

Blood RNA transcripts show changes in inflammation and lipid metabolism in Alzheimer's disease and mitochondrial function in mild cognitive impairment

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Abstract

Background: Abnormal immunity in the periphery has been reported in the pathogenesis of Alzheimer's disease (AD). **Objective:** In this study, blood transcriptome analyses of patients with AD, those with mild cognitive impairment (MCI) due to AD, and healthy controls were performed to elucidate immune-related pathophysiology.

Methods: The sample included 63 participants from a complete enumeration study of elderly people in Nakayama town (the Nakayama Study), who were over 65 years of age, diagnosed as (1) healthy controls (N = 21, mean age: 83.8 years), (2) having MCI due to AD (N = 20, mean age: 82.6 years), or (3) having AD (N = 21, mean age: 84.2 years). Every participant underwent blood tests, magnetic resonance imaging, and questionnaires about lifestyle and cognitive function. With transcriptome analysis, differential gene expressions in the blood of the three groups were evaluated by gene ontology, pathway enrichment, and ingenuity pathway analyses, and quantitative real-time PCR was performed.

Results: Neutrophil extracellular trap signaling was increased, and lipid metabolism (FXR/RXR activation, triacylglycerol degradation) was decreased in AD, whereas MCI showed protective responses via decreased neutrophil extracellular trap signaling and mitochondrial functions such as upregulation of the sirtuin pathway and downregulation of oxidative stress.

Conclusions: Based on these findings and consistent with other published studies, immune cells appear to have important roles in the pathogenesis of AD, and the transcriptome in blood may be useful as a biomarker for diagnosis via monitoring immunity in MCI and AD.

Keywords

Alzheimer's disease, blood, mild cognitive impairment, immune, lipid, mitochondria, transcriptome

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Introduction

In recent years, disease-modifying drugs for Alzheimer's disease (AD) have emerged, and the importance of biomarkers for early detection and treatment assessment has been reported.¹ Positron emission tomography and spinal fluid testing for amyloid- β and phosphorylated tau have been used for its biomarkers and are highly useful, but they have not yet reached the stage of routine use due to a lack of available facilities, high cost, and high invasiveness.² In contrast, blood biomarkers have the advantage of being less invasive, and their validity has been actively examined in recent years with the aim of generalizing their use.³ Use of whole blood transcriptomic analysis has increased in

recent years due to its advantages in terms of minimal invasiveness and repeatable measurement of a large number of gene expressions. For example, pathway and gene-set

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enrichment analyses of the blood of AD showed differences in immune response-related pathways enriched throughout the analysis, despite different underlying gene sets for different races.^{4,5} The use of small sets of blood transcripts from inflammation, stress, and epigenetics has also shown the potential to distinguish between patients with neurodegenerative diseases, such as AD, and healthy controls.⁶ Inflammation and redox genes selected from whole blood transcriptomic analysis have also been suggested as potentially useful biomarkers for monitoring anti-inflammatory therapy in mild AD.⁷ Although still small, the number of studies of whole blood transcriptomic analysis of AD is increasing and mainly detects immune abnormalities, but it is hoped that AD of varying stages in different races will be studied cross-sectionally and longitudinally.

Recently, it has been suggested that immune cells are not only biomarkers for AD, but are also involved in the pathogenesis of the disease.^{8,9} For example, the migration of peripherally derived immune cells, such as T cells and neutrophils, to the brain has been shown to be involved in brain aging and neurodegeneration.^{10–12} Serum C-reactive protein (CRP) and inflammatory cytokines, indicators of systemic inflammation, have been shown to be elevated in AD.^{13,14} We have recently reported that elevated serum high-sensitivity CRP levels were associated with AD prevalence and temporal lobe atrophy in a large cohort study.¹⁵ It has also been suggested that anti-inflammatory agents may slow the progression of AD.^{16,17} Recent data suggest that peripheral blood may be an appropriate proxy for gene expression profiles identified in AD, such as aberrant

immune and inflammatory system function and mitochondrial dysfunction.^{9,18–23} Transcriptomic studies using post-mortem AD brains also showed alterations in pathways including cell proliferation, apoptosis, immune response, synaptic transmission, and energy metabolism.^{24,25} The genetic etiology of AD using genome-wide association study (GWAS) data also confirmed the involvement of immunity in AD.²⁶ These results suggest that abnormal immunity in the periphery is involved in the pathogenesis of AD, and blood gene expression analysis is useful both for identifying biomarkers and for studying the pathogenesis of AD.

In this study, blood transcriptome analyses of patients with AD, those with mild cognitive impairment (MCI) due to AD, and healthy controls were performed to elucidate immune-related pathophysiology.

Methods

Participants

The sample included 62 participants from a complete enumeration study of elderly people over 65 years of age in Nakayama town, Ehime prefecture, Japan (the 5th Nakayama Study, in which 927 community-dwelling older persons living in the area participated)^{27–29} who were clinically diagnosed as (1) healthy controls (N = 21), (2) having MCI due to AD (N = 20), or (3) having AD (N = 21) (Table 1). There were 20 samples of patients diagnosed with MCI due to AD. Samples of 21 patients with

Table 1. Participants' characteristics.

	Control	MCI	AD	P
N	21	20	21	
Age (SD) y	83.8 (4.6)	82.6 (4.9)	84.2 (4.6)	0.50
Male (%)	10 (47.6)	8 (40.0)	11 (52.4)	0.73
Height (SD) cm	150.0 (7.6)	150.7 (8.5)	146.7 (9.9)	0.28
Weight (SD) kg	53.2 (9.0)	52.7 (10.6)	51.3 (8.0)	0.79
BMI (SD) kg/m ²	23.7 (3.8)	23.1 (3.3)	23.9 (3.0)	0.75
Education (N)				
Finished ≤ primary school	1	1	5	0.07
Finished ≤ junior high school	10	14	13	
Finished ≤ high school	8	5	3	
Finished ≤ college, university	2	0	0	
MMSE (SD)	29.1 (0.8)	25.5 (1.8)	19.0 (3.5)	<0.01
GDS15 (SD)	2.0 (2.1)	3.2 (2.6)	3.3 (3.2)	0.20
Medical history N (%)				
Hypertension	15 (71.4)	13 (65.0)	12 (57.1)	0.63
Diabetes mellitus	3 (14.3)	2 (10.0)	4 (19.0)	0.71
Hyperlipidemia	10 (47.6)	5 (25.0)	4 (19.0)	0.11
Drinking	3 (14.3)	5 (25.0)	4 (19.0)	0.69
Smoking N (%)				
Current	1 (4.8)	1 (5.0)	2 (9.5)	0.89
Never	15 (71.4)	16 (80.0)	14 (66.7)	
Past	5 (23.8)	3 (15.0)	5 (23.8)	

BMI: body mass index; MMSE: Mini-Mental State Examination; GDS15: Geriatric Depression Scale 15.

age- and sex-matched AD and 21 age- and sex-matched participants with normal cognitive function were selected. All samples were selected from participants with few physical complications that could affect gene expression in the blood. All subjects or their families gave written, informed consent using a format approved by the ethics committee of Ehime University Graduate School of Medicine (Approval Number: 1610004). This study was conducted in accordance with the Declaration of Helsinki.

Blood samples were generally taken 2 h after eating. A self-administered questionnaire was used to obtain data on education, tobacco and alcohol history, daily physical activity level, medical history, and use of medication. After data acquisition, the data were reviewed by trained interviewers. Height and weight were determined with the patients wearing light clothing, and the body mass index (BMI) was calculated from the measurements. The Mini-Mental State Examination (MMSE) and brain magnetic resonance imaging (MRI) were also conducted. All AD patients fulfilled the diagnostic criteria of the National Institute on Aging/Alzheimer's Association,³⁰ and a diagnosis of MCI due to AD was established for patients who satisfied the following criteria: (1) MMSE score ≥ 23 and normal general cognitive function; (2) Wechsler Memory Scale-Revised delayed recall test below the cut-off point for any cognitive impairment according to education status (≤ 8 points for 16 years of education, ≤ 4 points for 8–15 years, and ≤ 2 points for 0–7 years); (3) neuropsychiatric evaluation showing lack of dementia or depression as assessed by geriatric neuropsychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised (DSM-III R) (American Psychiatric Association, 1997); (4) no disability in activities of daily living; and (5) atrophy of the hippocampus and parietal lobes without apparent cerebrovascular disease on brain MRI.³¹ A joint meeting of the JPSC-AD²⁷ research group was then held, and a final diagnosis was made.

RNA isolation and complementary DNA synthesis

Blood was taken into PaxGene Blood RNA Systems tubes (BD, Tokyo, Japan), and RNA was isolated according to the manufacturer's protocol. RNA concentration and quality were assessed with the NanoDrop 1000 system (Thermo Fisher Scientific, Yokohama, Japan). The RNA integrity number was also determined with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and Agilent RNA6000 Nano kit (Agilent Technologies). All samples fulfilled the following conditions: A260/A280 ≥ 1.8 , A260/A230 ≥ 2.0 , and RNA integrity number ≥ 7.0 . With the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Santa Clara, CA, USA), 1.0 μg of RNA was used to synthesize 40- μL reaction mixtures of complementary DNA (cDNA).

RNA-Sequencing (transcriptome analysis)

RNA-Seq library preparation was performed using 145 ng of total RNA from each sample. An RNA-seq library of each sample was prepared using the QuantiSeq 3'mRNA-Seq Library Prep Kit for Illumina (LEXOGEN, Greenland, NH, USA) in combination with the Globin Block Modules for QuantSeq (LEXOGEN) according to the manufacturer's protocol. Then, 75-bp single-end sequencing was performed using the NextSeq 500 (Illumina, San Diego, CA, USA). Approximately 2.2 to 3.6 million reads were obtained for each sample. All data analysis was conducted using QIAGEN CLC Main Workbench 23.0 (QIAGEN, Germantown, MD, USA) with default parameters. Briefly, trimming of the adapter sequence, low-quality base of the 3' end, polyA sequence, and the 5' end first 12 bases derived from the random primer of fastq, was performed according to the library construction manual. Trimmed reads were aligned to the human reference data GRCh38, and significantly differentially expressed genes (DEGs) were detected based on Trimmed Mean of M-values (TMM) normalization and the negative binomial generalized linear model (GLM). A p-value of 0.05 was set as the threshold for DEGs, as no genes fulfilled $p < 0.05$ and FDR $q < 0.05$. All RNA-Seq data were deposited in the GEO database (accession number GSE 249477).

Gene ontology analysis and pathway enrichment analysis of the DEGs

Gene ontology (GO) analysis with default settings was performed to analyze the main functions of DEGs using DAVID tools (<https://david.ncifcrf.gov/home.jsp>). DAVID tools help researchers understand the biological implications behind many genes by delivering a comprehensive set of functional annotation tools.^{32,33} Significant pathways and GO items, including biological processes (BPs), cellular components (CCs), molecular functions (MFs), and KEGG pathway (PW) were defined as pathways with $p < 0.05$.

Ingenuity pathway analysis

Ingenuity pathway analysis (IPA) with default settings was conducted to further explore relevant pathways associated with DEGs (QIAGEN) (<https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>).³⁴ IPA is a web-based software application for the analysis, integration, and interpretation of data from RNA-Seq, microRNA, and SNP microarrays, metabolomics, proteomics, and gene and chemical listings, including small-scale experiments that generate downstream effects analyses to predict cellular functions, disease processes, and other

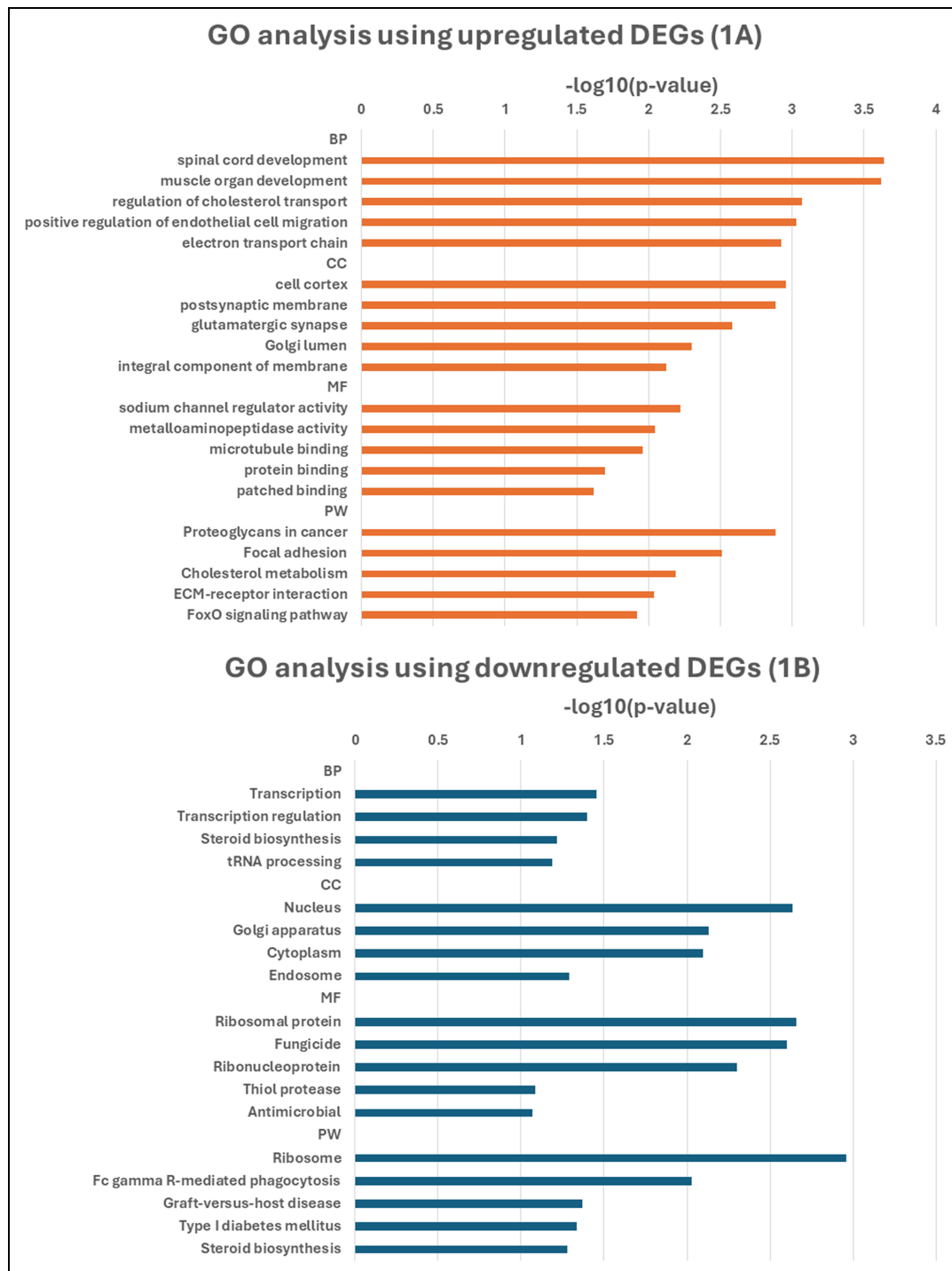


Figure 1. Gene ontology (GO) analysis (AD versus Control). The x-axis shows the $-\log_{10}$ p-value. GO and pathway analyses of upregulated DEGs (1A: Orange bars). GOs of regulation of cholesterol transport and cholesterol metabolism are significantly enriched. GO and pathway analysis of downregulated DEGs (1B: Blue bars). GOs of steroid biosynthesis, fungicide, and Fc gamma R-mediated phagocytosis are significantly enriched.

phenotypes affected by patterns in the analyzed dataset. In addition, upstream regulator analyses were conducted to identify regulators (e.g., transcription factors, cytokines, kinases, etc.) that are directly related to targets in the

analyzed data and whose activation or inhibition may explain the observed changes, with a positive Z-score meaning that the pathway was promoted and a negative Z-score meaning that the pathway was inhibited.

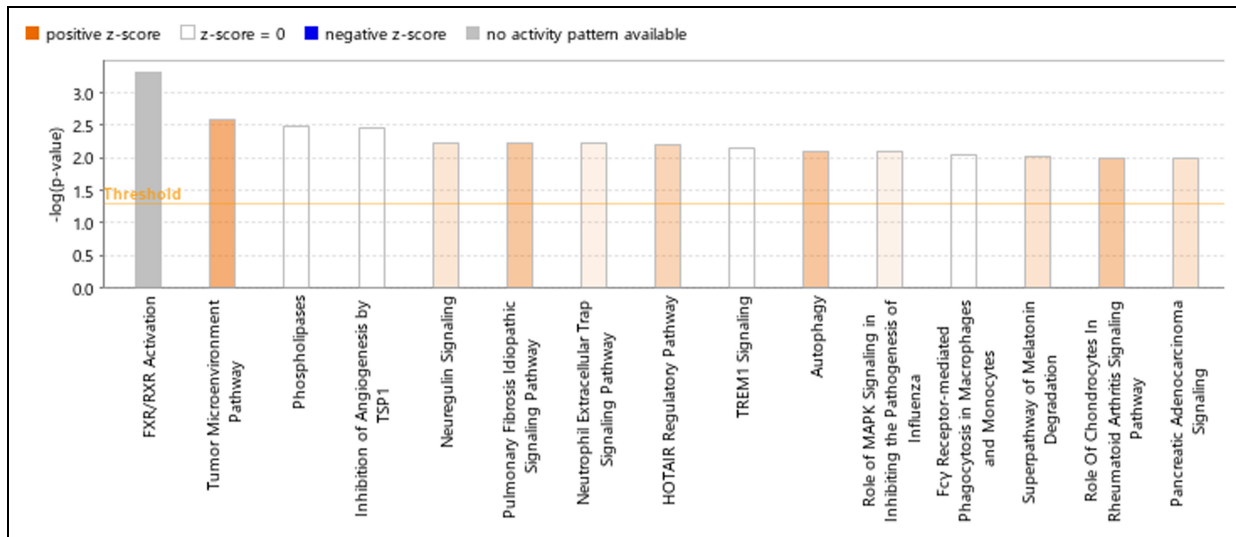


Figure 2. IPA analysis of DEGs (AD versus control). The y-axis shows the $-\log_{10}$ p-value. The top 15 pathways are presented. FXR/RXR activation has the highest Z-score. Tumor microenvironment and neutrophil extracellular trap signaling have a highly positive Z-score.

Quantitative polymerase chain reaction analysis

To validate microarray data, the expression levels of various mRNAs were determined by quantitative reverse transcription-polymerase chain reaction (qPCR) using the StepOnePlus Real-Time PCR System (Applied Biosystems). The mRNA expression levels were measured in duplicate using a StepOnePlus Real-Time PCR System with the PrimeTime Gene Expression Master Mix (Integrated DNA Technologies, Inc., Coralville, IA, USA) and mRNA-specific probes. The following were used as mRNA-specific probes: Hs.PT.58.4982621 for ITGB2, Hs.PT.58.20344216 for NFKB1, Hs.PT.58.25125759 for EGF, and Hs.PT.58.20622816 for CHRFAM7A. Hs.PT.39a.22214836 for GAPDH was used as an internal standard. RT-qPCR was conducted in 10- μ L mixtures with 0.5 μ L of cDNA. Following the manufacturer's protocol, the thermal cycling conditions were as follows: initial denaturation step (50°C for 2 min, and 95°C for 10 min), and 40 cycles of denaturation steps (95°C for 15 s, 60°C for 60 s annealing and elongation). The expression levels were calculated using the $\Delta\Delta C_t$ method. To adjust for differences between plates, the same sample was placed in all plates as a calibration.

Statistical analyses

Continuous variables with a normal distribution are represented as mean $\pm$ SD. Normality was assessed by the Kolmogorov-Smirnov test. Equal variance was assessed using the F test. Mean differences among three groups were examined by one-way analysis of variance. In qPCR analysis, comparisons between two groups of continuous variables were made using the Student's *t*-tests.

Categorical variables are presented as proportions (%). Comparisons between categorical variables were performed using the chi-squared test. Differences were considered significant if the two-tailed test yielded $p < 0.05$. All data were statistically analyzed using GraphPad Prism software (version 10.0.2; GraphPad Software, Boston, MA, USA).

Results

Characteristics of the participants

Characteristics of the participants including age, sex, height, weight, BMI, education, MMSE scores, Geriatric Depression Scale 15 (GDS15), medical history, and smoking status are presented in Table 1. The MMSE scores were significantly lower in AD (Control versus MCI versus AD: 29.1 versus 25.5 versus 19.0, $p < 0.01$). For the other characteristics, including medical history, there were no significant differences. There were no genes with $p < 0.05$ and FDR $q < 0.05$, indicating that gene expression in blood was not markedly different among AD, MCI, and Control participants. To ensure that the number of genes could withstand downstream analysis to detect slight changes, genes fulfilling only $p < 0.05$ were considered DEGs.

Transcriptome and DEG analysis

A total of 1091 genes were found to differentially distinguish patients with AD from Control, 938 genes were found to distinguish MCI from Control, and 788 genes were found to distinguish AD from MCI using $p < 0.05$ as the threshold for DEGs (Supplemental Table 1).

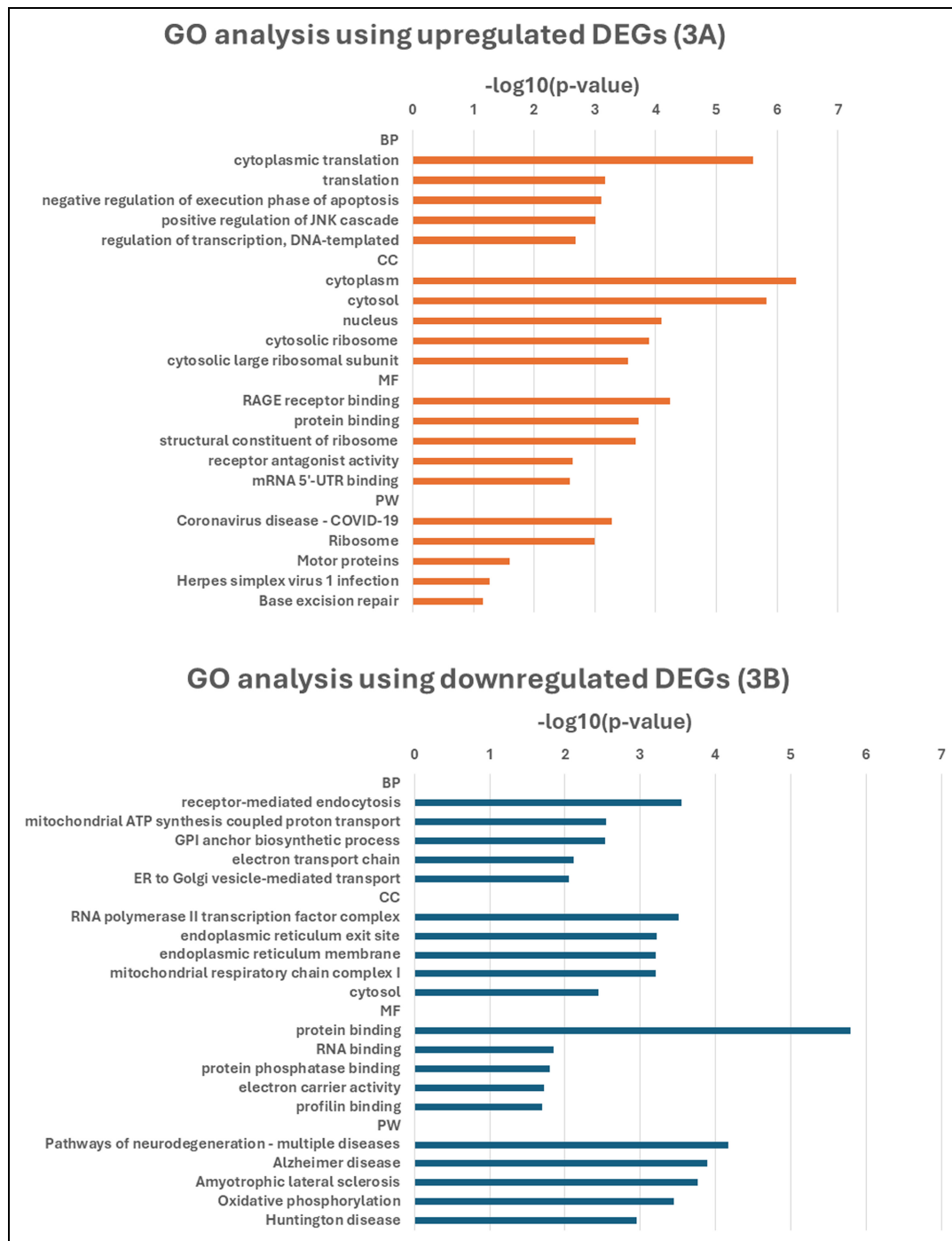


Figure 3. Go analysis (MCI versus control). The y-axis shows the $-\log_{10}$ p-value. GO and pathway analyses of upregulated DEGs (3A: Orange bars). GOs of negative regulation of the execution phase of apoptosis, positive regulation of the JNK cascade, and RAGE receptor binding are significantly enriched. GO and pathway analyses of downregulated DEGs (3B: Blue bars). GOs of the receptor-mediated endocytosis, mitochondrial ATP synthesis-coupled proton transport, pathways of neurodegeneration - multiple diseases, AD, and oxidative phosphorylation are significantly enriched.

Gene ontology and pathway enrichment analyses (AD versus control)

DEGs were further analyzed using DAVID tools, which were also used to analyze BPs, CCs and MFs. As shown

in Figure 1, GO analysis using upregulated DEGs identified several important BPs and PWs, including regulation of cholesterol transport and cholesterol metabolism. Significant GO downregulation in AD included steroid biosynthesis, fungicide, and Fc gamma R-mediated

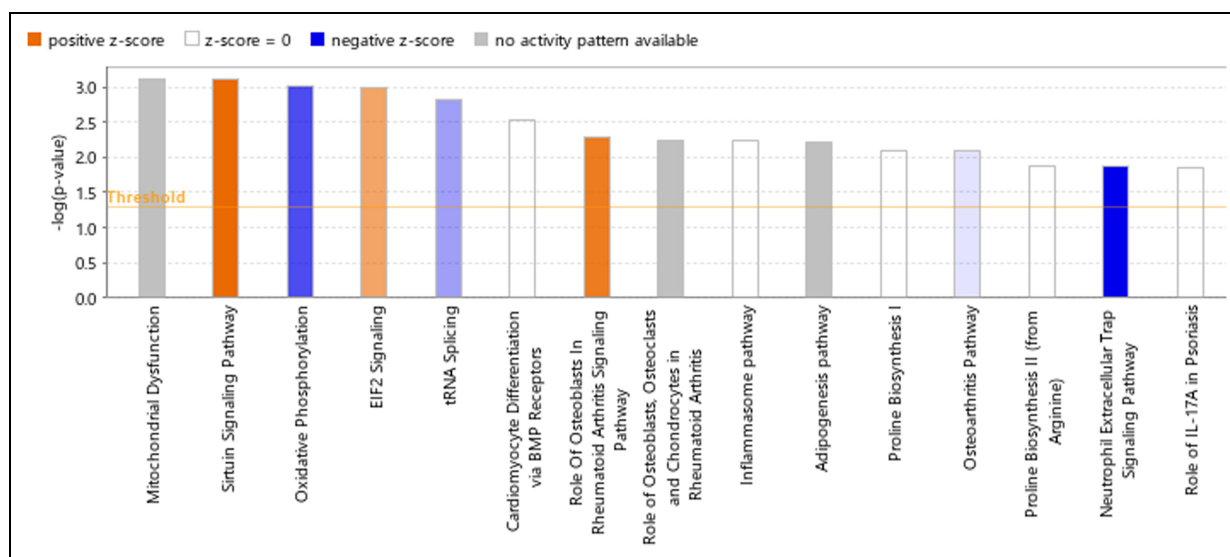


Figure 4. IPA analysis of DEGs (MCI versus control). The y-axis shows the $-\log_{10}$ p-value. The top 15 pathways are presented. The sirtuin pathway has a positive Z-score, and oxidative phosphorylation has a positive Z-score. Neutrophil extracellular trap signaling has a highly negative Z-score.

phagocytosis. Of these, GOs related to immunity and cholesterol metabolism were significantly accumulated in both upregulated and downregulated genes, indicating that the dysregulation of cholesterol metabolism and the immune system plays an important part in the process of AD.

IPA analysis (AD versus control)

Bar graphs of the enrichment of canonical pathways of DEGs were plotted using the IPA tool. The y-axis represents the $-\log_{10}$ p-value of the significance of the enrichment of the IPA pathways on Fisher's exact test. Most pathways were positively regulated (Figure 2). FXR/RXR activation had the highest Z-score. Tumor microenvironment and neutrophil extracellular trap signaling had a positive Z-score. Top upstream regulators included ESR2, beta-estradiol, aflatoxin B1, CCN5, and trans-hydroxytamoxifen, and the causal network included BECN1, natorazepide, PTPN13, CYP3A4, and PRMT5.

Gene ontology and pathway enrichment analyses (MCI versus control)

GO analysis using upregulated DEGs showed several important BPs and MFs, as shown in Figure 3, including negative regulation of the execution phase of apoptosis, positive regulation of the JNK cascade, and RAGE receptor binding. Significant GO downregulation in MCI included receptor-mediated endocytosis, mitochondrial ATP synthesis-coupled proton transport, pathways of neurodegeneration - multiple diseases, AD, and oxidative phosphorylation. Of these, GOs associated with mitochondrial function and neurodegeneration,

including AD, were significantly enriched in both up- and downregulated genes, suggesting that mitochondrial function, immune response, and neurodegeneration have important roles in the process of MCI.

IPA analysis (MCI versus control)

Mitochondrial dysfunction had the highest Z-score in MCI versus CT (Figure 4). The sirtuin pathway had a positive Z-score, and oxidative phosphorylation had a positive Z-score. Neutrophil extracellular trap signaling had a highly negative Z-score. Top upstream regulators included HNF4A, CD3, LARP1, RICTOR, and PRMT8, and the causal network included CMA1, miR-381-3p, ARG2, and mir-210.

Gene ontology and pathway enrichment analyses (AD versus MCI)

As shown in Figure 5, GO analysis using upregulated DEGs showed several important BPs and PWs, including membrane disruption in other organisms, innate immune response in mucosa, viral myocarditis, and allograft rejection. Significant GO downregulation in AD included telencephalon development, malaria, and human papillomavirus infection. Of these, GOs associated with the immune response were significantly clustered in both upregulated and downregulated genes. This suggests that the immune response is important in the conversion from MCI to AD.

IPA analysis (AD versus MCI)

Most pathways were negatively regulated (Figure 6). Triacylglycerol degradation had a negative Z-score,

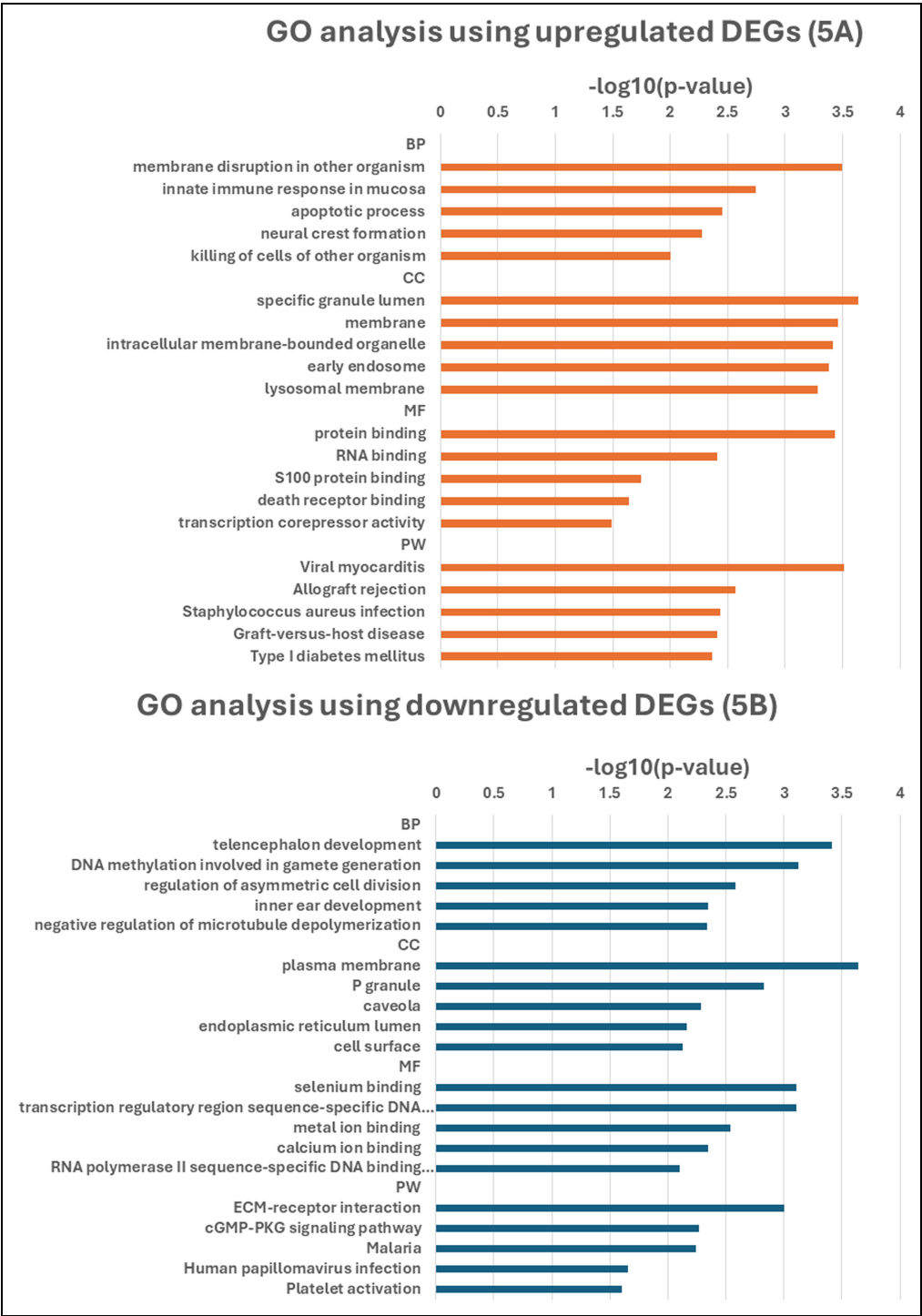


Figure 5. Go analysis (AD versus MCI). The y-axis shows the $-\log_{10}$ p-value. GO and pathway analyses of upregulated DEGs (5A: Orange bars). GOs of membrane disruption in other organisms, innate immune response in mucosa, viral myocarditis, and allograft rejection are significantly enriched. GO and pathway analysis of downregulated DEGs (5B: Blue bars). GOs of telencephalon development, malaria, and human papillomavirus infection are significantly enriched.

whereas PPAR α /RXR α activation and neutrophil extra-cellular trap signaling had positive Z-scores. Top upstream regulators included tretinoin, MRTFB, TGFBR2, IL6, and filgrastim, and causal network included ZNF703, ERBB2, WNT3A, zidovudine, and GATA3.

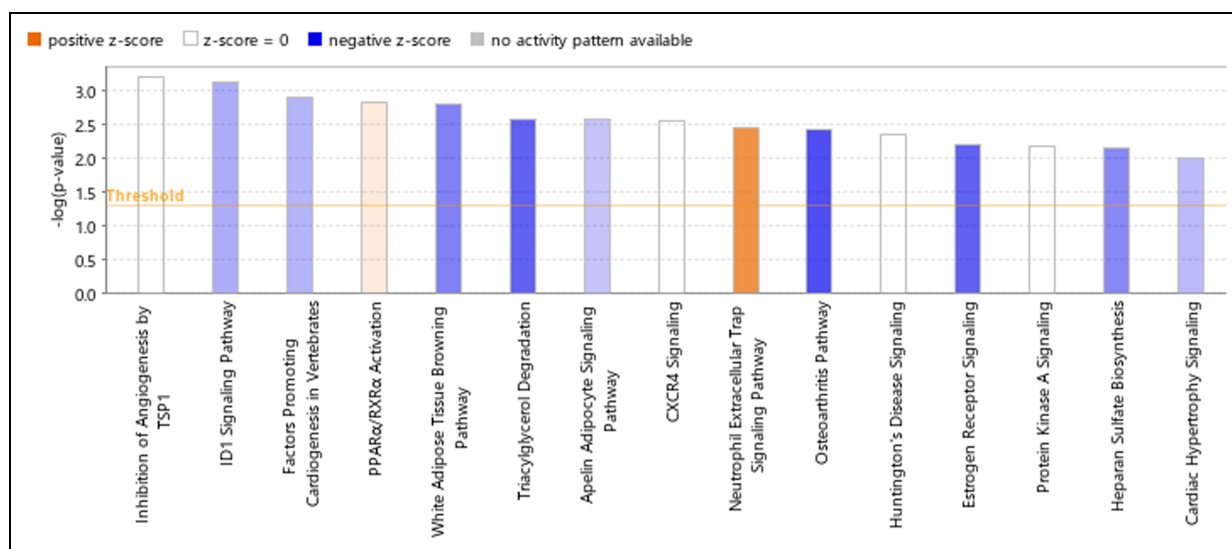


Figure 6. IPA analyses of DEGs (AD versus MCI). The y-axis shows the $-\log_{10}$ p-value. The top 15 pathways are presented. Triacylglycerol degradation has a negative Z-score, and PPAR α /RXR α activation and neutrophil extracellular trap signaling have positive Z-scores.

Real-time PCR for the validation of DEGs in transcriptome analysis

To check the reliability of the transcriptome analysis, ITGB2 (neutrophil extracellular trap signaling pathway), NFKB1 (sirtuin signaling pathway and neutrophil extracellular trap signaling pathway), EGF (tumor microenvironment pathway), and CHRFAM7A (ID1 signaling pathway) were selected (Figure 7). ITGB2 expression was lower in MCI than in Control, but there were no significant differences (Control versus MCI = 0.94 ± 0.19 versus 0.82 ± 0.20 , Student's *t*-test $p = 0.06$). NFKB1 expression was significantly lower in MCI than in Control (Control versus MCI = 0.83 ± 0.23 versus 0.68 ± 0.14 , Student's *t*-test $p = 0.02$). EGF expression was significantly higher in AD than in Control (Control versus AD = 0.97 ± 0.48 versus 1.36 ± 0.67 , Student's *t*-test $p = 0.04$). CHRFAM7A expression was lower in AD than in MCI, but there were no significant differences (MCI versus AD = 1.33 ± 0.77 versus 0.94 ± 0.48 , Student's *t*-test $p = 0.06$).

Discussion

There are three findings in this study.

First, activation of FXR/RXR, tumor microenvironment, and neutrophil extracellular trap signaling was detected in AD. FXR/RXR are receptors involved in cholesterol regulation, and IPA analysis identified FXR/RXR activation in 5XFAD and Trem2 KO AD model mice, highlighting the significance of energy dysregulation and inflammatory processes in the progression of AD-related pathology.³⁵ Retinoids are thought to be involved in AD through their impact on oxidative stress (which may be related to

mitochondrial dysfunction), amyloid- β deposits, inflammation, and neurotransmission.³⁶ Previous studies have shown that cholesterol and its derivatives affect T cell aging.³⁷ The tumor microenvironment consists of the cancer cells, the cytokine milieu, the extracellular matrix, subsets of immune cells, and other components. Not only do tumors manage to escape the host immune system, they also efficiently take advantage of invading cells by altering their function to create a microenvironment favorable to tumor progression.³⁸ The tumor microenvironment may be similar, with immune responses to pathogens such as amyloid plaques in AD.^{39,40}

Second, upregulation of the sirtuin pathway and downregulation of oxidative stress were detected in MCI. Sirtuins can alleviate neuroinflammation, whatever its cause, by inhibiting an intrinsic inflammatory pathway dependent on NF- κ B,⁴¹ and they also regulate many AD-related events, including APP and tau processing, mitochondrial activity, oxidative damage, and neuroinflammation. Mitochondrial DNA (mtDNA) defects in AD can lead to energy dysfunction (1) caused by defective complexes I, III, IV, and V (2), promotion of amyloid- β deposition (3), and elevated oxidative stress (4), which in turn worsens mtDNA damage and increases the production of reactive oxygen species, creating a perpetual cycle of malfunctioning and injured mitochondria.⁴² Blood gene expression of people with MCI may reflect protective or resistant responses to the pathogenesis of AD via sirtuins and mitochondria.

Third, triacylglycerol degradation had a negative Z-score, whereas PPAR α /RXR α activation and neutrophil extracellular trap signaling had a positive Z-score in the comparison of AD and MCI on IPA. GO analysis also

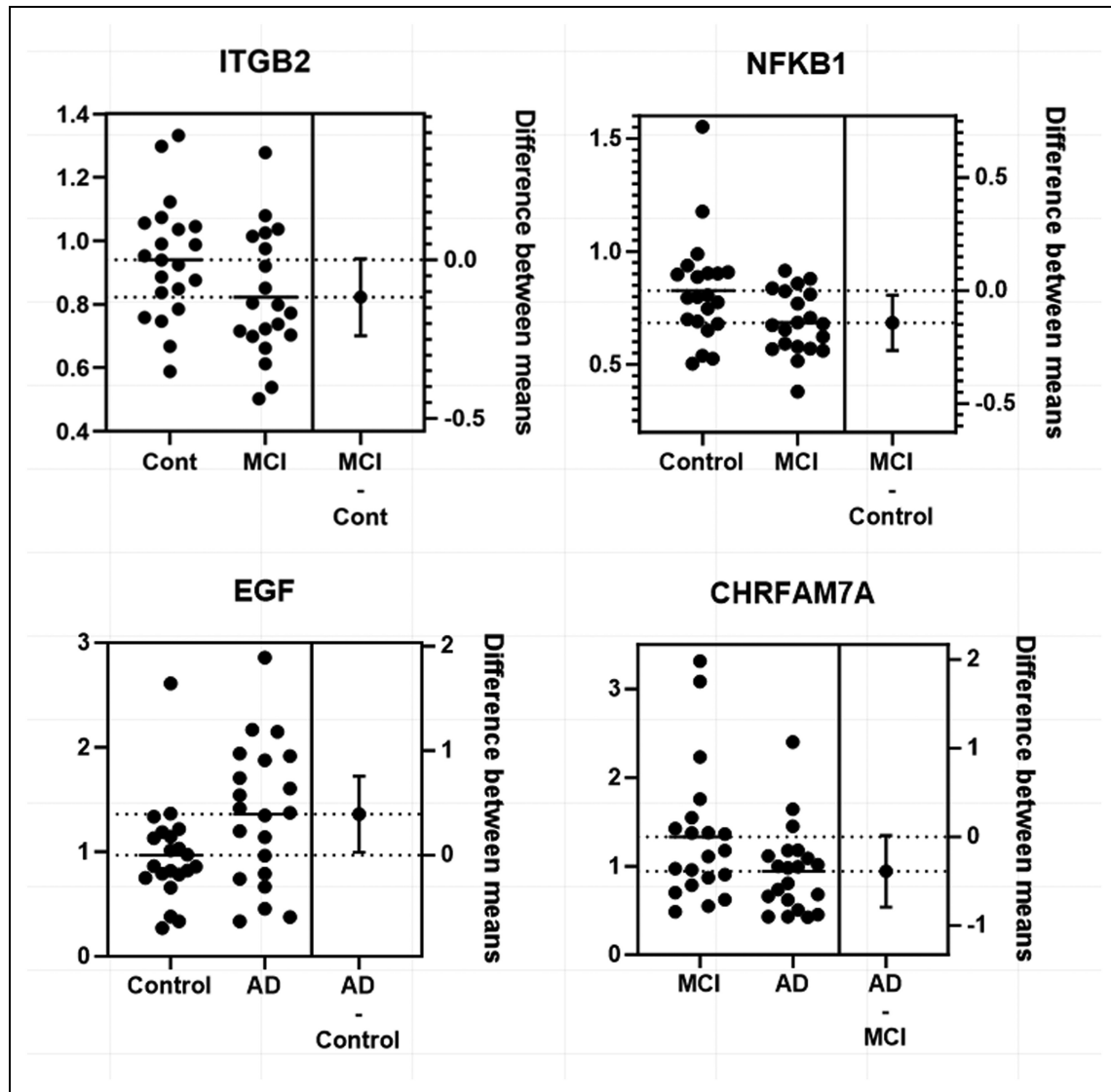


Figure 7. ITGB2, NFKB1, EGF, and CHRFAM7A expression levels. ITGB2 expression is lower in MCI (Control versus MCI = 0.94 ± 0.19 versus 0.82 ± 0.20 , Student's *t*-test $p = 0.06$). NFKB1 expression is significantly lower in MCI (Control versus MCI = 0.83 ± 0.23 versus 0.68 ± 0.14 , Student's *t*-test $p = 0.02$). EGF expression is significantly lower in AD (control versus AD = 1.36 ± 0.67 versus 0.97 ± 0.48 , Student's *t*-test $p = 0.04$). CHRFAM7A expression is higher in AD (Control versus AD = 0.94 ± 0.48 versus 1.31 ± 0.76 , Student's *t*-test $p = 0.07$).

showed that the immune response is altered in comparisons of AD and MCI. A higher triglyceride level was related to higher dementia risk in patients aged <60 years, but the inverse was observed for patients aged ≥ 60 years.⁴³ Current evidence suggests that the presence of increased blood total cholesterol (TC) or triglycerides (TG) is linearly related to an increase in the relative risk of AD, supporting the idea that high levels of TC and TG appear to have a causal effect in the pathogenesis of AD.⁴⁴ Triglycerides and fatty acids are important ingredients of the plaque environment to which T cells from the circulation are exposed, and there is growing evidence that fatty acids affect T cell activity.⁴⁵ In AD, neutrophils adhere to and spread within

brain vasculature and are present in the parenchyma, as well as neutrophil extracellular traps.⁴⁶ In AD, neutrophil-vascular interactions cause myeloperoxidase accumulation in the brain.¹¹

This study may help to link several independent fields of research, such as diabetes, hypertension, obesity, lipid droplet formation, and age-related mitochondrial dysfunction, not only in the brain, but also in the blood.^{9,19,47} Insulin resistance is involved in the progression of AD and is an important turning point in this disease.^{28,48} For example, the SGLT2 inhibitor empagliflozin improves endothelial function and thereby reduces related pathologies.^{49,50} In diabetes, glucose metabolism abnormalities

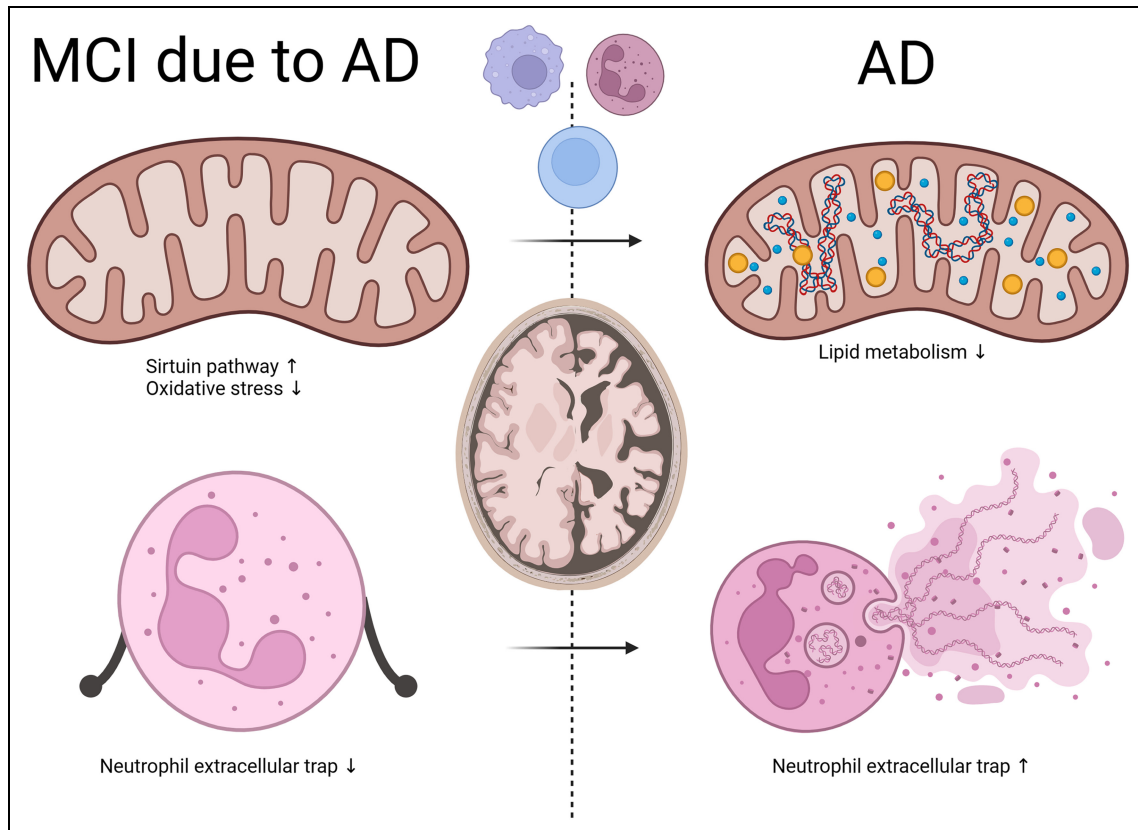


Figure 8. A pictorial representation of the main results. In AD, neutrophil extracellular trap signaling is increased, and lipid metabolism is decreased, whereas in MCI, neutrophil extracellular trap signaling is decreased, and mitochondrial function is enhanced. Created with BioRender.com.

and insulin resistance negatively affect cognitive function.⁵¹ Empagliflozin reduces oxidative stress and improves frailty in hypertensive and diabetic patients.⁵² Abnormal mitochondrial dynamics lead to neurodegeneration, but normalizing these dynamics may prevent its progression.⁵³ Metformin improves cognitive function in hyperglycemic patients and reduces oxidative stress in brain microvascular endothelial cells.⁵⁴ Mitochondrial quality control is crucial in neurodegenerative diseases, and mitophagy plays a key role in treatment.⁵⁵ Mitochondrial oxidative stress provides important insights into frail diabetic patients.^{56,57} Muscle weakness and cognitive decline are associated with factors related to aging, such as mitochondrial dysfunction.⁵⁸ Mitophagy plays an important role in mitochondrial quality control, providing important insights into the mechanisms and potential treatments for neurodegenerative diseases.^{59,60} Obesity is a risk factor for AD, and it destroys the microenvironment of adipose tissue, causing chronic inflammation and mitochondrial dysfunction, and inducing oxidative stress.⁶¹ Obesity contributes to cognitive decline through brain mitochondrial dysfunction and insulin resistance.⁶² There is a paradox regarding the role of adiponectin in AD, suggesting a relationship with amyloid formation.^{63,64} A clinical trial has demonstrated that L-arginine

supplementation, which may improve hypertension and endothelial function is effective in improving cognitive impairment in frail older adults with hypertension.⁶⁵

The strength of our research is that the clinical characteristics that are important in the pathology of dementia, such as the age and sex of the samples and physical complications such as diabetes, are neatly aligned between the three groups. In addition, since we are analyzing gene expression in the blood using PaxGene Blood RNA Systems tubes, it is easy to use as a diagnostic biomarker in clinical settings, and it can also detect abnormalities in the immune system. On the other hand, the present study had limitations. First, the sample size was relatively small, although age, sex, and rates of physical comorbidity were adequately controlled. Second, the blood samples were obtained from a cross-sectional survey, so no causal relationships can be inferred. The diagnosis of AD or MCI due to AD is clinical and does not involve confirmation of AD pathology through cerebrospinal fluid tests or amyloid PET scans. Not all MCI due to AD will develop into AD. Third, the limitations of generalizability should be pointed out, because this study intentionally selected an age- and sex-matched sample with relatively few physical complications from a large number of cohort samples.

Fourth, it should be noted that no correction for multiple comparisons was made because no genes met the criteria for $p < 0.05$ and FDR $q < 0.05$, and that this study may be interpreted as a negative study and there may be many false positives. Gene expression in blood showed that there were no marked differences among AD, MCI, and Control, but even small changes may be important in the pathology. In the future, it will be necessary to observe longitudinal changes to clarify the relationships between the present results and the pathophysiology of AD and MCI.

In summary, neutrophil extracellular trap signaling was increased, and lipid metabolism (FXR/RXR activation, triacylglycerol degradation) was decreased in AD, whereas MCI showed protective responses via decreased neutrophil extracellular trap signaling and mitochondrial functions such as upregulation of the sirtuin pathway and downregulation of oxidative stress (Figure 8). The present study's results are consistent with previous reports that whole blood transcriptome analysis shows immune and inflammatory abnormalities and mitochondrial abnormalities, but the changes were small and, when corrected for multiple comparisons, were not significant (Supplemental Figure 1). These findings show that immune cells may play an important role in the development of AD, and that it is possible to monitor the immune system in MCI and AD through the blood transcriptome.

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Statements and declarations

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

All of the data that were generated or analyzed during this study are included in this published article and in the supplementary information files. All RNAseq data were deposited in the GEO database (accession number GSE249477).

Supplemental material

Supplemental material for this article is available online.

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