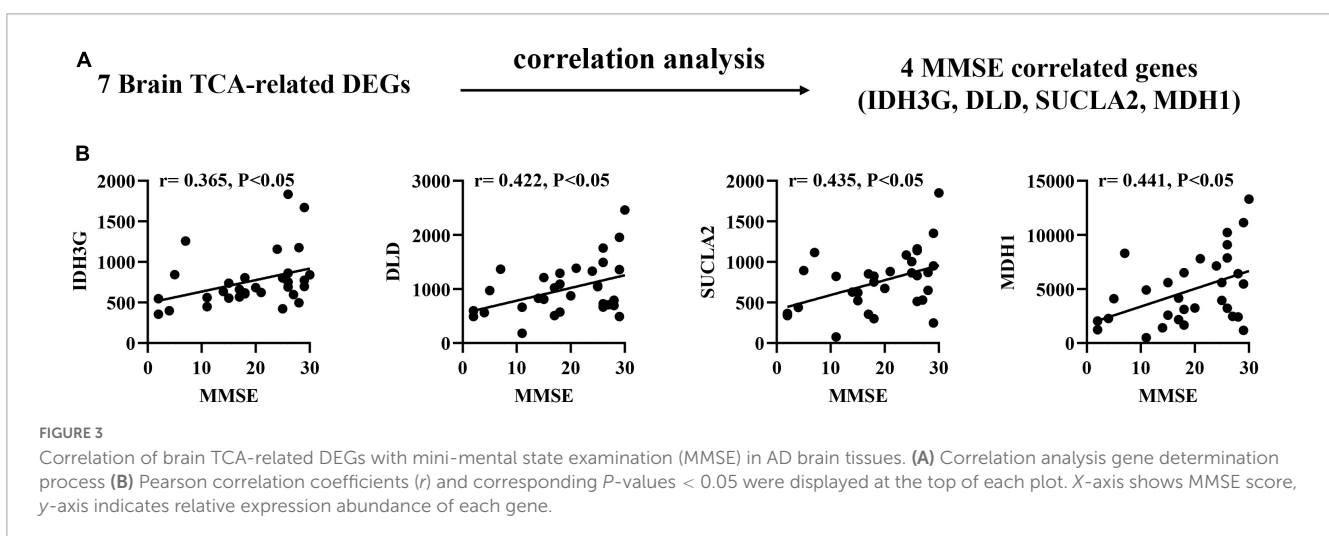
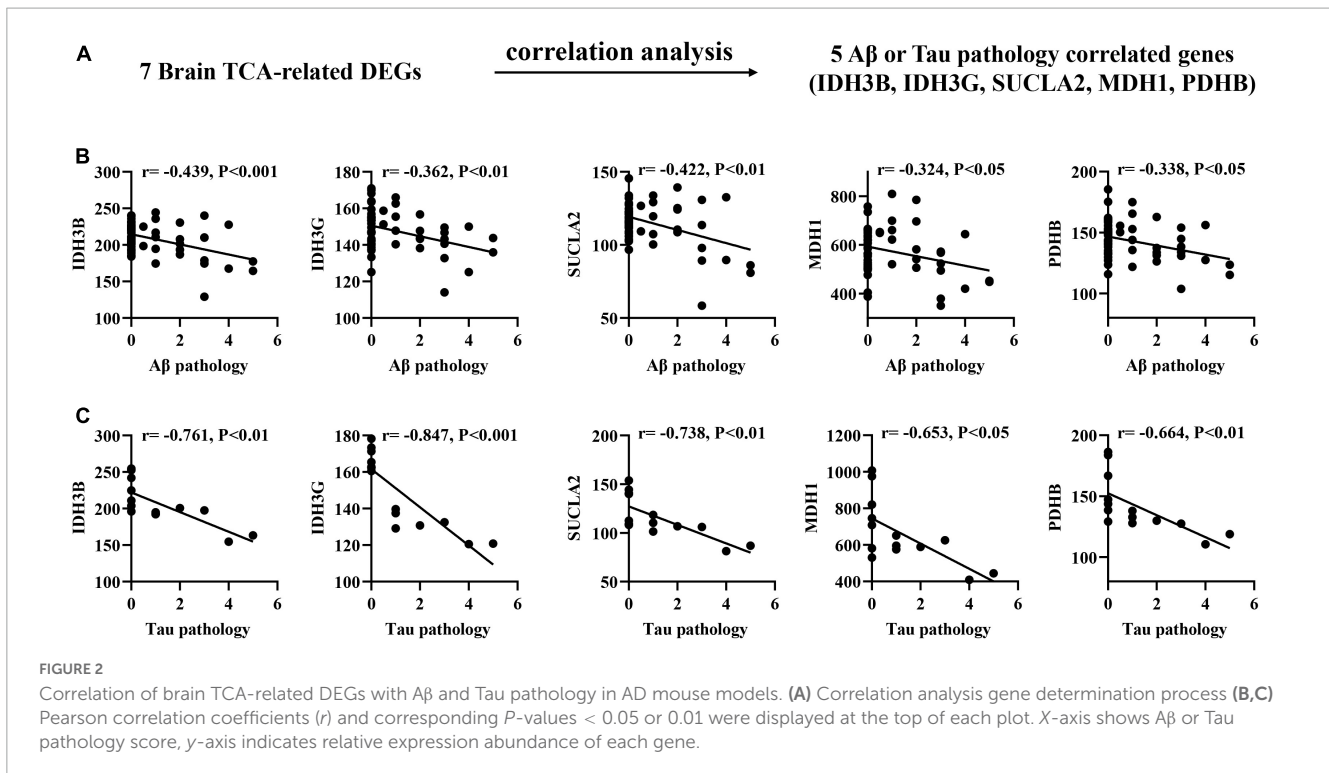
Brain region	Entorhinal cortex			Hippocampus			Temporal cortex			Frontal cortex		
Gene	log2 FC	p-value	adj p-value	log2 FC	p-value	adj p-value	log2 FC	p-value	adj p-value	log2 FC	p-value	adj p-value
Pyruvate dehydrogenase												
PDHA2	0.17	1.81E-01	3.57E-01	0.22	5.80E-02	2.20E-01	0.09	7.28E-01	8.59E-01	0.07	4.72E-01	8.34E-01
PDHA1	-0.11	1.95E-01	3.74E-01	-0.32	3.24E-05	3.00E-03	-0.59	5.96E-05	1.00E-03	-0.2	4.32E-04	6.00E-03
PDHB	-0.37	4.00E-03	3.70E-02	-0.3	1.00E-03	1.60E-02	-0.68	2.97E-05	1.00E-03	-0.2	1.00E-03	1.00E-02
PDHX	-0.15	1.37E-01	2.99E-01	-0.21	1.70E-02	1.07E-01	-0.39	1.00E-03	1.00E-02	-0.15	3.20E-02	1.05E-01
PDK1	-0.14	2.15E-01	3.97E-01	-0.22	5.00E-03	5.50E-02	-0.39	9.00E-03	4.50E-02	-0.04	5.69E-01	7.14E-01
PDK2	-0.32	5.00E-03	3.80E-02	-0.09	3.64E-01	6.14E-01	-0.41	1.59E-04	3.00E-03	-0.12	2.90E-02	9.90E-02
PDK3	-0.14	3.15E-01	5.04E-01	-0.14	8.00E-02	2.64E-01	-0.56	2.74E-05	1.00E-03	-0.19	2.20E-02	3.38E-01
PDK4	0.22	3.04E-01	4.94E-01	0.11	4.68E-01	6.97E-01	0.35	1.25E-01	2.97E-01	0.09	4.69E-01	6.29E-01
PDP1	NA	NA	NA	-0.13	1.76E-01	4.13E-01	-0.74	1.27E-04	2.00E-03	-0.05	7.23E-01	9.27E-01
PDP2	-0.22	2.43E-01	4.27E-01	0.07	6.30E-01	8.10E-01	0.04	8.68E-01	9.38E-01	NA	NA	NA
Pyruvate dehydrogenase phosphatase regulatory subunit (PDPB)	0.12	2.71E-01	4.59E-01	-0.07	5.27E-01	7.40E-01	0.26	2.30E-02	9.00E-02	0.06	4.05E-01	5.70E-01
DLAT	-0.14	3.94E-01	5.80E-01	-0.14	1.12E-01	3.22E-01	-0.43	5.72E-05	1.00E-03	-0.19	9.00E-03	4.60E-02
Citrate synthase												
CS	-0.34	1.00E-03	1.20E-02	-0.21	1.00E-03	1.40E-02	-0.27	1.50E-02	6.80E-02	-0.03	5.37E-01	6.87E-01
ACLY	-0.44	3.00E-03	2.90E-02	-0.23	2.00E-03	3.10E-02	-0.63	4.90E-05	1.00E-03	-0.28	1.83E-05	1.00E-03
Aconitase												
ACO1	0.01	9.11E-01	9.49E-01	-0.12	2.21E-01	4.69E-01	0.15	2.18E-01	4.28E-01	-0.03	3.74E-01	5.37E-01
ACO2	-0.45	4.50E-05	3.00E-03	-0.17	1.39E-01	3.63E-01	-0.24	1.45E-01	3.29E-01	-0.08	1.49E-01	2.92E-01
Isocitrate dehydrogenase												
IDH1	0	9.64E-01	9.81E-01	0.14	3.20E-02	1.58E-01	0.29	1.30E-02	6.00E-02	0.09	6.20E-02	1.65E-01
IDH2	-0.13	2.83E-01	4.71E-01	-0.12	3.07E-01	5.60E-01	0.06	5.96E-01	7.75E-01	-0.13	4.70E-02	1.37E-01
IDH3A	-0.19	5.20E-02	1.62E-01	-0.3	1.00E-03	1.80E-02	-0.48	1.71E-04	3.00E-03	-0.08	1.37E-01	2.77E-01
IDH3B	-0.36	2.00E-03	2.10E-02	-0.23	2.00E-03	2.60E-02	-0.47	3.72E-05	1.00E-03	-0.13	2.60E-02	9.20E-02
IDH3G	-0.79	7.72E-06	1.00E-03	-0.26	6.00E-03	5.70E-02	-0.73	1.65E-07	4.32E-05	-0.15	2.00E-03	2.00E-02

(Continued)

TABLE 1 (Continued)

Brain region	Entorhinal cortex			Hippocampus			Temporal cortex			Frontal cortex		
Gene	log2 FC	<i>p</i> -value	adj <i>p</i> -value	log2 FC	<i>p</i> -value	adj <i>p</i> -value	log2 FC	<i>p</i> -value	adj <i>p</i> -value	log2 FC	<i>p</i> -value	adj <i>p</i> -value
2-oxoglutarate dehydrogenase												
OGDHL	-0.48	4.69E-04	1.00E-02	-0.79	8.03E-07	1.00E-03	-0.4	1.50E-02	6.50E-02	-0.14	9.30E-02	2.11E-01
OGDH	0.19	1.20E-01	2.74E-01	-0.15	1.36E-01	3.59E-01	0	9.87E-01	9.95E-01	0.08	2.03E-01	3.60E-01
DLST	NA	NA	NA	-0.11	8.80E-02	2.79E-01	0.01	8.86E-01	9.46E-01	-0.04	5.24E-01	8.59E-01
DLD	-0.32	2.60E-02	1.07E-01	-0.24	5.00E-03	5.50E-02	-0.52	6.80E-05	1.00E-03	-0.23	2.00E-03	2.00E-02
Succinyl-CoA ligase												
SUCLA2	-0.54	3.00E-03	2.70E-02	-0.28	9.00E-03	7.50E-02	-0.59	1.00E-03	8.00E-03	-0.29	3.00E-03	2.30E-02
SUCLG1	-0.21	3.60E-02	1.31E-01	-0.3	1.97E-05	2.00E-03	-0.46	1.00E-03	6.00E-03	-0.05	5.44E-01	6.93E-01
SUCLG2	NA	NA	NA	0.07	4.52E-01	6.86E-01	0.57	1.00E-03	1.20E-02	0.1	3.82E-01	7.87E-01
Succinate dehydrogenase												
SDHA	-0.14	1.21E-01	2.77E-01	-0.34	1.00E-03	1.70E-02	-0.49	1.10E-04	2.00E-03	-0.15	2.00E-03	1.90E-02
SDHB	-0.16	8.90E-02	2.26E-01	-0.23	2.40E-02	1.32E-01	-0.52	2.00E-03	1.30E-02	-0.06	3.35E-01	5.00E-01
SDHC	-0.02	7.96E-01	8.81E-01	-0.08	2.44E-01	4.94E-01	-0.31	4.00E-03	2.60E-02	-0.14	2.00E-03	1.50E-02
Succinate dehydrogenase [ubiquinone] cytochrome b small subunit (SDHD)	-0.05	6.48E-01	7.84E-01	-0.03	7.26E-01	8.66E-01	-0.15	2.80E-01	5.02E-01	0.02	7.53E-01	8.46E-01
Fumarase												
FH	-0.08	4.48E-01	6.28E-01	-0.29	4.00E-03	4.40E-02	-0.56	6.61E-05	1.00E-03	-0.27	4.79E-04	1.22E-01
Malate dehydrogenase												
MDH1	-0.37	5.00E-03	3.80E-02	-0.25	4.00E-03	4.30E-02	-0.8	3.94E-06	2.40E-04	-0.21	2.20E-02	8.30E-02
MDH2	-0.4	1.00E-03	1.80E-02	-0.38	7.97E-06	1.00E-03	-0.57	1.79E-05	1.00E-03	-0.14	5.90E-02	1.60E-01

The mRNA expression levels of TCA cycle genes in brain tissues were obtained from the Alzdata database (<http://www.alzdata.org/>) (Xu et al., 2018). Log2FC, log2 fold change.



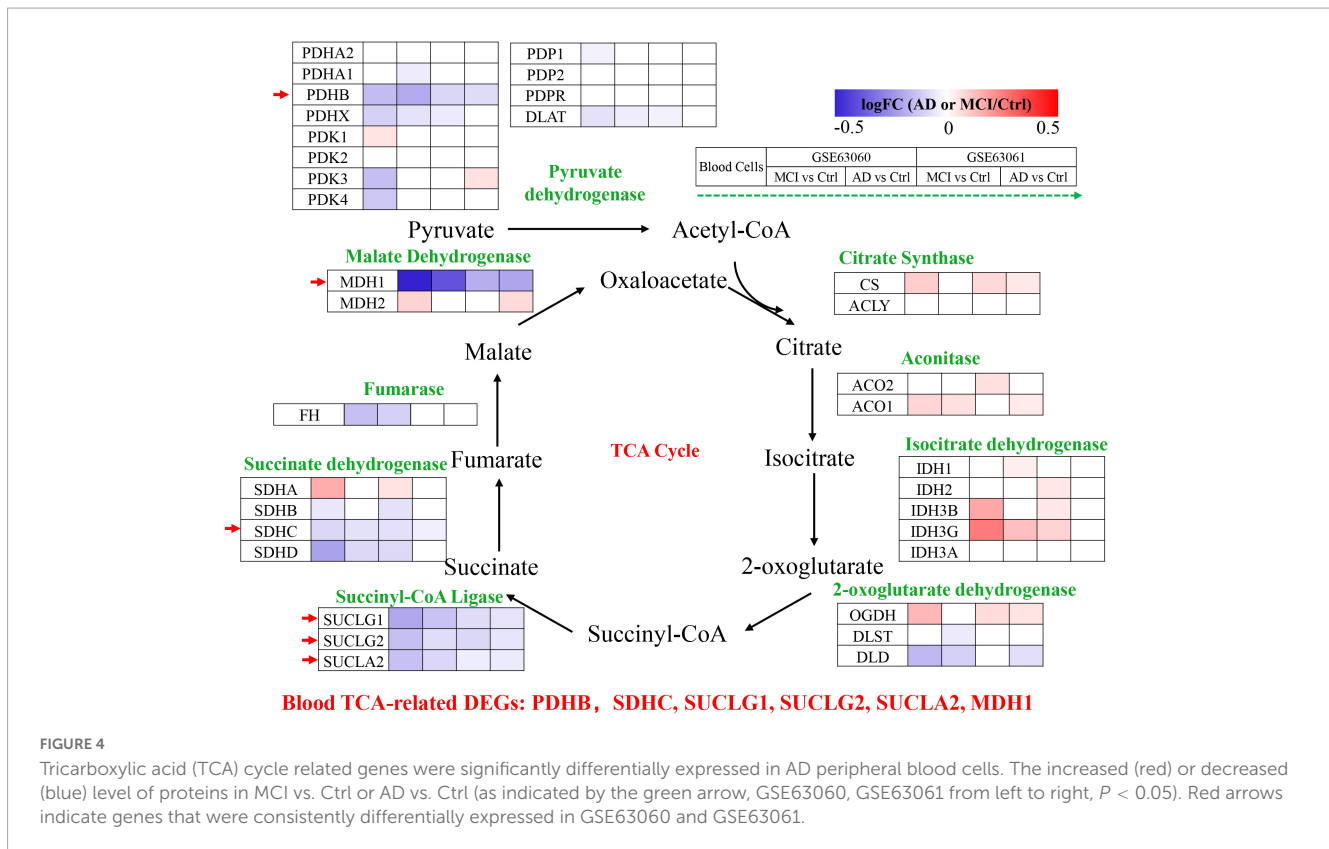
dehydrogenase (IDH1, IDH2, IDH3A, IDH3B) were significantly increased. Pyruvate dehydrogenase (PDHB), succinyl-CoA ligase (SUCLG1, SUCLG2, SUCLA2), succinate dehydrogenase (SDHC), and malate Dehydrogenase (MDH1) showed consistent decrease in peripheral blood cells of MCI and AD in both GSE63060 and GSE63061 cohorts, which may be good peripheral blood biomarkers for early diagnosis of AD.

The mRNA expression of TCA cycle genes in AD patients was systematically revealed by brain and peripheral blood cells transcriptome data, and combined with MMSE, A β /Tau correlation analysis to confirm that SUCLA2, MDH1, and PDHB genes may be candidate biomarkers that can effectively track the pathological and clinical manifestations of AD patients (Figure 5A). By analyzing the transcriptome data of GSE63060 and GSE63061 peripheral blood cells, we found that pyruvate dehydrogenase (PDHB),

succinyl-CoA ligase (SUCLA2) and malate dehydrogenase (MDH1) showed consistent decrease in peripheral blood cells of MCI and AD (Figures 5B, C), which were consistent with the trend in the central system.

Discussion

Energy metabolism disorder is one of the earliest symptoms in AD and MCI, and it may precede the appearance of clinical cognitive impairment (Cunnane et al., 2011; Ding et al., 2013). Positron emission tomography (PET) imaging combined with 18F-2-deoxy-2-fluoro-d-glucose have shown that regional glucose utilization in AD patients is significantly decreased, and it was significantly correlated with MMSE scores, indicating that



cognitive impairment was closely related to metabolic impairment in AD (de Leon et al., 1983). The susceptible areas of glucose metabolism decline in the brain of AD patients, including the posterior cingulate, parietal, and temporal cortices (Reiman et al., 1996; Small et al., 2000). Here, we systematically analyzed the expression of TCA cycle-related genes through large-scale mRNA expression profiling data in AD brain tissues, and showed that the overall expression of TCA cycle-related genes was decreased, suggesting that the TCA cycle was impaired in AD brain. Then, correlation analysis of A β , Tau and MMSE was performed to identify the molecules closely related to AD pathology and clinical manifestations. Finally, combined with the transcriptomics of peripheral blood cells from multiple AD cohorts, we found that PDHB, SUCLA2, and MDH1 can be used as candidate biomarkers for early diagnosis of AD.

Previous studies have shown that TCA cycle related genes were significantly decreased in AD brain (Bubber et al., 2005; Sang et al., 2022). Glucose is the main energy source for the brain, PDHC can convert pyruvate oxidative decarboxylation to acetyl-CoA, and reduced levels of PDHC result in less carbon entering the TCA cycle (Garabadu et al., 2019). Decreased PDHC leads to decreased acetyl-CoA production, which is a necessary raw material for acetylcholine production, and the correlation between acetyl-CoA and acetylcholine has been well established (Perry et al., 1980), so that decreased PDHC leads to decreased acetylcholine production. The loss of acetylcholine, which leads to cholinergic neuron loss, may further contribute to the loss of TCA cycle enzymes. TCA cycle enzymes deficiency leads to an increase in reactive oxygen species (ROS), which accelerates neurodegeneration (Klivenyi et al., 2004). In addition, pyruvate dehydrogenase deficiency is known to

cause abnormal mitochondrial metabolism, lactate accumulation, and thus neuropathy (Patel et al., 2012). Here, we found that pyruvate dehydrogenase related genes PDHA1, PDHB, PDHX, DLAT showed consistent down-regulation in AD brain and blood cells, suggesting that pyruvate dehydrogenase deficiency may participate in AD-like nerve injury by regulating acetyl-CoA production leading to TCA cycle and acetylcholine production disorder.

Alpha-ketoglutarate dehydrogenase complex (α KGDHc), a key rate-limiting enzyme in the TCA cycle, can convert alpha-ketoglutarate, coenzyme A and NAD + to succinyl-coenzyme A, NADH and CO₂, and its activity is significantly reduced in AD (Sheu et al., 1994; Stempler et al., 2014). Cholinergic neurons are crucial for α KGDHc defects are extremely sensitive, and α KGDHc mutations lead to increased ROS production and plays an important role in neurotoxicity (Sheu and Blass, 1999). Studies have shown that the decreased activity of α KGDHc is significantly correlated with cognitive decline in AD (Bubber et al., 2005; Shi et al., 2008). DLD deficient mice have aggravated mitochondrial damage and reduced neurogenesis (Klivenyi et al., 2004), and DLD inhibition can exacerbate Tau phosphorylation, thereby promoting the development of AD pathology (Ahmad, 2018). Dihydrolypoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex (DLST) deficiency can exacerbate amyloid protein deposition and memory damage in amyloid-beta precursor protein (APP)/PS1 mice (Dumont et al., 2009). In addition, the expression of OGDHL was significantly reduced in 3xTg-AD mice, while overexpression of OGDHL could reduce neuroinflammation, amyloid plaques, and Tau phosphorylation (Yao et al., 2022). Here, we found that DLD

TABLE 2 The mRNA expression levels of TCA cycle genes in peripheral blood cells of patients with MCI and AD and Ctrl.

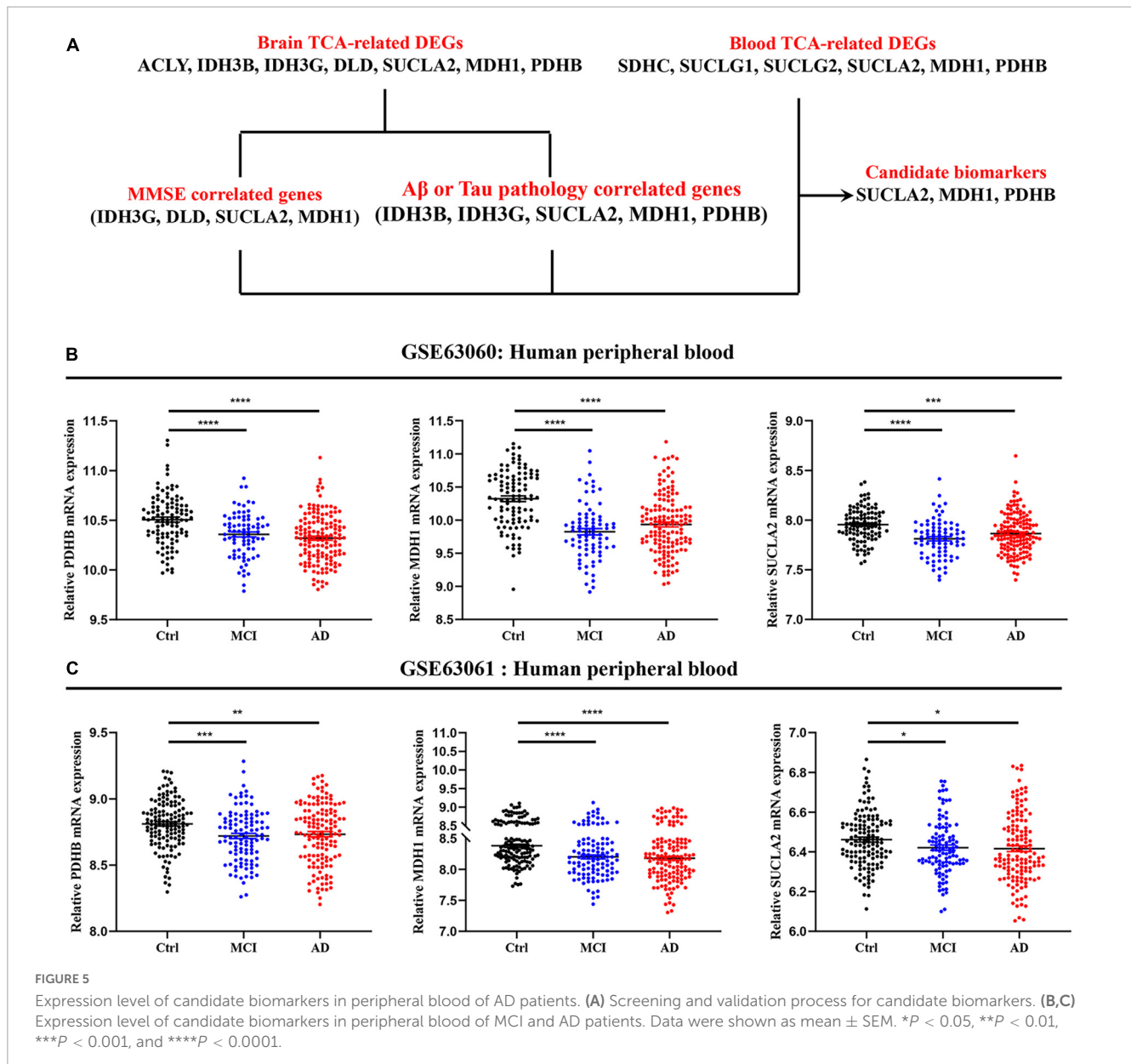
Blood cells	GSE63060						GSE63061					
	MCI vs. Ctrl			AD vs. Ctrl			MCI vs. Ctrl			AD vs. Ctrl		
Gene	logFC	p-value	adj p-value	logFC	p-value	adj p-value	logFC	p-value	adj p-value	logFC	p-value	adj p-value
Pyruvate dehydrogenase												
PDHA2	0.010	2.59E-01	5.16E-01	-0.008	3.15E-01	6.73E-01	-0.008	1.14E-01	3.74E-01	0.002	6.63E-01	8.86E-01
PDHA1	-0.037	8.72E-02	2.57E-01	-0.042	1.57E-02	1.16E-01	0.004	8.50E-01	9.50E-01	0.006	7.26E-01	9.13E-01
PDHB	-0.149	4.64E-05	5.60E-04	-0.187	1.48E-08	1.08E-06	-0.091	2.60E-04	9.11E-03	-0.077	2.33E-03	3.15E-02
PDHX	-0.103	1.13E-06	2.34E-05	-0.063	9.57E-04	1.31E-02	-0.045	3.59E-03	4.40E-02	-0.025	9.70E-02	3.73E-01
PDK1	0.057	4.39E-03	2.59E-02	0.030	7.99E-02	3.38E-01	0.001	9.47E-01	9.83E-01	0.007	5.20E-01	8.20E-01
PDK2	0.000	9.88E-01	9.96E-01	-0.007	4.91E-01	8.03E-01	0.013	3.78E-01	6.86E-01	0.002	8.83E-01	9.68E-01
PDK3	-0.146	7.89E-05	8.83E-04	0.050	5.40E-02	2.67E-01	0.041	5.90E-02	2.54E-01	0.060	1.50E-02	1.17E-01
PDK4	-0.122	1.12E-03	8.41E-03	-0.055	1.11E-01	4.07E-01	-0.060	9.48E-02	3.37E-01	-0.017	6.23E-01	8.69E-01
PDP1	-0.031	1.90E-03	1.30E-02	-0.013	1.66E-01	5.00E-01	-0.005	7.05E-01	8.87E-01	-0.010	4.34E-01	7.66E-01
PDP2	-0.011	3.29E-01	5.91E-01	-0.008	4.28E-01	7.63E-01	-0.011	1.72E-01	4.64E-01	-0.009	8.91E-02	3.55E-01
PDPR	0.021	1.09E-01	2.99E-01	0.010	3.85E-01	7.32E-01	-0.005	5.20E-01	7.91E-01	-0.005	5.46E-01	8.32E-01
DLAT	-0.067	5.84E-04	4.85E-03	-0.040	1.85E-02	1.30E-01	-0.030	3.26E-02	1.78E-01	-0.024	7.96E-02	3.33E-01
Citrate synthase												
CS	0.099	3.48E-04	3.11E-03	0.016	5.35E-01	8.29E-01	0.075	5.45E-03	5.76E-02	0.046	4.74E-02	2.44E-01
ACLY	0.057	5.99E-02	1.97E-01	0.039	1.64E-01	4.98E-01	0.028	1.44E-01	4.23E-01	0.021	2.68E-01	6.26E-01
Aconitase												
ACO1	0.084	2.39E-04	2.25E-03	0.061	1.98E-03	2.34E-02	0.026	1.31E-01	4.01E-01	0.044	7.21E-03	7.00E-02
ACO2	0.037	2.28E-01	4.78E-01	0.005	8.57E-01	9.60E-01	0.061	4.01E-03	4.71E-02	0.015	4.78E-01	7.94E-01
Isocitrate dehydrogenase												
IDH1	-0.048	1.35E-01	3.43E-01	0.037	4.75E-02	2.46E-01	-0.011	4.91E-01	7.71E-01	0.025	2.70E-01	6.28E-01
IDH2	0.03	3.56E-01	6.17E-01	-0.015	6.07E-01	8.62E-01	0.048	1.46E-02	1.07E-01	0.03	9.51E-02	3.68E-01
IDH3A	0.008	7.38E-01	8.82E-01	-0.02	3.32E-01	6.89E-01	0.0086	5.68E-01	8.19E-01	-0.016	2.54E-01	6.11E-01
IDH3B	0.174	9.53E-09	4.26E-07	0.049	8.94E-02	3.62E-01	0.05	2.88E-02	1.66E-01	-0.006	7.83E-01	9.34E-01
IDH3G	0.262	3.93E-10	3.13E-08	0.129	3.97E-04	6.47E-03	0.089	7.68E-04	1.74E-02	0.047	5.83E-02	2.78E-01

(Continued)

TABLE 2 (Continued)

Blood cells	GSE63060						GSE63061						
	MCI vs. Ctrl			AD vs. Ctrl			MCI vs. Ctrl			AD vs. Ctrl			
	Gene	logFC	p-value	adj p-value	logFC	p-value	adj p-value	logFC	p-value	adj p-value	logFC	p-value	adj p-value
2-oxoglutarate dehydrogenase													
OGDHL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OGDH	0.141	1.69E-04	1.68E-03	0.047	1.40E-01	4.62E-01	0.069	3.66E-03	4.44E-02	0.055	8.00E-03	7.49E-02	
DLST	-0.017	4.34E-01	6.84E-01	-0.046	1.10E-02	8.95E-02	-0.008	6.24E-01	8.49E-01	-0.0045	7.60E-01	9.25E-01	
DLD	-0.158	2.27E-08	8.73E-07	-0.108	6.49E-06	2.07E-04	-0.039	8.51E-02	3.16E-01	-0.07	8.04E-04	1.48E-02	
Succinyl-CoA ligase													
SUCLA2	-0.141	2.18E-07	5.75E-06	-0.089	2.28E-04	4.14E-03	-0.04	2.44E-02	1.49E-01	-0.045	1.77E-02	1.30E-01	
SUCLG1	-0.197	6.50E-13	2.62E-10	-0.135	4.03E-10	4.66E-08	-0.077	1.40E-04	6.53E-03	-0.059	3.41E-03	4.11E-02	
SUCLG2	-0.142	1.32E-05	1.89E-04	-0.076	1.55E-02	1.15E-01	-0.09	1.04E-04	5.52E-03	-0.058	9.54E-03	8.49E-02	
Succinate dehydrogenase													
SDHA	0.163	1.93E-07	5.20E-06	0.057	5.54E-02	2.71E-01	0.058	6.30E-03	6.32E-02	0.025	1.96E-01	5.39E-01	
SDHB	-0.053	3.65E-02	1.37E-01	0.008	7.33E-01	9.16E-01	-0.067	2.36E-04	8.59E-03	-0.009	6.07E-01	8.63E-01	
SDHC	-0.089	6.69E-05	7.66E-04	-0.065	4.74E-04	7.49E-03	-0.068	2.18E-06	6.36E-04	-0.036	9.77E-03	8.63E-02	
SDHD	-0.209	2.03E-05	2.75E-04	-0.087	4.42E-02	2.36E-01	-0.084	3.39E-03	4.23E-02	-0.012	6.54E-01	8.82E-01	
Fumarase													
FH	-0.143	6.50E-04	5.30E-03	-0.105	5.33E-03	5.16E-02	0.011	5.39E-01	8.03E-01	0.022	1.94E-01	5.36E-01	
Malate dehydrogenase													
MDH1	-0.499	3.29E-13	1.71E-10	-0.389	4.35E-11	6.46E-09	-0.18	1.15E-05	1.71E-03	-0.201	1.51E-06	1.43E-04	
MDH2	0.089	1.12E-02	5.51E-02	0.06	6.28E-02	2.93E-01	0.035	1.71E-01	4.63E-01	0.074	1.87E-03	2.69E-02	

Peripheral blood cells transcriptomes were obtained from GSE63060 (Ctrl = 104, MCI = 80, AD = 145) and GSE63061 (Ctrl = 134, MCI = 109, AD = 139). Ctrl, normal cognition controls group; MCI, mild cognitive impairment; Log2FC, log2 fold change.



was significantly decreased in the brain and blood cells of AD patients, and significantly positively correlated with MMSE scores, suggesting that it may not only be involved in the pathological development of AD, but also may mediate clinical cognitive impairment.

Succinyl-CoA ligase (SCL) catalyzes substrate level phosphorylation, and the related subunits are significantly reduced in AD, especially SUCLA2 (Sang et al., 2022). Succinyl-CoA and Glycine, as precursors, are involved in the synthesis of heme (Ponka, 1999). The levels of enzymes involved in the TCA cycle decrease, leading to a decrease in succinyl-CoA, which in turn decreases the ability to synthesize heme (Atamna and Frey, 2007). When heme deficiency occurs, key cellular pathological phenotypes of AD are produced (Atamna et al., 2001, 2002; Scheuermann et al., 2001), such as decreased levels of complex IV, iron deposition, mitochondrial dysfunction, APP dimerization, and neuronal death. Here, we found that SUCLA2 was significantly

decreased in AD brain and blood cells, and correlated with A β , Tau and MMSE scores, suggesting that SUCLA2 may be closely related to the pathological development and cognitive impairment of AD, and it may be a potential diagnostic and therapeutic target.

Malate dehydrogenase is involved in two major metabolic processes, namely malate-aspartate shuttle and tricarboxylic acid cycle, which are essential for mitochondrial respiration and adenosine 5'-triphosphate (ATP) production (Friedrich et al., 1988). Previous studies have found that MDH1, as a hub gene, participates in the network regulation of AD and may be crucial for its pathological development (Wang et al., 2017; Liu et al., 2020). MDH1 deficiency can cause severe metabolic disorders, thereby promoting delayed neural development (Broeks et al., 2019). Here, we found that MDH1 was significantly decreased in AD, and correlated with the typical pathologies of AD, and parallel with clinical cognitive decline.

In sum, we systematically analyzed the expression of TCA cycle related genes in the central and peripheral of AD, revealing molecules closely related to AD pathological development and clinical cognitive decline, providing valuable resources for further exploring the specific mechanisms between TCA cycle and AD progression. PDHB, SUCLA2, and MDH1 may be potential diagnostic and therapeutic targets.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

HY and DJ: experimental design and manuscript-writing. HY, DJ, and FW: experimental methods and data analysis. All authors contributed to the article and approved the submitted version.

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