Received: 5 October 2024 | Revised: 25 November 2024 | Accepted: 27 November 2024

DOI: 10.1002/alz.70019

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

RESEARCH ARTICLE

Posterior cingulate cortex microRNA dysregulation differentiates cognitive resilience, mild cognitive impairment, and Alzheimer's disease

Scott E. Counts[1,2] | **John S. Beck**[1] | **Bryan Maloney**[3] | **Michael Malek-Ahmadi**[4,5] | **Stephen D. Ginsberg**[6,7] | **Elliott J. Mufson**[8] | **Debomoy K. Lahiri**[3]

[1]Department of Translational Neuroscience, Michigan State University College of Human Medicine, Grand Rapids, Michigan, USA

[2]Department of Family Medicine, Michigan State University College of Human Medicine, Grand Rapids, Michigan, USA

[3]Departments of Psychiatry and Medical and Molecular Genetics, Indiana Alzheimer's Disease Research Center, Indiana University School of Medicine, Indianapolis, Indiana, USA

[4]Banner Alzheimer's Institute, Phoenix, Arizona, USA

[5]Department of Biomedical Informatics, University of Arizona College of Medicine-Phoenix, Phoenix, Arizona, USA

[6]Center for Dementia Research, Nathan Kline Institute, Orangeburg, New York, USA

[7]Departments of Psychiatry, Neuroscience & Physiology, and the NYU Neuroscience Institute, New York University Grossman School of Medicine, New York, New York, USA

[8]Departments of Translational Neuroscience and Neurology, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, Arizona, USA

Correspondence
Scott E. Counts, MSU Grand Rapids Research Center, 400 Monroe Ave NW, Ste 2008, Grand Rapids, MI 49503, USA.
Email: countssc@msu.edu

Funding information
National Institutes of Health, Grant/Award Number: P30 AG072975; Michigan Alzheimer's Disease Research Center,

Abstract

INTRODUCTION: MicroRNA (miRNA) activity is increasingly appreciated as a key regulator of pathophysiologic pathways in Alzheimer's disease (AD). However, the role of miRNAs during the progression of AD, including resilience and prodromal syndromes such as mild cognitive impairment (MCI), remains underexplored.

METHODS: We performed miRNA-sequencing on samples of posterior cingulate cortex (PCC) obtained *post mortem* from Rush Religious Orders Study participants diagnosed *ante mortem* with no cognitive impairment (NCI), MCI, or AD. NCI subjects were subdivided as low pathology (Braak stage I/II) or high pathology (Braak stage III/IV), suggestive of resilience. Bioinformatics approaches included differential expression, messenger RNA (mRNA) target prediction, interactome modeling, functional enrichment, and AD risk modeling.

RESULTS: We identified specific miRNA groups, mRNA targets, and signaling pathways distinguishing AD, MCI, resilience, *ante mortem* neuropsychological test performance, *post mortem* neuropathological burden, and AD risk.

DISCUSSION: These findings highlight the potential of harnessing miRNA activity to manipulate disease-modifying pathways in AD, with implications for precision medicine.

KEYWORDS
dementia, microRNA, mild cognitive impairment, non-coding RNA, resilience

Highlights

- MicroRNA (MiRNA) dysregulation is a well-established feature of Alzheimer's disease (AD).
- Novel miRNAs also distinguish subjects with mild cognitive impairment and putative resilience.

Grant/Award Number: P30 AG072976; Indiana Alzheimer's Disease Research Center; the Arizona Alzheimer's Consortium at Barrow Neurological Institute; National Institute on Aging, Grant/Award Numbers: AG014449, AG017617, AG072599, AG074004, AG077103, AG081286, AG072931

- MiRNAs correlate with cognitive performance and neuropathological burden.
- Select miRNAs are associated with AD risk with age as a significant covariate.
- MiRNA pathways include insulin, prolactin, kinases, and neurite plasticity.

1 | BACKGROUND

Alzheimer's disease (AD) is a devastating neurodegenerative dementing disorder defined by neuron and synapse loss in higher order cognitive brain regions and the pathological accumulation of cerebral amyloid plaques and neurofibrillary tangles (NFTs).[1] Therapeutic options have traditionally been limited to symptomatic relief through treatments such as cholinesterase inhibitors.[2] However, more recently, the prospect of mild disease modification through amyloid immunotherapies has emerged,[3] albeit with significant risk to subsets of the population including those carrying an apolipoprotein E (*APOE*) ε4 allele.[4] A prevailing sentiment in the field is that robust disease modification will require combinatorial therapy of potentially personalized treatment options to promote neuroprotection. Hence, the field's primary goal remains to identify upstream molecular and biochemical regulatory pathways that drive or protect against AD pathogenesis. One compelling upstream regulatory pathway involves small non-coding RNAs (ncRNAs) such as microRNAs (miRNAs), which bind to messenger RNAs (mRNAs) and usually reduce their stability and expression.[5] These miRNAs regulate diverse brain functions, and perturbations in their expression have been linked to AD.[6–10] Our group and others have identified miRNAs regulating the metabolism of amyloid beta (Aβ) precursor protein (APP), β-site APP-cleaving enzyme 1 (BACE1), and tau—thereby potentially mediating amyloid plaque and NFT pathology—as well as neuroinflammation, circuit-based synaptic transmission, energy metabolism, and neuronal survival.[11–26] Moreover, miRNAs have emerged as promising cerebrospinal fluid and blood-based diagnostic biomarkers for AD.[27–33] However, the extent to which specific miRNAs are dysregulated in vulnerable brain regions during the onset of AD, including individuals with mild cognitive impairment (MCI), a prodromal AD stage,[34–38] remains largely underexplored. Further research on the role of specific miRNAs in AD pathogenesis would advance the potential promise for microRNA-based therapies as part of a disease-modifying regimen for AD.

To help understand which miRNAs—as well as their putative mRNA targets and associated functional pathways—are altered in AD progression, we sequenced miRNA transcripts in *post mortem* tissue obtained from the posterior cingulate cortex (PCC), a key hub of the resting-state default mode network (DMN) that mediates autobiographical memory retrieval, emotional memory, and attention[39–41] and displays hypometabolic changes,[42–45] synaptic loss,[46] and tau pathology[47] in the earliest stages of AD. *Post mortem* PCC samples were obtained from Rush Religious Orders Study (RROS) participants who came to autopsy with a clinical diagnosis of no cognitive impairment (NCI), MCI, or AD and who received a *post mortem* neuropathological evaluation.[48–51] Over the last several years, we[48, 52–54] and others[55, 56] have reported that older adults with an *ante mortem* diagnosis of NCI often display high Braak NFT scores upon *post mortem* neuropathological evaluation suggestive of cognitive resilience to AD pathology. To test for potential miRNA markers of cognitive resilience[48] within the context of AD progression, we also examined differences in miRNA expression between NCI subjects who came to autopsy with either a *post mortem* diagnosis of low AD pathology (NCI-LP; e.g., Braak stage I/II) versus NCI subjects with high AD pathology (NCI-HP, e.g., Braak stage III/IV).[57–60] We also correlated specific miRNA levels with *ante mortem* clinical test scores and *post mortem* diagnostic variables. Finally, we used regression analysis to identify potential miRNAs associated with dementia risk within the current cohort. Given the multifactorial role of miRNAs in regulating neuronal function, these data will aid in determining specific miRNAs operating within select signaling networks in the PCC that are critical to the onset of AD pathogenesis and/or that relate to cognitive resilience in the elderly. The present findings may potentially reveal key upstream mechanisms leading to therapeutic interventions and biomarker strategies for AD as well as preservation of cognition in the elderly.

2 | METHODS

2.1 | Subjects and clinical pathologic assessments

Post mortem PCC tissue samples ($n = 39$) were obtained from participants in the RROS, a longitudinal clinical pathologic study of aging and dementia in elderly Catholic clergy (Tables 1 and 2).[48] Subjects were classified *ante mortem* as NCI ($n = 20$) and subdivided as NCI-LP (Braak stage I/II, $n = 8$) or NCI-HP/resilience (Braak stage III/IV, $n = 12$),[57–60] MCI ($n = 10$), or mild/moderate AD ($n = 9$).

Details of RROS clinical and neuropathologic evaluations and diagnostic criteria have been published extensively.[48–51] Briefly, RROS participants undergo an annual neurological examination and cognitive performance testing using the Mini-Mental State Examination (MMSE) and 19 additional neuropsychological tests referable to five cognitive domains: orientation, attention, memory, language, and perception.[49] Composite scores of episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability, as well as a composite global cognitive *z* score (GCS), were derived from this test battery for each subject; NCI subjects did not reveal impairment in any of these domains within a year of death.[49, 61] Exclusion criteria included

a history of major depressive disorder, chronic alcoholism, and/or neuropathological evidence of Parkinson's disease, Lewy body disease, TAR DNA-binding protein 43 proteinopathy, hippocampal sclerosis, or large strokes. *APOE* genotyping was performed as reported.[48]

Brain slabs were immersion fixed in 4% paraformaldehyde, cryoprotected, cut at 40 μm, and sections immunostained with antibodies against APP and Aβ (6E10, 1:400 dilution) and phosphorylated tau (AT8, 1:250 dilution) for neuropathological evaluation.[48] A board-certified neuropathologist evaluated all cases while blinded to clinical diagnosis.[51] Designations of "normal" (with respect to AD or other dementing processes), "possible AD," "probable AD," or "definite AD" were based on semi-quantitative estimation of neuritic plaque density, an age-related senile plaque score, and presence or absence of dementia as established by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD).[62] Braak scores based on the staging of NFT pathology were established for each case.[63] Cases also received a National Institute on Aging (NIA)-Reagan likelihood of AD diagnosis based on neuritic plaque and tangle pathology.[64] Semi-quantitative estimates of global diffuse plaques, neuritic plaques, and NFTs were also derived from summary evaluations of entorhinal cortex; hippocampus; and midtemporal, inferior parietal, and midfrontal cortices.[61] The "ABC" algorithm for the diagnosis of AD[65] is currently being applied to all RROS cases.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using PubMed and identified rigorous studies from human brain samples, rodent models, and in vitro constructs demonstrating a potentially critical upstream role of microRNA (miRNA) dysregulation in the pathophysiology of Alzheimer's disease (AD; cited in the present article). However, the extent to which miRNA dysregulation is involved in prodromal and preclinical stages of AD such as mild cognitive impairment (MCI) and resilience was not well established.
2. **Interpretation**: Our findings from human *post mortem* posterior cingulate cortex samples validate the concept that select groups of miRNAs are dysregulated in AD, yet also show that other groups of miRNAs distinguish MCI, resilience, associations with cognitive and neuropathological variables, and AD risk.
3. **Future directions**: The key miRNAs identified in this study are candidates for further functional studies to delineate their mechanistic impact on AD progression and their potential as therapeutic targets. They can also be explored further as fluid biomarkers for AD.

2.2 | Preparation of tissue, miRNA-seq, and post-processing

PCC samples were collected free of white matter using fiduciary landmarks defined by the corpus callosum inferiorly, Brodmann area 24 anteriorly, and precuneal cortex dorsally[66] and then flash frozen and stored at −80°C until processing for miRNA-seq. Total RNA (≈ 1 μg) was extracted using the mirVana miRNA Isolation Kit (Ambion) to enrich for small RNAs.[67,68] RNA quality was assessed using an Agilent Bioanalyzer and all samples selected for analysis displayed RNA Integrity Number values ≥ 7. RNA samples were sequenced using 2 × 50 paired-end configuration on the Illumina platform (GeneWiz Next Generation Sequencing). Sequence reads were trimmed to remove possible adapter sequences at the 3′ end. After trimming, sequence reads with 15 to 31 nucleotides were retained for subsequent analysis. Raw data were extracted from FastQ files using a custom R script and short sequences identified in the samples were searched against miRBase 21 for annotation. The hit counts for each small RNA were used as quantitative expression values. The experimenters were blinded to all subject data.

2.3 | Data analysis

Demographic, clinical, and pathological variables among the subject groups were screened by the Shapiro–Wilk test for normality and then compared by either one-way analysis of variance (ANOVA) or the Kruskal–Wallis test with post hoc corrections for multiple comparisons. Fisher exact test was used to compare sex and *APOE* ε4 allele distribution across the groups. Differential gene expression analysis was conducted using R (v3.3.2). A false discovery rate (FDR) was set at 0.4 and level of significance was set at $\alpha = 0.05$. Spearman correlation was used to test for associations between miRNA expression level and clinical pathologic criteria. Ordinal logistic regression[69] was used to relate the probability of AD, MCI, and NCI associated with high or low pathology to levels of selected miRNAs, along with potential covariates of age, sex, and presence of at least one *APOE* ε4 allele. Competing models with different covariates were compared by second-order Akaike information criterion (AICc),[70] with entropically favored models selected for analysis by ANOVA.

2.4 | mRNA target identification and pathway enrichment

TarBase v9.0 was used as a conservative approach to identify verified and manually curated, direct human miRNA–mRNA interactions with an algorithm-generated prediction score of 1.0 (highest confidence).[71] Putative interaction networks of miRNA-regulated gene/protein products were created using K means clustering in the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) V11 database.[72] Unconnected genes were filtered out of the final STRING interactome diagrams. Pathway enrichment was performed using Pathview data integration and visualization software[73] with Kyoto Encyclopedia of

TABLE 1 Subject demographic and clinical neuropathologic characteristics.

	Diagnosis					
	NCI-LP ($n = 8$)	NCI-HP ($n = 12$)	MCI ($n = 10$)	AD ($n = 9$)	*p* value	Pair-wise comparison
Age (years) at death	83.9 ± 4.7 (75–90)	89.1 ± 5.5 (82–95)	87.0 ± 4.3 (81–91)	90.2 ± 4.3 (87–94)	0.01[a]	(NCI-HP, MCI, AD) > NCI-LP
Number (%) of males	4 (50%)	4 (33%)	5 (50%)	3 (33%)	0.24[c]	–
Years of education	17.4 ± 3.8 (12-21)	18.3 ± 2.4 (14–23)	17.0 ± 2.7 (12–20)	17.8 ± 2.3 (14–20)	0.67[a]	–
% with *APOE* ε4 allele	2 (25%)	3 (25%)	3 (30%)	3 (33%)	0.54[c]	–
MMSE	28.6 ± 1.3 (27–30)	28.3 ± 1.4 (27–30)	25.0 ± 3.1 (23–29)	17.3 ± 5.1 (10–24)	< 0.0001[a]	(NCI-LP, NCI-HP, MCI) > AD
Global cognitive score	0.2 ± 0.3 (−0.2–0.4)	0.0 ± 0.3 (−0.5–0.4)	−0.6 ± 0.3 (−1.2– −0.2)	−1.6 ± 0.6 (−2.6– −0.7)	< 0.0001[a]	(NCI-LP, NCI-HP) > (MCI, AD)
Episodic memory	0.4 ± 0.3 (−0.2–0.8)	0.3 ± 0.4 (−0.4–0.9)	−0.7 ± 0.4 (−0.2–1.5)	−2.1 ± 1.0 (−3.7– −0.7)	< 0.0001[a]	(NCI-LP, NCI-HP) > (MCI, AD)
Semantic memory	0.3 ± 0.3 (0.1–1.0)	0.0 ± 0.4 (−0.2–0.8)	−0.3 ± 0.6 (−1.6-0.1)	−1.3 ± 0.8 (−3.0– −0.4)	< 0.0001[a]	(NCI-LP, NCI-HP) > (MCI, AD)
Working memory	−0.1 ± 0.6 (−1.0–0.7)	−0.1 ± 0.5 (−0.8–0.5)	−0.6 ± 0.5 (−1.3–0.4)	−1.2 ± 0.8 (−2.8– −0.1)	0.002[a]	(NCI-LP, NCI-HP) > AD
Perceptual speed	−0.2 ± 0.8 (−1.2-1.2)	−0.3 ± 0.6 (−1.2-0.5)	−0.9 ± 0.6 (−1.8–0.2)	−1.6 ± 0.8 (−3.0– −0.6)	0.001[a]	(NCI-LP, NCI-HP) > AD
Visuospatial Ability	−0.1 ± 0.4 (−0.5-0.7)	−0.2 ± 0.6 (−0.6-0.7)	−0.5 ± 0.5 (−1.4–0.4)	−1.3 ± 0.5 (−2.6– −0.9)	0.0003[b]	(NCI-LP, NCI-HP) > AD
PMI (hours):	6.6 ± 2.5 (3.0–10.7)	5.1 ± 1.7 (3.1–8.0)	5.8 ± 2.7 (2.0–10.4)	5.1 ± 2.0 (2.9–8.2)	0.42[b]	–
CERAD:					0.003[b]	AD > (NCI-LP, MCI)
No AD	5	3	3	0		
Possible	1	1	0	0		
Probable	1	6	6	2		
Definite	1	2	1	7		
Braak scores:					< 0.0001[b]	(NCI-HP, MCI, AD) > NCI-LP
0	0	0	0	0		
I/II	8	0	0	0		
III/IV	0	12	8	4		
V/VI	0	0	2	5		
NIA Reagan:					0.0008[b]	AD > NCI-LP
No	0	0	0	0		
Low	7	4	3	1		
Intermediate	1	8	6	5		
High	0	0	1	4		
Diffuse plaque load	33.6 ± 51.2 (0–118)	58.3 ± 60.4 (0–191)	53.5 ± 70.3 (0–291)	76.4 ± 28.9 (29–121)	0.09[b]	–

(Continues)

Genes and Genomes (KEGG) functional annotation.[74] Pathview parameters were set to produce pathway diagrams that strongly linked to our miRNA–mRNA target genes at $p < 0.01$. To maintain a conservative approach, only target mRNAs confirmed by at least two independent published experiments via TarBase were analyzed by STRING and Pathview.

3 | RESULTS

3.1 | Subject characteristics

Demographic variables, *ante mortem* clinical data including neuropsychological test scores, and *post mortem* neuropathology diagnostic

TABLE 1 (Continued)

	Diagnosis					
	NCI-LP ($n = 8$)	NCI-HP ($n = 12$)	MCI ($n = 10$)	AD ($n = 9$)	*p* value	Pair-wise comparison
Neuritic plaque load	19.5 ± 37.1 (0–106)	35.3 ± 32.4 (0–108)	40.4 ± 42.1 (0–135)	103.3 ± 45.2 (49–196)	0.002[b]	AD > (NCI-LP, NCI-HP, MCI)
NFT tangle load	5.6 ± 3.9 (1–12)	47.5 ± 25.1 (27–110)	41.0 ± 23.7 (20–88)	110.0 ± 66.7 (18–237)	< 0.0001[a]	(NCI-HP, AD) > NCI-LP

Note: Data shown as mean ± standard deviation (range).

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; MCI, mild cognitive impairment; miRNAs, microRNAs; MMSE, Mini-Mental State Examination; mTOR, mammalian target of rapamycin; NCI-HP, no cognitive impairment patients with high AD pathology; NCI-LP, no cognitive impairment patients with low AD pathology; NFT, neurofibrillary tangle; NIA, National Institute on Aging; PCC, posterior cingulate cortex; PMI, *post mortem* interval.

[a]One-way analysis of variance with Bonferroni correction for multiple comparisons.

[b]Kruskal–Wallis test with Dunn test for multiple comparisons.

[c]Fisher's exact test.

criteria for the subjects ($n = 39$) are shown in Table 1. There were no significant differences in sex, years of education, *post mortem* interval (PMI), or possession of at least one *APOE* ε4 allele across groups. By contrast, the NCI-LP group was younger in age than the NCI-HP, MCI, and AD groups ($p = 0.01$). Subjects with AD had significantly lower MMSE scores ($p < 0.0001$), whereas the MCI and AD groups displayed a lower GCS than the NCI groups ($p < 0.0001$). Comparisons of *ante mortem* performance on composite measures of episodic, semantic, and working memory, as well as perceptual speed and visuospatial ability, are shown in Table 1. Neuropathologically, the NCI group was quite heterogeneous and the NCI-HP subgroup overlapped with the MCI group, suggesting the presence of resilience.[52,59,75] For instance, NCI-LP subjects met the criteria for Braak NFT stages I/II (100%), whereas the NCI-HP subjects were categorized as Braak NFT stages III/IV (100%), whereas MCI subjects were categorized as either Braak NFT stages III/IV (80%) or V/VI (20%). Distribution of Braak scores were significantly different between the AD and NCI/MCI groups ($p < 0.0001$). By contrast, CERAD neuritic plaque scores were higher in the AD group compared to the NCI-LP and MCI groups ($p = 0.003$); ≈ 62% of NCI-LP subjects were classified as "No AD," while ≈ 66% of the NCI-HP subjects were classified as "Probable AD" or "Definite AD." The AD group displayed a significantly greater degree of amyloid and NFT pathology than the NCI-LP group based on NIA-Reagan criteria ($p = 0.0008$, Table 1). Comparisons of global diffuse plaque, neuritic plaque, and NFT load (see Methods) among the groups are also shown in Table 1.

3.2 | PCC miRNA dysregulation during the progression of AD

Differential gene expression analysis of the miRNA sequencing dataset identified 36 individual miRNAs or miRNA gene families that were differentially expressed within the PCC comparing the subjects by clinical status (Table 2), which fell into two main categories: (1) miRNAs with significantly decreased expression levels in AD compared to NCI/MCI, and (2) miRNAs upregulated in MCI compared to NCI/AD, which may represent limbic cortical cellular plasticity to mounting AD pathology within the PCC.[50,76–78] In addition, a secondary differential gene expression analysis of the NCI group, as well as separate Spearman correlation and linear ordinal regression analyses of the entire dataset, revealed three more notable miRNA groupings: (3) miRNAs differentiating NCI-HP from NCI-LP subjects, which may be related to resilience,[48,53,57–60] (4) miRNAs with altered expression levels associated with *ante mortem* neuropsychological test scores and/or *post mortem* neuropathological diagnostic criteria, and (5) miRNAs with expression levels related to AD risk, with age, sex, and *APOE* status as covariates.[53] Sequencing data files have been submitted to the GEO database and are available from the corresponding author.

3.3 | Group 1: PCC miRNAs involved in the onset of AD

The preponderance of significant miRNA expression level changes (22/36; ≈ 61%) were identified as decreased in mild/moderate AD compared to NCI/MCI, including let-7d ($p = 0.004$), miR-504 ($p = 0.009$), and miRs-664a/b ($p = 0.005$; Table 2). The predicted mRNA targets for the miRNAs are shown in Table S1 in supporting information. The stringency of the target prediction analysis precluded representation of all the AD-related miRNAs. However, STRING analysis of the most highly validated mRNA targets revealed a large potential functional interactome with hubs including insulin receptor substrate 1 (IRS1), transforming growth factor beta receptor 1 (TGFBR1), and mitogen-activated protein kinase kinase kinase 1 (MAP3K1), suggesting miRNA regulation of multiple cell signaling pathways including those mediated by insulin and transforming growth factor beta (TGF-β) receptors, both of which recruit mitogen-activated protein kinase (MAPK) members as second messengers (Figure 1A).[79,80]

Pathview functional enrichment analysis[73] revealed that AD-related miRNAs may be regulating mRNA targets operating in five

TABLE 2 Differentially expressed miRNAs among the diagnostic groups.

miRNAs	NCI	MCI	AD	*p* value	Groupwise comparisons
let-7a.1_let-7a.2_let-7a.3	118,829.50 ± 68,581.73[a]	169,457.60 ± 64,857.85	87,134.78 ± 50,012.67	0.03[b]	MCI > AD; $d = 1.42$[c]
let-7d	652.90 ± 207.35	891.90 ± 349.01	469.89 ± 138.13	0.004	MCI > AD; $d = 1.59$
let-7f.1	44.55 ± 16.85	57.60 ± 19.18	34.78 ± 12.20	0.02	MCI > AD; $d = 1.42$
miR-1229	19.15 ± 14.02	22.10 ± 12.62	10.22 ± 9.32	0.02	MCI > AD; $d = 1.07$
miR-126	24,009.60 ± 11,454.72	35,203.10 ± 12,328.70	19,531.33 ± 9,903.46	0.03	MCI > AD; $d = 1.40$
miR-1271	378.25 ± 175.46	543.90 ± 321.18	268.89 ± 131.95	0.02	MCI > AD; $d = 1.12$
miR-146a	543.80 ± 225.91	837.40 ± 216.75	574.89 ± 139.00	0.005	MCI > NCI; $d = 1.33$
miR-16.1_miR-16.2	11,750.35 ± 5,017.05	13,380.90 ± 3,974.12	8,222.22 ± 2,545.36	0.04	MCI > AD; $d = 1.55$
miR-191	50,885.60 ± 21,107.10	68,616.70 ± 21,789.84	38,856.22 ± 15,781.66	0.01	MCI > AD; $d = 1.56$
miR-192	5,611.25 ± 2,097.24	7,578.70 ± 2,388.20	4,493.56 ± 1,612.70	0.02	MCI > AD; $d = 1.51$
miR-1983	47.15 ± 22.13	72.20 ± 27.84	36.56 ± 29.53	0.009	MCI > AD; $d = 1.24$
miR-204	12,330.95 ± 4,566.45	17,452.60 ± 5,437.25	9,216.33 ± 3,501.52	0.004	MCI > AD; $d = 1.80$
miR-218.1	16.20 ± 10.51	21.40 ± 8.33	8.78 ± 3.38	0.01	MCI > AD; $d = 1.99$
miR-2467	98.95 ± 48.24	136.90 ± 42.10	81.22 ± 42.48	0.03	MCI > AD; $d = 1.32$
miR-3074	1,313.05 ± 482.43	1,968.40 ± 887.54	1,139.00 ± 344.00	0.03	MCI > AD; $d = 1.23$
miR-30a	2,135.95 ± 764.45	3,197.10 ± 874.54	1,863.11 ± 716.87	0.006	MCI > NCI, AD; $d = 1.28, 1.65$
miR-30e	2,031.90 ± 736.03	2,798.00 ± 716.56	1,771.00 ± 673.88	0.01	MCI > AD; $d = 1.48$
miR-3117	37.30 ± 21.89	56.00 ± 19.94	26.11 ± 8.65	0.008	MCI > AD; $d = 1.94$
miR-3560	8.15 ± 4.02	13.50 ± 4.79	10.56 ± 5.64	0.03	MCI > NCI; $d = 1.21$
miR-362	84.85 ± 43.98	183.20 ± 87.82	105.00 ± 68.49	0.01	MCI > NCI; $d = 1.42$
miR-374a	353.05 ± 144.34	547.10 ± 139.98	337.89 ± 113.52	0.004	MCI > NCI, AD; $d = 1.36, 1.64$
miR-374b	520.70 ± 303.93	753.50 ± 238.09	554.00 ± 173.48	0.02	MCI > NCI; $d = 0.85$
miR-501	489.75 ± 149.66	771.80 ± 242.91	447.89 ± 184.34	0.005	MCI > NCI, AD; $d = 1.40, 1.50$
miR-504	263.25 ± 163.89	354.20 ± 147.63	143.78 ± 91.14	0.009	MCI > AD; $d = 1.72$
miR-589	183.40 ± 84.54	280.10 ± 83.55	181.78 ± 75.54	0.02	MCI > NCI, AD; $d = 1.15$
miR-6511a.1_miR-6511a.2_miR-6511a.3 miR-6511a.4	116.80 ± 45.30	146.50 ± 43.32	82.00 ± 71.10	0.02	MCI > AD; $d = 1.10$
miR-664a	303.50 ± 133.08	418.00 ± 141.88	196.67 ± 81.99	0.005	MCI > AD; $d = 1.91$
miR-664b	26.60 ± 9.78	31.40 ± 11.89	14.89 ± 6.64	0.005	NCI, MCI > AD; $d = 1.40, 1.71$
miR-99a	11,476.30 ± 3,965.20	16,165.50 ± 4,077.75	10,164.44 ± 3,665.41	0.01	NCI, MCI > AD; $d = 0.34, 1.55$
miRNAs associated with resilience		**NCI-LP**[d]	**NCI-HP**		
miR-211		2.00 (2.00, 9.00)	59.00 (11.00, 73.00)	0.003	
miR-429		6.00 (2.25, 8.00)	3.50 (2.00, 7.00)	0.01	
miR-193a		44.00 (29.25, 74.50)	68.00 (18.50, 74.25)	0.01	
let-7D		637.00 (383.75, 724.25)	660.00 (578.25, 835.50)	0.02	
miR-467		124.00 (65.75, 176.50)	82.00 (49.00, 126.75)	0.02	

(Continues)

TABLE 2 (Continued)

miRNAs associated with resilience	NCI-LP[d]	NCI-HP	
miR-32	6.00 (4.00, 6.00)	5.50 (4.00, 7.00)	0.02
miR-190a_1	13.00 (8.25, 15.00)	11.00 (7.00, 15.25)	0.03
miR-320c_	15.50 (11.00, 21.00)	11.00 (6.00, 14.75)	0.04
miR-499a	69.00 (40.50, 90.25)	49.00 (27.25, 55.00)	0.04
miR-887	343.00 (140.25, 373.75)	247.00 (114.25, 286.00)	0.04
miR-99b	11.00 (6.25, 13.75)	14.00 (7.75, 27.25)	0.04

Abbreviations: AD, Alzheimer's disease; FDR, false discovery rate; miRNAs, microRNAs; NCI, no cognitive impairment; NCI-HP, no cognitive impairment patients with high AD pathology; NCI-LP, no cognitive impairment patients with low AD pathology; NFT, neurofibrillary tangle.
[a]Mean ± standard deviation.
[b]FDR significance level = 0.04.
[c]Between-group effect sizes via Cohen *d*.
[d]Median (25th percentile, 5th percentile).

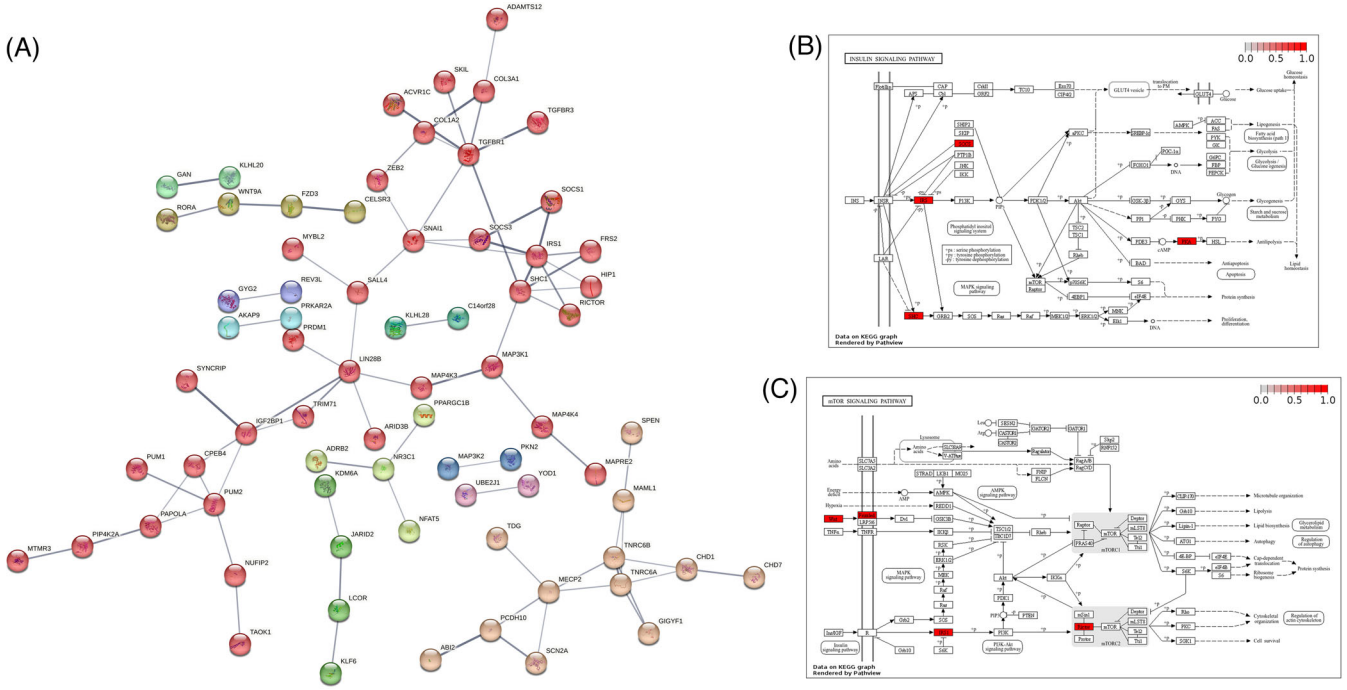

FIGURE 1 Putative protein–protein interactions and pathways of genes targeted by differentially expressed miRNAs PCC of AD subjects. A, STRING interactome diagram shows putative functional relationships among mRNAs targeted by AD-specific miRNAs. Pathview diagrams show functionally enriched (B) insulin and (C) mTOR signaling pathways. Genes/proteins in red are functional interaction nodes for mRNA targets of AD-related miRNAs in PCC. AD, Alzheimer's disease; miRNAs, microRNAs; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; PCC, posterior cingulate cortex; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins

different signaling pathways: insulin, mammalian target of rapamycin (mTOR), MAPK, neurotrophin, and prolactin (Table S2 in supporting information). Each of these pathways has been implicated in AD,[81–85] and the present dataset suggests that altered miRNA regulatory networks play a critical upstream role in mediating the integrity of these pathways during disease progression. Nodes of interaction for AD-related miRNA targets within the two most strongly connected pathways—insulin and mTOR—are shown in Figure 1B and Figure 1C, respectively.

3.4 | Group 2: MCI-specific PCC miRNA dysregulation—putative plasticity responses

A second class of differentially expressed miRNAs comprising miR-30a ($p = 0.006$), miR-374a ($p = 0.004$), miR-501 ($p = 0.005$), and miR-589 ($p = 0.02$) was specifically upregulated in MCI compared to NCI and AD (Table 2). The predicted mRNA targets for the miRNAs are shown in Table S3 in supporting information. STRING interactome analysis of the mRNA targets identified heat-shock protein 90 alpha family class

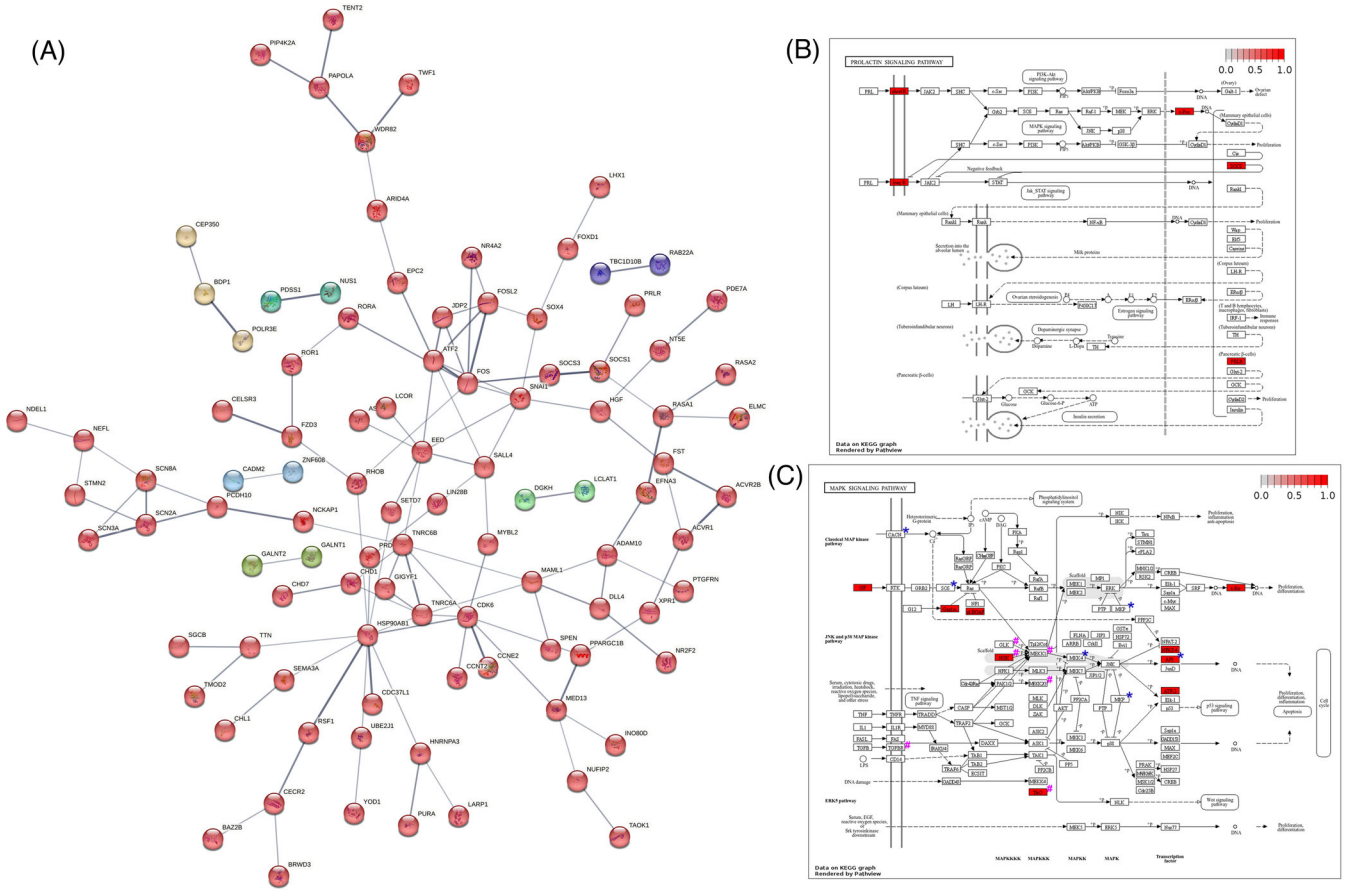

FIGURE 2 Putative protein–protein interactions and pathways of genes targeted by miRNAs upregulated in PCC of MCI compared to NCI and AD subjects. A, STRING interactome diagram show putative functional relationships among mRNAs targeted by MCI-specific miRNAs. Pathview diagrams show functionally enriched (B) prolactin and (C) MAPK signaling pathways. Genes/proteins in red are functional interaction nodes for mRNA targets of miRNAs upregulated in PCC of MCI subjects. *, MAPK pathway interaction nodes for AD-related miRNAs; #, MAPK pathway interaction nodes for resilience-related miRNAs. AD, Alzheimer's disease; MAPK, mitogen-activated protein kinase; MCI, mild cognitive impairment; miRNAs, microRNAs; mRNA, messenger RNA; NCI, no cognitive impairment; PCC, posterior cingulate cortex; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins.

B member 1 (HSP90AB1) as a major hub (Figure 2A), suggesting a role for this chaperone in mediating MCI-related miRNA responses in PCC. STRING analysis also identified a cluster of transcription regulatory genes including activating transcription factor 2 (ATF2), also known as cyclic AMP-responsive element-binding protein 2 (CREB2); Fos proto-oncogene, AP-1 transcription factor subunit (FOS); embryonic ectoderm development (EED); and snail family transcriptional repressor 1 (SNA1), suggesting an additional upstream reorganization of miRNA expression that redirects the transcriptional machinery in PCC during MCI (Figure 2A).

In tandem with the functional clues to MCI-specific miRNA alterations provided by STRING, Pathview functional enrichment analysis uncovered interactions with several cell signaling pathways including prolactin, MAPK, phosphatidylinositol 3-kinase/AKT-serine/threonine kinase (PI3K-AKT), TGF-β, and estrogen (Table S4 in supporting information). Nodes of interaction for the MCI-related miRNA targets within the two most strongly connected pathways—prolactin and MAPK—are shown in Figure 2B and Figure 2C, respectively.

3.5 | Group 3: PCC miRNAs related to resilience in NCI subjects

We performed a secondary analysis comparing miRNA levels in NCI-LP (e.g., Braak NFT stages I/II) and NCI-HP (e.g., Braak NFT stages III/IV) to identify potential miRNA alterations in PCC associated with resilience in NCI-HP subjects. Bidirectional changes in specific miRNAs were identified between the two subgroups (Table 2). miR-211 ($p = 0.003$) and miR-193a ($p = 0.01$) were downregulated, whereas miR-429 ($p = 0.01$) and miR-467 ($p = 0.02$) were upregulated, in NCI-HP subjects compared to NCI-LP subjects. PCC levels of let-7d, which were downregulated in AD compared to MCI, were also downregulated in NCI-HP versus NCI-LP subjects ($p = 0.02$, Table 2). The predicted mRNA targets for the NCI/resilience-related miRNAs are shown in Table S5 in supporting information. STRING interactome analysis of the targets identified Jun proto-oncogene, AP-1 transcription factor subunit (JUN) as a major hub (Figure 3A), suggesting a role for this transcription factor in orchestrating the functional outcome of

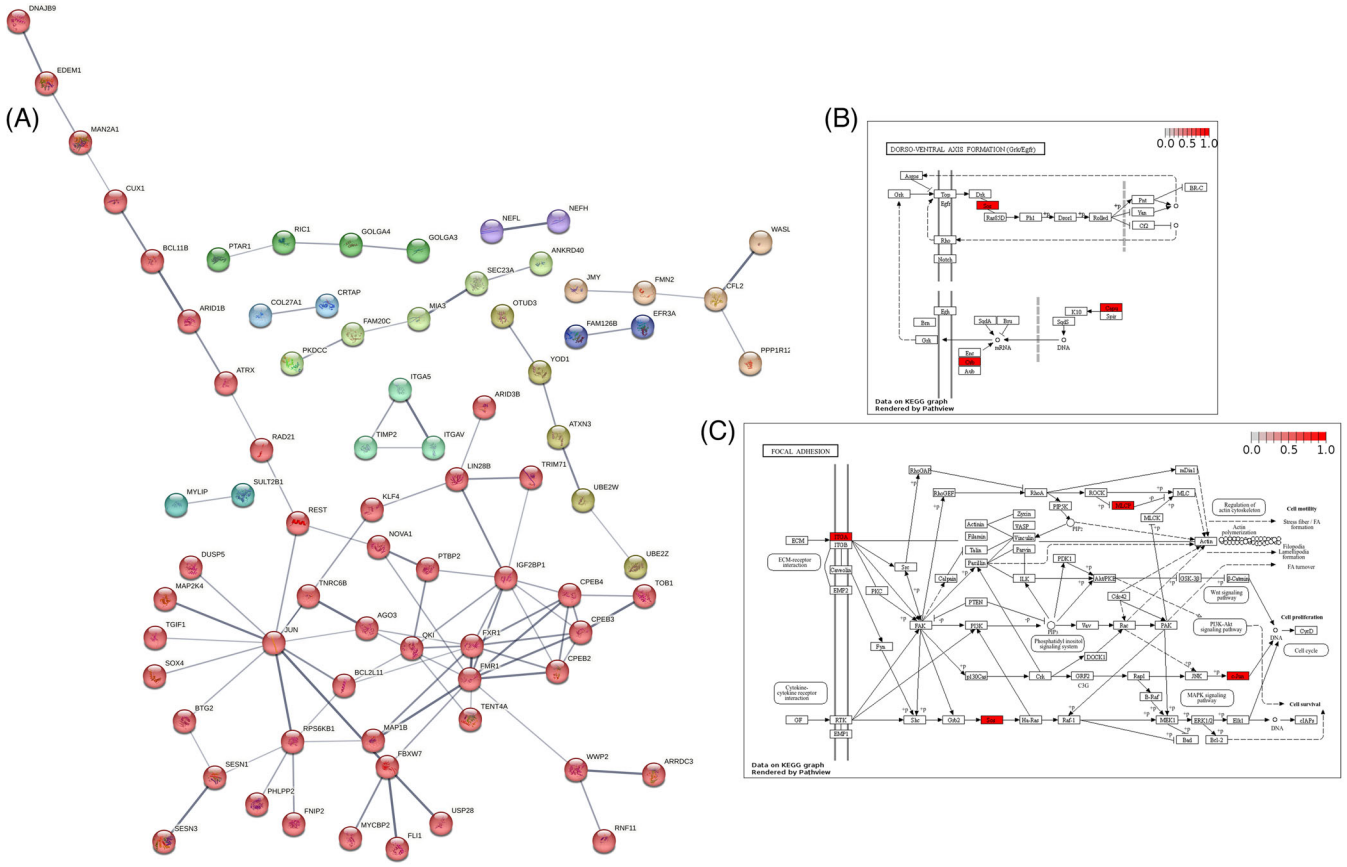

FIGURE 3 Putative protein–protein interactions and pathways of genes targeted by resilience-related miRNAs differentially expressed in PCC of NCI-HP compared to NCI-LP subjects. A, STRING interactome diagram show putative functional relationships among mRNAs targeted by resilience-associated miRNAs. Pathview diagrams show functionally enriched (B) Dorso-ventral axis formation and (C) focal adhesion pathways. Genes/proteins in red are functional interaction nodes for mRNA targets of miRNAs differentially expressed in NCI-HP versus NCI-LP subjects. NCI-HP, no cognitive impairment patients with high AD pathology; NCI-LP, no cognitive impairment patients with low AD pathology; miRNAs, microRNAs; mRNA, messenger RNA; PCC, posterior cingulate cortex; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins.

miRNA alterations within PCC in the face of increasing AD pathology. JUN activation is elicited by neurotransmitter, neurotrophin, and cytokine receptors,[86] resulting in heterodimerization with FOS family members and other binding partners to form AP-1 and ATF–CREB transcription factor complexes, which regulate immediate–early gene expression, particularly during cell differentiation, stress responses, and apoptosis.[87–89] Also of interest was that JUN displayed connectivity with the repressor element 1-silencing transcription factor (REST), which is reduced in MCI and AD and is neuroprotective against various neuronal stressors such as Aβ and oxidative stress.[90] Three additional small interactomes included the dendritic spine protein cofilin 2 (CFL2), the cell adhesion molecules Integrin Subunit Alpha 5/V (ITAG5/V) and TIMP metallopeptidase inhibitor 2 (TIMP2), and neurofilament heavy and light chains (NEFL/NEFH; Figure 3A).

Pathview functional enrichment analysis revealed several mRNA target pathways with potential relevance to the NCI-HP/resilience-associated miRNAs, including dorso-ventral axis formation, focal adhesion, regulation of actin cytoskeleton, and extracellular matrix (ECM)-receptor interaction, along with MAPK, PI3K-AKT, and ErbB signaling pathways (Table S6 in supporting information). Nodes of interaction for the NCI/resilience-related genes within the two most strongly connected pathways–dorso-ventral axis formation and focal adhesion—are shown in Figure 3B and Figure 3C, respectively.

3.6 | Group 4: PCC miRNAs associated with clinical pathologic variables

We also tested for associations between PCC miRNA alterations and either *ante mortem* neuropsychological test scores or *post mortem* neuropathological variables across the NCI, MCI, and AD subjects. Significant associations are shown in Table 3. Decreasing levels of let-7d, which was downregulated in AD and NCI-HP versus NCI-LP cases, were associated with poorer performance on semantic memory tests ($r = 0.45$, $p = 0.004$) and GCS ($r = 0.38$, $p = 0.02$), whereas decreasing levels of miR-664b, which was also downregulated in AD, were associated with poorer performance on tests of semantic memory ($r = 0.44$, $p = 0.004$), episodic memory ($r = 0.41$, $p = 0009$), and visuospatial orientation ($r = 0.45$, $p = 0.004$) in addition to GCS ($r = 0.45$, $p = 0.004$). The predicted mRNA targets for the cognition-related miRNAs are shown

TABLE 3 Correlation of PCC miRNAs with neuropsychological test scores and AD neuropathology.

	miRNA	Correlation	*p* value
Global cognitive score	let-7d	$r = 0.38$	$p = 0.02$
	miR-4634	$r = 0.45$	$p = 0.005$
	miR-664b	$r = 0.45$	$p = 0.004$
Episodic memory	miR-4634	r = 0.41	$p = 0.01$
	miR-664b	r = 0.41	$p = 0.009$
Semantic memory	let-7d	$r = 0.45$	$p = 0.004$
	miR-6511a	$r = 0.33$	$p = 0.04$
	miR-664b	$r = 0.44$	$p = 0.004$
Working memory	miR-218_1	$r = 0.33$	$p = 0.04$
	miR-4634	r = 0.45	$p = 0.005$
Visuospatial	miR-504	$r = 0.33$	$p = 0.04$
	miR-664b	$r = 0.45$	$p = 0.004$
CERAD stage	miR-218_1	$r = 0.34$	$p = 0.03$
Braak NFT stage	miR-211	$r = -0.33$	$p = 0.04$
	miR-3587	$r = 0.40$	$p = 0.01$
	miR-3968	$r = -0.43$	$p = 0.006$
	miR-4634	$r = -0.47$	$p = 0.003$
	miR-4705	$r = 0.35$	$p = 0.03$
NIA Reagan	miR-4634	$r = -0.36$	$p = 0.03$
Diffuse plaque load	miR-1983	$r = -0.38$	$p = 0.02$
	miR-218_1	$r = -0.33$	$p = 0.04$
	miR-30a	$r = -0.34$	$p = 0.03$
	miR-4634	$r = -0.45$	$p = 0.005$
	miR-501	r = 0–0.38	$p = 0.02$
Neuritic plaque load	miR-1229	$r = -0.37$	$p = 0.02$
	miR-1983	$r = -0.37$	$p = 0.02$
	miR-218_1	$r = -0.37$	$p = 0.02$
	miR-664b	$r = -0.33$	$p = 0.04$
NFT load	let-7a1/let-7a2	$r = -0.33$	$p = 0.04$
	let-7d	$r = -0.51$	$p = 0.001$
	miR-16_1/miR-16_2	$r = -0.34$	$p = 0.03$
	miR-191	$r = -0.38$	$p = 0.02$
	miR-204	$r = -0.37$	$p = 0.02$
	miR-211	$r = -0.37$	$p = 0.02$
	miR-3968	$r = -0.43$	$p = 0.007$
	miR-4634	$r = -0.44$	$p = 0.006$
	miR-504	$r = -0.45$	$p = 0.004$
	miR-6511a	$r = -0.41$	$p = 0.001$
	miR-664a	$r = -0.46$	$p = 0.003$
	miR-664b	$r = -0.41$	$p = 0.01$

Abbreviations: AD, Alzheimer's disease; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; miRNAs, microRNAs; NFT, neurofibrillary tangle; NIA, National Institute on Aging; PCC, posterior cingulate cortex.

in Table S7 in supporting information and reveal that only putative mRNA targets of let-7d and miR-664b were identified. Pathway enrichment analysis revealed that the pathway most strongly linked with miRNA alterations associated with cognitive test scores was protein processing in endoplasmic reticulum (ER); nodes of interaction within this pathway are shown in Figure S1A in supporting information.

Among the multiple miRNAs associated with neuropathology (Table 3), decreasing levels of miR-4634 in PCC, which were not differentially expressed across the diagnostic groups, were inversely associated with increasing NFT load ($r = -0.44$, $p = 0.006$) and Braak stage ($r = -0.47$, $p = 0.003$) as well as increasing diffuse plaque load ($r = -0.45$, $p = 0.005$) and NIA-Reagan likelihood of AD scores ($r = -0.36$, $p = 0.03$). Levels of miR-4634 were also associated with episodic memory ($r = 0.41$, $p = 0.01$), working memory ($r = 0.45$, $p = 0.005$), and GCS ($r = 0.45$, $p = 0.005$), suggesting this miRNA is a prime target for future exploration. The predicted mRNA targets for the pathology-related miRNAs are shown in Table S8 in supporting information. Pathway enrichment analysis revealed that the pathway most strongly linked with miRNAs associated with plaque and NFT neuropathology scores was Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling, with interaction nodes concentrated at the level of membrane-bound JAK non-receptor protein tyrosine kinases (Figure S1B). Enrichment pathways for cognition- and pathology-related miRNAs are shown in Table S9 in supporting information.

3.7 | Group 5: PCC miRNAs related to AD risk

We leveraged ordinal logistic regression techniques to determine which miRNAs displayed expression changes associated with the likelihood of AD, with potential covariates such as age, sex, and *APOE* ε4 status. We identified seven miRNAs associated with differences in probabilities of the four diagnostic groups (NCI-LP, NCI-HP, MCI, and AD): miR-32, miR-3560, miR-6500, miR-101a, miR-183, miR-142b, and miR-374 (Table 4). Of these, miR-32 was increased in NCI-HP compared to NCI-LP subjects ($p = 0.02$) and miR-3560 was upregulated in MCI compared to NCI ($p = 0.03$, Table 2). When we analyzed miRNA effects on the likelihood of each diagnosis, along with potential covariates, AICc comparison revealed that age was the most influential. We found that increasing miR-32 levels were associated with increased AD probability and reduced NCI-LP probability at ages 75 to 85 (Figure 4A). However, elevated miR-32 levels were associated with decreased AD probability and elevation of NCI-LP probability in subjects ≥ 85 years (Figure 4B,C). By contrast, increasing miR-3560 levels were associated with elevated AD probability at all ages and an accompanying reduction in the probability of NCI-LP and other diagnoses (Figure 4D–F). We also analyzed the relationships between miR-6500, miR-101a, miR-183, miR-142b, and miR-374 levels and AD risk. When we tested the potential effects of *APOE* ε4 status alongside the selected miRNAs, we found that *APOE* ε4 status had no significant effect (Figures S2–S4 in supporting information). By contrast, sex influenced miR-183 associations with AD risk, with a potential protective role in females across age groups (Figure S3, Table S4).

The predicted mRNA targets for the AD risk-related miRNAs are shown in Table S10 in supporting information. Pathway enrichment analysis revealed that the pathways most strongly linked with the predicted mRNA targets of these miRNAs were the PI3K-AKT signaling pathway and dorso-ventral axis formation (Table S11 in supporting information). Intriguingly, these pathways were also enriched for the resilience-related miRNAs (Table S6). Nodes of interaction of mRNAs/proteins targeted by the AD risk miRNAs within the PI3K-AKT signaling pathway are shown in Figure S5 in supporting information.

TABLE 4 miRNAs associated with AD risk.

Effect	χ^2 (df)	*p*	R^2	Effect	χ^2 (df)	*p*	R^2
miR-32							
Age	11.998 (1)	< 0.001	0.405	Age	11.986 (1)	< 0.001	0.386
miR-32	0.513 (1)	0.474	0.244	miR-32	0.463 (1)	0.496	0.233
Age × miR-32	10.647 (1)	0.001	0.193	*APOE*	0.000 (1)	0.990	< 0.001
				Age × miR-32	9.746 (1)	0.002	0.174
miR-3560							
Age	11.494 (1)	< 0.001	0.240	Age	11.865 (1)	< 0.001	0.242
miR-3560	7.959 (1)	0.005	0.158	miR-3560	8.607 (1)	0.003	0.168
				APOE	1.128 (1)	0.288	0.020
miR-6500							
Age	12.691 (1)	< 0.001	0.274	Age	13.332 (1)	< 0.001	0.282
miR-6500	4.307 (1)	0.038	0.200	miR-6500	4.414 (1)	0.036	0.205
Age × miR-6500	4.561 (1)	0.033	0.082	*APOE*	0.946 (1)	0.331	0.016
				Age × miR-6500	4.850 (1)	0.028	0.085
miR-101a							
Age	9.168 (1)	0.002	0.390	Age	9.221 (1)	0.002	0.383
miR-101a	0.247 (1)	0.619	0.188	miR-101a	0.259 (1)	0.611	0.178
Age × miR-101a	8.602 (1)	0.003	0.165	*APOE*	0.066 (1)	0.797	0.001
				Age × miR-101a	8.173 (1)	0.004	0.156
miR-183							
miR-183	4.711 (1)	0.030	0.236	*APOE*	0.037 (1)	0.847	0.196
Sex	0.437 (1)	0.509	0.205	Age	10.692 (1)	0.001	0.172
Age	10.695 (1)	0.001	0.196	miR-183	4.694 (1)	0.030	0.149
miR-183 × Sex	10.931 (1)	< 0.001	0.201	Sex	0.460 (1)	0.498	< 0.001
				miR-183 × Sex	10.261 (1)	0.001	0.141
miR-142b							
Age	9.688 (1)	0.002	0.297	*APOE*	0.167 (1)	0.683	0.294
miR-142b	6.738 (1)	0.009	0.176	Age	9.624 (1)	0.002	0.175
Age × miR-142b	4.613 (1)	0.032	0.086	miR-142b	6.436 (1)	0.011	0.003
				Age × miR-142b	4.425 (1)	0.035	0.088
miR-3574							
Age	10.789 (1)	0.001	0.238	Age	14.312 (1)	< 0.001	0.301
miR-3574	5.465 (1)	0.019	0.112	miR-3574	6.277 (1)	0.012	0.118
				APOE	0.675 (1)	0.411	0.071
				Age × APOE	3.244 (1)	0.072	0.059

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; miRNAs, microRNAs.

4 | DISCUSSION

We show that select miRNAs are altered within the PCC, a critical region for episodic memory and attentional function,[91] during the onset of AD, prodromal MCI, and in NCI cases displaying putative cognitive resilience. We also demonstrate that several miRNA clusters are associated with *ante mortem* cognitive performance and *post mortem* neuropathological burden and that others may influence AD risk. These miRNA groups appear to regulate a wide variety of functional pathways, as discussed below.

4.1 | PCC miRNAs dysregulated during the onset of AD: involvement in insulin and mTOR signaling

Insulin expression has been noted in rodent and human cortex and hippocampus[92–94] and AD progression is characterized by defective brain insulin signaling, as evidenced by reduced insulin receptor binding and subsequent loss of IRS1 (Figure 1A) activation and downstream PI3K-AKT (Figure S5) and MAPK (Figure 2C) signaling.[83, 95, 96] A key manifestation is impaired cellular energy metabolism and oxidative stress, which further perturbates defective insulin signaling.[97]

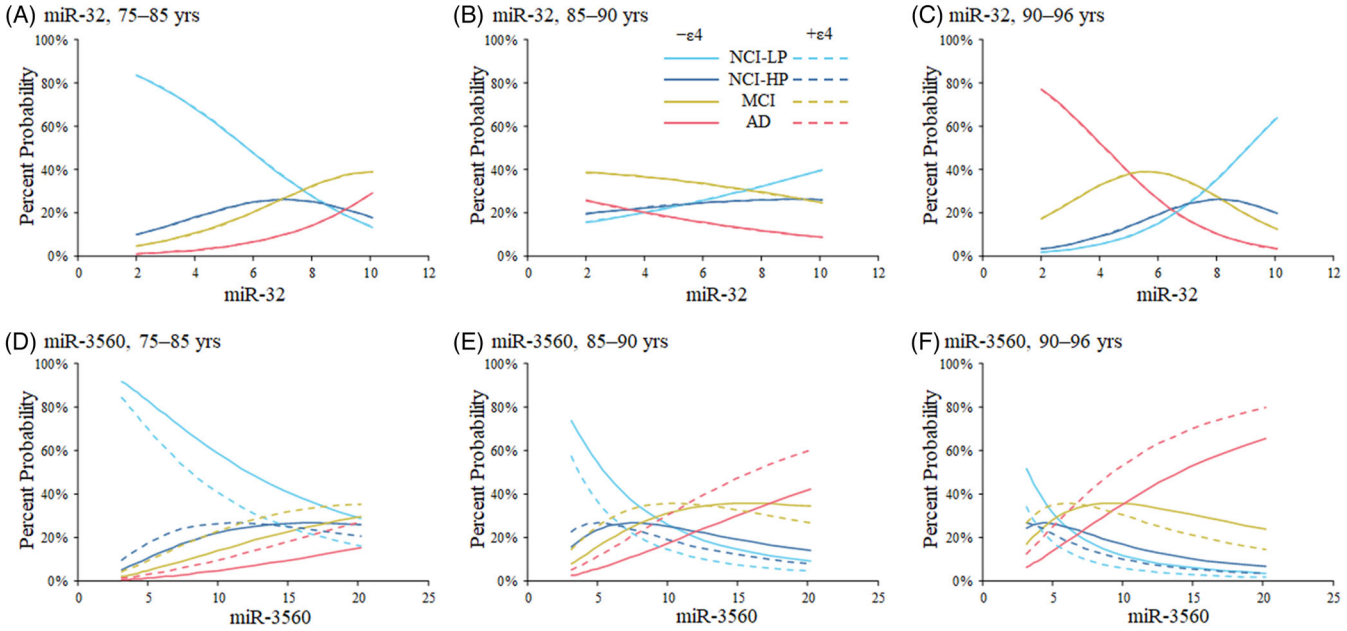

FIGURE 4 Relationship between miR-32 (A–C) and miR-3560 (D–F) miRNA levels and percent probabilities of NCI-LP (——), NCI-HP (——), MCI (——), and AD (——) at ages 75–85 (A,D), 85–90 (B,E), and 90–96 (C,F). AD, Alzheimer's disease; MCI, mild cognitive impairment; miRNAs, microRNAs; NCI-HP, no cognitive impairment patients with high AD pathology; NCI-LP, no cognitive impairment patients with low AD pathology.

Moreover, type 2 diabetes mellitus is a risk factor for dementia, and insulin-sensitizing drugs have shown therapeutic promise for the disease.[98–100] In this regard, given the rising popularity of glucagon-like peptide-1 (GLP-1) receptor agonists and similar incretin mimetics for diabetes and weight loss, it will be interesting to gauge AD risk reduction in those patients in future studies.[100]

The association of mTOR signaling pathways in disrupting autophagy in AD has also been well described.[81, 101] However, it is notable that sustained mTOR activation is also associated with IRS1 inhibition, thus playing a role in insulin pathway impairments.[102] Furthermore, neurons co-expressing tau pathology and activated mTOR display decreased mitochondrial antioxidant enzymes and higher levels of oxidative damage.[103] These data suggest that miRNA perturbations during the onset of AD are linked to promoting or preventing cellular damage and impaired energy metabolism through insulin and mTOR signaling pathways.

4.2 | PCC miRNAs dysregulated during MCI: involvement of prolactin and MAPK signaling

While prolactin is best known for its role in lactation, prolactin receptors are expressed by neurons and glia in the cortex and hippocampus,[104] where prolactin signaling regulates the expression of glutamatergic (e.g., vesicular glutamate transporter 1 [*SLC17A7*]), cholinergic (e.g., choline acetyltransferase [*CHAT*]), and catecholaminergic (e.g., adrenoceptor alpha 2 [*ADRA2A*] and dopamine receptor 2 [*DRD2*]) genes, axonal guidance genes (e.g., semaphore 3A [*SEMA3A*], which interacts with HSP90AB1 [Figure 2A]);[82, 105, 106] and hippocampal neurogenesis.[107] A prospective cerebrospinal fluid biomarker study of control and AD subjects found that increased prolactin levels were significantly associated with decreased $A\beta_{1\text{-}42}$ levels,[108] and increases in prolactin levels are associated with insulin resistance.[109, 110] These findings suggest a role for miRNA expression alterations in regulating energy metabolism within PCC during MCI and AD via their co-regulation of interacting prolactin and insulin pathways.

The role of MAPK serine/threonine kinases such as those comprising the extracellular-regulated kinase (ERK), p38 MAPK, and stress-activated c-Jun N-terminal kinase (JNK) families in AD has been appreciated for decades.[111] In addition to their physiological role in cognitive behavior as downstream second messengers of glutamatergic, cholinergic, noradrenergic, dopaminergic, and neurotrophin receptors,[86] these kinases have been implicated in tau hyperphosphorylation, $A\beta$ aggregation, neuroinflammation, and synaptic deficits.[85] Small molecule modulators of ERK, p38 MAPK, and JNK are active areas of therapeutic investigation for AD.[85]

Notably, MAPK signaling emerged as the only functional enrichment pathway shared by the AD-, MCI-, and NCI/resilience-related miRNA target genes (see below and Figure 2C), and MAPK signaling is an essential mediator for most of the functionally enriched cell signaling pathways in these datasets.[112] Other miRNA modulators of MAPKs have been observed in different datasets. In particular, the miR-132/212 specifically targets *ERK1* and *ERK2* as well as tau (*MAPT*) and sirtuin 1 (*SIRT1*) transcripts,[113] and we previously showed that miR-132/212 transcripts are transiently downregulated in the frontal cortex of MCI subjects.[78] Follow-up in vitro studies revealed that experimental downregulation of miR-132/212 protected human neu-

ronotypic cultures from Aβ-induced cell death via a SIRT1-dependent manner.[78]

4.3 | PCC miRNAs dysregulated during cognitive resilience: involvement of dorso-ventral axis formation and focal adhesion pathways

There is little data on dorso-ventral axis formation as a functional pathway in AD as it is more commonly associated with embryonic development,[114] although evidence exists for developmental radial and transverse axis differences in innervation patterns, gene expression, and sector-specific mediation of cognitive function in the hippocampus.[115–117] In addition, this functional enrichment category has been linked to miRNA alterations in response to Aβ in primary neurons and increasing pathology in mouse and Drosophila models of AD.[118–120] Hence, while this pathway remains underexplored in AD, it is intriguing in the context of potential morphological compensatory pathways associated with resilience.[48, 53, 57–60]

By contrast, focal adhesion pathways mediating communication between ECM cues and cytoskeletal proteins have been more broadly implicated in AD.[121] Physiologically, this pathway is required for growth cone formation, neurite outgrowth, and axon pathfinding via focal adhesion kinase (FAK) receptors,[122, 123] which activate focal adhesions through integrin clustering and ECM mobilization.[124] In contrast, Aβ can bind FAK receptors to induce tau phosphorylation, dystrophic changes, synaptic loss, and toxicity in primary neurons via focal adhesion proteins such as integrins and paxillins.[124–126]

Remarkably, none of the miRNAs associated with resilience in the present dataset aligned with miRNAs related to resilience in our previous total RNA sequencing study of PCC in NCI subjects.[48] Although 19 of the 20 NCI cases used in the present study matched with the 26 cases used in our previous study, these divergent findings could be due to several factors including differences in sample size, library preparation, sequencing strategies, trimming for sequence reads, or bioinformatic approaches. Then again, the resilience-related miRNAs identified in our previous dataset were functionally enriched for axon guidance, glutamatergic synapse, long-term potentiation, and extracellular structure pathways (adherens junction), suggesting a commonality of resilience-related miRNA changes among the two datasets for morphological responses and perhaps reflecting upstream regulation of DMN connectome reorganization within PCC during the preclinical stages of AD. [48, 57, 60]

4.4 | PCC miRNAs associated with clinical pathologic variables: involvement of protein processing in the ER and JAK-STAT signaling

To our knowledge, this is the first study to report miRNA associations with individual neuropsychological test scores during the progression of AD. The most significantly linked miRNA pathway associated with *ante mortem* cognitive test scores was ER-mediated protein metabolism. Nodes of interaction were associated with N-linked glycosylation (e.g., oligosaccharyltransferase complex subunit [OST]) in the secretory pathway and with deubiquitination (e.g., OTU deubiquitinase 1 [OTU1]) during ER-associated protein catabolism involving the ubiquitin/proteasome pathway. The role of ubiquitin/proteasomal system dysfunction and potential therapeutic implications of targeting this system in AD have been reviewed extensively,[127–130] and we have demonstrated the dysregulation of gene families associated with ubiquitin/proteasome function and other mechanisms of protein turnover in pre-tangle bearing neurons.[131]

The most significantly linked miRNA pathway associated with *post mortem* neuropathological diagnostic criteria was JAK-STAT signaling, which is a driver of neuroinflammation in AD and vascular contributions to cognitive impairment and dementia.[132–136] In particular, pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukins 1 beta (IL-1β), IL-6, and interferon gamma (INF-γ), produced by innate immune cells such as microglia,[137] stimulate intracellular JAK-STAT pathways upon binding their cognate receptors to amplify the inflammatory cascade, ultimately leading to oxidative damage, impaired cellular metabolism, and the promotion of tau and amyloid pathology.[134, 136, 138, 139] These data suggest miRNA-mediated pathways regulate proteostasis and neuroinflammatory pathways that influence cognitive status and pathological burden during the progression of AD.

4.5 | PCC miRNAs associated with AD risk: involvement of PI3K-AKT signaling

A potential role for the PI3K-AKT pathway in AD has been widely reported[85, 140, 141] and, similar to the MAPK pathway, PI3K-AKT second messenger signaling is stimulated by ligand binding to glutamatergic, cholinergic, noradrenergic, dopaminergic, and neurotrophin receptors. This pathway also mediates putative miRNA-associated mechanisms discussed above, including insulin signaling, mTOR signaling, metabolic, morphologic, and proteostasis pathways.[85, 140, 141] Interestingly, these miRNAs appear to exert upstream regulation of PI3K-AKT signaling at the level of growth factors (GF) and receptor tyrosine kinases (RTK), integrin receptors (ITGA), and $G_{\beta\gamma}$ proteins[142] (Figure S5). Therefore, miRNA regulation of diverse signaling functions related to growth factor (e.g., neurotrophin) and G protein-coupled receptors, as well as focal adhesion pathways, may also influence the risk of AD onset.[84, 121]

4.6 | Study limitations and conclusions

There are several study caveats to consider. First, miRNA databases are populated primarily by studies from cancer research, where miRNA pathways have long been recognized as essential regulators of cell transformation and therapeutic targeting,[143] so these databases are likely returning incomplete information about miRNA–mRNA targeting in neural circuits, especially those associated with cortico–cortical

vulnerability. In addition, incomplete information on miRNA targets precluded unequivocal conclusions about the directionality of change in the affected functional pathways. While downregulation of the AD-associated miRNAs implies that insulin and mTOR pathways were upregulated, this may be a simplistic view because multiple positive and negative regulators of these pathways are being targeted. Future studies can be designed to model specific miRNA–mRNA coordinate interactions to gain a better mechanistic understanding of target pathway alterations that underlie resilience, MCI, or AD. Moreover, as miRNA databases are constantly being updated with new target prediction and functional validation data, the sequencing dataset generated in this study will provide an invaluable resource for developing more refined analyses of the intricate regulation of protective and pathogenic protein expression orchestrated by miRNAs during the progression of AD, with critical insights into PCC functionality within the DMN.[41] Functional validation in in vitro and animal models[13, 19] is also needed to gauge the potential for targeting these regulatory interactions as protective therapies. Although we did not note sex differences related to differential expression in *post hoc* analyses (not shown), the sample sizes of the groups were not powered for such an analysis, warranting further study. Also warranting further study is the extent to which the differentially expressed miRNAs are dysregulated in other brain regions in this cohort. Finally, it will be important to understand how miRNA expression and function are moderated by external factors affecting AD risk, such as lifestyle variables and epigenetic responses to environmental exposures,[144, 145] and how these interactions might impact miRNA-mediated pathways to promote or prevent neurodegeneration. Nonetheless, these data from a key, vulnerable limbic region of the DMN provide the field with new insights into putative molecular mechanistic roles of miRNAs in AD risk, pathophysiology, and resilience. They add to a growing body of literature underscoring the potential of harnessing miRNA activity, as well as that of other ncRNAs such as long ncRNAs,[146] circular RNAs,[147] PIWI-interacting RNAs,[148] tRNA fragments,[149] and natural antisense transcripts,[150] to manipulate disease-modifying pathways associated with AD and other neurodegenerative disorders, with implications for precision medicine.[70, 151, 152]

ACKNOWLEDGMENTS

The authors thank the RROS participants for their altruism and the Rush ADRC (National Institutes of Health grant P30 AG072975). This study was supported by National Institute on Aging grants: PO1 AG014449, PO1 AG017617, AG072599, AG074004, AG077103, RFAG081286, P30 AG072931 (Michigan Alzheimer's Disease Research Center), P30 AG072976 (Indiana Alzheimer's Disease Research Center), and the Arizona Alzheimer's Consortium at Barrow Neurological Institute.

CONFLICT OF INTEREST STATEMENTs

The authors report no competing interests. Author disclosures are available in the supporting information.

CONSENT STATEMENT

De-identified *post mortem* tissue was obtained from RROS subjects who provided informed consent for both annual clinical evaluations and brain donation upon death.

ORCID

Scott E. Counts https://orcid.org/0000-0003-2851-9763

REFERENCES

1. Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer's disease. *Lancet*. 2021;397(10284):1577-1590.
2. Lopez OL, Becker JT, Wisniewski S, Saxton J, Kaufer DI, DeKosky ST. Cholinesterase inhibitor treatment alters the natural history of Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2002;72(3):310-314.
3. Golde TE, Levey AI. Immunotherapies for Alzheimer's disease. *Science*. 2023;382(6676):1242-1244.
4. Foley KE, Wilcock DM. Three major effects of APOE(epsilon4) on abeta immunotherapy induced ARIA. *Front Aging Neurosci*. 2024;16:1412006.
5. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science*. 2001;294(5543):853-858.
6. Hebert SS, De Strooper B. Alterations of the microRNA network cause neurodegenerative disease. *Trends Neurosci*. 2009;32(4):199-206.
7. Nelson PT, Wang WX, Rajeev BW. MicroRNAs (miRNAs) in neurodegenerative diseases. *Brain Pathol*. 2008;18(1):130-138.
8. Kaur S, Verma H, Kaur S, et al. Understanding the multifaceted role of miRNAs in Alzheimer's disease pathology. *Metab Brain Dis*. 2024;39(1):217-237.
9. Wang L, Shui X, Diao Y, Chen D, Zhou Y, Lee TH. Potential implications of miRNAs in the pathogenesis, diagnosis, and therapeutics of Alzheimer's disease. *Int J Mol Sci*. 2023;24(22):16259.
10. Lukiw WJ. MicroRNA (miRNA) complexity in Alzheimer's disease (AD). *Biology*. 2023;12(6):788.
11. Absalon S, Kochanek DM, Raghavan V, Krichevsky AM. MiR-26b, upregulated in Alzheimer's disease, activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons. *J Neurosci*. 2013;33(37):14645-14659.
12. Banzhaf-Strathmann J, Benito E, May S, et al. MicroRNA-125b induces tau hyperphosphorylation and cognitive deficits in Alzheimer's disease. *The EMBO Journal*. 2014;33(15):1667-1680.
13. Chopra N, Wang R, Maloney B, et al. MicroRNA-298 reduces levels of human amyloid-beta precursor protein (APP), beta-site APP-converting enzyme 1 (BACE1) and specific tau protein moieties. *Mol Psychiatry*. 2021;26(10):5636-5657.
14. Cui JG, Li YY, Zhao Y, Bhattacharjee S, Lukiw WJ. Differential regulation of interleukin-1 receptor-associated kinase-1 (IRAK-1) and IRAK-2 by microRNA-146a and NF-kappaB in stressed human astroglial cells and in Alzheimer disease. *J Biol Chem*. 2010;285(50):38951-38960.
15. Hebert SS, Horre K, Nicolai L, et al. Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. *Proc Natl Acad Sci*. 2008;105(17):6415-6420.
16. Hebert SS, Papadopoulou AS, Smith P, et al. Genetic ablation of Dicer in adult forebrain neurons results in abnormal tau hyperphosphorylation and neurodegeneration. *Hum Mol Genetics*. 2010;19(20):3959-3969.

17. Li YY, Alexandrov PN, Pogue AI, Zhao Y, Bhattacharjee S, Lukiw WJ. miRNA-155 upregulation and complement factor H deficits in Down's syndrome. *Neuroreport*. 2012;23(3):168-173.
18. Lukiw WJ, Cui JG, Yuan LY, et al. Acyclovir or Abeta42 peptides attenuate HSV-1-induced miRNA-146a levels in human primary brain cells. *Neuroreport*. 2010;21(14):922-927.
19. Wang R, Chopra N, Nho K, et al. Human microRNA (miR-20b-5p) modulates Alzheimer's disease pathways and neuronal function, and a specific polymorphism close to the MIR20B gene influences Alzheimer's biomarkers. *Mol Psychiatry*. 2022;27(2):1256-1273.
20. Wang WX, Rajeev BW, Stromberg AJ, et al. The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. *J Neurosci*. 2008;28(5):1213-1223.
21. Pratico D. The functional role of microRNAs in the pathogenesis of tauopathy. *Cells*. 2020;9(10):2262.
22. Rashidi SK, Kalirad A, Rafie S, Behzad E, Dezfouli MA. The role of microRNAs in neurobiology and pathophysiology of the hippocampus. *Front Mol Neurosci*. 2023;16:1226413.
23. Saleem A, Javed M, Akhtar MF, et al. Current updates on the role of MicroRNA in the diagnosis and treatment of neurodegenerative diseases. *Curr Gene Ther*. 2024;24(2):122-134.
24. Yang R, Yang B, Liu W, Tan C, Chen H, Wang X. Emerging role of non-coding RNAs in neuroinflammation mediated by microglia and astrocytes. *J Neuroinflam*. 2023;20(1):173.
25. Long JM, Lahiri DK. MicroRNA-101 downregulates Alzheimer's amyloid-beta precursor protein levels in human cell cultures and is differentially expressed. *Biochem Biophys Res Comm*. 2011;404(4):889-895.
26. Long JM, Maloney B, Rogers JT, Lahiri DK. Novel upregulation of amyloid-beta precursor protein (APP) by microRNA-346 via targeting of APP mRNA 5'-untranslated region: implications in Alzheimer's disease. *Mol Psychiatry*. 2019;24(3):345-363.
27. Arora T, Prashar V, Singh R, et al. Dysregulated miRNAs in progression and pathogenesis of Alzheimer's disease. *Mol Neurobiol*. 2022;59(10):6107-6124.
28. Han SW, Pyun JM, Bice PJ, et al. miR-129-5p as a biomarker for pathology and cognitive decline in Alzheimer's disease. *Alzheimers Res Ther*. 2024;16(1):5.
29. Kumar A, Su Y, Sharma M, et al. MicroRNA expression in extracellular vesicles as a novel blood-based biomarker for Alzheimer's disease. *Alzheimers Dement*. 2023;19(11):4952-4966.
30. Kumar S, Reddy PH. Elevated levels of MicroRNA-455-3p in the cerebrospinal fluid of Alzheimer's patients: a potential biomarker for Alzheimer's disease. *Biochim Biophys Acta Mol Basis Dis*. 2021;1867(4):166052.
31. Pereira JD, Teixeira LCR, Mamede I, et al. miRNAs in cerebrospinal fluid associated with Alzheimer's disease: a systematic review and pathway analysis using a data mining and machine learning approach. *J Neurochem*. 2024;168:977-994.
32. Sandau US, Wiedrick JT, McFarland TJ, et al. Analysis of the longitudinal stability of human plasma miRNAs and implications for disease biomarkers. *Sci Rep*. 2024;14(1):2148.
33. Siedlecki-Wullich D, Minano-Molina AJ, Rodriguez-Alvarez J. microRNAs as early biomarkers of Alzheimer's disease: a synaptic perspective. *Cells*. 2021;10(1):113.
34. Bennett DA, Schneider JA, Bienias JL, Evans DA, Wilson RS. Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. *Neurology*. 2005;64(5):834-841.
35. Markesbery WR, Schmitt FA, Kryscio RJ, Davis DG, Smith CD, Wekstein DR. Neuropathologic substrate of mild cognitive impairment. *Arch Neurol*. 2006;63(1):38-46.
36. Morris JC, Storandt M, Miller JP, et al. Mild cognitive impairment represents early-stage Alzheimer disease. *Arch Neurol*. 2001;58(3):397-405.
37. Mufson EJ, Binder L, Counts SE, et al. Mild cognitive impairment: pathology and mechanisms. *Acta Neuropathol*. 2012;123(1):13-30.
38. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. 2004;256(3):183-194.
39. Leech R, Sharp DJ. The role of the posterior cingulate cortex in cognition and disease. *Brain*. 2014;137:12-32.
40. Maddock RJ, Garrett AS, Buonocore MH. Remembering familiar people: the posterior cingulate cortex and autobiographical memory retrieval. *Neuroscience*. 2001;104(3):667-676.
41. Raichle ME. The brain's default mode network. *Annu Rev Neurosci*. 2015;38:433-447.
42. Bergeron D, Beauregard JM, Soucy JP, et al. Posterior cingulate cortex hypometabolism in non-amnestic variants of Alzheimer's disease. *J Alzheimers Dis*. 2020;77(4):1569-1577.
43. Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann Neurol*. 1997;42(1):85-94.
44. Neth BJ, Graff-Radford J, Mielke MM, et al. Relationship between risk factors and brain reserve in late middle age: implications for cognitive aging. *Front Aging Neurosi*. 2019;11:355.
45. Zhou Y, Dougherty JH Jr., Hubner KF, Bai B, Cannon RL, Hutson RK. Abnormal connectivity in the posterior cingulate and hippocampus in early Alzheimer's disease and mild cognitive impairment. *Alzheimers Dement*. 2008;4(4):265-270.
46. Scheff SW, Price DA, Ansari MA, et al. Synaptic change in the posterior cingulate gyrus in the progression of Alzheimer's disease. *J Alzheimers Dis*. 2015;43(3):1073-1090.
47. Vogt BA, Vogt LJ, Vrana KE, et al. Multivariate analysis of laminar patterns of neurodegeneration in posterior cingulate cortex in Alzheimer's disease. *Exp Neurol*. 1998;153(1):8-22.
48. Kelley CM, Ginsberg SD, Liang WS, Counts SE, Mufson EJ. Posterior cingulate cortex reveals an expression profile of resilience in cognitively intact elders. *Brain Commun*. 2022;4(4):fcac162.
49. Bennett DA, Wilson RS, Schneider JA, et al. Natural history of mild cognitive impairment in older persons. *Neurology*. 2002;59(2):198-205.
50. Counts SE, Nadeem M, Lad SP, Wuu J, Mufson EJ. Differential expression of synaptic proteins in the frontal and temporal cortex of elderly subjects with mild cognitive impairment. *J Neuropathol Exp Neurol*. 2006;65(6):592-601.
51. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol*. 2009;66(2):200-208.
52. Mufson EJ, Malek-Ahmadi M, Perez SE, Chen K. Braak staging, plaque pathology, and APOE status in elderly persons without cognitive impairment. *Neurobiol Aging*. 2016;37:147-153.
53. Kelley CM, Maloney B, Beck JS, et al. Micro-RNA profiles of pathology and resilience in posterior cingulate cortex of cognitively intact elders. *Brain Commun*. 2024;6(2):fcae082.
54. Mahady LJ, He B, Malek-Ahmadi M, Mufson EJ. Telomeric alterations in the default mode network during the progression of Alzheimer's disease: selective vulnerability of the precuneus. *Neuropathol Appl Neurobiol*. 2021;47(3):428-440.
55. Arnold SE, Louneva N, Cao K, et al. Cellular, synaptic, and biochemical features of resilient cognition in Alzheimer's disease. *Neurobiol Aging*. 2012;34(1):157-168.
56. Azarpazhooh MR, Avan A, Cipriano LE, et al. A third of community-dwelling elderly with intermediate and high level of Alzheimer's neuropathologic changes are not demented: a meta-analysis. *Ageing Res Rev*. 2020;58:101002.

57. King D, Holt K, Toombs J, et al. Synaptic resilience is associated with maintained cognition during ageing. *Alzheimers Dement*. 2023;19(6):2560-2574.
58. Perez-Nievas BG, Stein TD, Tai HC, et al. Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. *Brain*. 2013;136:2510-2526.
59. Montine TJ, Cholerton BA, Corrada MM, et al. Concepts for brain aging: resistance, resilience, reserve, and compensation. *Alzheimers Res Ther*. 2019;11(1):22.
60. Gomez-Isla T, Frosch MP. Lesions without symptoms: understanding resilience to Alzheimer disease neuropathological changes. *Nature Rev Neurol*. 2022;18(6):323-332.
61. Bennett DA, Buchman AS, Boyle PA, Barnes LL, Wilson RS, Schneider JA. Religious orders study and Rush Memory and Aging Project. *J Alzheimers Dis*. 2018;64(s1):S161-S189.
62. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology*. 1991;41(4):479-486.
63. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;82(4):239-259.
64. Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J Neuropathol Exp Neurol*. 1997;56(10):1095-1097.
65. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol*. 2012;123(1):1-11.
66. Vogt BA, Laureys S. Posterior cingulate, precuneal and retrosplenial cortices: cytology and components of the neural network correlates of consciousness. *Prog Brain Res*. 2005;150:205-217.
67. Jiang YJ, Cao SQ, Gao LB, et al. Circular ribonucleic acid expression profile in mouse cortex after traumatic brain injury. *J Neurotrauma*. 2019;36(7):1018-1028.
68. Sekar S, Geiger P, Cuyugan L, et al. Identification of Circular RNAs using RNA Sequencing. *J Vis Exp*. 2019(153).
69. Ananth CV, Kleinbaum DG. Regression models for ordinal responses: a review of methods and applications. *Int J Epidemiol*. 1997;26(6):1323-1333.
70. Lahiri, DK. An Integrated Approach to Genome Studies. *Science*. 2011;331(6014):147. doi:10.1126/science.331.6014.147-a
71. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. *Nucleic Acids Res*. 2018;46(D1):D239-D245.
72. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607-D613.
73. Luo W, Pant G, Bhavnasi YK, Blanchard SG, Brouwer C. Pathview Web: user friendly pathway visualization and data integration. *Nucleic Acids Res*. 2017;45(W1):W501-W508.
74. Kanehisa M, Sato Y, Kawashima M. KEGG mapping tools for uncovering hidden features in biological data. *Protein Sci*. 2022;31(1):47-53.
75. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology*. 2006;66(12):1837-1844.
76. Bell KF, Bennett DA, Cuello AC. Paradoxical upregulation of glutamatergic presynaptic boutons during mild cognitive impairment. *J Neurosci*. 2007;27(40):10810-10817.
77. Counts SE, Alldred MJ, Che S, Ginsberg SD, Mufson EJ. Synaptic gene dysregulation within hippocampal CA1 pyramidal neurons in mild cognitive impairment. *Neuropharmacology*. 2014;79:172-179.
78. Weinberg RB, Mufson EJ, Counts SE. Evidence for a neuroprotective microRNA pathway in amnestic mild cognitive impairment. *Front Neurosc*. 2015;9:430.
79. Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol*. 2014;6(1):a009191.
80. Zhang YE. Non-Smad pathways in TGF-beta signaling. *Cell Res*. 2009;19(1):128-139.
81. Davoody S, Asgari Taei A, Khodabakhsh P, Dargahi L. mTOR signaling and Alzheimer's disease: what we know and where we are?. *CNS Neurosci Ther*. 2024;30(4):e14463.
82. Duc Nguyen H, Pal Yu B, Hoang NHM, Jo WH, Kim MS, Prolactin and its altered action in Alzheimer's disease and Parkinson's disease. *Neuroendocrinology*. 2022;112(5):427-445.
83. Kellar D, Craft S. Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. *Lancet Neurol*. 2020;19(9):758-766.
84. Mufson EJ, Counts SE, Ginsberg SD, et al. Nerve growth factor pathobiology during the progression of Alzheimer's disease. *Front Neurosci*. 2019;13:533.
85. Li Z, Yin B, Zhang S, Lan Z, Zhang L. Targeting protein kinases for the treatment of Alzheimer's disease: recent progress and future perspectives. *Eur J Med Chem*. 2023;261:115817.
86. Medina JH, Viola H. ERK1/2: a key cellular component for the formation, retrieval, reconsolidation and persistence of memory. *Front Mol Neurosci*. 2018;11:361.
87. Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta*. 1991;1072(2-3):129-157.
88. Hai T, Wolfgang CD, Marsee DK, Allen AE, Sivaprasad U. ATF3 and stress responses. *Gene Expr*. 1999;7(4-6):321-335.
89. Schlingensiepen KH, Wollnik F, Kunst M, Schlingensiepen R, Herdegen T, Brysch W. The role of Jun transcription factor expression and phosphorylation in neuronal differentiation, neuronal cell death, and plastic adaptations in vivo. *Cell Mol Neurobiol*. 1994;14(5):487-505.
90. Lu T, Aron L, Zullo J, et al. REST and stress resistance in ageing and Alzheimer's disease. *Nature*. 2014;507(7493):448-454.
91. Heintz N. Cell death and the cell cycle: a relationship between transformation and neurodegeneration?. *Trends Biochem Sci*. 1993;18(5):157-159.
92. Csajbok EA, Kocsis AK, Farago N, et al. Expression of GLP-1 receptors in insulin-containing interneurons of rat cerebral cortex. *Diabetologia*. 2019;62(4):717-725.
93. Grunblatt E, Salkovic-Petrisic M, Osmanovic J, Riederer P, Hoyer S. Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein. *J Neurochem*. 2007;101(3):757-770.
94. Mehran AE, Templeman NM, Brigidi GS, et al. Hyperinsulinemia drives diet-induced obesity independently of brain insulin production. *Cell Metab*. 2012;16(6):723-737.
95. Ferreira ST. Brain insulin, insulin-like growth factor 1 and glucagon-like peptide 1 signalling in Alzheimer's disease. *J Neuroendocrinol*. 2021;33(4):e12959.
96. Talbot K, Wang HY, Kazi H, et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest*. 2012;122(4):1316-1338.
97. Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. *Free Radic Biol Med*. 2011;50(5):567-575.
98. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nature Rev Neurol*. 2018;14(3):168-181.
99. Hayden MR, Grant DG, Aroor AR, DeMarco VG. Empagliflozin ameliorates type 2 diabetes-induced ultrastructural remodeling of the

neurovascular unit and neuroglia in the female db/db mouse. *Brain Sci*. 2019;9(3):57.
100. Kopp KO, Glotfelty EJ, Li Y, Lahiri DK, Greig NH. Type 2 diabetes mellitus/obesity drugs: a neurodegenerative disorders savior or a bridge too far?. *Ageing Res Rev*. 2024;98:102343.
101. Nixon RA. The role of autophagy in neurodegenerative disease. *Nature Med*. 2013;19(8):983-997.
102. Norambuena A, Wallrabe H, McMahon L, et al. mTOR and neuronal cell cycle reentry: how impaired brain insulin signaling promotes Alzheimer's disease. *Alzheimers Dement*. 2017;13(2):152-167.
103. Majd S, Power JHT. Oxidative stress and decreased mitochondrial superoxide dismutase 2 and peroxiredoxins 1 and 4 based mechanism of concurrent activation of AMPK and mTOR in Alzheimer's disease. *Curr Alzheimer Res*. 2018;15(8):764-776.
104. Hamilton K, Harvey J. Leptin regulation of hippocampal synaptic function in health and disease. *Vitam Horm*. 2021;115:105-127.
105. Cabrera-Reyes EA, Vanoye-Carlo A, Rodriguez-Dorantes M, et al. Transcriptomic analysis reveals new hippocampal gene networks induced by prolactin. *Sci Rep*. 2019;9(1):13765.
106. Mayor D, Tymianski M. Neurotransmitters in the mediation of cerebral ischemic injury. *Neuropharmacology*. 2018;134(Pt B):178-188.
107. Walker TL, Vukovic J, Koudijs MM, et al. Prolactin stimulates precursor cells in the adult mouse hippocampus. *PLoS One*. 2012;7(9):e44371.
108. Leung YY, Toledo JB, Nefedov A, et al. Identifying amyloid pathology-related cerebrospinal fluid biomarkers for Alzheimer's disease in a multicohort study. *Alzheimers Dement*. 2015;1(3):339-348.
109. Lopez-Vicchi F, De Winne C, Brie B, Sorianello E, Ladyman SR, Becu-Villalobos D. Metabolic functions of prolactin: physiological and pathological aspects. *J Neuroendocrinol*. 2020;32(11):e12888.
110. Park S, Kang S, Lee HW, Ko BS. Central prolactin modulates insulin sensitivity and insulin secretion in diabetic rats. *Neuroendocrinology*. 2012;95(4):332-343.
111. Zhu X, Lee HG, Raina AK, Perry G, Smith MA. The role of mitogen-activated protein kinase pathways in Alzheimer's disease. *Neurosignals*. 2002;11(5):270-281.
112. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. *Biochim Biophys Acta*. 2010;1802(4):396-405.
113. Hernandez-Rapp J, Rainone S, Goupil C, et al. microRNA-132/212 deficiency enhances abeta production and senile plaque deposition in Alzheimer's disease triple transgenic mice. *Sci Rep*. 2016;6:30953.
114. Moussian B, Roth S. Dorsoventral axis formation in the drosophila embryo–shaping and transducing a morphogen gradient. *Curr Biol*. 2005;15(21):R887-R899.
115. Alldred MJ, Pidikiti H, Ibrahim KW, et al. Hippocampal CA1 pyramidal neurons display sublayer and circuitry dependent degenerative expression profiles in aged female down syndrome mice. *J Alzheimers Dis*. 2024;100:S341-S362.
116. Masurkar AV, Srinivas KV, Brann DH, Warren R, Lowes DC, Siegelbaum SA. Medial and lateral entorhinal cortex differentially excite deep versus superficial CA1 pyramidal neurons. *Cell Rep*. 2017;18(1):148-160.
117. Mizuseki K, Diba K, Pastalkova E, Buzsaki G. Hippocampal CA1 pyramidal cells form functionally distinct sublayers. *Nat Neurosci*. 2011;14(9):1174-1181.
118. Kong Y, Wu J, Yuan L. MicroRNA expression analysis of adult-onset drosophila Alzheimer's disease model. *Curr Alzheimer Res*. 2014;11(9):882-891.
119. Schonrock N, Ke YD, Humphreys D, et al. Neuronal microRNA deregulation in response to Alzheimer's disease amyloid-beta. *PLoS One*. 2010;5(6):e11070.
120. Zhou H, Zhang R, Lu K, et al. Deregulation of miRNA-181c potentially contributes to the pathogenesis of AD by targeting collapsin response mediator protein 2 in mice. *J Neurol Sci*. 2016;367:3-10.
121. Caltagarone J, Jing Z, Bowser R. Focal adhesions regulate abeta signaling and cell death in Alzheimer's disease. *Biochim Biophys Acta*. 2007;1772(4):438-445.
122. Davis-Lunn M, Goult BT, Andrews MR. Clutching at guidance cues: the integrin-FAK axis steers axon outgrowth. *Biology*. 2023;12(7):954.
123. Robles E, Gomez TM. Focal adhesion kinase signaling at sites of integrin-mediated adhesion controls axon pathfinding. *Nat Neurosci*. 2006;9(10):1274-1283.
124. Williamson R, Scales T, Clark BR, et al. Rapid tyrosine phosphorylation of neuronal proteins including tau and focal adhesion kinase in response to amyloid-beta peptide exposure: involvement of Src family protein kinases. *J Neurosci*. 2002;22(1):10-20.
125. Grace EA, Busciglio J. Aberrant activation of focal adhesion proteins mediates fibrillar amyloid beta-induced neuronal dystrophy. *J Neurosci*. 2003;23(2):493-502.
126. Shulman JM, Imboywa S, Giagtzoglou N, et al. Functional screening in drosophila identifies Alzheimer's disease susceptibility genes and implicates tau-mediated mechanisms. *Hum Mol Genetics*. 2014;23(4):870-877.
127. Davidson K, Pickering AM. The proteasome: a key modulator of nervous system function, brain aging, and neurodegenerative disease. *Front Cell Dev Biol*. 2023;11:1124907.
128. Jones CL, Tepe JJ. Proteasome activation to combat proteotoxicity. *Molecules*. 2019;24(15):2841.
129. Song S, Jung YK. Alzheimer's disease meets the ubiquitin-proteasome system. *Trends Mol Med*. 2004;10(11):565-570.
130. Weng FL, He L. Disrupted ubiquitin proteasome system underlying tau accumulation in Alzheimer's disease. *Neurobiol Aging*. 2021;99:79-85.
131. Tiernan CT, Ginsberg SD, Guillozet-Bongaarts AL, et al. Protein homeostasis gene dysregulation in pretangle bearing nucleus basalis neurons during the progression of Alzheimer's disease. *Neurobiol Aging*. 2016;42:80-90.
132. Planas AM, Gorina R, Chamorro A. Signalling pathways mediating inflammatory responses in brain ischaemia. *Biochem Soc Trans*. 2006;34(Pt 6):1267-1270.
133. Qin H, Buckley JA, Li X, et al. Inhibition of the JAK/STAT pathway protects against alpha-synuclein-induced neuroinflammation and dopaminergic neurodegeneration. *J Neurosci*. 2016;36(18):5144-5159.
134. Rusek M, Smith J, El-Khatib K, Aikins K, Czuczwar SJ, Pluta R. The role of the JAK/STAT signaling pathway in the pathogenesis of Alzheimer's disease: new potential treatment target. *Int J Mol Sci*. 2023;24(1):864.
135. Taylor JM, Minter MR, Newman AG, Zhang M, Adlard PA, Crack PJ. Type-1 interferon signaling mediates neuro-inflammatory events in models of Alzheimer's disease. *Neurobiol Aging*. 2014;35(5):1012-1023.
136. Yan Z, Gibson SA, Buckley JA, Qin H, Benveniste EN. Role of the JAK/STAT signaling pathway in regulation of innate immunity in neuroinflammatory diseases. *Clin Immunol*. 2018;189:4-13.
137. Roy ER, Wang B, Wan YW, et al. Type I interferon response drives neuroinflammation and synapse loss in Alzheimer disease. *J Clin Invest*. 2020;130(4):1912-1930.
138. Murray PJ. The JAK-STAT signaling pathway: input and output integration. *J Immunol*. 2007;178(5):2623-2629.
139. Zhang W, Xiao D, Mao Q, Xia H. Role of neuroinflammation in neurodegeneration development. *Signal Transduct Target Ther*. 2023;8(1):267.
140. Kumar M, Bansal N. Implications of Phosphoinositide 3-Kinase-Akt (PI3K-Akt) pathway in the pathogenesis of Alzheimer's disease. *Mol Neurobiol*. 2022;59(1):354-385.
141. Razani E, Pourbagheri-Sigaroodi A, Safaroghli-Azar A, Zoghi A, Shanaki-Bavarsad M, Bashash D. The PI3K/Akt signaling axis in

Alzheimer's disease: a valuable target to stimulate or suppress?. *Cell Stress Chaperones*. 2021;26(6):871-887.
142. Clapham DE, Neer EJ. G protein beta gamma subunits. *Annu Rev Pharmacol Toxicol*. 1997;37:167-203.
143. Peng Y, Croce CM. The role of MicroRNAs in human cancer. *Signal Transduct Target Ther*. 2016;1:15004.
144. Baker LD, Snyder HM, Espeland MA, et al. Study design and methods: U.S. study to protect brain health through lifestyle intervention to reduce risk (U.S. POINTER). *Alzheimers Dement*. 2024;20(2):769-782.
145. Maloney B, Lahiri DK. Epigenetics of dementia: understanding the disease as a transformation rather than a state. *Lancet Neurol*. 2016;15(7):760-774.
146. Zhang Y, Zhao Y, Ao X, et al. The role of non-coding RNAs in Alzheimer's disease: from regulated mechanism to therapeutic targets and diagnostic biomarkers. *Front Aging Neurosci*. 2021;13:654978.
147. Zhou M, Li S, Huang C. Physiological and pathological functions of circular RNAs in the nervous system. *Neural Regen Res*. 2024;19(2):342-349.
148. Huang X, Wang C, Zhang T, et al. PIWI-interacting RNA expression regulates pathogenesis in a caenorhabditis elegans model of Lewy body disease. *Nature Comm*. 2023;14(1):6137.
149. Shulman D, Dubnov S, Zorbaz T, et al. Sex-specific declines in cholinergic-targeting tRNA fragments in the nucleus accumbens in Alzheimer's disease. *Alzheimers Dement*. 2023;19(11):5159-5172.
150. Lahiri DK, Maloney B, Wang R, et al. The seeds of its regulation: natural antisense transcripts as single-gene control switches in neurodegenerative disorders. *Ageing Res Rev*. 2024;99:102336.
151. Wang, R, Maloney, B, Nho, K, Beck, J, Counts, SE, Lahiri, DK. Human microRNA-153-3p targets specific neuronal genes and is associated with the risk of Alzheimer's disease. 2024. doi:10.1101/2024.09.07.611728
152. Wang, R, Lahiri, DK. Effects of microRNA-298 on APP and BACE1 translation differ according to cell type and 3′-UTR variation. *Sci Rep*. 2022;12(1). doi:10.1038/s41598-022-05164-4

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Counts SE, Beck JS, Maloney B, et al. Posterior cingulate cortex microRNA dysregulation differentiates cognitive resilience, mild cognitive impairment, and Alzheimer's disease. *Alzheimer's Dement*. 2025;21:e70019. https://doi.org/10.1002/alz.70019