GENES AND AGING: FROM BENCH SIDE TO BED SIDE

EDITED BY: Wael M. Y. Mohamed, Chenju Yi, Lilach Soreq and Toshihide Yamashita

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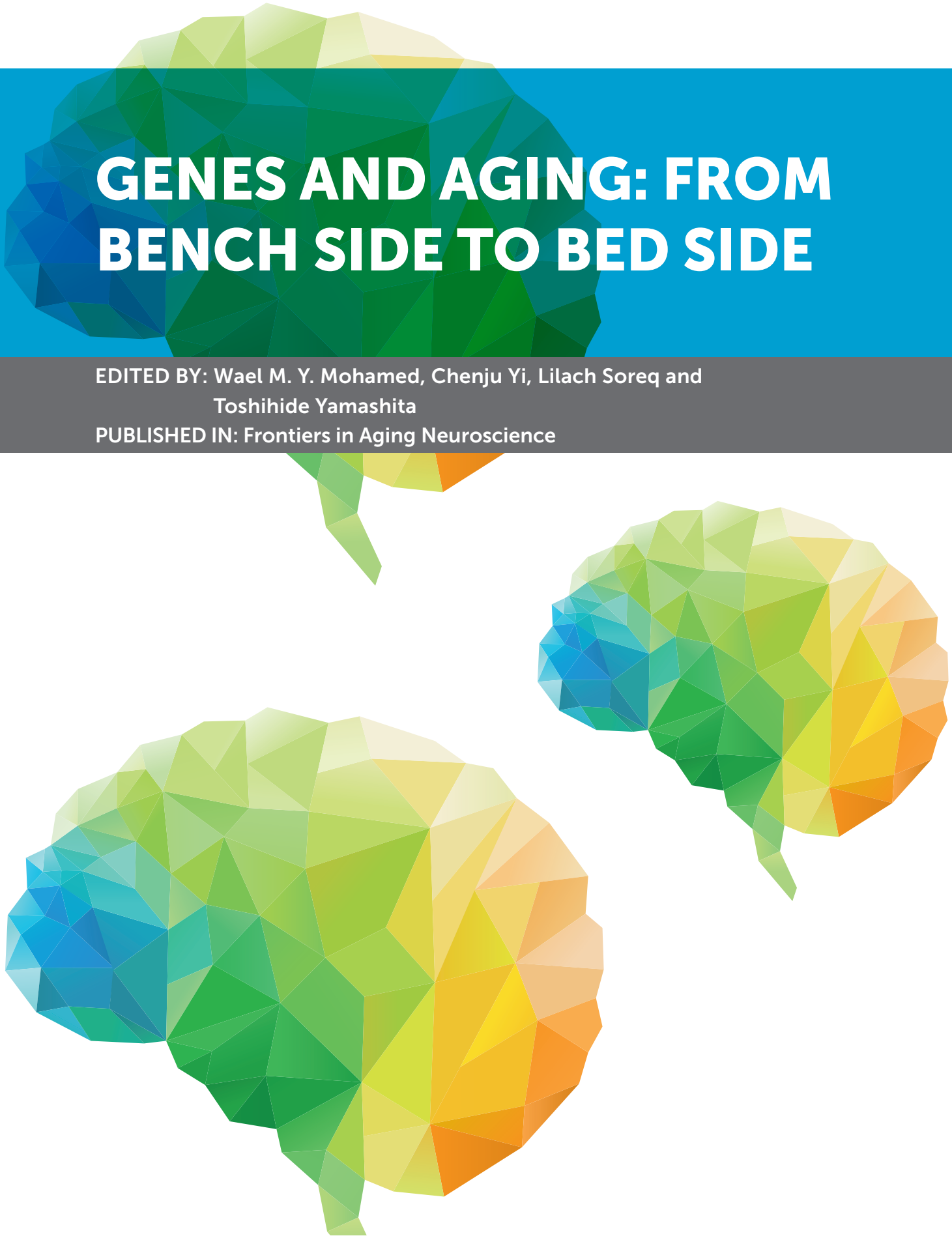

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GENES AND AGING: FROM BENCH SIDE TO BED SIDE

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Table of Contents

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EDITORIAL
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Editorial: Genes and Aging: From Bench-to-Bedside

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Keywords: genes, aging, neurodegeneration, dementia, AD

Editorial on the Research Topic

Genes and Aging: From Bench-to-Bedside

Aging is inevitable, and several structural and functional changes occur in the body with advanced aging. Aging affects the brain in a variety of ways, the most obvious of which are changes in motor, sensory, and cognitive function (Jeromin and Bowser, 2017; Ibrahim et al., 2020). Aging has becoming a remarkable public health issue worldwide: by 2050, the proportion of the world's population over 60 years will reach 22%. This increase in the number of populations aging calls for attention to aging-associated diseases, including Neurodegenerative disorders such as Alzheimer's Disease (AD) and Parkinson's Disease (PD) (Gazzaley et al., 2005). In the brain, different cell types, including neurons, astrocytes, oligodendrocytes, as well as microglia, respond to aging differently (von Bartheld et al., 2016; Chapman and Hill, 2020). Studying the transcriptome changes in different cell types during aging, and identification of cell type-specific aging markers would enable us to move the therapeutic window to earlier stages of aging-associated disease progression/premature aging. In addition, understanding the mechanism of how cell-type specific aging genes are involved in the aging process could further shed light on the prevention and therapy of the aging-related diseases (Soreq et al., 2017, 2021).

The present article collection aims at giving a reader the most up-to-date perspective on how the interaction between genes and neuropsychological processes leads to neurological disorders in normal aging and minor and major neurocognitive disorders. It is intended to provide an opportunity for researchers of different perspectives to discuss recent progress in this field. This volume aimed to provide an overview of contributions from basic, and clinical aspects to understand the interplay between genes and aging from Bench-to-Bedside. The authors from different research fields identified numerous existing and emerging genes/aging related topics that provide new and unique perspectives.

In this volume, several articles focus on Alzheimer's Disease. For instance, a review by Abubakar et al. discussed the updates on pathophysiology of Alzheimer's Disease that introduces a better understanding of novel signaling pathways associated with neural and glial mechanisms involved in AD, elaborates potential links between vascular dysfunction and AD, and recent developments in "omics"-based biomarkers in AD. Also, Sabaie et al. carried out a scoping review according to sic-stage methodology structure and PRISMA guideline to analyse validated loops of competing endogenous (ce)RNA in AD and focus on ceRNA axes associated with lncRNA with the therapeutic potential in AD. In parallel with this, Abuelezz et al. presented an updated analysis of miRNAs role in regulating signaling processes that are involved in AD-related pathologies. They discussed the current challenges against wider use of miRNAs and the future promising capabilities of miRNAs as diagnostic and therapeutic means for better management of AD. Further, this volume discussed AD dementia from different perspectives. Liu L et al. investigated the frequency of Chinese Han

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patients harboring genetic Frontotemproal Dementia (FTD) variants. They concluded that there was a high prevalence of genetic variants in Chinese bvFTD patients, highlighting the necessity of genetic testing for bvFTD. Similarly, Sun et al. analyzed the correlations of genetic features with clinical symptoms in patients with degenerative dementia. They recruited a group of 84 Chinese dementia patients and conducted the whole exome sequencing (WES). The data were analyzed focusing on 153 dementia-related causing and susceptible genes. They concluded that the new variants in dementia-related genes indicated heterogeneity in pathogenesis and phenotype of degenerative dementia. WES could serve as an efficient diagnostic tool for detecting intractable dementia. It is worth mentioning that the current volume contains a basic study conducted by Ba et al. they modeled chronic sleep fragmentation (SF) in young wild-type mice and detected pathological hyperphosphorylated-tau (Ser396/Tau5) and gliosis in the SF hippocampus. In summary, 1.5-month sleep fragmentation could generate AD-like pathological changes including tauopathy and gliosis, mainly linked to stress, as the incremented glucose metabolism observed with PET imaging suggested. Thus SF could eventually lead to chronic neurodegeneration if the stress condition is prolonged in time. This is supported by Zhang et al. as they summarized present knowledge about dynamic changes of oligodendroglial lineage cells during normal aging and discussed their potential roles in age-related functional decline. Especially, they focused on declined myelinogenesis during aging and underlying mechanisms. Their article was clarifying those oligodendroglial (OLG) changes and their effects on neurofunctional decline may provide new insights in understanding aging associated brain. Jin et al. examined the associations of four single nucleotide polymorphisms (SNPs) on aldehyde dehydrogenase (*ALDH*) and alcohol dehydrogenase (*ADH*) genes (i.e., *ALDH2* rs671, *ADH1B* rs1229984, *ADH1B* rs1042026, and *ADH1C* rs1693482) and cognitive impairment among the oldest-old. They examined data from the Chinese Longitudinal Healthy Longevity Survey genetic sub-study, including 1,949 participants aged over 90 years. They did not observe a significant interaction between those SNPs and alcohol consumption. Finally, Liu Y-H et al. discussed a case report leukoencephalopathy syndrome and they identified a novel homozygous mutation (NM_012268.3: c.186C>G/ p.Y62X) of *PLD3* in a consanguineous family with white matter lesions, hearing and vision loss, and kidney disease by whole exome sequencing. This may be the first case report on the homozygous mutation of PLD3 in patients worldwide.

Overall, this volume provides a rare opportunity to promote awareness and creativity for neurological disorders using animal models of different species, while stimulating testable theories and establishing a strategic research agenda to facilitate their integration into clinical studies.

AUTHOR CONTRIBUTIONS

WM wrote the original draft and incorporated suggestions from the co-authors. LS edited the paper draft. The other co-authors edited the manuscript. All authors contributed to the article and approved the submitted version.

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We would like to thank our colleagues for dedicating their time, resources and energies to deliver creative and critical perspectives. Their important contributions have provided a comprehensive foundation for this theme of study. It is also a privilege to recognize and thank the editorial team for their expert help and support in creating and producing this volume. Finally, we wish to dedicate this book to all medical practitioners and researchers, with our utmost appreciation and deep respect. We are indebted to their sacrifices and to their unfailing spirit of hope that is our greatest source of inspiration.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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Case Report: A Homozygous Mutation (p.Y62X) of *Phospholipase D3* May Lead to a New Leukoencephalopathy Syndrome

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Leukodystrophies are a heterogeneous group of inherited disorders with highly variable clinical manifestations and pathogenetic backgrounds. At present, variants in more than 20 genes have been described and may be responsible for different types of leukodystrophies. Members of the phospholipase D family of enzymes catalyze the hydrolysis of membrane phospholipids. Meanwhile, phospholipase D3 (PLD3) has also been found to exhibit single stranded DNA (ssDNA) acid 5′ exonuclease activity. Variants in *phospholipase D3* (*PLD3*) may increase the risk of Alzheimer's disease and spinocerebellar ataxia, but this hypothesis has not been fully confirmed. In this study, we identified a novel homozygous mutation (NM_012268.3: c.186C>G/ p.Y62X) of *PLD3* in a consanguineous family with white matter lesions, hearing and vision loss, and kidney disease by whole exome sequencing. Real-time PCR revealed that the novel mutation may lead to non-sense-mediated messenger RNA (mRNA) decay. This may be the first case report on the homozygous mutation of PLD3 in patients worldwide. Our studies indicated that homozygous mutation of *PLD3* may result in a novel leukoencephalopathy syndrome with white matter lesions, hearing and vision loss, and kidney disease.

Keywords: leukoencephalopathy, white matter lesions, hearing and vision loss, PLD3, Homozygous mutation, chronic kidney disease

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INTRODUCTION

Leukoencephalopathy (LE) is a structural alteration of the cerebral white matter in which myelin suffers the most damage (Kohler et al., 2018). Leukodystrophies can be broadly subdivided into hypomyelinating leukodystrophies, which are characterized by primary deficits in myelin development, and demyelinating leukodystrophies, where myelin develops normally but subsequently undergoes progressive disruption (Vanderver et al., 2015). At present, ∼20 distinct disorders are defined as adulthood leukodystrophies (Tillema and Renaud, 2012; Kohler et al., 2018), such as Pelizaeus–Merzbacher disease, adult polyglucosan body disease, and X-linked adrenoleukodystrophy.

The human *PLD3* gene, which encodes a single-pass type II membrane protein with two phospholipase D (PLD) phosphodiesterase domains, is located on chromosome 19q13.2 and consists of 13 exons spanning 32 kb. As a member of the phospholipase D family of enzymes that catalyze the hydrolysis of membrane phospholipids, PLD3 has been proven to be involved in the processing of amyloid-beta precursor protein (Fazzari et al., 2017). Recently, two proteins from the PLD family, namely, phospholipase D3 (PLD3) and phospholipase D4 (PLD4), were found to exhibit single stranded DNA (ssDNA) acid 5′ exonuclease activity (Gavin et al., 2018; Cappel et al., 2020). Previous studies revealed that a heterozygous mutation of *PLD3* might increase the risk of Alzheimer's disease and spinocerebellar ataxia (Wang et al., 2015; Nibbeling et al., 2017). However, the effect of homozygous mutations in PLD3 is still not clear.

In this study, we identified a novel homozygous mutation (NM_012268.3: c.186C>G/ p.Y62X) of *PLD3* by whole exome sequencing in a patient from a consanguineous family with white matter lesions, hearing and vision loss, and kidney disease.

CASE PRESENTATION

Ethics Approval

This study was carried out in accordance with the guidelines of the institutional ethics committee of the Affiliated Hospital of Yangzhou University in China. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

We enrolled a consanguineous family from the Han-Chinese population (**Figure 1A**). The proband (IV-1), a 57-year-old woman, was admitted to our hospital due to sudden onset of lightheadedness and vertigo accompanied by nausea and vomiting. Magnetic resonance imaging (MRI) testing detected brain lesions in the proband. T2 image showed high intensity of white matter and thalamus, high intensity of brain stem and thalamus, high intensity of bilateral white matter, high intensity of bilateral thalamus, and high intensity of brain stem and bilateral cerebellum (**Figure 1B**). The Mini-Mental State Examination (MMSE) suggested a normal cognitive state (score, 27). Except for several episodes of lightheadedness and vertigo, the patient did not present with other motor or cognitive impairment during the hospital stay. A medical history investigation found that the proband suffered from sudden hearing and vision loss. The computed tomography (CT) image of the proband indicated tapering of bilateral optic nerves (**Figure 1C**). Eye examination revealed normal eye movement but a significant low vision (left, 0.3; right, 0.1). Pure-tone audiometry (PTA) showed severe hearing loss (**Figure 1D**). In addition, the patient also suffered from chronic kidney disease (CKD) and was diagnosed with focal segmental glomerulosclerosis by renal biopsy in another hospital 10 years ago (**Figure 1E**). The body mass index of the proband was 21.7, and the blood pressure was 121/77 mmHg. Blood lipid and glucose levels, as well as cerebrovascular CT of the proband, did not display any abnormalities. Upon further interviews on the family history, we found that her parents married consanguineously. The parents (III-1 and III-2) and her brother (IV-3) presented with normal vision, hearing, and kidney function. Brain MRI of the proband's parents and her brother also showed no obvious lesions (**Supplementary Figure 1**).

We then performed whole exome sequencing in an effort to identify the genetic lesions responsible for the disease phenotype of the proband. The central part of the whole exome sequencing was provided by the Novogene Bioinformatics Institute (Beijing, China). The exomes were captured using Agilent SureSelect Human All Exon V6 kits (Agilent Technologies, Sta Clara, CA, USA), and high-throughput sequencing was performed using Illumina HiSeq X-10 (Illumina, San Diego, CA, USA). The necessary bioinformatics analyses, including reads, mapping, variant detection, filtering, and annotation, were also carried out by Novogene Bioinformatics Institute as previously described (Fan et al., 2019).

The strategies of data filtering are as follows (Wang et al., 2020): (a) non-synonymous single-nucleotide polymorphisms (SNPs) or frameshift-causing INDELs with an alternative allele frequency >0.05 in the NHLBI Exome Sequencing Project Exome Variant Server (ESP6500), dbSNP152 (http://www.ncbi.nlm.nih.gov/projects/SNP/index.html), the 1000 Genomes project (http://www.1000genomes.org/), the ExAC database (http://exac.broadinstitute.org), or in-house exome databases of Novogene (2500 exomes) were excluded; (b) the filtered single nucleotide variants (SNVs) and INDELs, predicted to be damaging by SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), and MutationTaster (http://www.mutationtaster.org/) were remained; (c) all the homozygous mutations were retained; and (d) cosegregation analysis was conducted in the family.

After data filtering and American College of Medical Genetics and Genomics (ACMG) guideline assessment (Richards et al., 2015), only a novel homozygous mutation (NM_012268.3: c.186C>G/ p.Y62X) of *PLD3* identified in the proband met the likely pathogenic criteria (**Supplementary Table 1**). The novel non-sense mutation, resulting in a premature stop codon in exon 5 of the *PLD3* gene, was validated in the proband in a homozygous form and existed in the proband's parents and her brother in a heterozygous form (**Figure 2A**). We then decided to determine whether the novel variant of *PLD3* is sensitive to non-sense-mediated messenger RNA (mRNA) decay. According to the GTEx database, PLD3 is expressed in white blood cells. Hence, we isolated the total RNA from peripheral white blood cells derived from three groups or donors (five healthy controls, three heterozygote carriers, and one homozygote patient). After synthesizing complementary DNA (cDNA), real-time PCR found that the mRNA level of PLD3 in heterozygous carriers (III-1, III-2, and IV-3) was decreased by ∼43% compared with that in healthy controls (five healthy people without PLD3 mutations), and the expression of PLD3 in homozygous patients (IV-1, repeated three times) was reduced by ∼90% compared to that in healthy controls (**Figure 2B**), which indicated that the non-sense mutation may have led to non-sense-mediated mRNA decay. Concurrently, we isolated the total proteins from white blood cells obtained from two healthy people, two heterozygote carriers (III-1 and IV-3), and the proband (IV-1). Western blot analysis found that the expression of PLD3 in heterozygote group was decreased dramatically compared to

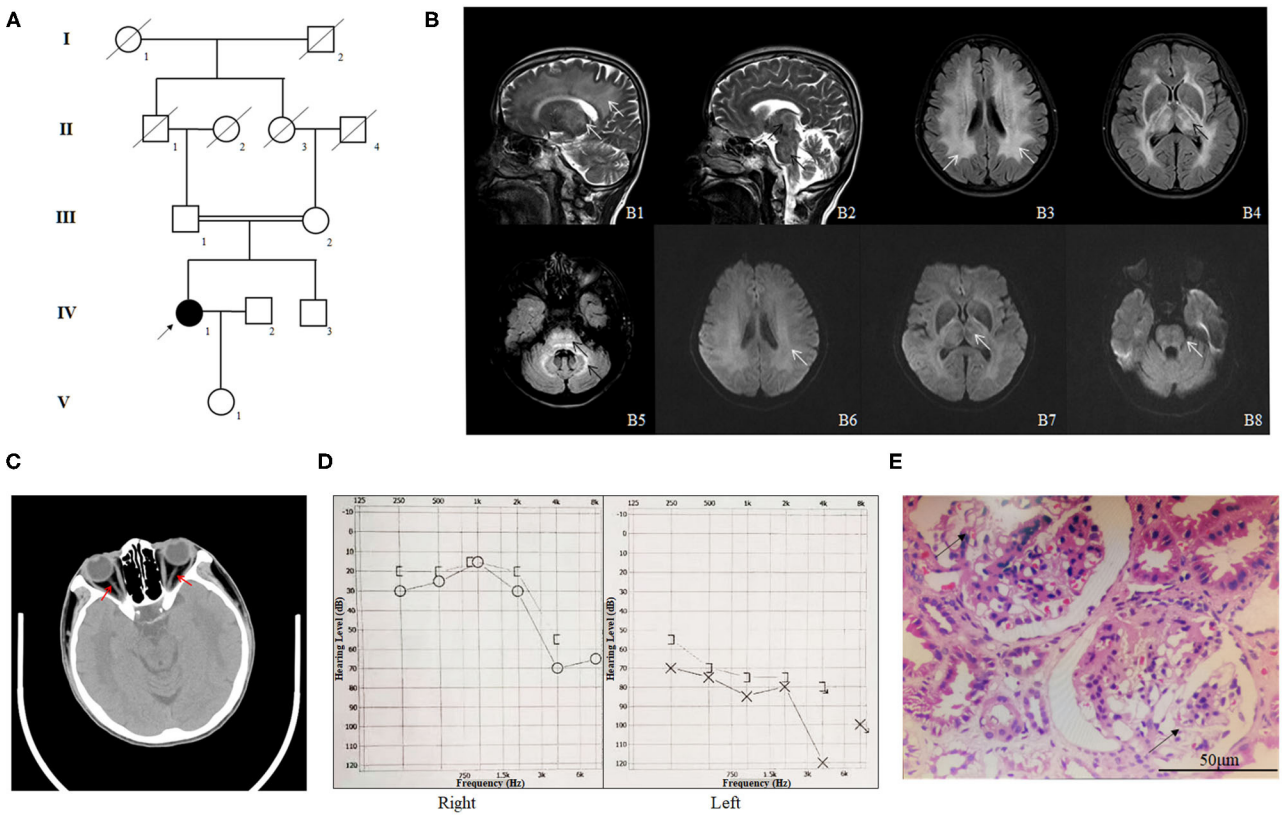

FIGURE 1 | The clinical profile of the family. **(A)** Pedigree of the phospholipase D3 (PLD3)-deficient family. The pedigree chart shows five generations of the family. Roman numerals refer to generations. Circles refer to female subjects. Squares refer to male subjects. Solid symbols refer to affected subjects. Crossed-out symbols refer to deceased subjects. The arrow indicates the proband. **(B)** The MR images of the proband. T2 image shows high intensity of white matter and thalamus (**B1**, white arrows), high intensity of brain stem and thalamus (**B2**, black arrows), high intensity of bilateral white matter (**B3**, white arrows), high intensity of bilateral thalamus (**B4**, black arrows), and high intensity of brain stem and bilateral cerebellum (**B5**, black arrows). **B6–8** are the diffusion weighted image (DWI) corresponding to **B3–5** and shows slightly higher intensity. **(C)** The CT image of the proband indicating tapering of bilateral optic nerves (red arrows). **(D)** Pure-tone audiometry (PTA) results of the proband. **(E)** HE staining of the renal biopsy specimen of the proband (IV-1); the arrows indicate glomerulosclerosis.

healthy controls, and PLD3 was almost absent in the proband (IV-1) (**Figure 2C**).

DISCUSSION

In this study, a novel homozygous mutation (NM_012268.3: c.186C>G/ p.Y62X) of *PLD3* was identified in a consanguineous family with leukoencephalopathy syndrome by whole exome sequencing. Furthermore, real-time PCR and Western blot assays confirmed that the homozygous mutation might have led to non-sense-mediated mRNA decay and resulted in loss of function in PLD3. PLD3 is highly expressed in neurons and may regulate early neuronal development in the central nervous system (Wang et al., 2015). Meanwhile, studies found that the function of PLD3 was to catalyze the hydrolysis of membrane phospholipids. In leukoencephalopathy, neurons are damaged, and myelin is also disrupted (Kohler et al., 2018). Myelin, a phospholipids, might also be regulated by PLD3. Recently, PLD3 was found to exhibit ssDNA acid 5′ exonuclease activity (Gavin et al., 2018; Cappel et al., 2020), which was also associated with brain diseases such as Alzheimer's disease and spinocerebellar ataxia (SCA) (Gavin et al., 2018). Hence, in our case, the pathological changes in the patient who presented with high intensity white matter may have resulted from PLD3 deficiency.

Mutations in *PLD3* may also lead to endoplasmic reticulum (ER) stress and reduced phospholipase activity (Nibbeling et al., 2017). Further studies revealed that *PLD3* mutation can impair O-glycosylation at pT271 in PLD3, which is essential for normalizing antioxidative phospholipid levels and protecting the brain (Demirev et al., 2019). In addition, variants in *PLD3* can reduce PLD3 activity and affect amyloid-β levels in a cellular model of Alzheimer's disease, possibly via the autophagy-dependent mTOR signaling pathway (Tan et al., 2019). Recently, PLD3 has also been found to play a crucial role in regulating inflammatory cytokine responses (Gavin et al., 2018). Macrophages from PLD3-deficient mice had exaggerated TLR9 responses (Gavin et al., 2018). Here, in our study, the patient who carried a homozygous mutation of *PLD3* presented with white matter lesions, hearing and vision loss,

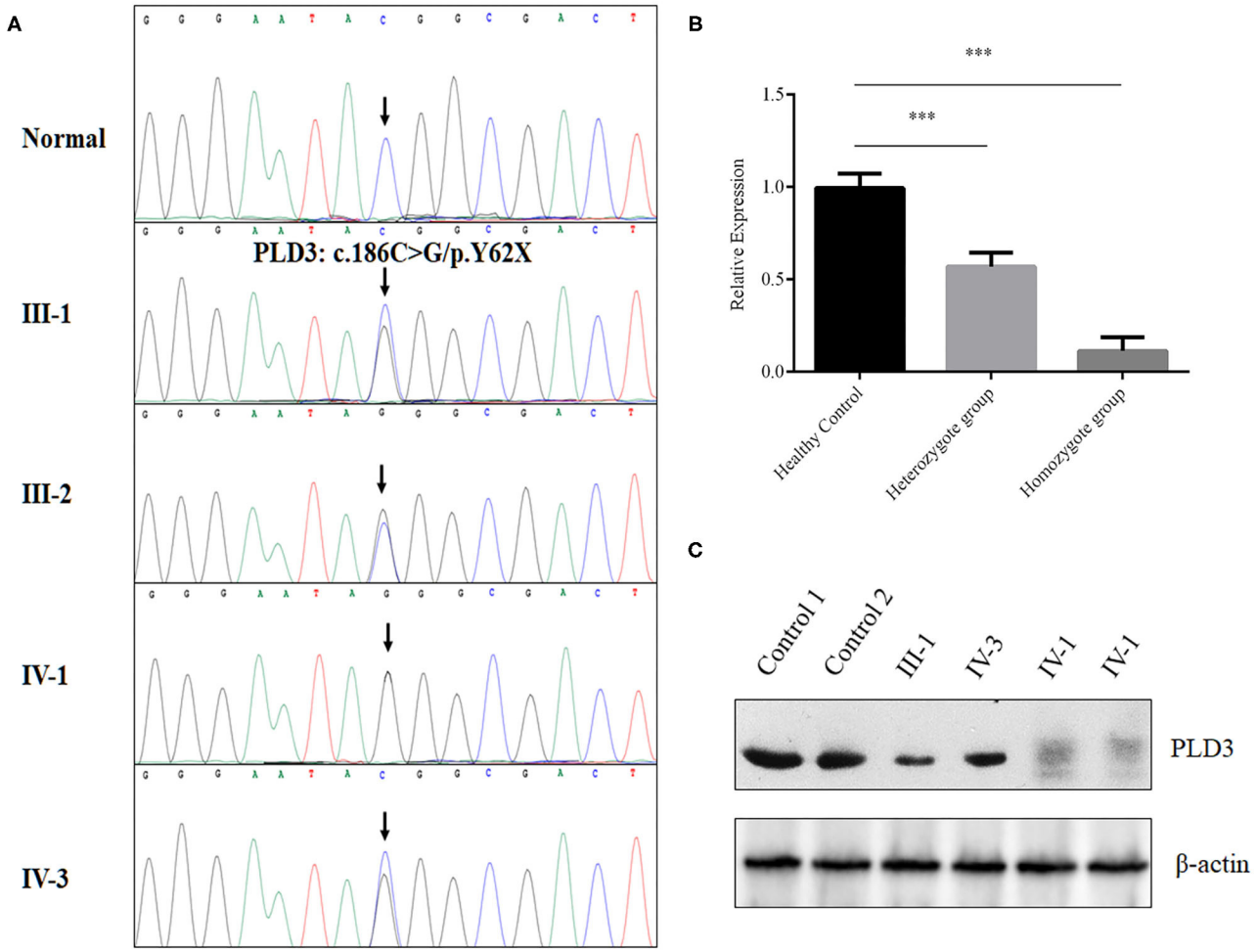

FIGURE 2 | Genetic analysis of the family. **(A)** Sanger sequencing analysis of the *PLD3* gene in the patients, their family members, and the controls. **(B)** Real-time PCR determined the messenger RNA (mRNA) levels of phospholipase D3 (PLD3) in white blood cells from healthy controls (five samples), heterozygotes (III-1, III-2, and IV-3), and a homozygote (IV-1, repeated three times). ***$p < 0.01$. **(C)** Western blot analysis of the expression of PLD3 in white blood cells from healthy controls (two samples), the heterozygote group (III-1 and IV-3), and the homozygote group (IV-1, repeated two times).

and focal segmental glomerulosclerosis. We speculated that the homozygous mutation (NM_012268.3: c.186C>G/ p.Y62X) of *PLD3* may lead to PLD3 deficiency, which may induce ER stress and reduce phospholipase and exonuclease activities in neurons, as well as loss of O-glycosylation at pT271 of PLD3, ultimately damaging neuros in the central nervous system and optic and vestibulocochlear nerves. Simultaneously, PLD3 deficiency may also induce inflammatory cytokine responses in the kidney. Hence, the proband presented with phenotypes not only in the nervous system but also in the kidney. Our studies indicated that homozygous mutation of *PLD3* may result in a novel leukoencephalopathy syndrome including white matter lesions, hearing and vision loss, and kidney disease.

Previous genetic studies revealed that variants in *PLD3* may increase the risk for late-onset Alzheimer's disease (van der Lee et al., 2015; Tan et al., 2018). However, studies in Belgium found that rare variants in *PLD3* do not raise the risk for early-onset Alzheimer's disease (Cacace et al., 2015). Subsequently, Nibbeling et al. identified novel genes (*FAT2*, *PLD3*, *KIF26B*, *EP300*, and *FAT1*) in autosomal dominant SCA patients by whole exome sequencing. Functional studies revealed that PLD3 is located in the ER and that the missense mutation p. Leu308Pro of *PLD3* may lead to loss of function, which can induce ER stress and reduce phospholipase activity in COS-7 cells (Nibbeling et al., 2017). However, Gonzalez et al. discovered that PLD3 was located in lysosomes but not in the ER and acted as a 5′ exonuclease in HeLa cells. In addition, they also found that loss of PLD3 did not disrupt lipid catabolism and that *PLD3* knockout mice did not present cerebellar ataxia phenotypes, which challenged the interpretation of *PLD3* mutations as the causative SCA46 gene (Gonzalez et al., 2018). Hence, the identification of additional

patients carrying *PLD3* mutations will further strengthen the role of PLD3 in brain disease.

In our study, four heterozygous mutation carriers (III-1, III-2, IV-3, and V-1) showed normal physical features, which indicated that heterozygous non-sense mutation of *PLD3* might not be the responsible genetic lesion of SCA and Alzheimer's disease. However, homozygous non-sense mutation of PLD3 can lead to white matter lesions, which may develop into Alzheimer's disease in the future. Certainly, we cannot exclude the genetic heterogeneity and incomplete appearance of PLD3. In fact, we enrolled almost 181 patients with white matter lesions in the past 5 years, but we only detected one homozygous mutation of PLD3 in this family. This may be the first case report on a homozygous mutation of PLD3 in patients with leukoencephalopathy syndrome. Our study may reveal a relationship between leukoencephalopathy syndrome and PLD3 homozygous mutation in patients.

In summary, we identified a novel homozygous mutation (NM_012268.3: c.186C>G/ p.Y62X) of *PLD3* in a consanguineous family with white matter lesions, hearing and vision loss, and kidney disease. This may be the first case report on a homozygous mutation of PLD3 in patients worldwide. Our study also provided new insights into function of PLD3 in human diseases.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://ncbi.nlm.nih.gov/, PRJNA723675.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Affiliated Hospital of Yangzhou University in China. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Y-HL and H-FZ enrolled the samples and performed the Sanger sequencing. J-YJ performed the real-time PCR and Western blot. Y-QW and C-YW enrolled the clinical data. L-LF and LL revised the manuscript and support the project. Y-HL, H-FZ, and J-YJ wrote the draft. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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The Frequency of Genetic Mutations Associated With Behavioral Variant Frontotemporal Dementia in Chinese Han Patients

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Background: Behavioral variant frontotemporal dementia (bvFTD) is a clinically heterogeneous syndrome with high heredity. However, the frequencies of mutations associated with bvFTD have yet to be determined. The aim of the current study was to investigate the frequency of Chinese Han patients harboring genetic bvFTD variants.

Methods: A total of 49 bvFTD patients selected from our frontotemporal lobar degeneration database, including 14 familial cases belonging to eight families and 35 sporadic cases were consecutively recruited from July 2014 to December 2019 at Xuanwu Hospital (Beijing, China). Whole-exome sequencing (WES) was performed and repeat-primed PCR was used to test samples for the C9orf72 hexanucleotide repeat expansion mutation. The frequency of genetic variants and the pathogenicity of the novel variants were analyzed.

Results: Ten pathogenic or likely pathogenic variants were identified in 17 bvFTD patients, including C9orf72 repeat expansions, six previously reported mutations and three novel mutations (MAPT p. R5C, p. D54N, GRN p. P451L). Genetic mutations accounted for 27.9% (12/43) of total cases, 87.5% (7/8) of patients with familial bvFTD, and 14.3% (5/35) with sporadic bvFTD. Pathogenic variants mostly occurred in MAPT gene (20.9%, 9/43), followed by C9orf72 repeat expansions (2.3%, 1/43), GRN gene (2.3%, 1/43) and FUS gene (2.3%, 1/43).

Conclusion: There was a high prevalence of genetic variants in Chinese bvFTD patients, highlighting the necessity of genetic testing for bvFTD.

Keywords: Chinese Han ethnicity, behavioral variant frontotemporal dementia, genetics, MAPT, GRN, C9orf72

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INTRODUCTION

Frontotemporal lobar degeneration (FTLD) is a heterogeneous clinical syndrome with high heredity, which mainly includes behavioral variant frontotemporal dementia (bvFTD), non-fluent variant primary progressive aphasia (nfvPPA), and semantic variant primary progressive aphasia (svPPA) (Onyike and Diehl-Schmid, 2013; Ferrari et al., 2019; Seeley, 2019; Sirkis et al., 2019).

Atypical forms of FTLD present an overlap with Parkinsonian disorders (PD), corticobasal syndrome (CBS) and progressive supranuclear palsy (PSP), and amyotrophic lateral sclerosis (FTD-ALS) (Ferrari et al., 2019). BvFTD, which presents as progressive abnormal changes in personality and social-emotional behavior, is the most common subtype of FTLD, and accounts for approximately 60% of all FTLD patients (Onyike and Diehl-Schmid, 2013; Seeley, 2019). Compared with the other two language variants, bvFTD has a stronger genetic component, almost 50% of patients with bvFTD have a positive family history compared to only 12% of patients with PPA (Greaves and Rohrer, 2019; Ramos et al., 2019a; Pytel et al., 2021). The genetics of FTLD have been studied widely, but the genetics of bvFTD remain elusive.

Known disease-causing genetic variants currently account for 10–40% of FTLD (Onyike and Diehl-Schmid, 2013; Sirkis et al., 2019). Mutations in hexanucleotide expansion repeats of C9orf72, microtubule-associated protein tau (MAPT), and granulin (GRN) have been definitively proven to be the most common types of pathogenic variants of FTLD, accounting for 6–30%, 3–14%, and 1–16%, respectively, in North American and European cohorts (Rohrer et al., 2009; Olszewska et al., 2016; Sirkis et al., 2019). In previous studies investigating FTLD genetics in Chinese populations the prevalence of pathogenic variants was comparatively lower (4.9–7.7%) (Tang et al., 2016; Che et al., 2017), but whether this discrepancy derives from geography and ethnicity factors remains to be clarified. Although bvFTD was included and analyzed in those aforementioned studies as the most important phenotype with the highest genetic mutation frequency, its genetic pathogenicity has never been focused on as an independent disease entity. In order to enhance understanding of bvFTD, specific genetic research investigating bvFTD is urgently needed.

In the current study whole-exome sequencing was performed, and the frequencies of pathogenic variants were analyzed in 49 Chinese Han bvFTD patients to investigate genetic features associated with bvFTD in this population.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Ethics Committees of the Xuanwu Hospital of Capital Medical University, China, and was conducted in accordance with the principles stated in the Declaration of Helsinki. Written informed consent was obtained from each patient or their guardian.

Participants

An FTLD database was established at the Department of Neurology of Xuanwu Hospital, China, which included 77 patients with FTLD who were consecutively recruited between July 1, 2014 and December 31, 2019. All patients underwent detailed clinical interviews, physical examinations, neuropsychological assessments, cerebral 18F-fluorodeoxyglucose positron emission tomography/magnetic resonance imaging examinations (18F-FDG PET/MRI), and genetic testing within 1 month of recruitment. The diagnosis was performed according to the consensus criteria for probable bvFTD published in 2011, which requires three out of six clinically discriminating features (disinhibition, apathy/inertia, loss of sympathy/empathy, perseverative/compulsive behaviors, hyperorality, and dysexecutive neuropsychological profile), functional disability, and characteristic neuroimaging (Rascovsky et al., 2011). Each patient was followed up for at least 1 year. In total 49 patients enrolled in the final analysis met the diagnostic criteria for probable bvFTD. The family history of each patient was analyzed by assigning a modified Goldman score between 1 and 4, as described previously (Goldman et al., 2005).

Genetic Screening

We extracted genomic DNA from all patients enrolled in the study from fresh peripheral blood leukocytes and used an Agilent SureSelect Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA, United States) to generate a sequencing library for whole exome sequencing (WES). The prepared libraries were sequenced using the HiSeq-2000 platform (Illumina, San Diego, CA, United States). The sequenced reads were aligned to the human genome (GRCh37/hg19). Reads were then aligned to the targeted regions and collated for single nucleotide polymorphism (SNP) calling and subsequent analysis using Burrows-Wheeler Aligner software. All potential variants were verified via Sanger sequencing, which was performed on an ABI3730xl Genetic Analyzer (Applied Biosystems). Repeat primed PCR was performed as previously described to obtain a qualitative estimation of the presence of C9orf72-expanded repeats (Tang et al., 2016). Next, ANNOVAR software and Realigner Target Creator in Genome Analysis Toolkit were used to annotate the variants (McKenna et al., 2010; Wang et al., 2010).

We explored variants from genes associated with "dementia" according to several databases: Human Gene Mutation Database (HGMD[1]), Online Mendelian Inheritance in Man (OMIM[2]), Clinvar[3], and GeneCards[4]. All shortlisted genes that were associated with dementia were verified by UniProt[5], a web resource which curates comprehensive, high-quality, annotated information of a gene with its corresponding protein functions. The selected genes were further confirmed by MalaCards[6], an integrated database of public literatures relating to human disease and disorders. Our final analysis included 42 genes that were associated with FTD and other neurodegenerative diseases. **Supplementary Table 1** provides further details of the genes selected for analysis.

Variant Assessment

Variants were filtered for missense, nonsense, splice site, frameshift, non-frameshift. The splicing site was defined as ± 1 or 2 bp of the splicing donor or acceptor sequence. To prioritize

[1]http://www.hgmd.cf.ac.uk/ac/search.php
[2]https://www.omim.org/
[3]https://www.ncbi.nlm.nih.gov/clinvar
[4]https://www.genecards.org
[5]http://www.uniprot.org/
[6]http://www.malacards.org/

variants, we used a stringent minor allele frequency filter (<1%) in several public databases: the single-nucleotide polymorphism database[7], the 1000 Genomes Project[8], the ExAC database[9], and the genome aggregation database[10]. *In silico* prediction of the functional effects of missense mutations was conducted using Polymorphism Phenotyping v.2 (PolyPhen2) (Adzhubei et al., 2010), Sorting Intolerant From Tolerant (SIFT) (Choi, 2012), MutationTaster (Schwarz et al., 2014), and the likelihood ratio test (LRT) (Chun and Fay, 2009). Protein sequence alignment was performed with UniProt[11] to determine whether sequences were evolutionarily conserved across different species including *Mus musculus* (mouse), *Rattus norvegicus* (rat), *Equus caballus* (horse), *Felis catus* (cat), *Bos taurus* (bovine), and *Macaca mulatta* (rhesus macaque). All analyses were performed on the Seqmax platform[12] and Pubvar platform[13] platforms. Significant findings were comprehensively assessed by considering minor allele frequency (MAF), predicted pathogenicity, disease association, and family history.

Statistical Analysis

The frequency of genetic mutations was calculated as the number of patients with mutations relative to the total number of samples with and without mutations. The frequency of each mutation was equal to the number of patients with the mutation of interest relative to the total sample size with and without a given mutation. Statistical analysis was performed using GraphPad Prism v.8.0. Categorical variables were compared by the Chi-squared test. Data are represented as means ± the standard deviation. Two-tailed p values < 0.05 were considered statistically significant.

RESULTS

Demographic Features of the Subjects

Of the 49 bvFTD patients identified in our final dataset (25 males; 51.0%), 14 patients from eight families were identified as having familial bvFTD (f-bvFTD), while 35 were considered to have sporadic bvFTD (s-bvFTD). The demographic features of these patients are shown in **Supplementary Table 2**. Family history was positive in 14 out of 49 cases (28.6%), with modified Goldman scores of 1.0–3.5 and a mean modified Goldman score of 1.9. There were no significant differences between the f-bvFTD and the s-bvFTD patients in terms of gender, age at onset, years of education, onset-diagnosis interval, MMSE, MoCA, CDR, NPI-Q, MBI-C, or FBI scores between f-bvFTD and s-bvFTD patients.

Genetic Screening

Whole-exome sequencing was conducted in all 49 bvFTD patients in the study. The mean sequencing depth for target regions was 125.48×. On average, per sequencing individual, 99.87% of targeted bases were covered by at least 1 × coverage and 99.50% of the targeted bases had at least 10 × coverage. Many rare variants were identified in bvFTD patients. However, we focused particularly on variants in genes with known functions that were potentially associated with FTD and other neurodegenerative diseases, such as PD, ALS, and AD. After filtering (as described above) and confirmed via Sanger sequencing, nine possible pathogenic variants were identified in 15 patients, including six known pathogenic variants (MAPT p. P301L, p. N279K, p. V337M, p. N296N, p.P513A; FUS p. G231del), and 3 novel variants (MAPT p. R5C, p. D54N; GRN p. P451L). None of these pathogenic variants had been reported previously in the public databases described above. C9orf72 repeat expansions (>52 repeats) were observed in the two additional patients. All of the detected pathogenic variants and clinical features are shown in **Supplementary Table 3**.

Variant Assessment

Details relating to novel variants, along with pathogenic prediction are summarized in **Table 1** and **Figure 1C**. Three novel variants were absent in the general population. These missense variants affected an amino acid that had been highly conserved during evolution, and were classified as "possibly damaging," "damaging," or "disease causing" by the three prediction programs PolyPhen2, SIFT, and MutationTaster, respectively. In addition, the MAPT R5C variant was a missense mutation in the same amino acid location as other pathogenic variant (MAPT R5H/R5L) that had been reported previously (Lin et al., 2017).

Frequency of Mutations

Of the 49 patients, 2 carried a pathogenic C9orf72 repeat expansion, 12 carried known gene mutations, 3 carried novel mutations, and 32 did not carry any mutations. Ten pathogenic or likely pathogenic variants were identified, accounting for 27.9% (12/43) of total cases, 87.5% (7/8) patients with f-bvFTD and 14.3% (5/35) with s-bvFTD (**Figures 1A,B**).

Mutations in MAPT were the most common genetic determinant, with a mutation frequency of 20.9% (9/43) in all bvFTD patients, 75% (6/8) in f-bvFTD patients, and 8.6% (3/35) in s-bvFTD patients. C9orf72 repeat expansion was detected in 2.3% (1/43) of bvFTD patients. In addition, GRN and FUS mutation was responsible for 1 bvFTD case (2.3%), respectively. Nevertheless, no mutations in CHCHD10, VCP, TARDBP, or TBK1 genes were detected.

Of the patients with MAPT mutations, six patients (46.2%) from three families carried P301L mutation, and two patients (15.4%) from one family had an N279K mutation. V337M, N296N, R5C, D54N, and P513A were also detected in five patients with MAPT mutations, respectively (7.7%).

Clinical Features of Patients Carrying Mutations

Genetic, clinical, and imaging features of the seventeen clinical probable bvFTD patients with mutations identified in this study are summarized in **Supplementary Table 3**. There were

[7]https://www.ncbi.nlm.nih.gov/snp/

[8]https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/

[9]https://exac.broad.institute.org/

[10]http://gnomad.Broad.institute.org/

[11]https://www.uniprot.org

[12]https://www.seqmax.com/ accessed March 2020

[13]https://www.pubvar.com accessed March 2020

TABLE 1 | Pathogenicity prediction analyses of three novel missense variants identified in the study.

Mutation	Family history	Genomic location (hg19)	Location in the gene	Reference sequence	Reference SNP (rs) ID	Amino acid change	MAF	Database			Pathogenicity				
								Genome Aggregation Database	1000 Genomes Project	Exome Aggregation Consortium	Polyphen2	SIFT	Mutation Taster	LRT	CADD score
MAPT c.160G > A	–	17:44049251	Exon 3	NM_016835.4	–	p. D54N	absent	NR	NR	NR	Probably damaging	Damaging	Disease causing	Deleterious	26.6
MAPT c.13C > T	–	17:44039716	Exon 1	NM_016835.4	rs7661 66210	p. R5C	absent	NR	NR	NR	Probably damaging	Damaging	Disease causing	Neutral	34.0
GRN c.1352C > T	–	17:42429555	Exon 11	NM_002087.2	rs7524 28000	p. P451L	absent	NR	NR	NR	Probably damaging	Damaging	Disease causing	Deleterious	34.0

MAF, minor allele frequency; CADD, combined annotation-dependent depletion; LRT, likelihood ratio test; NR, not reported; Polyphen2, Polymorphism Phenotyping v.2; SIFT, Sorting Intolerant From Tolerant.

statistically significant differences in terms of age at onset when comparing patients with mutations and those who did not have any of these mutations (57.76 ± 9.17 versus 64.81 ± 9.08; $t = 2.56$; $p = 0.014$). Most patients (12, 70.6%) exhibited personality changes and inappropriate behaviors at onset, and five (29.4%) patients presented with memory decline (**Supplementary Table 3**).

Of the 17 variant carriers, extrapyramidal signs occurred in 1 MAPT P301L variant, 2 MAPT N279K variants, and 2 C9orf72 repeat expansions, which were from three families, respectively. The first one belonged to the bvFTD family, in which 2 generations of 4 patients with bvFTD were found to be associated with a known mutation in MAPT p.P301L. All patients exhibited typical symptoms of bvFTD, except for one male patient who developed the disease at the age of 67 years, with Parkinsonism as the main manifestation, accompanied by mild cognitive decline. Two affected individuals suffering from bvFTD with parkinsonism were found in the pedigree with C9orf72 repeat expansion, in which more than 50 repeats were observed. The proband was a 66-year-old woman that presented with Parkinsonism as the initial symptom of onset and was misdiagnosed as Parkinson's disease. Her younger brother developed behavioral changes and cognitive impairment with gradual onset at 62 years-of-age. One year later, the patient experienced progressive bradykinesia and rigidity.

DISCUSSION

To the best of our knowledge this is the first investigation of genetic features in well-characterized Chinese Han bvFTD patients. In this study potentially pathogenic variants were detected in 27.9% of the bvFTD patients, and the most frequently affected gene was MAPT. In total, we identified two novel MAPT mutations and one novel GRN mutation were identified. These findings indicate that genetic mutations are relatively common in Chinese bvFTD patients.

Two novel MAPT variants (p. R5C, p. D54N) and one novel GRN variant (p. P451L) were identified in the current study. All three missense mutations were absent from the gnomAD, 1000 Genomes Project, and Exome Aggregation Consortium databases. Amino acids in this region are highly conserved across several species, and the variants identified were predicted to be damaging by four *in silico* analysis tools. All of the patients harboring these novel variants exhibited the classic manifestations of bvFTD, including personality change, inappropriate behaviors, and significant hypometabolism in frontal and temporal lobes as determined by 18F-FDG-PET. Apart from these, the MAPT R5C variant was located in exon 1 of the MAPT gene, where the other two known mutations (MAPT R5H and R5L) have been found in Japanese and Taiwanese bvFTD patients (Lin et al., 2017). Collectively, three novel variants may be associated with bvFTD in the Chinese population, further expanding the known mutational spectrum of bvFTD.

The frequency of mutations in Chinese bvFTD patients was higher than expected. In the present bvFTD cohort genetic

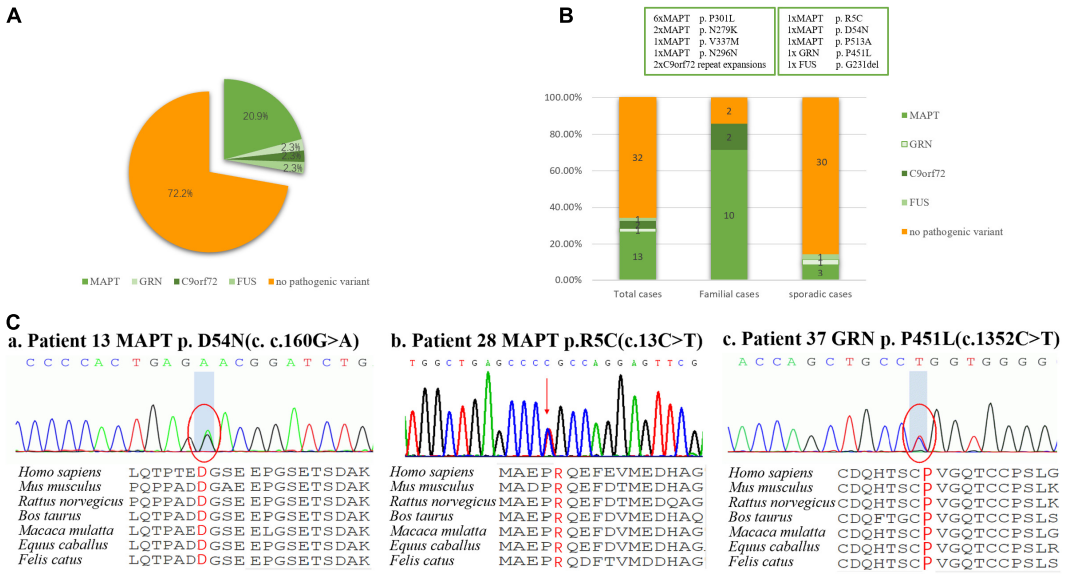

FIGURE 1 | Frequency of mutations in a consecutive series of 49 subjects with behavioral variant frontotemporal dementia. **(A)** 27.9% of subjects carried mutations, including those of MAPT, GRN, FUS, and C9orf72 repeat expansion, but surprisingly not CHCHD10. **(B)** Mutations were found in 87.5% of familial subjects and 14.3% of sporadic subjects. **(C)** Sanger sequencing revealed two novel missense mutations of MAPT (p. R5C and p. D54N), and one novel missense mutation of GRN (p. P451L). These missense mutations were present at a highly conserved position, as indicated by a comparison of the corresponding sequences of seven vertebrate species.

mutations accounted for 27.9% of bvFTD cases, and mutations were more likely to be detected in patients with a definite family history of bvFTD (87.5%). Mutations were also detected in sporadic cases (14.3%). In previous studies, bvFTD was usually included and analyzed as the most important phenotype of FTLD (summarized in **Table 2**), which did not specifically focus on bvFTD. Consequently, the prevalence of pathogenic variants in Chinese bvFTD patients has not been reported. In the only three existing studies based on Chinese frontotemporal dementia (FTD) cohorts, the prevalence of pathogenic variants was comparatively low (4.9–7.7%) (Shi et al., 2016; Tang et al., 2016; Che et al., 2017). A number of explanations may account for the discrepancy between these previously reported rates and the comparatively higher frequency of genetic mutations observed in the current study. The high proportion of f-bvFTD (28.6%) identified in the present study, compared to the 13.5% reported previously (Tang et al., 2016) was likely to have been contributory, because a higher frequency of mutations was detected in bvFTD patients with a family history. The discrepancy may also be the result of pure bvFTD phenotype screening criteria depending on FDG-PET in the present study, which was more heritable than the language syndromes nfvPPA and svPPA. There may also be differences between subjects from Northern and Southern China, as notably most of the aforementioned previous studies were based in South China (Tang et al., 2016; Che et al., 2017; Jiang et al., 2021). In any event, in view of the substantial proportion of mutations discovered in the present cohort, genetic screening should be considered in all bvFTD patients – even those without a family history of dementia – because underlying genetic causes of bvFTD cannot be excluded.

The frequency of MAPT variants in the present cohort was 20.7%, and they were more common in bvFTD patients with a positive family history, followed by C9orf72 repeat expansions (2.3%), disease-associated variants of GRN (2.3%) and FUS (2.3%). High variability in mutation prevalence across different geographical regions has been reported for C9orf72, MAPT, and GRN genes, and hexanucleotide repeat expansions in C9orf72 are the most common genetic cause of bvFTD in western populations (Seeley, 2019). The most common genetic determinant in the present bvFTD cohort was MAPT mutations, and C9orf72 repeat expansions were identified with a low frequency, which differ from reports derived from Europe and America (Greaves and Rohrer, 2019; Oijerstedt et al., 2019; Ramos et al., 2019b, 2020), but are similar to previous studies involving Chinese patients with FTD (Shi et al., 2016; Tang et al., 2016; Che et al., 2017; Jiang et al., 2021). Notably, the frequency of MAPT mutations was much higher than described in previous studies of Chinese FTD patients (2.8%) (Jiang et al., 2021). The most common MAPT mutation in the present study was P301L, which was not clear in a previous study in Chinese FTD patients. This may be a consequence of the high proportion of f-bvFTD patients in the current study since the P301L mutation was only detected in subjects with a family history of bvFTD.

Interestingly, Jiao et al. (2016) reported that rare variants in the CHCHD10 gene were detected at high frequencies in Chinese sporadic FTD patients, some of which were present in subjects with bvFTD. No mutations in CHCHD10 were identified in the current cohort, indicating that they are likely to be either not causative or a rare cause of bvFTD. The relatively small sample size, pure bvFTD phenotypes, and different geographical study

TABLE 2 | Identified mutations in Chinese bvFTD patients.

References	Gene	Nucleic acid change	Amino acid change	Area	Phenotype			
					Behavioral changes	Cognitive impairment	Parkinsonism	Motor syndrome
Che et al., 2017	MAPT	c.14G > A	p.R5H	Shanghai	NA	NA	NA	NA
Tang et al., 2016		c.837T > G	p.N279K	Hunan/Beijing	+	+	+	–
He et al., 2018		c.902C > T	p.P301L	Henan/Tianjin	+	+	–	–
Xu et al., 2018		c.1165G > A	p.G389R	Shanghai	+	+	+	–
Che et al., 2017	GRN	c.750C > A	p.D250E	Shanghai	+	NA	NA	NA
Tang et al., 2016		c.898C > T	p.Q300X	Hunan	+	+	–	–
Jiao et al., 2014	C9orf72	GGGGCC hexanucleotide repeat expansion		Hunan	+	+	–	–
Jiao et al., 2016	CHCHD10	c.64C > T	p.H22Y	Hunan	+	+	–	–
Jiao et al., 2016; Che et al., 2017		c.67C > T	p.P23S	Hunan/Shanghai	+	+	–	–
Jiao et al., 2016		c.95C > A	p.A32D	Hunan	+	NA	–	–
Jiao et al., 2016		c.170T > A	p.V57E	Hunan	+	+	–	–
Che et al., 2017		c.266C > T	p.P89L	Hunan	NA	NA	NA	NA
Shi et al., 2016	VCP	c.379A > G	p.T127A	Tianjin	+	+	+	–
Yu et al., 2019	TBK1	c.1001T > C	p.I334T	Shanghai	–	+	–	–
Tsai et al., 2016		c.1330C > T	p.R444X	Taiwan	+	+	–	+

region in the present study may also have contributed to this discrepancy. It is notable that these populations and geographical differences have to be interpreted with caution, because they may also be partly influenced by between-center and between-region differences in subject recruitment. Therefore, further screening of larger bvFTD cohorts needs to be undertaken in different geographical areas.

Though there were no significant differences in terms of age at onset between f-bvFTD and s-bvFTD patients, the frequency of mutations was significantly higher in a definite family history of bvFTD compared with sporadic cases (87.5% versus 14.3%). Moreover, age at onset was earlier in bvFTD patients with mutations than those without mutations. A younger age at onset and a strong positive family history were particularly strong predictors for the presence of a pathogenic mutation, which may be relevant in guiding the priority of genetic testing for this population in clinical practice.

Notably, prominent parkinsonism was found for the first time in Chinese bvFTD patients with the P301L MAPT and C9orf72 gene mutation, respectively. It is well documented that the P301L MAPT mutation and C9orf72 repeat expansion were are predominantly associated with FTD in the western population, often accompanied by Parkinsonism (Siuda et al., 2014). In the Chinese cohort, a variety of phenotypes with MAPT mutation were observed, which was associated with mutation location to some extent (Shi et al., 2016; Tang et al., 2016; Che et al., 2017; Mao et al., 2021). However, prominent Parkinsonism has not been reported previously for the P301L MAPT mutation (Shi et al., 2016; He et al., 2018). In the present cohort, one of four affected individuals in the pedigree with P301L MAPT mutation presented with Parkinsonism. This demonstrated the phenotypic variability associated with the P301L mutation in individuals with the same MAPT mutation, even within the same family. GGGGCC repeat expansions in the C9orf72 gene is rarely detected in Chinese FTD patients. A search of the existing literature showed that C9orf72 repeat expansion has only previously been identified in a patient with sporadic bvFTD and a family with FTD-ALS (Jiao et al., 2014). Interestingly, GGGGCC repeat expansions in the C9orf72 gene was found in a family with FTD and Parkinsonism in the present study, thus suggesting that parkinsonism in affected individuals may be attributable to dopaminergic dysfunction of the putamen resulting from C9orf72 repeat expansion. In contrast with Europe, parkinsonism is relatively rare in the FTD patients possessing the P301L MAPT mutation or the C9orf72 repeat expansion in China, thus suggesting that genetic heterogeneity is associated with different geographical regions and ethnicities. These results indicate that parkinsonism might be present in the pure bvFTD phenotype with P301L MAPT mutation or C9orf72 repeat expansion in China, thus suggesting that more attention should be paid to parkinsonism in patients with bvFTD in clinical practice.

The current study had some limitations. One was the relatively small sample size, and another was that gene recognition was based on clinical diagnosis of bvFTD patients rather than postmortem autopsy-based diagnosis, which affected the acquisition of conclusive information relating to the clinical manifestations and pathogenesis of mutations. We will address

this issue via pathological evaluation and mutation function analysis in the future.

CONCLUSION

In summary, in the present study potentially pathogenic variants were detected in approximately 28% of bvFTD patients, and MAPT variants were the most common causative genetic factors. Three novel bvFTD-related pathogenic variants were identified (MAPT p. R5H, p. D54N, and GRN p. P451L), broadening the known mutation diversity of bvFTD. The study provides evidence of a high prevalence of pathogenic variants in Chinese bvFTD patients, and highlights the necessity of genetic screening for bvFTD.

DATA AVAILABILITY STATEMENT

According to national legislation/guidelines, specifically the Administrative Regulations of the People's Republic of China on Human Genetic Resources (http://www.gov.cn/zhengce/content/2019-06/10/content_5398829.htm, http://english.www.gov.cn/policies/latest_releases/2019/06/10/content_281476708945462.htm), no additional raw data is available at this time. Data of this project can be accessed after an approval application to the China National Genebank (CNGB, https://db.cngb.org/cnsa/). Please refer to https://db.cngb.org/, or email: CNGBdb@cngb.org for detailed application guidance. The accession code CNP0001883 should be included in the application.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Xuanwu Hospital of Capital Medical University, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LL and LW were responsible for study concept and design. YC, DJ, DL, KX, YK, and TX were responsible for clinical data collection. MC and KX were responsible for sequencing and quality control. YC, MC, and CW analyzed gene results. LL and BC were mainly responsible for writing the manuscript. CW and LW were responsible for revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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frontiers in Aging Neuroscience

REVIEW
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MicroRNAs as Potential Orchestrators of Alzheimer's Disease-Related Pathologies: Insights on Current Status and Future Possibilities

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Alzheimer's disease (AD) is a progressive and deleterious neurodegenerative disease, strongly affecting the cognitive functions and memory of seniors worldwide. Around 58% of the affected patients live in low and middle-income countries, with estimates of increasing deaths caused by AD in the coming decade. AD is a multifactor pathology. Mitochondrial function declines in AD brain and is currently emerging as a hallmark of this disease. It has been considered as one of the intracellular processes severely compromised in AD. Many mitochondrial parameters decline already during aging; mitochondrial efficiency for energy production, reactive oxygen species (ROS) metabolism and the *de novo* synthesis of pyrimidines, to reach an extensive functional failure, concomitant with the onset of neurodegenerative conditions. Besides its impact on cognitive functions, AD is characterized by loss of synapses, extracellular amyloid plaques composed of the amyloid-β peptide (Aβ), and intracellular aggregates of hyperphosphorylated Tau protein, accompanied by drastic sleep disorders, sensory function alterations and pain sensitization. Unfortunately, till date, effective management of AD-related disorders and early, non-invasive AD diagnostic markers are yet to be found. MicroRNAs (miRNAs) are small non-coding nucleic acids that regulate key signaling pathway(s) in various disease conditions. About 70% of experimentally detectable miRNAs are expressed in the brain where they regulate neurite outgrowth, dendritic spine morphology, and synaptic plasticity. Increasing studies suggest that miRNAs are intimately involved in synaptic function and specific signals during memory formation. This has been the pivotal key for considering miRNAs crucial molecules to be studied in AD. MicroRNAs dysfunctions are increasingly acknowledged as a pivotal contributor in AD via deregulating genes involved in AD pathogenesis. Moreover, miRNAs have been proved to control pain sensitization processes and regulate circadian clock system that affects the sleep process. Interestingly, the differential expression of miRNA panels implies their emerging potential as diagnostic AD biomarkers. In this review, we will present an updated

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analysis of miRNAs role in regulating signaling processes that are involved in AD-related pathologies. We will discuss the current challenges against wider use of miRNAs and the future promising capabilities of miRNAs as diagnostic and therapeutic means for better management of AD.

Keywords: Alzheimer's, mitochondria, microRNAs, synaptic plasticity, sleep disorder, pain

INTRODUCTION

Alzheimer's: A Peculiar Case of Brain Disease

"Alois Alzheimer," The German Bavarian psychiatrist, and neurologist was the first to report Alzheimer's disease (AD) in 1906 as "A peculiar severe disease of the cerebral cortex" (Hippius and Neundörfer, 2003). Today, Alzheimer's is acknowledged as a common neurodegenerative disease affecting elderly population. It is the most common form of dementia and may account to 60–70% of cases (Plassman et al., 2007), with increasing numbers of people getting AD every year, especially in middle and low-income countries (Alzheimer's Disease Facts and Figures, 2020).

AD is an irreversible, progressive brain disorder and the most common cause of dementia among older people. It is heterogeneous in every aspect, such as the relation between the presence of plaques and tangles of Aβ and Tau in the brain, clinical symptoms, and genetic background that causes memory loss, language problems, and impulsive or unpredictable behavior. Tau tangles block the transport of nutrients and other essential molecules inside neurons. Recently, it was found that Tau signal emerges first in the rhinal cortex independently of Aβ. Tau pathology begins focally but expands catastrophically under the influence of Aβ pathology to mediate neurodegeneration and cognitive decline. Subsequent Tau elevation in the temporal neocortex is associated with age, Aβ, and APOE status (Sanchez et al., 2021). This indicates a loss of connection between the nerve cells, or neurons, in the brain. At the earlier stages of AD, patient's daily routine is impacted due to disruptions in the entorhinal cortex and hippocampus that affects memory, executive cognition, and visuospatial awareness. Meanwhile, during later AD stages, personality, behavior, and language impairments arise due to escalating damage in frontal, temporal, and parietal lobes. Such drastic damages are associated with continuous decline in independence, culminating in patients' complete dependence on their caregivers by the latest stages of the disease. Consequently, AD is one of the most debilitating disorders as it impacts both patients and families on mental, psychological, and socio-economic aspects.

Alzheimer's: What We Know So Far

Scientists do not yet fully understand the exact causes of AD. AD is a multifactorial disorder in which genetic and environmental risk factors interact to increase the rate of normal aging. It is so tightly associated with old age that there was a speculation it is a normal part of aging (Masters et al., 2015). Various causes probably contribute to AD etiology including a combination of age-related changes in the brain; brain proteins fail to function normally and the presence of toxic oligomeric species of Amyloid peptides (Aβ) and Tau within the AD brain. Recent data confirms the view that such species can propagate and spread within neural circuits, disrupting the work of brain cells (neurons) and triggering a series of toxic events (Chen and Mobley, 2019). Also, mitochondrial dysfunction, a compromised blood brain barrier, immune system dysfunction, and infectious agents probably contribute to the etiology of AD (Fulop et al., 2021). AD is a progressive neurologic disorder that causes the brain to shrink (atrophy) and brain cells to die, culminating into continuous decline in thinking, behavioral and social skills that affect a person's ability to function independently. Clinical manifestations, which are insidious in onset, include memory loss and cognitive decline (Scheltens et al., 2016). The most accepted theory is that AD is caused by misfolded proteins and the culprit in this misfolding is Aβ peptides, that aggregate or clump, killing brain cells and giving rise to the symptoms of memory loss and reduced cognition.

Current research identifies three stages of AD: preclinical Alzheimer's disease, mild cognitive impairment (MCI) due to Alzheimer's disease, and dementia due to Alzheimer's disease (Sperling et al., 2011). In the last two stages, symptoms are present, but to varying degrees. Early signs include difficulty remembering recent events or conversations. As the disease progresses, memory impairments worsen, and other symptoms develop.

Abbreviations: BACE1, Beta-Secretase 1; HEY2, Hairy/Enhancer-Of-Split Related With YRPW Motif Protein 2; APP, Amyloid beta precursor protein; MAPK, Mitogen-activated protein kinase; DUSP6, Dual specificity phosphatase 6; PPP1CA, Protein Phosphatase 1 Catalytic Subunit Alpha; PTBP2, Polypyrimidine Tract Binding Protein 2; *MeCP2*, Methyl-CpG Binding Protein 2; PTEN, Phosphatase And Tensin Homolog; *Fyn*, Proto-oncogene tyrosine-protein kinase Fyn; ERK1, extracellular signal-regulated kinase 1; ERK2, extracellular signal-regulated kinase 2; CREB, cAMP response element-binding protein; BDNF, Brain-derived neurotrophic factor; *EphB2*, Ephrin type-B receptor 2; *GRIA2*, Glutamate ionotropic receptor AMPA type subunit 2; *GRIN2B,* Glutamate Ionotropic Receptor NMDA Type Subunit 2B; *Syt-1,* Synaptotagmin1; *Syt-1A,* Syntaxin 1A; *NPTX1,* Neuronal Pentraxin 1; *AMPAR,* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionicacid receptor; *GRIN2A*, Glutamate Ionotropic Receptor NMDA Type Subunit 2A; *EPHA4*, Ephrin type-A receptor 4; *Nrn1*, Neuritin 1; *Arpc3*, Actin Related Protein 2/3 Complex Subunit 3; *CPLX1,* Complexin 1; *CPLX2,* Complexin2; *STIM2,* Stromal Interaction Molecule 2; REST, RE1 Silencing Transcription Factor; *CX3CL1*, C-X3-C Motif Chemokine Ligand 1; CX3CR1, C-X3-C Motif Chemokine Receptor 1; *NPAS2,* Neuronal PAS Domain Protein 2; *DBP,* D-Box Binding PAR BZIP Transcription Factor; *Mycs*, MYC Proto-Oncogene; *YAP1*, Yes1 Associated Transcriptional Regulator; *C-MAf,* C musculoaponeurotic fibrosarcoma; *CCM1*, Cerebral cavernous malformations 1; *NOX2*, NADPH oxidase 2; *NOX4*, NADPH oxidase 4; *C/EBP-α*, CCAAT/enhancer-binding protein alpha; TREM2, Triggering receptor expressed on myeloid cells 2.

People with MCI due to Alzheimer's disease have biomarker evidence of an Alzheimer's-related brain change (for example, elevated levels of Aβ) and greater cognitive decline than expected for their age, but this decline does not significantly interfere with everyday activities (Roberts and Knopman, 2013). In MCI, changes in thinking abilities may be noticeable to family members and friends but may not be noticeable to others.

Sadly till date, there is no cure for AD. Currently, the only approved drugs for AD merely alleviate some of the symptoms -partially and temporarily- but do not stop the disease from progressing.

Alzheimer's Hallmarks: Aβ and Phosphorylated Tau

The neuropathology of AD is characterized by an abnormal build-up of extracellular amyloid-β (Aβ) peptide as neuritic plaques, pathological extracellular aggregates formed around a core of Aβ and are a hallmark of AD (Stratmann et al., 2016), accompanied by intracellular hyperphosphorylated (p)-Tau fibrils which accumulate as neurofibrillary tangles (NFTs) within neurons (Stratmann et al., 2016). Amyloid peptide (Aβ) is derived from the amyloid precursor protein (APP), α-secretase ("normal" cleavage), or β-secretase ("abnormal" cleavage) cleaves APP, and a second cleavage of the β-secretase product, by γ-secretase, cleaves APP further to produce Aβ (Scheuner et al., 1996; Di Carlo et al., 2012). Depending on the cleavage site by γ-secretase, Aβ40 or Aβ42 are produced. Aβ40 is the most common form, while the 42-amino-acid–long fragment, Aβ42, is less abundant and more associated with AD. The proportion of Aβ40 to Aβ42 is important in AD because Aβ42 is far more prone to oligomerize and form fibrils than Aβ40 peptide (Sun et al., 2015). Recently, Alexandra Grubman's group isolated amyloid plaque-containing (using labeling with methoxy-XO4, $XO4^+$) and non-containing ($XO4^-$) microglia from an AD mouse model. Transcriptomics analysis identified different transcriptional trajectories in aging and AD mice. Where $XO4^+$ microglial transcriptomes demonstrated dysregulated expression of genes associated with late onset AD (Grubman et al., 2021). Recent genome-wide association studies have established that the majority of AD risk loci are found in or near genes that are highly and sometimes uniquely expressed in microglia, the resident macrophages of the central nervous system. This leads to the concept of microglia being critically involved in the early steps of the disease and identified them as important potential therapeutic targets. Changes in microglial morphology, the resident macrophages of the central nervous system, and signaling is also evident in AD brains, contributing to the pathology (Hemonnot et al., 2019).

The neuropathological changes of AD brain, based on brain imaging studies, show slow and progressive cerebral atrophy, where the frontal and temporal cortices often have enlarged sulcal spaces with atrophy of the gyri, while primary motor and somatosensory cortices most often appear unaffected (Perl, 2010). Neuropathological studies in combination of MRI approaches also showed early pathological alterations to the locus coeruleus (LC), a tiny nucleus in the brainstem and the principal site of noradrenaline synthesis. The LC undergoes significant neuronal loss in AD, with postmortem studies showing as much as 80% reduction in cell number in people with AD compared to age-matched controls associated with tauopathy in AD (German et al., 1992; Hoogendijk et al., 1995; Beardmore et al., 2021). Another macroscopic feature commonly observed in AD is the loss of neuromelanin pigmentation in the locus coeruleus with age and is proposed to be toxic and inflammatory when released into the extracellular environment (Serrano-Pozo et al., 2011).

Another characteristic feature in AD pathology is the neurofibrillary tangles of hyperphosphorylated microtubule-associated protein (Tau). Microtubules (MTs) are hollow cylinders composed of parallel protofilaments of α and β tubulin subunits. Tau is a neuronal microtubule associated protein whose main biological functions are to promote microtubule self-assembly by tubulin and to stabilize those already formed. MTs dynamics are regulated by Tau proteins that stabilize or destabilize them (van der Vaart et al., 2009) and protect MTs against depolymerization by decreasing the dissociation of tubulin at both MT ends, resulting in an increased growth rate and decreased catastrophe frequency (Trinczek et al., 1995). Disruption of microtubules, as are observed in patients with AD, interrupts axonal transport which prevents vesicles and organelles from reaching the synapses. These result in the slow and steady deterioration of the synapses and retrograde degeneration of the neurons.

Genes of AD

Multiple studies reported different genes to be involved in AD development. Although twin studies support the existence of a genetic component in late onset Alzheimer's disease (LOAD), no one particular causative gene has been identified yet. Familial AD is mainly associated with mutations in the Aβ precursor protein (APP) gene and presenilin genes *PSEN1* and *PSEN2* that are responsible for γ-secretase cleavage of APP. Still, Familial AD comprises only the minor subclass of AD. Apolipoprotein E (APOE) on chromosome 19 is another polymorphic protein and the allele APOE4 is the strongest genetic risk factor for Sporadic AD (Giri et al., 2016). As the strong affinity of APOE for Aβ affects its production, hydrolysis, and elimination (Reitz and Mayeux, 2014). Yet, although around 80% of LOAD is associated with APOE, apolipoprotein E (APOE)-4 allele confers only a 20% risk for developing the disease. Similarly, increasing evidence confirms that Sporadic AD has a more underlying complex etiology that includes both genetic and environmental factors (Bekris et al., 2010).

The most successful approach to identifying the genetic architecture of AD is the genome-wide association studies (GWAS) which identified and confirmed 19 genome-wide-significant common variant signals in addition to APOE. Together with GWAS, Whole Exome Sequencing (WES), and Whole Genome Sequencing (WGS) defined no fewer than 20 additional genes whose variants contribute to increased risk of AD (Jun et al., 2017; Liu et al., 2017). Kunkle et al. (2019), confirmed 20 previous LOAD risk loci and identified five new genome-wide loci (*IQCK, ACE, ADAM10, ADAMTS1, and WWOX*), two of which (*ADAM10 and ACE*) were identified

in a recent genome-wide association (GWAS)-by-familial-proxy of AD or dementia. Fine-mapping of the human leukocyte antigen (HLA) region confirms the neurological and immune-mediated disease haplotype HLA-DR15 as a risk factor for LOAD (Kunkle et al., 2019). Other implicated genes are Clusterin *(CLU)*, Sortilin-related receptor-1 *(SORL1)*, ATP-binding cassette subfamily A member 7 *(ABCA7)*, Bridging integrator 1 *(BIN1),* phosphatidylinositol binding clathrin assembly protein *(PICALM)*, CD2 associated protein *(CD2AP)*, Complement component (3b/4b) receptor 1 *(CR1)*, CD33, triggering receptor expressed on myeloid cells 2 *(TREM2),* and phospholipase D3 *(PLD3)* (Karch and Goate, 2015). These variants define possible contributions in AD from genes that regulate endocytosis, inflammation and the brain's innate immune system, and cholesterol/sterol metabolism (Karch and Goate, 2015).

Alzheimer's Pathophysiology: Multiple Hypotheses

The complexity of AD led to the generation of multiple hypotheses, in trials to unravel AD pathogenesis. Illustration of the different hypotheses of AD development and progression is shown in **Figure 1**.

Aβ Cascade Hypothesis

For long, Aβ has been seen as the main causative agent in AD pathology that is followed by neurofibrillary tangles, vascular damage and neuronal loss as direct consequences of Aβ deposition (Murphy and LeVine, 2010). Where Aβ deposits in the hippocampus and basal segment, provoking Aβ to form insoluble aggregates, and inducing mitochondrial damage (Lustbader et al., 2004), and synaptic dysfunction (Hunt and Castillo, 2012). This cascade of events is followed by microglia and astrocytes activation, which arise inflammation and oxidative stress, causing synaptic loss and neuronal death (Fan et al., 2020). Recent studies show that elevated levels of Aβ plaque do not always correlate with magnified AD pathology. Whereas, increasing evidence reveals that Aβ oligomers (Aβ Prions) might be more neurotoxic as they are easily transmitted and released in the synapses (Mucke and Selkoe, 2012; Amin and Harris, 2021). Consequently, and considering the accumulated body of AD research, the majority of data still supports Aβ role as the principal initiator of AD complicated pathology in the early stages. Yet obviously, Aβ is not the only contributor to AD late stages.

Tau Hyperphosphorylation Hypothesis

Tau is mainly found in neuronal axons of the brain. It maintains microtubule structure, synaptic structure, function (Kimura et al., 2014) and regulates neuronal signaling. Tau is also a phosphoprotein that depends on protein kinase and protein phosphatase activities. Hyperphosphorylated Tau in AD patients' brains causes configuration changes and loss of tubulin polymerization capacity (Grundke-Iqbal et al., 1986) that result in defective microtubule functioning. Moreover, increased levels of cytosolic Tau induce polymerization of phosphorylated Tau to form NFTs (Iqbal et al., 2010), that contribute to reduced synapses numbers, and cell dysfunction (Callahan et al., 1999). Tau hyperphosphorylation is positively correlated with the pathological severity of AD and is apparently more detrimental to cognitive impairment than Aβ (Mocanu et al., 2008).

Cholinergic Hypothesis

The cholinergic hypothesis suggests that disruptions of acetylcholine-containing neurons contribute to the cognitive decline in Alzheimer's disease (AD). This hypothesis is supported by the fact that severity of dementia in AD, is positively correlated with the extent of cholinergic loss (Francis et al., 1985). Furthermore, multiple reports imply that cholinergic loss may be an early sign of cognitive decline in AD, and can therefore have a more crucial role in Aβ depositions, Tau phosphorylation, and neuroplasticity (Terry and Buccafusco, 2003; Hampel et al., 2018). Currently, inhibitors of acetylcholinesterase are widely used in AD management, and they show some tangible results concerning symptomatic improvement in AD patients.

Inflammation Hypothesis

Neuroinflammation is a hallmark of AD and increasing evidence shows that microglia is a central player in AD. As in early AD stage, microglia, *TREM2* and complement system are responsible for synaptic disruptions (Paolicelli et al., 2011; Hong et al., 2016). Meanwhile, as the disease progresses, reactive microglia and astrocytes surround amyloid plaques and secrete numerous pro-inflammatory cytokines that drastically impact synaptic functions and neuroplasticity over the course of AD (Du et al., 2018).

Oxidative Stress Hypothesis

Likewise, oxidative stress is a significant player in AD pathogenesis. Normally, the brain utilizes more oxygen than other tissues and undergoes mitochondrial respiration, which increases the potential for ROS exposure. AD is highly associated with cellular oxidative stress that contributes to increased protein oxidation, glycol oxidation, lipid peroxidation and Aβ accumulation. In line, advanced glycation end products (AGE) and malondialdehyde have been detected in the neuro tangles and senile plaques of AD (Markesbery, 1997). Moreover, multiple studies show that Aβ is capable of generating free radicals that mediate neuron degeneration and death (Cheignon et al., 2017).

Mitochondrial Damage and Glucose Hypometabolism Hypothesis

Increasing body of evidence implies that mitochondrial impairments play fundamental roles in AD pathology. Healthy mitochondria support neuronal activity, provide proper energy supply to neurons by regulating glucose metabolism, and minimizing oxidative damage. In the neurons, mitochondria are vital for biosynthesis of iron and heme. They are also involved in presynaptic transmission, and they regulate calcium concentration during signal transduction (Picone et al., 2014).

Emerging reports spot glucose hypometabolism as an early pathogenic event in preclinical stages of AD concurrently with cognitive and functional decline. Using fluoro-2-deoxyglucose positron-emission tomography (FDG-PET), reveal that glucose hypometabolism is consistently detected in hippocampus and cortex of AD brain compared to normal individuals. In line,

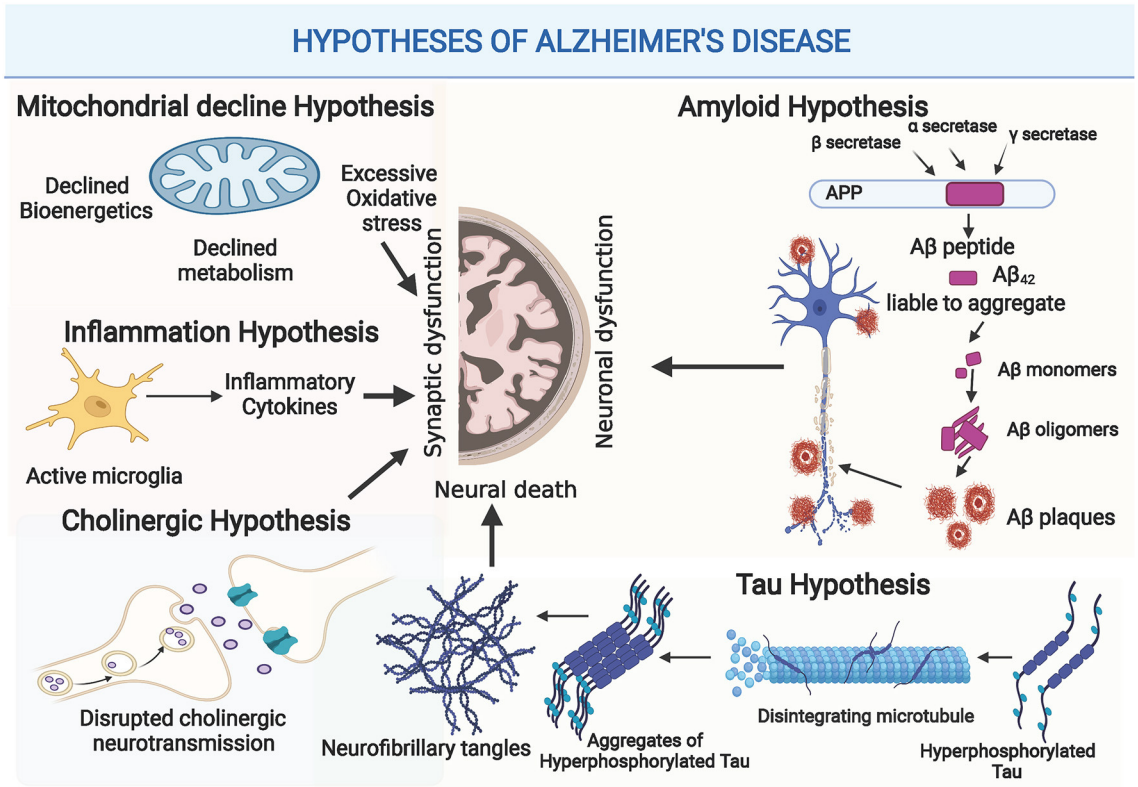

FIGURE 1 | Illustration of the different hypotheses of Alzheimer's Disease (AD) Development and Progression: Amyloid hypothesis: as disturbed secretase enzymes increase production of Aβ42 eventually forming Aβ plaques that impact synaptic functionality, and neuronal dysfunction. Tau Hypothesis: Where increased production of hyperphosphorylated tau provokes disintegration of microtubules and accumulation of hyperphosphorylated tau fibrils causing neuronal death. Cholinergic hypothesis: where disturbed Acetylcholine (ACH) impacts synaptic function. Inflammation hypothesis: where increased release of inflammatory modulators provoke exaggerated immune response that disrupts synaptic functions and plasticity. Mitochondrial hypothesis: where disrupted mitochondrial functions causes glucose hypometabolism, poor ATP production and increased production of ROS, eventually causing synaptic dysfunction. (Created with BioRender.com).

impaired mitochondrial bioenergetics, increased oxidative stress and disrupted mitochondrial genome are consistent features of mitochondrial abnormalities in AD and are interconnected to amplify the debilitating pathologies of AD (Wang W. et al., 2020).

Till date, it is not totally clear whether any of these characteristic deficits is the primary initiator or just a contributor in the contemporary multifactorial AD pathology. A challenge confirmed by the little therapeutic success achieved against AD so far. Consequently, we obviously need new approaches for AD diagnosis at its earliest stages before neuronal damage becomes irreversibly established. It is also crucial to concurrently target multiple axes in AD pathologies for more tangible therapeutic feasibility.

MicroRNAs: Origin, Maturation, and Role

MicroRNAs production excels with formation of a long primary transcript (pri-miRNA) via RNA polymerase II. Inside the nucleus, pri-miRNAs are cleaved by Drosha protein and DiGeorge Syndrome Critical region 8 (DGCR8) protein which dimerize together to form a functional microprocessor complex. These dimerized proteins cleave pri-miRNAs into precursor miRNAs (pre-miRNA), which are then transported to the cytoplasm and digested by Dicer and TAR RNA binding proteins (TRBP) to release a double-stranded miRNA duplex. Helicase enzyme unwinds the duplex to form mature miRNA strands. One of these strands is usually degenerated, while the other associates with Ago2 protein to form miRNA-induced Silencing Complex (miRISC). Each mature miRNA contains a sequence of 7 or 8 nucleotides that binds to its complementary region(s) on target mRNAs. Mature miRNAs bind to the 5′- or 3′-untranslated regions (UTR) of target mRNAs and rarely, both strands can serve as mature functional miRNA. Generally, functional miRNAs induce gene silencing using two different mechanisms, depending on the complementarity between the miRNA and its mRNA target (Amakiri et al., 2019; Kou et al., 2020). If mature miRNA binds perfectly to the complementary regions of the target mRNA, it induces mRNA degradation via de-adenylation, cap removal, and exonucleolytic digestion of mRNA. Meanwhile, if miRNA binds with imperfect complementarity to target mRNA, it causes a translation block by repression of translation during the initial phase or the elongation phase. Alternatively, miRNAs can repress translation by inducing premature ribosome detachment (Silvestro et al., 2019). One functional miRNA can interact with hundreds of target mRNAs to exert various levels of

regulatory effects, and a single mRNA can be targeted by several miRNAs as well.

Around 70% of miRNAs are found in the human brain, where miRNAs are responsible for regulating synaptic functions, neurotransmitter release, and neuronal development. In the last few years, the significance of balanced miRNAs expression proved to be crucial for proper functionality and homeostasis in the body. The differential expression of miRNAs and/or single nucleotide polymorphism (SNP) of miRNAs are implicated in multiple diseases (Reddy et al., 2017). Likewise, the dysregulation of miRNAs emerged as a key contributor in AD pathology, as it leads to altered protein expressions and impairment of the complicated signaling network balance in the brain. The next section will cover the main updated findings concerning miRNAs role in AD pathogenesis and its progressive events.

MICRORNAS AND AD COGNITIVE IMPAIRMENT

Cognition refers to the brain's ability to think, learn, remember, and process information. The loss of neuronal connections in AD brain is basically attributed to disrupted signaling pathways that affect both synaptic plasticity and dendritic functions, the two crucial controllers of cognitive processes. On the molecular level, updated studies show that Aβ and Tau pathologies cause progressive axonal degeneration and drastic downstream impairments in the synaptic processes (Pereira et al., 2021). Moreover, Aβ and Tau aggregates induce exacerbated immune microglial response, as well as disrupted astrocytes functionality, that eventually contribute to cognitive decline in AD (Fakhoury, 2018). Over the past few years, miRNAs have emerged as significant regulators of Aβ and Tau metabolism, glial functionality, and synaptic plasticity. Simultaneously, increasing studies report the drastic impact of miRNA dysregulations on cognitive functions in AD, through targeting key genes and activity-mediated protein synthesis at the synaptic level. Comprehensive illustration of the modulatory role of miRNAs in AD-related cognitive impairment is presented in **Figure 2**.

MicroRNAs and Aβ Pathway Disruptions

Although amyloid deposition alone is not able to produce full AD-pathology, studies that used Aβ PET in cognitively normal elderly individuals, mild cognitive impairment (MCI) and AD patients found significant relationships between cognitive deficits and increased brain fibrillar Aβ (Wang F. et al., 2015). MicroRNAs are closely related to the synaptic dysfunction induced by abnormal Aβ metabolism. Increasing body of work shows that cognitive impairment caused by Aβ can be restored by manipulation of miRNAs, which strongly supports the belief that disrupted miRNA expressions are critical in cognitive impairment in AD patients (Weldon Furr et al., 2019). MicroRNA dysregulations are repeatedly reported in association with key genes that regulate Aβ synthesis, cleavage, and clearance. **Table 1** illustrates an updated comprehensive summary of the human studies showing differentially expressed miRNAs in AD samples and their relation to Aβ pathway regulations.

Regarding Aβ metabolism, several miRNAs including miR-9, miR-29, miR-135, and miR-186 are significant regulators of Beta-Secretase 1 (*BACE1)* enzyme levels which is central in Aβ generation (Wang et al., 2019).

MiR-200-3p particularly has been grabbing increased attention for its role in Aβ pathology in AD. MiR-200-3p is repressed in AD animal and cell models. Mechanistically, miR-200-3p modulates translocation of BACE1 enzyme and ribosomal protein S6 kinase B1 (S6K1), hence it suppresses cell apoptosis, decreases Aβ1-42 and Tau phosphorylation in cell experiments (Samadian et al., 2021). To evaluate the effect of miRNA-200b/c *in vivo*, Tg2576 mice were treated with miRNA-200b/c by intracerebroventricular injection. This experiment confirmed that upregulating miR-200 reduced secretion of Aβ. Moreover, the treated mice were relieved of memory impairments induced by intracerebroventricular injection of oligomeric Aβ. They also demonstrated proper spatial learning, suggesting that miRNA-200b and miRNA-200c are potential therapeutic targets in AD (Higaki et al., 2018). These data are strongly supported by clinical studies that showed decreased miR-200b in serum and cerebrospinal fluid of AD patients, compared to healthy subjects (Silvestro et al., 2019).

Recent studies have recently shown that miR-137 and miR-15 b can reverse the neurotoxicity induced by Aβ abnormal metabolism in animal and cell lines (He et al., 2017; Li and Wang, 2018). In the updated pilot study of Vergallo et al. (2021), the protective anti-Aβ effect of miR-15b is reported in asymptomatic at-risk population for AD, as there were significant associations between plasma concentrations of miR-15b, with core neuroimaging biomarkers of AD pathophysiology in the hippocampus (Vergallo et al., 2021).

Several miRNAs as miR-98 and miR-124 modulate Aβ production via notch signaling pathway. Specifically, miR-98 suppresses Amyloid accumulation as it inhibits *HEY2* protein levels which inactivates the notch signaling pathway responsible for Aβ production (Amakiri et al., 2019).

Furthermore, miRNAs that directly target *APP,* confirm the role of miRNAs in AD pathogenesis. Downregulated levels of miR-101 are reported in AD brain and are consistent with *in vitro* studies where inhibition of miR-101 increased APP levels (Siedlecki-Wullich et al., 2021). *In vitro* and *in vivo* studies also show that downregulation of miR-137 determines an increase in $Ca2^{+}$ levels and a reduction of Aβ1-40 and Aβ1-42. These results indicate that an increase in miR-137 could cause a decrease in $Ca2^{+}$ levels in neurons, improving neuronal dysfunctions of AD (Davare and Hell, 2003).

Another approach of AD Aβ pathology is the Aβ clearance from the brain to the circulation. Aβ clearance from the brain requires adequate balance of Aβ phagocytosis, glymphatic clearance and healthy system of ABC transporters *ABCB1* and *ABCG2*. Recently, miR-34a and miR-29b have been found to interfere with at least three pathways of Aβ clearance (Weldon Furr et al., 2019). In adult mammalian brain, miR-34a and miR-29b are highly expressed and have been implicated in a range of neurodevelopmental and neuropathological processes.

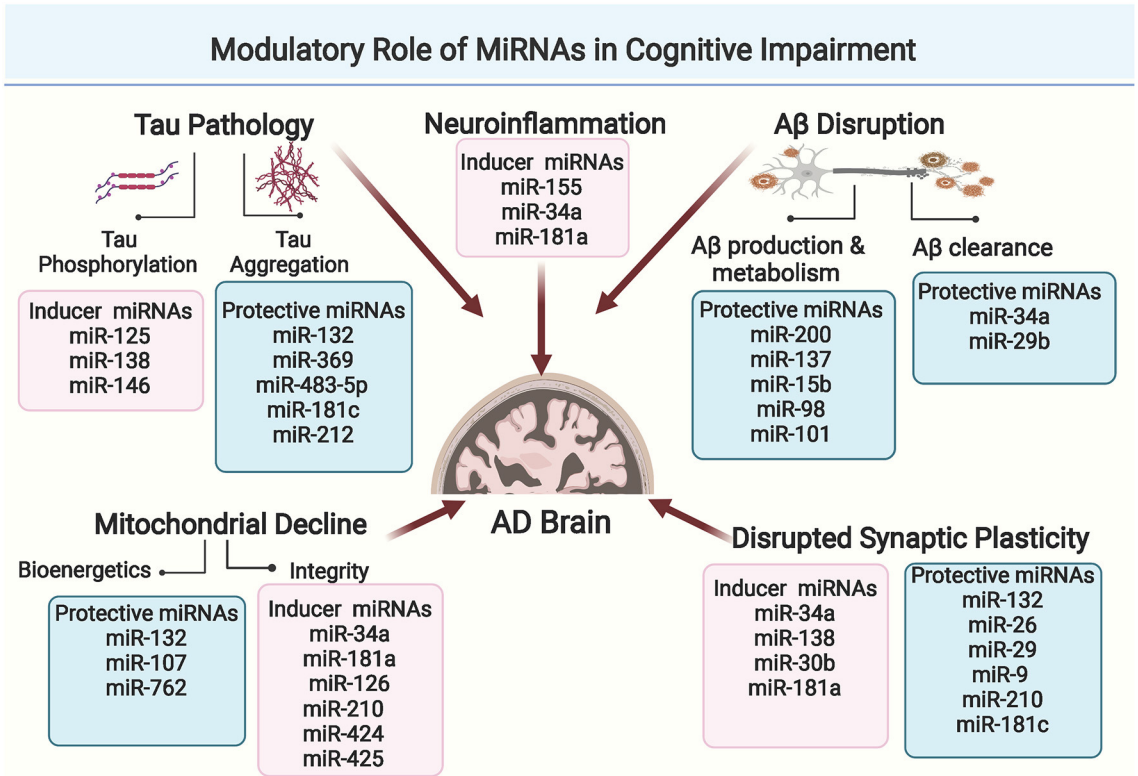

FIGURE 2 | Comprehensive illustration of the modulatory role of miRNAs in cognitive impairment in AD through affecting Aβ and Tau metabolism, mitochondrial functionality, neuroinflammation or synaptic plasticity. (Created with BioRender.com).

Both miR-34a and miR-29b are dysregulated in brain and serum samples of AD patients (Madadi et al., 2019).

Taken together, these cumulative studies show that AD disease can disrupt miRNA coordinated expression. Simultaneously, miRNA altered expression contributes to Progressive AD pathogenesis through disrupting key genes in Aβ pathway. Unleashing the mechanism of microRNA Aβ regulating pathways, can identify novel therapeutic targets for better AD management.

MicroRNAs and Tau Pathology in AD

In the last few years, abnormal phosphorylated Tau has proved to be detrimental in cognitive decline (Di et al., 2016). Moreover, extracellular soluble Tau oligomers have been recognized as a possible cause of memory loss and synaptic dysfunction (Biundo et al., 2018). Experimental AD studies helped to identify several miRNAs that are linked to Taupathy in AD. Among the most prominent miRNAs, miR-125b, and miR-138 are upregulated in AD and have been shown to induce Tau hyper phosphorylation and tangling in neuronal cultures. Subsequently their upregulation disrupts associative learning and cognition in AD mice models (Banzhaf-Strathmann et al., 2014; Wang X. et al., 2015). MiR-125 upregulation in AD promotes Tau hyperphosphorylation through activating Mitogen-activated protein kinase (*MAPK)* kinases, most likely by down-regulating its target phosphatases genes: *DUSP6* and *PPP1CA*. Whereas, direct hippocampal delivery of miR-125b mimic improved learning, memory and inhibited Tau phosphorylation and expression of DUSP6, and PPP1CA in C57BL/6 mice (Banzhaf-Strathmann et al., 2014). Other miRNAs are reported to modulate Tau affinity for microtubule, regulate the maintenance of microtubule network, and affect Tau aggregation/deposition in NFTs (Siedlecki-Wullich et al., 2021). MiR-22-3p affects Tau phosphorylation through regulating Sirtuin 1 *SIRT1* gene. Meanwhile, miR132-3p regulates Tau phosphorylation via *PTBP2* gene and Tau splicing via modulating *MeCP2* and *PTEN* genes (Praticò, 2020). In 3xTg mice, loss of miR-132 increased total and phosphorylated Tau levels and provoked Tau aggregation. Consistently, restoring miR-132 to normal levels improved Tau pathology and long-term memory (Smith et al., 2015). Similarly, miR146a-5p is reported to regulate Tau phosphorylation via *ROCK1* gene.

Clinically and in support of these translational experiments, miR-125, miR-138, miR-146 and miR- 132 are significantly dysregulated in the cerebrospinal fluid of AD patients (Galimberti et al., 2014; Lee et al., 2016; Wei et al., 2020). MiR-369 is one of the most recently studied miRNAs. Knocking out miR-369 in 3xTg AD mice aggravated cognitive impairment and promoted hyperphosphorylation of Tau, through upregulating kinases *Fyn* and serine/threonine-protein kinase 2 *(SRPK2)* as the upstream molecules. Meanwhile, Restoring miR-369 reversed the hyperphosphorylation of Tau and downregulated *Fyn* and

TABLE 1 | Disturbed miRNAs reported in human AD studies and their relation to Aβ pathways.

miRNA	Sample	Regulation	Relevant pathway	References
miR-15a miR-15 b	Serum	Downregulation	Downregulates BACE1 expression	Satoh et al., 2015; Lang et al., 2016; Li and Wang, 2018
miR-29a	Brain CSF Serum	Down Up Down	BACE1	Hébert et al., 2008; Geekiyanage et al., 2012; Müller et al., 2016
miR-29b	Blood	Down	BACE1	Satoh et al., 2015
miR-29c	Brain	Down	BACE1	Lei et al., 2015
miR-20b	Brain	Down	APP	Nunez-Iglesias et al., 2010
miR-21-5p	Brain	Up	APP	Giuliani et al., 2021
miR-16	CSF	Down	APP	Müller et al., 2014
miR-9	Brain	Down	APP	Hébert et al., 2008
miR-30c	Brain	Down	APP	Cogswell et al., 2008
miR-101a-3p	Brain	Down	APP	Hébert et al., 2008
miR-188	Brain, Serum	Down	APP	Kou et al., 2020
miR-106a	Brain Serum	Down Up	APP	Wang et al., 2011; Cheng et al., 2015
miR-106b	Brain Whole blood	Down Up	APP	Hébert et al., 2008; Cheng et al., 2015
miR-138-5p	Plasma Exosomes	Up	APP	Lugli et al., 2015
miR-138	Brain, CSF	Variant	APP	Boscher et al., 2019
miR-135b	Blood	Down	BACE1	Zhang et al., 2016
miR-124	CSF	Down	APP	Burgos et al., 2014
miR-155	Peripheral Blood mononuclear cells	Upregulated	Inhibits Aβ catabolism	Guedes et al., 2016
miR-34	Peripheral Blood mononuclear cells	Upregulated	Aβ clearance	Basavaraju and de Lencastre, 2016
miR-181b	Peripheral Blood mononuclear cells	Upregulated	Aβ clearance	Kumar and Reddy, 2016
miR-181c	Serum	Downregulated	APP	Geekiyanage et al., 2012
miR-186	Brain	Downregulated	BACE1	Ben Halima et al., 2016
miR-346	Human Neuron Enriched culture	Upregulated	APP	Long et al., 2019
miR-338	Hippocampus	Downregulated	APP	Qian et al., 2019
miR-200	Brain	Upregulated	SIRT1, Aβ accumulation, Apoptosis	Zhang et al., 2017

SRPK2, implying the possible therapeutic potential of miR-369 in AD (Yao et al., 2020).

Similarly, miR-483-5p is recently reported to regulate *ERK1* and *ERK2* kinases at both mRNA and protein levels, resulting in reduced phosphorylation of Tau protein associated with Tau neurofibrillary pathology in AD. Taking these observations together, suggests the neuroprotective action of miR-483-5p in AD pathology (Nagaraj et al., 2021).

Besides Tau phosphorylation, miRNAs have a key role in Tau clearance. Where some post-translational modifications of Tau inhibit Tau ubiquitin binding which promote Tau aggregation. Moreover, acetylation at specific sites of Tau provokes autophosphorylation, and aggregation. This acetylation process is dependent on the balance between acetyltransferase p300 (an acetylase) and sirtuin 1 (SIRT1, a deacetylase). Recent studies demonstrated that *SIRT1* gene could be directly inhibited by miR-9, miR-212, and miR-181c to imply their potential role in Tau regulation and consequent AD events (Zhao et al., 2017; Praticò, 2020).

MicroRNAs and Synaptic Plasticity in AD

Dendrites and dendritic spines are the loci of long-term synaptic plasticity that facilitates cognitive processes such as learning and memory. On the cellular level, synaptic plasticity is mediated by structural changes and functionality of dendritic spines. The dendritic spines have specialized subdomains that contain scaffolding proteins, signal transduction molecules, ion channels, and cytoskeleton components which collectively regulate spine morphology, synaptic transmission, and plasticity. Assembly and remodeling of neuronal circuit are generally affected by alterations in density and properties of ionic channels and proteins of the dendritic spines (Bosch and Hayashi, 2012; Reza-Zaldivar et al., 2020).

Growing evidence confirms that alterations of spine morphology and dendritic spine (DS) loss are correlated with AD cognitive decline even before neuronal loss (Dorostkar et al., 2015). Furthermore, the characteristic Aβ and Tau pathologies in AD, suppress synaptic plasticity, which simultaneously provokes changes in dendritic morphology, synaptic maturation

and synaptic loss (Pereira et al., 2021). Moreover, updated postmortem examination of AD brains shows that cognitive impairment correlates with synaptic loss better than the number of extracellular plaques or NFTs in AD, making synaptic failure a hallmark of AD (Kumar and Reddy, 2020).

Interestingly, microRNAs are now established as principal regulators of synaptic plasticity during neuronal circuit formation and integration. Moreover, changes in neuronal microRNA expression contribute to synaptic function modification via modulating dendritic spine morphology and/or regulating local protein translation to synaptic transmission. These mechanisms are proved determinant for both synapse formation and synaptic plasticity (Reza-Zaldivar et al., 2020). **Figure 3** spots the prominent regulatory role of miRNAs in synaptic plasticity.

Multiple studies reported miRNAs that contribute to impaired synaptic plasticity in AD. **Table 2** illustrates an updated summary of the miRNAs involved in regulating target genes of synaptic functions, morphology and dendritic spine alterations in AD studies. Among the interesting miRNAs, miR-34a overexpression is found to impact synaptic functionality and cognitive decline in AD mice models (Sarkar et al., 2019). On the molecular level, miR-34a targets *SIRT1*, *CREB* and *BDNF* genes that have multiple roles in AD progression via increasing Tau Phosphorylation, altering spine morphology and spine functions. Loss of miR-101 in hippocampal neurons was found to cause cognitive decline and modulation of AD-related genes in mice. Where miR-101 knockdown in the hippocampus of C57BL/6 J mice showed AMPK hyperphosphorylation, upregulation of miR-101 target genes associated with AD such as *APP*, and *Rab5* and overproduction of Aβ42 levels (Barbato et al., 2020).

Similarly, overexpression of hippocampus miR-30b disrupts basal synaptic transmission, and reduces DS density, eventually leading to declined learning and memory (Song et al., 2019). This is accomplished as miR-30b modifies SIRT1 expression which regulates Tau phosphorylation. Interestingly miR-30b also targets *EphB2* that has a protective role against Aβ oligomers accumulation and disruption of glutamate receptors that are directly linked to synaptic plasticity and cognitive processes (Cissé et al., 2011). Another crucial target of miR-30b is the *GRIA2* gene, the predominant excitatory neurotransmitter receptors in mammalian brain (Siedlecki-Wullich et al., 2021), and an increasingly reported linker between Aβ clearance and synaptic disruptions in AD models (Hettinger et al., 2018).

On the clinical side, miR-138 is highly expressed in the dendrites of hippocampal neurons and it acts to regulate dendritic spine size and structure. Functional screening demonstrates that Acyl Protein Thioesterase1 (APT1)-induced palmitoylation of G protein α13 (*Gα13*) is important for the regulatory function of miR-138 during dendritic spine development. Whereas, high level of miR-138 significantly reduces APT1 level which leads to dendritic spine shrinkage and concomitant reduction in synaptic transmission (Siegel et al., 2009). Recently a panel of studies focusing on miR-138 function during the process of learning and memory showed a close association with local plasticity-related protein synthesis. Examination of human postmortem brain tissue showed expression of miR-138 and decapping mRNA 1B *(DCP1B)* in hippocampus and frontal cortex. Furthermore, it was found that a human memory-associated single nucleotide polymorphism could interfere with miR-138 binding to the transcripts of DCP1B, implying that miR-138 is a strong modulator of human memory performance (Ye et al., 2016).

Contrarily, some miRNAs have protective effects against synaptic disruptions in AD. For instance, miR-132 inhibits extra synaptic gene Matrix metallopeptidase 9 *(MMP-9)*, whose overexpression promotes formation of immature DS. Consequently, MMP-9 inhibition by miR-132 supports DS head widening that potentiates synaptic plasticity (Jasińska et al., 2016). In line, a recent study showed that upregulating miR-132 by an enriched environment, enhanced hippocampal synaptic plasticity and prevented DS impairments induced by Aβ oligomers (Wei et al., 2020). Furthermore, miR-26a and miR-384-5p have been found as significant regulators of dendritic spine growth and targeting endogenous ribosomal S6 kinase 3 (*RSK3*). Inhibition of miR-26a is reported to attenuate neurite outgrowth and neuronal morphogenesis (Gu et al., 2015).

Collectively, these data spot the significant role of miRNAs as key players in synaptic plasticity and the impact of miRNAs dysregulations on synaptic homeostasis and functionality in AD pathology. Getting deeper understanding of miRNAs and their targets concerning synaptic modeling in AD may provide new approach to earlier diagnosis and therapeutic management of AD cognitive pathology.

MicroRNAs and Glia Cells Role in AD-Cognitive Impairment

Microglia are brain-resident myeloid cells that mediate innate immune responses in the CNS. Under normal conditions, microglia exist in a "resting" state where they "monitor" the surrounding microenvironment and maintain brain homeostasis via synapse organization, removal of debris by phagocytosis and release of neurotrophic factors (Fan and Pang, 2017). Meanwhile, activation of microglia is accompanied by morphological changes that permit motility and phagocytosis. Microglia can differentiate into either M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotypes depending on the provoking signals. M2 microglia release anti-inflammatory and protective cytokines such as IL-10, TGF-β, IL-4, and IL-13, which promote repair (Guedes et al., 2013). Whereas, M1 microglia release inflammatory mediators such as ROS, MMP-9 and pro-inflammatory cytokines such as TNFα, IL-6 and IL-1β.The balance between these different microglial phenotypic states promotes inflammation or tissue repair and influences the progression of neuroinflammatory disorders (Guedes et al., 2014).

Currently, "Microglia" is an increasing hot topic in AD research, and cumulative studies spot the multifaceted role of microglia as beneficial or detrimental in AD. Recent genome-wide association studies confirm that most of AD risk loci are present in or close to genes that are highly and/or uniquely expressed in microglia. This strongly implies the significant involvement of microglia in early steps of AD (Hemonnot et al., 2019). Among the well-known genes are *Cd33* and *TREM2*

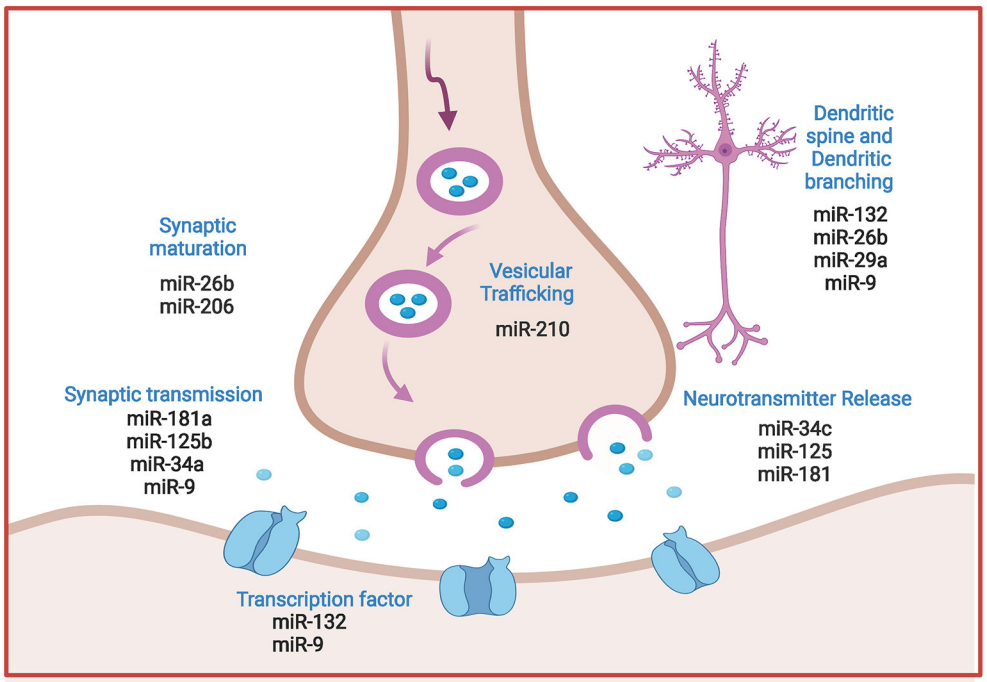

FIGURE 3 | Comprehensive illustration of the regulatory role of prominent miRNAs in synaptic plasticity in AD. MicroRNAs regulate vesicle formation and trafficking, ACH release and neurotransmission, post synaptic transcription processes and dendrites morphology. (Created with BioRender.com).

which are linked to Aβ phagocytosis and regulation of microglial inflammatory interaction with Tau tangles (Onuska, 2020).

Additionally, the CX3CR1 receptor is predominantly expressed in microglia. Its ligand CX3CL1 is constitutively expressed by neurons, and it helps maintaining microglia in a resting state. CX3CL1-CX3CR1 is a critical signaling pathway that is disrupted in neurodegenerative conditions and is associated with a strong microglial toxicity (Keren-Shaul et al., 2017). The involvement of the CX3CL1/CX3CR1 signaling pathway in AD is confirmed by an elevated plasma concentration of CX3CL1 in AD patients compared to healthy control subjects.

Other interesting contributors are the complement proteins C1q and CR3 (Veerhuis et al., 2011) which are highly produced in the microglia as crucial factors in synapse pruning and are highly present in CSF of AD patients with emerging evidence about their role in Aβ pathogenesis (Fatoba et al., 2021). Furthermore, Numerous studies demonstrate that microglial Aβ phagocytosis contributes to degeneration by triggering NLR family pyrin domain containing 3 receptor *(NLRP3)* and lysosomal cathepsin-B that subsequently releases IL-1β and disrupts autophagosome degradation.

Growing body of evidence shows that miRNAs dysregulation impacts microglial hyper-activation, neuroinflammation, and alters macrophage polarization in the brain. Mechanisms that are closely implicated in AD pathology. **Table 3** illustrates updated data about miRNAs that regulate key genes in the microglia.

Concerning AD studies, miR-34a is reported as a major target of *TREM2*, a microglial receptor that mediates Aβ42 clearance via phagocytosis in the CNS. Simultaneously, multiple clinical reports showed miR-34a level is dysregulated in AD patients (Bhattacharjee et al., 2016).

In line, both miR-155 and miR-146a are upregulated in the CNS during AD and both regulate the excessive inflammatory signaling observed in AD disease course (Su et al., 2016). MiR-155 is now established as a crucial pro-inflammatory factor in microglia, as it represses suppressor of cytokine signaling 1 gene (*SOCS-1*). Increased miR-155 expression was recently reported in 3xTg AD mice brains (Guedes et al., 2014), together with enhanced microglial activation. Meanwhile, knockdown of miR-155, induced *SOCS-1* expression and led to downregulation of iNOS and nitric oxide production.

MiR-146 is another multi-faceted miRNA that is implicated in the pathogenesis of AD (Jayadev et al., 2013). In microglia, miR-146 is reported to target Presenilin 2 (*PS2*), a membrane associated protease that regulates proinflammatory microglial behavior (Wang and Wang, 2018).

Astrocytes are another class of glial cells that affect inflammatory response in the CNS. In healthy conditions, astrocytes regulate neuronal metabolism, synaptogenesis, intracellular calcium levels and interact with neuronal signaling (Vasile et al., 2017). Under pathological conditions, astrocytes participate in shaping the CNS response to stress and disease. Where neuroinflammation can be either promoted or restricted by astrocytes through release of pro-inflammatory or anti-inflammatory molecules, leukocyte recruitment and forming functional barriers for CNS parenchyma (Sofroniew, 2015).

In the brain of AD patients, the inflammatory response aggravates astrocytes number, volume and activity

TABLE 2 | MicroRNAs involved in modulating key target genes of synaptic plasticity in AD.

miRNA	Target gene	Function	References
miR-34a	*GRIN2B* *Syt-1* *Stx-1A*	Synaptic transmission reduced dendritic trees and alteration of DS morphology and function	Agostini et al., 2011; Xu et al., 2018; Sarkar et al., 2019
miR-210	*NPTX1 AMPAR*	Recruitment/clustering, synaptic transmission	Pulkkinen et al., 2008
miR-125b	*GRIN2A* *EPHA4*	Synaptic transmission Formation of long and narrow DS filopodia-like, with a subsequently synaptic transmission weakening	Alsharafi et al., 2016 Edbauer et al., 2010
miR-181a	*GRIA2*	Synaptic transmission	Rodriguez-Ortiz et al., 2020
miRNA-574	*Nrn1*	DS stabilization leading to cognitive impairment	Li F. et al., 2015
miRNA-29a/b	*Arpc3*	Reducing the mushroom-shaped DS formation, and DS head enlargement, a fundamental step in synaptic maturation	Lippi et al., 2011
miRNA-135	*CPLX1 CPLX2*	Impairment of the postsynaptic exocytosis of AMPA receptors leading to DS shrinkage	Hu et al., 2014
miRNA-124	*CREB*	Serotonin-induced long-term synaptic plasticity	Rajasethupathy et al., 2009
miR-128	*STIM2*	Negative modulator of intracellular calcium	Deng et al., 2020
miR-92, miR-137, miR-501	*GRIA1*	Synaptic transmission	Letellier et al., 2014; Hu et al., 2015; Olde Loohuis et al., 2015
miR-9	*REST*	Synaptogenesis, synaptic plasticity, and structural Remodeling	Giusti et al., 2014
miR-132, miR-134	*CREB1* *MMP-9* *BDNF*	Transcription factors involved in synaptic plasticity Alterations in DS morphology and maturation	Mellios et al., 2011; Jasińska et al., 2016
miRNA-206	*BDNF*	Decreasing DS density	Lee et al., 2012
miRNA-218	*GRIA2*	Increasing the amplitude of synaptic currents and formation of thin DS	Rocchi et al., 2019
miRNA-30b	*EphB2*, *SIRT1*, *GRIA2*	Reduced basal synaptic transmission, impaired spatial learning and memory retention and DS density reduction	Song et al., 2019

TABLE 3 | MicroRNAs and their key target genes in the microglia.

miRNA	Target Gene	Gene function	References
miR-124	*C/EBP-α*	Microglial Polarization to M2	Ponomarev et al., 2011
miR-155	*C-MAf, CCM1, NOX2, NOX4*	Microglial polarization to M2, microglial activation and increased count	Guo et al., 2019
miR-9	*NF-kB*	Microglial activation	Yao et al., 2014
miR-204	*SIRT1*	Repression of microglial activation	Li L. et al., 2015
miR-125b	*NF-kB*	Contributing in increased TNF-α release	Parisi et al., 2013
miR-34a	*TREM2*	Aβ42 aggregation and accumulation and microglial activation	Alexandrov et al., 2013

(Meraz-Rios et al., 2013). Interestingly, IL-1β, IL-6, and transforming growth factor-β (TGF-β) are upregulated before Aβ aggregation and Tau hyperphosphorylation. These Inflammatory factors activate astrocytes to over express BACE1 enzyme and produce excessive amounts of Aβ proteins (Blasko et al., 2000). In turn accumulated Aβ provokes astrocytes to release more cytokines as TNF-α, a crucial factor in AD-related cognitive impairment (Veeraraghavalu et al., 2014). Additionally, astrocyte dysfunction leads to a decrease in Aβ uptake and clearance (Rolyan et al., 2011). Moreover, astrocytes have been recently reported to promote Tau lesions and accelerate NFTs formation (Birch et al., 2014; Yang et al., 2019).

Increasing reports spot the disruption of miRNA expression in astrocytes during neuroinflammation and neurodegenerative processes accompanying AD. Among the characteristic miRNAs is miR-146 a. Cui et al. (2010) found that miR-146a was upregulated in human astrocytes when exposed to Aβ. The study found that miR-146a mediated down-regulation of interleukin-1 receptor-associated kinase-1 (IRAK-1). IRAK-I is coupled to NF-κB extensive sustained inflammatory response of astrocytes and downstream Toll like receptors proteins. Therefore, NF-κB inhibitors and miR-146a can present a treatment strategy against excessive immune response to Aβ in the brain (Cui et al., 2010).

Glutamate release, reuptake, and recycling are tightly regulated by astrocytes at tripartite synapses. Glutamate overload can trigger neuronal and synaptic loss (Marttinen et al., 2018). Glial glutamate transporter 1 (GLT-1) contributes to clearance and regulation of glutamate at synaptic clefts. In a 3xTg-

AD mouse model, increased levels of miR-181a downregulated synaptic proteins related to GLT-1, impacting the plasticity of glutamatergic synapses in astrocytes, and implying its key mediating role in synapses plasticity (Zumkehr et al., 2015). Generally, miR-181 family regulates neuroinflammatory signaling in astrocytes, and miR-181 family has been reported to be upregulated in AD mouse model, causing impaired synaptic plasticity through targeting *SIRT-1*.

MiR-155 is another significant astrocyte modulator, as it affects astrocytes density during inflammation. Furthermore, increased astrocytes levels of miR-155 were shown to target *SOCS1*, a negative regulator of the inflammatory gene response, in Aβ-treated astrocytes, causing prolonged expression of inflammatory cytokines (Guedes et al., 2014).

MicroRNAs and Mitochondrial Damage

A large body of research shows enormous mitochondria alterations in the brains of AD patients. Interestingly, mitochondrial alterations have been consistently observed before the clinical onset of AD (Swerdlow, 2018). As a result, mitochondrial dysfunction is now a hot topic in AD for its possible role in the earlier progression of the disease.

One important feature of mitochondrial alterations in AD is the impaired mitochondrial bioenergetic machinery. Glucose hypometabolism in AD brain is strongly linked to impaired oxidative phosphorylation. Moreover, it is closely correlated to impaired levels of blood thiamine diphosphate (TDP), a crucial coenzyme of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (KGDHC) enzymes allocated in the Krebs cycle (Sang et al., 2018).

Furthermore, Redox proteomics studies found that many antioxidant enzymes that are allocated in the mitochondria, including glutathione-S-transferase Mu, peroxiredoxin 6, GSH and ATP synthase are oxidized in AD, which might compromise their functions by increasing oxidative stress conditions that prevail in AD affected brain regions (Swomley and Allan Butterfield, 2015). Interestingly, levels of oxidized nucleic acids in mtDNA are reported to be significantly elevated in preclinical AD, again stressing mitochondrial abnormalities as an early event of AD progression (Wang W. et al., 2020). Additionally, emerging studies demonstrated impaired base-excision repair (BER) activity in both AD and MCI patients (Lillenes et al., 2016), suggesting significant contribution of replication error to increased mtDNA mutations in AD.

Only recently, a subset of microRNAs is found to be localized to human mitochondria (mitomiRs) and while mitomiRs functions are still far from being completely explored, recent findings relate mitomiRs to neurodegenerative diseases, including Alzheimer's. MiR-107 is one of the recently discovered mitomirs. It regulates oxidative abilities of mitochondria and downregulated miR-107 was found to decrease mitochondrial volume, cristae and mitochondrial membrane potential. Interestingly, decreased plasma levels of miR-107 correlated with abnormal cortical anatomy, common to AD patients, while injecting miR-107 mimic reversed spatial memory impairment, decreased phosphorylated Tau levels, and Aβ neurotoxicity (Shu et al., 2018; John et al., 2020).

MitomiR-34a was recently found to affect mitochondrial metabolism contributing to declining spatial memory (Sarkar et al., 2016). In support to the significant potential of mitomiRs in AD, miR-181a is recently reported to affect mitochondrial glucose metabolism and increase mitochondrial dysfunction, while clinically it is upregulated in MCI patients' plasma and has been reported as a promising early diagnostic marker to predict progression to AD (Ansari et al., 2019).

Table 4 illustrates updates about the recently discovered mitomiRs and their potential role in AD progression.

AD, SLEEP DISORDERS AND CIRCADIAN RHYTHM

Sleep: The Complex Process of Circadian Rhythm in Action

Circadian rhythms are 24 h cycles that maintain homeostasis in different body tissues. Circadian rhythm is controlled by the suprachiasmatic nucleus (SCN) which is found in the hypothalamus, and it synchronizes multiple functions as sleep/wake cycle, metabolism, thermoregulation, and hormonal regulation. This molecular clockwork involves genetically encoded autoregulatory feedback loops that provide a 24-h period of circadian oscillation (Park et al., 2020).

The core loop of this molecular clock is driven by a heterodimeric transcriptional activator that is composed of two clock genes: circadian locomotor output cycle kaput *(CLOCK)* and brain–muscle–arnt-like protein 1 *(BMAL1)*. These heterodimers accelerate E-box-mediated transcription and increase gene expression of negative regulators; [Periods *(PERs: PER1, PER2, and PER3)* and Cryptochromes *(CRYs: CRY1 and CRY2)*] and circadian output genes. Expressed and dimerized *PER: CRY* represses the transcriptional activity of CLOCK:BMAL1, hence, downregulate their own gene transcription. It takes 24 h to complete such loop cycle and the accurate generation of 24 h cycles is regulated by post-translational modifications that includes phosphorylation, ubiquitination, and acetylation. Majorly, the phosphorylation of *PER* proteins by casein kinase Iε *(CKI ε)* and glycogen synthase kinase-3β *(GSK-3 β)* promotes *PER* nuclear translocation, thereby provide proper completion of the cycle (Eide et al., 2005).

The SCN also contains gamma-aminobutyric acid (GABA) and arginine vasopressin (AVP) neurons that send inhibitory signals to the paraventricular nucleus of the hypothalamus (Reghunandanan and Reghunandanan, 2006). This activates melatonin secretion by the pineal gland and when it binds to MT1 and MT2 receptors, it inhibits the firing of the SCN. Hence, melatonin promotes sleep and resets the circadian pacemaker (Aulinas, 2000).

AD and Sleep Disturbances

Circadian rhythms that regulate sleep gradually weaken with aging causing disturbances in sleep quality and cognitive alterations. However, circadian rhythms are markedly disturbed in AD all through the disease course. Day-time agitation, night insomnia, restlessness and sun-downing are among the

TABLE 4 | Mitochondrial miRNAs in relation to mitochondrial dysfunctions.

miRNA	Role	Altered level	References
miR-34a	Allocated in the mitochondria and Promotes apoptosis and dysfunction of mitochondria	Upregulated	Sarkar et al., 2019
miR-107	Decreased mitochondrial functions and morphological changes	Downregulated	Rech et al., 2019
miR-126	Reduces aerobic respiration	Upregulated	Tomasetti et al., 2014
miR 23a/23b	Affects mitochondrial biogenesis	Downregulated	Tang, 2016
miR-132	Reduces aerobic respiration	Downregulated	Weinberg et al., 2015
miR-181a	Localized in mitochondria. Promotes apoptosis and dysfunction of mitochondria	Upregulated	Giuliani et al., 2018
miR-762	Reduces ATP production	Downregulated	Yan et al., 2019
miR-210	Clinical marker for MCI and AD Targets mitochondrial iron sulfur cluster homolog	Upregulated	Siedlecki-Wullich et al., 2019
miR-424	Suppression of ATP levels and mitochondrial integrity	Upregulated	Duarte et al., 2014
miR-425	Mitochondrial dysfunction and increased ROS production	Upregulated	Hu Y.-B. et al., 2019

characteristic changes observed in AD and they worsen with AD progression to affect around 42–52% of AD patients. Such sleep changes are majorly attributed to disruptions in the precise cascade of circadian rhythm (Todd, 2020).

AD is often associated with changes in the physiological parameters of sleep that include decrease in total sleep time and efficiency, prolonged sleep time stage 1 and stage 2 sleep, lesser time in deeper sleep, increased REM sleep latency and decreased REM sleep, together with decreased density of eye movement activity (Weldemichael and Grossberg, 2010).

Another major disruption of AD-sleep disturbance is the "Sundowning." This condition refers to a delirium-like status usually occurring at late-afternoon and till dawn. Behavioral components of sundowning can include loud vocalization, wandering, physical aggression, maladaptive physical behaviors, and overall agitation. The prevalence of sundowning in AD ranges from 12 to 25%. The increased frequency for AD agitation at night imply that these chronobiologic changes are affected by disruptions in "the timing" of physiological events (Weldemichael and Grossberg, 2010).

Causes and Deeper Look to miRNAs Role

The main reason for the sleep-wake disrupted cycle in AD is related to alterations in the suprachiasmatic nucleus (SCN) and melatonin secretion. Moreover, expression of MT1 receptors is decreased in the SCN of AD patients resulting in reduced melatonin production and disappearance of melatonin rhythm (Todd, 2020). Till date, the molecular mechanisms underlying these disturbances are still not fully resolved which leave a tight margin for effective therapeutic intervention. Interestingly, collected data from both human and animal studies show that sleep disturbances are not only a consequence of AD progression, but may also precede AD symptoms onset and may contribute to AD pathology through affecting Tau and Aβ deposition and clearance from the brain (Musiek et al., 2015). However, despite the high frequency of sleep disturbances during AD course, there is an obvious lack of data that specifically discusses the molecular basis of AD-sleep disorders, apart from other AD-disruptions.

In a recent study, deletion of the master gene BMAL1 abrogated all circadian functions, leading to complete loss of day-night rhythmicity of sleep (Musiek et al., 2015). Simultaneously, sleep deprivation was found to change the expression of clock genes and BMAL1/CLOCK heterodimers binding, thus altering clock function.

Growing evidence now confirm that mature miRNAs are crucial for the fine-tuning of circadian rhythm regulations that also include sleep (**Table 5** enlists some of the miRNAs reported to regulate Circadian Rhythm in different tissues).

Moreover, although differential expression of multiple miRNA panels has been detected in AD, it is of high interest that consensus miRNAs that regulate key genes in AD pathogenesis, are also involved in sleep-circadian disorders. For example, miR-219 is reported to be overexpressed in postmortem brain tissues of AD patients and interestingly, miR-219 regulates Tau phosphorylation and targets GSK-3 which is vital for phosphorylating PER genes (Kinoshita et al., 2020). Moreover, miR-219 modulates CLOCK-BMAL1 complex. Similarly, miR-132 modulates GSK-3 and Tau Phosphorylation and it is downregulated in AD neurons (El Fatimy et al., 2018).

MiR-125a, miR-125b, miR-146a have high diagnostic potential to predict AD progression, where they modulate Tau hyperphosphorylation (Nagaraj et al., 2019), inflammatory responses and autophagy in microglia and astrocytes, while regulating PER genes in the circadian clock (Lee et al., 2016; Liang et al., 2021). MiR-125 also regulates the cholinergic functions via modulating CLOCK gene. Meanwhile miR-146a is associated with short sleep (Davis et al., 2007; Karabulut et al., 2019) and shows rhythmic expression. It is worth mentioning that miR-146a shows remarkable potential as a diagnostic AD biomarker (Siedlecki-Wullich et al., 2019). Differential expression of miR-34a has also been detected in the brains and blood of AD patients, where it regulates genes involved in memory formation, amyloid precursor protein metabolism and Tau phosphorylation (Sarkar et al., 2016). Intersecting with these functions, miR-34a also regulates PER1 and PER2 genes. MiR-29 family members are established as potential indicators of

TABLE 5 | MicroRNAs regulating core clock components.

miRNA	Affected clock component	References
miR-449a	*PER* gene	Hansen et al., 2011
miR-103		Chen et al., 2019
miR-125a-3p		Ma et al., 2020
miR-192		Garufi et al., 2016
miR-194		Nagel et al., 2009
miR-24		Oyama et al., 2017
miR-29a/b/c		
miR-30a		
miR-34a		Ma et al., 2020
miR-211	*Ebox, NPAS2, CLOCK* Genes	
miR-107		
miR-124		
miR-141		Na et al., 2009
miR-17-5p		Gao et al., 2016
miR-182-5p		Ma et al., 2020
miR-19b		Na et al., 2009
miR-199b-5p		Yuan et al., 2020
miR-206		Garufi et al., 2016
miR-10	BMAL1 Gene	Zheng et al., 2021
miR-135b		Zheng et al., 2021
miR-142-3p		Tan et al., 2012; Shende et al., 2013
miR-155		Shende et al., 2011
miR-211		Ma et al., 2020
miR-27b-3p		
miR-494		Shende et al., 2011
miR-106b	*CRY* Genes	Zheng et al., 2018
miR-181a,d		Na et al., 2009
miR-185		Lee et al., 2013
miR-340		Zheng et al., 2018
miR-219	*ROR* output genes	Ma et al., 2020
miR-142-3p		Shende et al., 2013
miR-125a-3p	*(CKI ε) and (GSK-3 β)*	Zheng et al., 2018
miR-181a	*RORα Genes*	Zheng et al., 2018
miR-27a-3p		Zheng et al., 2018
miR183-5p		Dambal et al., 2015
miR-450-5p		Zheng et al., 2018
miR-19b		Linnstaedt et al., 2020
miR-503-5p		Zheng et al., 2018
miR-126a-3p	*E4BP4, DBP Genes*	Cheng et al., 2007

AD status. In line, miR-29a, b, c are also involved in regulating PER genes, β-secretase (BACE1) mRNA and Aβ accumulation (Müller et al., 2016). Similarly, miR-107 is down regulated in the temporal cortex and plasma of AD patients and it targets both *CLOCK* gene and *BACE1* expression.

MiR-155 is among the most well-studied microRNAs in AD-neuroinflammation. The high expression level of miR-155 in 3xTg AD animal model is accompanied with hyperactivation of microglia and astrocytes, to trigger inflammatory mediators. Moreover, miR-155 contributes to AD through activating different T cell functions during inflammation. Clinically, miR-155 is upregulated in human AD brains and it aggravates neuroinflammation (Kou et al., 2020). Similarly, miR-181 family impaired levels are also repeatedly reported in AD and are linked to accumulated plaque formation in the temporal complex and regulation of inflammation cytokine TNF-α and IL-6 (Kou et al., 2020). Both miR-155 and miR-181 have been linked to sleep disorders in AD as well as other neurodegenerative disorders (Slota and Booth, 2019).

Despite the scarcity of studies exploring miRNAs in AD- associated sleep disorders, the previously mentioned data strongly suggests the association between miRNAs dysregulations and sleep disorders together with other AD molecular disturbances. Clarifying this interconnected network with more studies, might unravel the molecular basis of sleep disturbances in AD and provide novel approaches for better and earlier management of AD.

PAIN: THE UNDERESTIMATED COMPANION IN ALZHEIMER'S DISEASE?

Alzheimer's is often co-morbid with chronic pain, where chronic pain prevalence is around 45.8% in AD (van Kooten et al., 2016). Pain that results from damage of body tissues is classified as nociceptive pain, while pain resulting from direct consequence of a lesion or disease that affects the somatosensory system" is classified as neuropathic pain (NP) (Merskey et al., 1994). Over the course of AD, AD patients can experience both nociceptive and neuropathic pain. And while, peripheral neuropathic lesions do not always cause chronic pain, central neuropathic pain is often chronic (Husebo et al., 2016).

Due to their inability to communicate and express their needs, pain feeling is often overlooked in AD patients. However, pain is more prevalent with severe dementia (Rajkumar et al., 2017), and its intensity is positively correlated with dementia severity (Whitlock et al., 2017). Impaired pain perception that is mediated by central nervous system is crucial in chronic pain. Simultaneously, central sensitization of pain processing pathways impacts cognition and emotional processing. These pathological interactions imply the presence of deeper link between chronic pain and AD progressiveness.

The "locus coeruleus" (LC) modulates pain and is a key player in chronic pain processing (Taylor and Westlund, 2017). It also innervates most brain areas and is the principal site of norepinephrine (NE) synthesis and neurotransmission in the CNS. The mechanistic processing underlying chronic pain is a complex issue that still needs to be resolved, nevertheless when experienced with AD.

Increasing evidence now shows that chronic pain and AD share disrupted function and structure of the LC. Moreover, both human and animal reports show that chronic pain induces microglial activation and neuroinflammation in hippocampus, anterior cingulate cortex, amygdala, nucleus accumbens, thalamus, and sensory cortex (Cao et al., 2019). This is associated with elevated release of inflammatory cytokines; TNF-α, IL-6, IL-1β that trigger disruptions in

synaptic remodeling, brain connectivity and network function. Interestingly, microglial activation and neuroinflammation are found to precede cognitive decline in AD patients, implying its participation in aggravating AD disease (Fan et al., 2017). The chronic activation of microglia induces synaptic loss, and this occurs in both AD and chronic pain. Moreover, the prolonged exposure to amyloid depositions, has also been found to activate microglia, resulting in excessive secretion of synaptic-toxic cytokines, and tau accumulation that eventually cause synaptic loss and neuronal death (Cao et al., 2019). Besides microglial activation, disrupted autophagy process has been increasingly reported as a significant contributor to both chronic pain and AD (Yin et al., 2018).

It is established that miRNAs are key players in modulating macromolecular complexes in neurons, glia, and immune cells. They regulate signals interconnecting neuro-immune network in the pain pathway and are crucial modulators of inflammation and autophagy pathways; two major factors in AD progression as well as chronic pain (López-González et al., 2017; Bernaus et al., 2020). As a result, miRNAs are now considered as significant "master switches" in chronic pain and AD. **Figure 4** highlights the overlapping role of some miRNAs in AD and pain.

For instance, miR-132 participates in regulating inflammation and is a negative regulator of the inflammatory response in PC12 cells. IL-1β, IL-6, TNF-α and TNF receptor associated factor 6 *(TRAF6)* are potential targets of miR-132 (Kou et al., 2020). Recently upregulation of miR-132 in WBCs of patients were associated with chronic neuropathic pain (Leinders et al., 2016). Interestingly, miR-132 is significantly reduced in the brains of AD patients and deletion of miR-132 in mice hastened Aβ accumulation, and tau pathology via modulating the synaptic proteins (Xu N. et al., 2019). In line, the down regulation of miR-132 was found to inhibit the level of hippocampal acetylcholinesterase *(AChE)*, impacting both cognitive function and synaptic plasticity. The downstream molecules responsible for miR-132 actions involve both p250 GTPase Activating Protein *(p250GAP)* and Methyl CpG-Binding Protein 2 *(MeCP2)* (Ye et al., 2016).

MiR-155 is one of the most prominent miRNAs that are differentially expressed in AD serum and brain. Recent reports confirm that miR-155 has significant impact on development of pain and pain hypersensitivity, where up-regulation of miR-155 is accompanied by enhanced activation of microglia and consequent production of inflammatory mediators (Kou et al., 2020). Moreover, miR-155 is a significant regulator of neuropathic pain via targeting serum and glucocorticoid regulated protein kinase 3 *(SGK3)*, an important protein involved in phosphorylation cascades (Liu et al., 2015).

Similarly, Let-7 is critical for maintaining microglial function in inflammation-mediated injury (Roush and Slack, 2008). Let-7a inhibits the expression of inflammatory cytokines via activation of apoptosis signal-regulating kinase 1 (ASK1), IL-10 and *Mycs* in microglia (Song and Lee, 2015). Meanwhile, let-7 miRNAs are found to be differentially and specifically released in CSF of AD patients (Derkow et al., 2018). MiR-124 is another brain-enriched miRNA involved in the regulation of neural development. Increasing reports spot its role as a remarkable alleviator of neuropathic pain, via inhibiting (IL-6, IL-1β, and TNF-α) protein expressions and direct targeting of Enhancer of zeste homolog 2 *EZH2* gene (Zhang et al., 2019). Moreover, miR-124 regulates BACE1 enzyme and is found to decrease gradually with AD progression (An et al., 2017). Most importantly, updated studies are now spotting miR-124 as a therapeutic target for its role in modulating inflammation in the central nervous system and brain injuries (Xu et al., 2021).

MiR-21 is another interesting miRNA regulator of pain that is increasingly reported as a significant contributor against AD progressive events. MiR-21 can inhibit cell apoptosis induced by $A\beta_{1-42}$ via modulating cell death protein 4 *(PDCD4)*/phosphatidylinositol 3-kinase PI3K/AKT/GSK-3β pathway in the CNS (Feng et al., 2018). Meanwhile circulating miR-21-5p was significantly upregulated in the plasma of AD patients and was negatively correlated to cognitive impairment (Giuliani et al., 2021). Simultaneously, disturbed level of miR-21 was observed in diverse neuropathic pain models (Zhong et al., 2019) where it targets metalloproteinase-3 *(TIMP3)* and chemokines C-C motif ligand 1 *(CCL1)* that consequently evoke cytokine production of TNF-α, IL-1β, and IL-6 and aggravate neuroinflammation.

MiR-146a-5p is reported to modulate immune response and reduce inflammation by targeting both *TRAF6* and interleukin-1 receptor-associated kinase 1 *(IRAK1) IRAK1* in macrophages, and astrocytes. Lu et al. (2015) reported the protective effect of miR-146a against SNL-induced neuropathic pain by suppressing *TRAF6* signaling in the spinal cord (Lu et al., 2015). Meanwhile, multiple studies linked miR-146a-5p with cognitive deterioration, where its upregulation in the CNS is associated with increased expression of Aβ, Taup38, and Reactive oxygen species (ROS) through targeting *MAPK* signaling (Alexandrov et al., 2014).

One of the emerging and promising miRNAs in both AD pathology and chronic pain is miR-218. Recently miR-218 upregulation was found to contribute to AD progression by enhancing Tau phosphorylation and disrupting mitochondrial respiratory chain through modulating *Wnt* signaling pathway (Gugliandolo et al., 2020; Wu et al., 2020). Meanwhile, downregulation of miR-218 was proved effective in suppressing central neuropathic pain via regulating cytokine signaling (Li and Zhao, 2016).

Elucidating the regulatory role of miRNAs on pain sensitization, neuropathic pain and the interplay with cognition and behavior alterations in AD, can unleash new resolutions on the pathophysiology of chronic pain in AD. Considering the common role of miRNAs in regulating chronic pain and its possible contribution in worsening cognitive impairment, miRNAs might also provide a prognostic tool to predict susceptible AD patients.

MicroRNAs as Diagnostic Biomarkers of AD

Current AD diagnostic markers and methods are applicable in the late stages of AD. Ultimately, they can be classified into (1) Neuropsychological tests: which are cognitive assessments

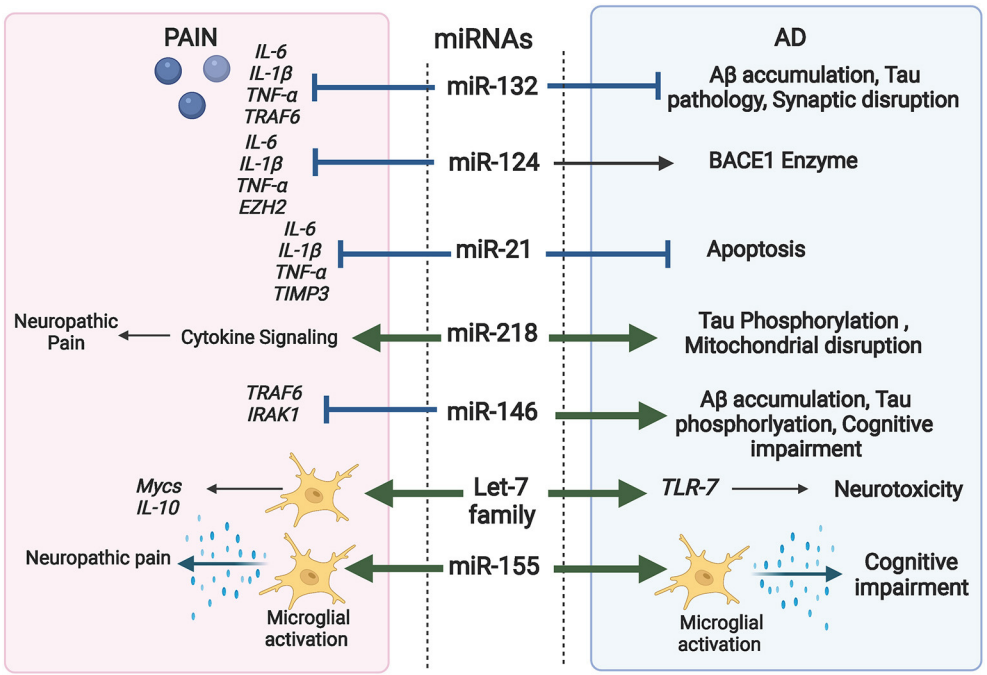

FIGURE 4 | Comprehensive illustration of the overlapping role of some miRNAs in both AD and Pain and their targeted genes. (Created with BioRender.com).

used to quantitatively assess the degree of cognitive impairment and its progression over time. This method, however, has limited specificity and sensitivity and is majorly affected by patients' educational levels. (2) Neuroimaging examination: such as Magnetic resonance imaging (MRI) and fluorodeoxyglucose (FDG)-positron emission tomography (PET) that monitor the pathological and functional alterations before severe appearance of cognitive impairment (Calvillo and Irimia, 2020). However, this method is hugely limited by the high cost.

Recent trials reported the high diagnostic potential of Amyloid and Phosphorylated Tau /Aβ ratio in CSF samples. Yet again, this technique is highly invasive and requires well-trained personnel for sample acquisitions. The detection of neuro filament light chains (NFL) in biological samples is emerging as good neuronal biomarkers, however, techniques and kits for reliable detection are still limited, and more research studies are needed to explore their ability to differentiate between different neurodegenerative diseases (Gaetani et al., 2019).

In the previous decade, the reported differential expressions of miRNAs in animal models of AD have opened the field to unleash the potential of miRNAs as promising diagnostic biomarkers for multiple neurodegenerative diseases. Moreover, continuous reports show that specific miRNAs are detected in the biofluids of AD patients with different levels from normal controls, along with their correlation to AD observed pathological and cognitive changes (Wei et al., 2020). A point that can be optimized to monitor AD progression. Furthermore, circulating miRNAs that are collected from serum or plasma resist environmental degradation and can provide a cheaper and less-invasive diagnostic means, compared to neuroimaging and CSF examinations. Ongoing research continuously reveals the remarkable diagnostic potential of miRNAs in AD. **Table 6** presents an updated summary for different miRNAs that have shown promising potential as AD diagnostic markers.

Spotting the most prominent miRNAs, recent studies have investigated the predictive potential of miRNAs in longitudinal studies over time. In an updated study, plasma miR-206 level stands out as a good prognostic biomarker to monitor MCI progression to AD over a period of 5 years (Kenny et al., 2019). In the same context, Ansari et al. (2019) showed the ability of miR-181a and miR-146a blood levels to predict whether MCI would progress to AD or remain stable after 2 years monitoring (Ansari et al., 2019).

Specific single miRNAs have shown consistent differentially expressed levels in various AD samples. For instance, Persistent miR-26b upregulation is reported in both serum and whole blood AD samples (Galimberti et al., 2014). Yang et al. (2018), recently spotted the diagnostic ability of exosomal miR-384, as its expression in serum of AD and non-AD patients differ significantly. Furthermore, serum level of exosomal miR-384 showed potent differential diagnostic ability for AD and Parkinson's dementia, as well as for AD and vascular dementia, with sensitivity/specificity indices of 97.2/100% and 99.1/100%, respectively (Yang et al., 2018).

One of the most emerging and promising miRNAs in AD diagnosis is miR-455-3p. Where serum miR-455-3p is upregulated in AD patients as compared to both MCI subjects and healthy controls. Interestingly, this finding is also confirmed in fibroblast cells, postmortem AD brains examination at

TABLE 6 | MicroRNAs with diagnostic potential in human AD studies.

miRNA	Sample	Expression in AD	Studied cohort	Specificity/sensitivity	References
miRNA-483-5p	Plasma	Increased	20 MCI 20 AD 20 Healthy controls	95, 90% 85, 90%	Sabry et al., 2020
miR-26a	Serum	Decreased	121 AD, 48 HC	57, 85%	Guo et al., 2017
miR-34a	Plasma CSF	Increased Decreased	25 AD, 27 HC 10 AD, 10 HC	74, 84%	Cosín-Tomás et al., 2017
miR-125b	Serum	Increased	105 AD, 155 HC	68, 80%	Tan et al., 2014a
miR-181a	Serum	Decreased	121 AD, 86 HC	73, 72%	Ansari et al., 2019
miR-181c	Serum	Decreased	150 HC, 105 AD	64, 75%	Manzano-Crespo et al., 2019
miR-206	Serum	Decreased	66 MCI, 33 HC	100, 70%	Xie et al., 2015
miR-342-3p	Serum	Decreased	158 AD, 155 HC	70, 81	Tan et al., 2014b
miR-34c	Blood mononuclear cells	Increased	110 AD, 123 HC	74, 84%	Bhatnagar et al., 2014
miR-133b	Serum	Decreased	98 AD, 105 HC	74.3, 90%	Yang et al., 2019
miR-433a	Serum	Decreased	32 AD, 12 HC	0.82 AUC	Wang and Zhang, 2020
miR-103	Plasma	Decreased	120 AD, 120 PD, 120 HC	84, 80%	Wang J. et al., 2020
miR-106	Serum	Decreased	56 AD, 50 HC	62, 94%	Madadi et al., 2020
miR-9	Serum	Decreased	36 AD female patients 38 HC females	Not available	Souza et al., 2020
miR-29cmiR-19b	Serum	Decreased	45 AD, 40HC	Not available	Wu et al., 2017
miR-455-3p	Serum	Increased	11 AD, 18 MCI, 20 Healthy	AUC 79%	Kumar et al., 2017
miR-let-7b	CSF	Increased	41 memory complaints, 36 MCI, 17 AD patients	Addition to total and *p- tau* panel led to improved AUC to 91.6%	Liu et al., 2018

different Braak stages (Kumar et al., 2017; Kumar and Reddy, 2018), as well as in CSF samples of sporadic AD subjects (Kumar and Reddy, 2021). Analyzing the mechanistic effects of miR-455-3p in AD showed that *APP* is a validated target of miR-455-3p. Whereas, elevated levels of miR-455-3p help to alleviate Aβ toxicity, improve mitochondrial dynamics and synaptic activity (Kumar and Reddy, 2019).

Another important concept is the utilization of miRNA panels of multiple miRNAs as a signature for AD. This can provide higher accuracy, specificity, and sensitivity. In this context, miR-181c, miR-92a-3p, and miR-210-3p showed remarkable ability to differentiate between AD and healthy controls. Moreover, this signature panel showed promising results in predicting MCI progression to AD after monitoring patients for 1 to 11 years (Siedlecki-Wullich et al., 2019). Additionally, a serum 9-miNRA signature panel including; miR-26a-5p, hsa-miR-181c-3p, hsa-miR-126-5p, hsa-miR-22-3p, hsa-miR-148b-5p, hsa-miR-106b-3p, hsa-miR-6119-5p, hsa-miR-1246, and hsa-miR-660-5p was recently reported to differentiate between AD and healthy controls with an AUC ROC reaching 85%, in a study that comprised one of the biggest cohorts of AD patients (Guo et al., 2017).

Although, miRNAs possess the properties of good diagnostic tools, miRNA use is still faced by several limitations. Namely, the parameters used for groups classification, subjects' inclusion and exclusion criteria, miRNAs extraction and quantification methods and reliable reference genes, vary widely among different labs. This is why finding a method to standardize miRNAs isolation and quantification protocols, as well as recruiting wider and more diverse populations can provide deeper knowledge about miRNA diagnostic potential.

The Therapeutic Potential of miRNAs in AD

The cumulative knowledge on miRNAs functions, strongly implies their potential as an emerging therapeutic possibility for multiple AD pathologies. Generally, therapeutic intervention using miRNAs can take three approaches: (1) Using natural or synthetic compounds to modulate the expression of miRNAs (2) inhibiting the function of a particularly targeted miRNA using complementary single stranded antisense oligonucleotide (ASO). (3) Readjusting the expression of the targeted miRNA using miRNA mimics. So far, several trials have been conducted to adjust miRNA expression in AD animal models as well as cell lines.

Numerous studies used natural or synthetic compounds to readjust miRNAs expressions. Among the most characteristic trials, Osthole is reported to modulate the expression of miR-9 and miR 101a-3p in APP/PS1 mice, SHSY-5Y cells as well as neural stem cells. Consequently, this resulted in decreased cellular apoptosis, improved cell growth, improved learning and memory capacities, together with prevention of

Aβ aggregation (Li et al., 2017). Berberine was also reported to remodulate the expression of miR-188 in BV2 cells, resulting in remarkable inhibition of Apoptosis (Chen et al., 2020). The Chinese herbal Tiaoxin recipe was recently reported to inhibit miR-34a expression in APPswe/PS1ΔE9 mouse AD model leading to decreased Aβ aggregation (Hu Y.-R. et al., 2019). A combination of resveratrol and curcumin was also successfully reported to readjust the expression of cortical let-7c in rats and PC-12 cells, which led to significant reduction of neuroinflammation along with readjustment of β-secretase and APP expressions (Zaky et al., 2017). In the same context, Sun et al. (2020), recently reported the ability of Dexmedetomidine to upregulate miR-129 in NIH Swiss mice, which led to cognitive improvement through targeting *YAP1* gene (Sun et al., 2020).

The continuous advances in drug delivery and biotechnology help in the rapid progress toward specific miRNA targeting and delivery. Interestingly, a recent study that used engineered exosomes to deliver miR-29 in a rat model of induced Aβ pathology, showed significant improvement of memory deficits (Jahangard et al., 2020). Meanwhile, photoactivation of targeted miRNAs, miRNAs sponges that sequester a specific miRNA and inclusion of miRNAs in labeled liposomes or cubosomes to cross the blood brain barrier are all emerging innovative means that can be optimized in the near future for miRNA delivery (López-González et al., 2017).

DISCUSSION AND OUTLINE

Current Hopes, Challenges, and Future Perspectives

There is no doubt miRNAs provide a novel opportunity to tackle AD disease, either through their potential as earlier diagnostic markers or through modulating their expression to reinstate the relevant AD target genes.

One interesting point is that along our research, numerous miRNAs apparently act as multi-faceted modulators of AD-interconnected signaling pathways. A point that can be advantageous in re-adjusting the different impaired pathways affected by miRNAs dysregulations. Furthermore, despite their nature as epigenetic modulators that can influenced by environmental and ethnic variations, multiple miRNA panels were repeatedly reported in different AD studies of variable regions and populations. For instance, miRNAs including but not restricted to; miR-155, miR-146a, miR-34a, miR-29, miR-132, miR-483-5p, and miR-181 are reported as differentially expressed in multiple AD samples of Chinese, American, European populations (Cheng et al., 2015; Wei et al., 2020; Huaying et al., 2021; Siedlecki-Wullich et al., 2021), as well as emerging studies from Africa and middle eastern regions (Sabry et al., 2020). At the same time, these miRNAs have also been introduced as crucial modulators of Aβ and Tau pathologies, inflammatory, mitochondrial, and synaptic dysfunctions that accompany AD progression.

An emerging hot topic in AD pathology is the role of mitochondrial miRNAs in mitochondrial dysfunction and mitophagy that are highly likely to precede actual AD neuronal damage (John et al., 2020). And while recent studies show miR-7, miR-155, miR-210 and miR-125 as crucial in mitochondrial impairments accompanying cancer (Ortega et al., 2020), we are still scratching the surface concerning mitomiRs in "inflamm-aging "axis and AD.

Another hot topic in AD dilemma is the promising role of lactate level in astrocytes and its possible effect on reinstating cognitive decline of AD. Updated studies spot the role of miRNAs; miR-34a (Sarkar et al., 2016), exosomal miR-137 (Thomas et al., 2020) and miR-124-3p (Xu S.-Y. et al., 2019) in controlling astrocytes lactate shuttle in different brain disorders and the consequent impact of adjusting their levels on cognitive improvement. Further future studies can definitely elucidate more facts about miRNAs role in astrocyte metabolism during AD and unravel ways to use this knowledge.

That being said, it is inevitable to state that till date, miRNAs bench studies are still faced by several obstacles that need to be resolved before being capable of efficiently serving AD clinical applications.

For a beginning, till now, there are limitations in comparing miRNA studies due to the basal differences in AD stages of the recruited patients and unclear specificity to AD. Moreover, communication difficulties with AD patients and their caregivers, comprise a major challenge by itself to recruit bigger populations of AD patients. To improve comparability in miRNAs studies, classifying the recruited patients in comparable groups according to their disease stages, followed by correlating their pathology with their miRNA's profiles can be more reliable.

Another challenge is the scarcity of data originating from middle and low-income countries and the relatively small populations size in AD clinical studies that majorly originate from well-developed countries. This can be resolved by enhancing the means of reaching out to affected communities, performing more longitudinal studies, together with utilizing advanced screening techniques, and raising awareness about the critical significance of participating in AD studies.

On the technical level, an important challenge in miRNAs studies, is the wide variability in sample collection, storage, standardization against reference controls, and analytical protocols (Mushtaq et al., 2016). Multi-center comparisons and universal standardization of the used techniques would to a large extent improve miRNAs reliability.

Indeed, efficient use of miRNAs for AD diagnosis, still needs to be preceded by precise standardization of sampling and quantification protocols and proper staging of AD. It might also be useful to rely on multiple panels of miRNA or combinations of miRNAs together with other fluid biomarkers. Progresses in this aspect are continuously accomplished. Interestingly, some registered clinical trials are already running

for example: (NCT03388242) that is using a combination of microRNAs and proteins with different expression patterns to distinguish between normal control, MCI and AD patients (Zuo, 2019).

Therapeutically, miRNAs use is challenged by some issues. Firstly, miRNAs target multiple genes and hence, manipulating one miRNA can result in unwanted effects on other sides (Siedlecki-Wullich et al., 2021). However, advances in *in silico* analysis and neuroinformatics can help face this problem. Besides, conducting more studies from diverse populations can contribute to getting more precise data that guide researchers to the proper beginning concerning their targeted population. Moreover, identification of the individual roles of specific miRNAs, as well as the collective role of multiple miRNAs in AD should be explored.

Another challenge is the difficulty of efficient miRNA delivery to the brain. However, the increasing advances in nanotechnology (Abdel-Mageed et al., 2021) and targeted delivery of biological materials have been successfully reported in some AD animal models.

Interestingly, miR-155 is already entering a clinical phase trial as a therapeutic compound registered by ©Miragen Therapeutics against Amyotrophic lateral sclerosis (Chakraborty et al., 2021). We believe, the near future can witness similar clinical successes to face the challenging pathologies of AD.

AUTHOR CONTRIBUTIONS

NA and FN drafted the manuscript. AZ and AB performed critical editing. NA, MA, and FN participated in constructive outline, discussions, and editing. All authors read and approved the final manuscript.

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Analysis of Genotype-Phenotype Correlations in Patients With Degenerative Dementia Through the Whole Exome Sequencing

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Background: Sporadic dementias generally occur in older age and are highly polygenic, which indicates some patients transmitted in a poly-genes hereditary fashion.

Objective: Our study aimed to analyze the correlations of genetic features with clinical symptoms in patients with degenerative dementia.

Methods: We recruited a group of 84 dementia patients and conducted the whole exome sequencing (WES). The data were analyzed focusing on 153 dementia-related causing and susceptible genes.

Results: According to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines, we identified four reported pathogenic variants, namely, *PSEN1* c.A344G, *APP* c.G2149A, *MAPT* c.G1165A, and *MAPT* c.G742A, one reported likely pathogenic variant, namely, *PSEN2* c.G100A, one novel pathogenic variants, *SQSTM1* c.C671A, and three novel likely pathogenic variants, namely, *ABCA7* c.C4690T, *ATP13A2* c.3135delC, and *NOS3* c.2897-2A > G. 21 variants with uncertain significance in *PSEN2*, *C9orf72*, *NOTCH3*, *ABCA7*, *ERBB4*, *GRN*, *MPO*, *SETX*, *SORL1*, *NEFH*, *ADCM10*, and *SORL1, etc.,* were also detected in patients with Alzheimer's disease (AD) and frontotemporal dementia (FTD).

Conclusion: The new variants in dementia-related genes indicated heterogeneity in pathogenesis and phenotype of degenerative dementia. WES could serve as an efficient diagnostic tool for detecting intractable dementia.

Keywords: Alzheimer's disease, frontotemporal lobe degeneration, dementia, next-generation sequencing, whole exome sequencing (WES)

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INTRODUCTION

Dementia currently affects an estimated 46.8 million people globally (Chaudhury et al., 2018). The most common dementia is Alzheimer's disease (AD), and others are less common, such as dementia with Lewy body (DLB), frontotemporal dementia (FTD), and Huntington's disease (HD), *etc.* The etiology of most neurodegenerative dementias is considered multifactorial, including genetic and environmental factors (Nicolas and Veltman, 2019). A proportion of dementias with a positive family history are consistent with a single gene pattern of inheritance, which provides

an opportunity to make a precise diagnosis in the very early stages of disease (Koriath et al., 2020). However, sporadic dementias generally occur in older age and are highly polygenic, which indicates some patients transmitted in a poly-genes hereditary fashion.

It is known that dormant inherited mutations relating to amyloid β (Aβ) synthesis in familial AD include *APP*, *PSEN1*, and *PSEN2*, and variants relating to the deposition of multiple abnormal proteins in FTD include *MAPT*, *GRN*, *SQSTM1*, *TARDBP*, *C9orf72*, and *ERBB4, etc* (Sun et al., 2020). However, mutations in these genes can only explain 13% of early-onset AD (EOAD) and 60% of familial FTD, respectively (Bettens et al., 2013; Olszewska et al., 2016). At present, the *APOE* ε4 allele is the strongest genetic factor of sporadic AD, which confers a 3- to 15-fold increased risk of AD (Seo et al., 2020). Through Genome-Wide Association Studies (GWASs) and next-generation sequencing, some genetic loci have been discovered, which affect the risk of sporadic AD and other dementias, including the *TREM2*, *ABCA7*, *NOTCH3*, *TRIP4*, *ATP13A2*, *and ABI3* genes (Chaudhury et al., 2018; Nygaard et al., 2019), while the clinical phenotypes relating to these variants are not fully known.

Recently, whole exome sequencing (WES) has been demonstrated to be an efficient tool for detecting novel pathogenic or risk variants in large samples and delivering novel insights with these selected patients (Xu et al., 2018). In this study, considering the limitation of WES to detect copy number variations (CNVs), we have screened the *C9orf72* gene in patients suspected of FTD. In this study, we discuss the correlations between genotype and clinical phenotype of degenerative dementias, which increases interest in the search for novel variants as candidate causal mechanisms in some patients with a sporadic presentation through WES.

MATERIALS AND METHODS

Subjects

A total of 84 patients were included in our study. All patients were assessed by specialists in the field of dementia. Of note, 55 patients fulfilled the diagnostic criteria for probable AD (McKhann et al., 2011), 14 patients met the clinical criteria for the FTD disease spectrum (Gorno-Tempini et al., 2011; Rascovsky et al., 2011), 6 patients fulfilled the diagnostic criteria for dementia but with uncertainty whether possible AD or FTD and nine patients could not be judged the dementia type or fulfilled other types of neurodegenerative dementia such as DLB. All available affected individuals were recruited from the Geriatric Psychiatry Department of Shanghai Mental Health Center. The research was approved by the Ethics Committee of Shanghai Mental Health Center. Written informed consents were obtained from all subjects.

Examinations

All patients received neuropsychological assessments including Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MoCA). Brain imaging and polymorphisms of *APOE* were analyzed in all patients. Blood tests (i.e., treponema pallidum hemagglutination assay, HIV assay, vitamin B12 levels, folic acid levels, thyroid function, and tumor markers) were conducted to exclude acquired causes of dementia.

Genetic Analysis

To comprehensively explore the potential genetic factors of the involved patients, we summarized 153 dementia-related causing and susceptible genes using PubMed database and Online Mendelian Inheritance in Man (OMIM) (**Table 1**). Total genomic DNA was prepared and amplified from the peripheral blood of all subjects according to the standard procedures. The quality of DNA was assessed by Qubit 3.0 (Thermo Fisher Scientific, United States) and agarose gel electrophoresis. WES was performed by HiSeq X Ten (Illumina, United States), and the sequencing library was carried out according to the SureSelectXT Target Enrichment System Manual (Agilent, United States). The average depth of coverage was 119.53. The data were analyzed for single nucleotide polymorphism (SNP) and insertion or deletion (INDEL) based on the genome analysis toolkit (GATK) best practice. The significant results were comprehensively evaluated in aspects, including minor allele frequency (MAF), conservation, predicted pathogenicity, disease association, confirmation with Sanger sequencing, and familial segregation. Alignment to the human genome assembly hg19 was carried out, followed by recalibration and variant calling. First, population allele frequencies compiled from public databases of normal human variation (dbSNP, National Center for Biotechnology Information Database of Single Nucleotide Polymorphisms 142; ESP6500, National Heart, Lung and Blood Institute Exome Sequencing Project 6500; gnomAD version 2.1.1, Genome Aggregation Database; ClinVar; HGMD, Human Gene Mutation Database; and 1000g, 1000 Genomes Project) were used to initially filter the data set to exclude all variants presenting in the population at greater than 5% frequency. A secondary filter for a paroxysmal movement disorder gene panel was then applied. A total of 200 normal Chinese individuals were set as control, and all the variants in this study could not be detected in the control group. The software of Mutation Taster, Plyphen2, and Mendelian Clinically Applicable Pathogenicity (M-CAP) Score were applied to predict the pathogenicity of the detected variants. These results were interpreted based on the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) standards and guidelines (Li et al., 2017).

Genotype-Phenotype Correlation Analysis and Protein Interaction Network Construction

Patients with gene variants were divided into three groups according to initial symptoms, which included hypomnesia, mood problems, and behavioral changes. Genotype-phenotype was analyzed through a contingency table in GraphPad Prism software. To understand the protein–protein interaction (PPI), a PPI network was created using the Search Tool for Retrieval of Interaction Genes (STRING) database.

TABLE 1 | Dementia-related causing and susceptible genes.

A2M	BSCL2	DNAJC5	GRIA2	MAP3K14	PLEKHG5	SLC5A7	TRPV4
ABCA7	C19orf12	DNMT1	GRID2	MAPT	PNPLA6	SMN1	TUBA4A
ABCD1	C9orf72	DPP6	GRIK1	MAPT	PRICKLE1	SMN2	TYROBP
ACE	CAMK1G	DYNC1H1	GRN	MATR3	PRKAR1B	SNCA	UBA1
ADAM10	CASP3	EFHD2	GSK3B	MEF2C	PRKN	SNCB	UBQLN2
ALS2	CD2AP	ELP3	HFE	MOB3B	PRNP	SOD1	UNC13A
ANG	CD33	EPHA1	HLA-DQB1	MPO	PRPH	SORL1	UNC5C
APBB2	CELF1	EPM2A	HNRNPA1	MS4A4E	PRPH2	SPAST	VAPB
APEX1	CHCHD10	ERBB4	HNRNPA2B1	MS4A6A	PSEN1	SPG11	VCP
APOE	CHMP2B	ETS1	HSPB1	MYH14	PSEN2	SQSTM1	VEGFA
APP	CLU	EWSR1	HSPB3	NEFH	PTK2B	SUSD2	VPS54
AR	COQ2	FBXO38	HSPB8	NHLRC1	RAB38	TAF15	WDR45
ASAH1	CR1	FERMT2	HTR7	NME8	REEP1	TARDBP	ZNF512B
ASCC1	CSF1R	FGGY	IGHMBP2	NOS3	REST	TBK1	TRPM7
ATP13A2	CTSC	FIG4	INPP5D	NOTCH3	SETX	TFG	SLC52A3
ATP7A	CYP27A1	FMNL1	ITM2B	NPC1	SIGMAR1	TMEM106B	PLAU
ATXN2	CYP2C19	FUS	ITPR2	OPTN	SLC1A1	TREM2	LRRK2
BICD2	DAO	GARS	KIF20B	PFN1	SLC1A2	TRIB3	GRB2
BIN1	DCTN1	GBA	LRP1	PICALM	SLC52A2	TRIP4	DNAJB2
BLMH							

RESULTS

Demographic and Clinical Variables

We identified five reported pathogenic/likely pathogenic variants in AD or FTD, one novel pathogenic variant, three novel likely pathogenic variants, and 21 variants with uncertain significance in AD or FTD (**Tables 2**, **3**). No pathogenic expansions in *C9orf72* were detected in suspectable patients with FTD. Among all patients, 75% (63/84) patients developed hypomnesia as an initial symptom, 10.71% (9/84) developed mood problems, and 14.29% (12/84) developed behavioral changes. *APOE*ε4 allele was not more common in the variant group. *APOE*ε4 alleles occurred in 27.60% of patients in the gene mutation group and 50.90% of patients in the non-gene mutation group.

Phenotypes of Patients With Alzheimer's Disease and Associated Variants

We identified three known pathogenic/likely pathogenic variants in *PSEN1*, *PSEN2*, and *APP* in patients with AD, respectively. No. 001 presented with short memory disturbance at the age of 49. In the following 2 years, he could not work as normal. He got lost, became irritable, and showed visual hallucinations at the age of 54. He suffered from slow gait and suspicion at the age of 55, had to stay in bed at the age of 60, and died of severe pneumonia at the age of 62. Brain MRI revealed that brain atrophy and his MMSE and MoCA scores were both 0/30 with 16 years of education at the age of 58. His mother and two sisters had developed similar symptoms. The known *PSEN1* variant (NM_000021: c.A344G, p.Y115C) (Cruts et al., 1998) was found in this patient. No. 002 was admitted at the age of 56 with memory impairment. In the following years, his activities of daily living declined gradually. He was easy to become irritable, got lost, and had incontinence at the age of 62. His father also had the same symptoms at the age of 50+. The known *APP* variant (NM_000484: c.G2149A, p.V717I) (Jiao et al., 2014) was detected in this patient. No. 007 with *PSEN2* (NM_000447: c.G100A, p.G34S) (Jia et al., 2020) developed memory deficit at the age of 74 and got lost at the age of 75. He walked unsteadily, could not get benefits from the anti-Parkinson's disease treatment at the age of 76, and had to stay in bed due to stiff limbs at the age of 78. Brain MRI showed multiple ischemic foci of the bilateral frontal-parietal lobe and brain atrophy (**Tables 2**, **3** and **Figure 1**).

We identified three novel likely pathogenic variants in *ABCA7*, *ATP13A2*, and *NOS3* in patients with AD. No. 014, carrying *ABCA7* variant (NM_019112: c.C4690T, p.R1564X), which produced a truncated protein with 583 amino acids less than normal protein, developed memory problem and speech deficit at the age of 62. Of note, 2 years after onset, her MMSE score was 16/30, and her MoCA was 14/30 with 9 years of education. Notably, 4 years later, she always wore one suit of clothes. At the age of 67, she often ignored her family members and could not look after herself. At the age of 68, she did not know her children but only her husband. Brain MRI revealed bilateral hippocampal atrophy, brain atrophy, and hyperintensity spots beside the left radiation coronal area. No. 018 developed hypomnesia at the age of 52 and was diagnosed with AD at the age of 54. Her MMSE score was 21/30 with 10 years of education, and brain MRI revealed mild demyelination of paraventricular white matter and brain atrophy at the age of 54. She was identified to harbor the variant *ATP13A2* (NM_022089: c.3135delC, p.Y1045X), which resulted in protein truncation with 136 amino acids less than the normal protein (**Table 3**). No. 020 became forgetful at the age of 68 and progressed to dementia at the age of 72. Her MMSE score was 20/30 with 12 years of education at the age of 72. She was identified to harbor *NOS3* (NM_000603: c.2897-2A > G), which existed at the junction of

TABLE 2 | Variants in cases with pathogenicity or uncertain significance.

Case	APOE	Gene	Transcript No. (NM)	Mutation (CDS)	Change of amino acid	Age onset	Sex	Family history	Clinical diagnosis	Frequency prediction				Software prediction			ClinVar	ACMG
										dbSNP	HGMD	1000g	gnomAD v2.1.1	Plyphen2	Mutation taster	M-CAP		
001	ε3/ε3	*PSEN1*	000021	A344G	Y115C	49	M	Yes	AD	Include	Include	NA	NA	Likely DC	DC	PP	P/LP	P
002	ε3/ε3	*APP*	000484	G2149A	V717I	56	M	Yes	AD	Include	Include	NA	NA	DC	DC	PP	P	P
003	ε3/ε3	*MAPT*	005910	G1165A	G389R	27	M	NA	FTD	Include	Include	NA	0.00005438	DC	DC	PP	LP	P
005	ε3/ε4	*SQSTM1*	003900	C671A	S224X	59	F	NA	FTD	NA	NA	NA	NA	/	DC	PP	NA	PATH
006	ε3/ε4	*PSEN2*	000447	T437C	I146T	59	F	Yes	AD/FTD	NA	NA	NA	0.00005437	DC	DC	PP	NA	VUS
007	ε3/ε3	*PSEN2*	000447	G100A	G34S	75	M	Yes	AD-PD	Include	NA	Include	0.006120	Benign	DC	PP	CIP	LP
008	ε3/ε3	*PSEN2*	000447	A785G	Y262C	81	M	Yes	AD*	NA	NA	NA	NA	DC	DC	PP	NA	VUS
009	ε3/ε3	*MAPT*	005910	G742A	V248M	57	M	NA	FTD#	Include	NA	Include	0.0001088	DC	DC	PP	P	P
010	ε2/ε4	*GRN*	002087	T617C	V206A	57	F	Yes	FTD#	NA	NA	NA	0.0002175	Likely DC	PM	PP	NA	VUS
011	ε3/ε3	*ERBB4*	005235	T2136G	I712M	56	F	NA	FTD-ALS	NA	NA	NA	NA	DC	DC	PP	NA	VUS
012	ε3/ε3	*C9orf72*	018325	T1442A	F481Y	63	M	NA	AD	NA	NA	NA	NA	DC	DC	PP	NA	VUS
013	ε2/ε3	*ABCA7*	019112	G2653C	V885L	44	F	NA	AD	Include	NA	NA	NA	Benign	PM	PP	NA	VUS
014	ε3/ε4	*ABCA7*	019112	C4690T	R1564X	62	F	Yes	AD	NA	NA	NA	0.00005438	DC	DC	PP	NA	LP
015	ε4/ε4	*ABCA7*	019112	T2933C	I978T	51	F	NA	AD/FTD	NA	NA	NA	0.0006522	DC	DC	PP	NA	VUS
016	ε3/ε3	*ADAM10*	001110	G502A	G168S	67	M	NA	AD/FTD	NA	NA	NA	NA	DC	DC	PP	NA	VUS
017	ε3/ε3	*ATP13A2*	001141973	A2281G	M761V	59	M	Yes	DLB	NA	NA	NA	NA	DC	DC	LB	NA	VUS
018	ε3/ε3	*ATP13A2*	022089	3135delC	Y1045X	52	F	NA	AD	NA	NA	NA	NA	DC	DC	/	NA	LP
019	ε3/ε4	*MPO*	000250	C1120T	R374W	63	M	Yes	AD*	NA	NA	NA	NA	DC	DC	PP	NA	VUS
020	ε2/ε3	*NOS3*	000603	2897-2A > G	/	68	F	NA	AD	NA	NA	NA	NA	DC	DC	/	NA	LP
021	ε3/ε3	*NOS3*	000603	1788dupT	S596fs	54	F	NA	AD*	Include	NA	Include	NA	/	DC	/	NA	VUS
022	ε3/ε3	*NOTCH3*	000435	G4240A	G1414S	53	M	NA	AD-NPH	NA	NA	NA	NA	DC	DC	PP	NA	VUS
024	ε3/ε4	*NOTCH3*	000435	G182T	R61L	64	F	NA	AD-VD	NA	NA	NA	NA	Benign	PM	PP	NA	VUS
026	ε3/3	*NOTCH3*	000435	C1715T	P572L	60	M	Yes	AD	NA	Include	NA	NA	DC	DC	PP	NA	VUS
027	ε3/ε3	*PTK2B*	004103	C1451T	P484L	65	M	Yes	AD-CAA	NA	NA	NA	0.0000544	DC	DC	PP	NA	VUS
028	ε3/ε3	*SETX*	015046	A3890G	Y1297C	69	F	NA	AD	NA	NA	NA	NA	/	PM	PP	NA	VUS
029	ε3/ε3	*SORL1*	003105	G1081C	V361L	28	F	NA	FTD#	NA	NA	NA	0.0003806	DC	DC	LB	NA	VUS
030	ε3/ε4	*SORL1*	003105	A296G	N99S	55	F	Yes	AD	NA	NA	NA	0.001033	DC	DC	LB	NA	VUS
031	ε3/ε3	*NEFH*	021076	C373G	L125V	62	F	Yes	AD	NA	NA	NA	0.0001449	DC	DC	PP	NA	VUS
032	ε3/ε4	*SYNJ1*	003895	T4664C	L1555P	72	M	NA	DLB	NA	NA	NA	NA	Benign	PM	LB	NA	VUS
		SYNJ1	003895	G317A	R106Q					NA	NA	NA	NA	Likely DC	DC	PP	VUS	VUS

**AD accurate diagnosis by Aβ biomarkers in CSF or PET.*

#Exclusion of AD diagnosis by Aβ biomarkers in CSF or PET.

CDS, coding sequence; Het, heterozygous; dbSNP, The Single Nucleotide Polymorphism Database; HGMD, Human Gene Mutation Database; 1000g, 1000 Genomics Projects; gnomAD, Genome Aggregation Database; M-CAP, Mendelian Clinically Applicable Pathogenicity; ACMG, American College of Medical Genetics and Genomics standards and guideline; DC, disease causing; PM, polymorphism; VUS, variant of uncertain significance; NA, not available; PP, possibly pathogenic; P, pathogenic; LP, likely pathogenic; CIP, conflicting interpretations of pathogenicity.

TABLE 3 | DNA sequence, brain MRI or CT, pedigree, initial symptoms, and clinical diagnosis of dementia patients with variants.

Case	DNA sequence	Pedigree	MRI	Initial symptoms	Diagnosis
001	*PSEN1*: c.344 A>G		7 years after onset	Hypomnesia	AD
007	*PSEN2* c.100 G>A		1 years after onset	Hypomnesia	AD-PD
008	*PSEN2*: c.785 A>G		1 years after onset	Hypomnesia	AD
013	*ABCA7*: c. 2653 G>C		3 years after onset	Hypomnesia	AD
014	*ABCA7*: c. 4690 C >T		2 years after onset	Hypomnesia, speech decrease	AD
024	*NOTCH3*: c. 182 G>T		3 years after onset	Hypomnesia, gait disturbance	AD-VD
026	*NOTCH3*: c. 1715 C>T		2 years after onset	Hypomnesia	AD
003	*MAPT*: c. 1165 G>A		3 years after onset	Inert, apathy	FTD
005	*SQSTM1*: c. 671 C>A		6 years after onset	Depression, hypomnesia	FTD
006	*PSEN2*: c. 437 T>C		10 years after onset	Hypomnesia, interest decline	AD/FTD
009	*MAPT*: c. 742 G>A		7 years after onset	Depression, laziness	FTD
010	*GRN*: c. 617 T>C		0.5 years after onset	Anxiety, hypomnesia	FTD
011	*ERBB4* c. 2136 T>G		2 years after onset	Repetitive/stereotype language, apathetic	FTD-ALS

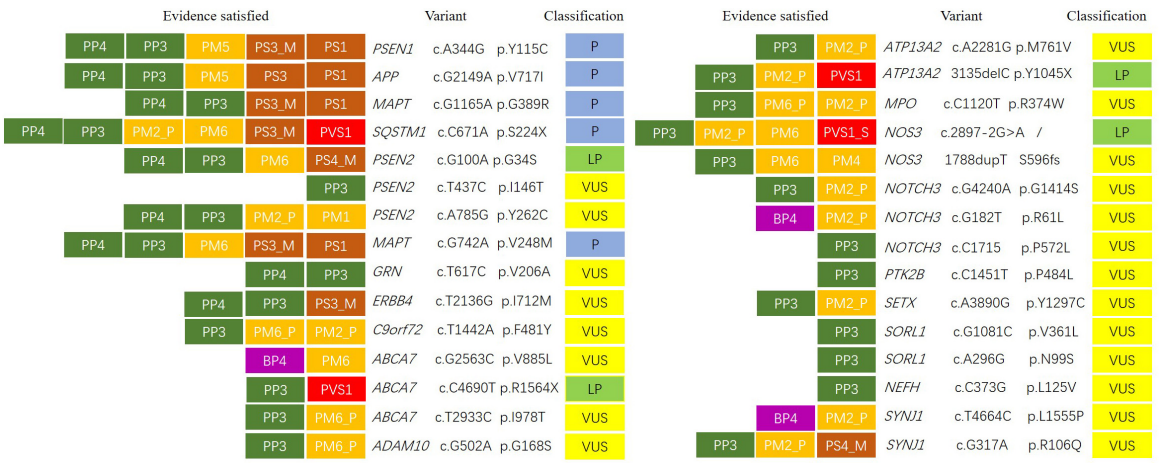

FIGURE 1 | Variant classification according to American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) categorical rules. The first letter of each evidence indicated support toward a pathogenic (P) or benign (B) classification, and the second letter indicated the assigned evidence strength: very strong (VS), strong (S), moderate (M), or supporting (P). Evidence boxes were colored by evidence strength. Final classification was determined by the combinations of evidence resulting in the following: pathogenic (P), likely pathogenic (LP), or variant of uncertain significance (VUS).

intron 23 and exon 24 and caused protein truncation (**Tables 2**, **3** and **Figure 1**).

We identified *PSEN2*, *C9orf72*, *NOTCH3*, and *NOS3* variants with uncertain significance in patients with AD and also detected six variants with uncertain significance in *ABCA7*, *MPO*, *SETX*, *SORL1*, and *NEFH*. No. 008 with *PSEN2* (NM_000447: c.A785G, p.Y262C) developed hypomnesia at the age of 81, got lost, and suffered from behavior disturbance after 6 months. His MMSE score was 23/30 and MoCA 18/30 with 16 years of education at the age of 65. On cerebrospinal fluid (CSF) examination, the level of Aβ (494.1 pg/ml) decreased and phosphorylated tau (p-tau; 110.23 pg/ml) increased. Brain MRI showed multiple cavity infarctions of bilateral radiation coronal area and frontal-parietal lobe, moderate leukoaraiosis, and brain atrophy. No. 012, carrying *C9orf72* variant (NM_018325: c.T1442A, p.F481Y), was admitted with memory disturbance at the age of 63. His MMSE score was 15/30 and MoCA 20/30 with 12 years of education at the age of 65. No. 021 presented with memory disturbance at the age of 54 and poor activities of daily living at the age of 59. On CSF examination, the level of Aβ decreased and p-tau increased. In the following 5 years, she suffered from eating difficulty and weight loss. She became receptive to aphasia and irritability. The *NOS3* variant (NM_001160110: c.1788dupT, p.S596fs), which resulted in the termination codon appearing ahead of schedule and a truncated protein with 18 amino acids less than the normal protein, was identified in this patient. No. 022, 024, and 026 were all identified to harbor the *NOTCH3* variants (NM_000435). No. 022 showed memory disturbance at the age of 53. He had difficulty in walking and dressing, as well as became urinary incontinent at the age of 61. His MoCA score was 1/30 with 6 years of education, and brain MRI revealed ischemic foci of bilateral basal ganglia, mild leukoaraiosis, and brain atrophy at the age of 63. Under CSF shunt operation, his function of walking and cognition were improved at the age of 64 but began to decline after 2 months. No. 024 presented with a memory problem at the age of 63 and an unsteady movement at the age of 65. She had difficulties in calculation at the age of 66, lost the way home, and became agnosia at the age of 70. Her MMSE and MoCA scores were both 0/30 with 6 years of education, and brain CT showed infarction of right basal ganglia, leukoaraiosis, and brain atrophy at the age of 66. No. 026 developed hypomnesia at the age of 60. He was in a delusion of being stolen, became grouchy, and often quarreled with others at the age of 63. His MoCA score was 6/30 with 12 years of education, and brain MRI revealed mild brain atrophy at the age of 62 (**Tables 2**, **3** and **Figure 1**).

Phenotypes of Patients With Frontotemporal Dementia and Associated Variants

We identified two reported pathogenic variants in *MAPT*, one novel pathogenic variant in *SQSTM1*, and three variants with uncertain significance in *ERBB4*, *GRN*, and *SORL1* in patients with FTD. No. 003 with *MAPT* (NM_005910: c.G1165A, p.G389R) (Bermingham et al., 2008) was identified and reported by our team in 2017 (Sun et al., 2017). Then, he had to stay in bed at the age of 30 and died of severe pneumonia at the age of 33. No. 009 with *MAPT* variant (NM_005910: c.G742A, p.V248M) depressed and suspected himself seriously ill at the age of 56. In the following 2 years, he was apathetic and irritable. He was forgetful, stubborn, and ate only one type of food at the age of 60, and urinated anywhere at the age of 63. Brain MRI showed brain atrophy, especially in the bilateral anterior temporal lobe and hippocampus. No. 005 with *SQSTM1* variant (NM_003900: c.C671A, p.S224X) was identified and reported by our team in 2018 (Sun et al., 2018), which resulted in the premature termination of protein synthesis and a predicted truncated protein. However, we did not detect truncated proteins in this variant overexpressing HEK-293T cells because of the degradability of truncated protein. We identified

the novel pathogenic *SQSTM1* S224X variant with loss of SQSTM1/p62 protein expression probably due to *SQSTM1* gene haploinsufficiency. Then, she exhibited hypertonia, unsteady gait, irritability, language dysfunction at the age of 67 and showed visual hallucination and dysphagia at the age of 68. No. 011 with *ERBB4* (NM_005235: c.T2136G, p.I712M) was identified and reported by our team in 2020 (Sun et al., 2020). Through follow-up, we learned that this patient died at home at the age of 62. No. 010 felt depressed, anxious, and forgetful at the age of 57. In the following 3 years, her condition gradually aggravated, and her abilities to work were influenced. Her MoCA score was 14/30 with 14 years of education. *GRN* variant (NM_002087: c.T617C, p.V206A) was identified in this patient (**Tables 2**, **3** and **Figure 1**).

Phenotypes of Patients With Uncertainty Whether Possible Alzheimer's Disease or Frontotemporal Dementia and Associated Variants

We identified three variants with uncertain significance in *PSEN2*, *ABCA7*, and *ADCM10*. No. 006 showed memory impairment at the age of 59, felt depressed and interest declined at the age of 61 and acted in a strange way, such as putting the jacket down in the close stool at the age of 64. In the following 7 years, she was suspicious and became urinary incontinent. The *PSEN2* (NM_000447: c.T437C, p.I146T) was identified in this patient. No. 015 with *ABCA7* (NM_019112: c.T2933C, p.I978T) became irritable at the age of 49, often forgot recent things, and suffered from poor ability in writing at the age of 51. She could not take care of herself and lose weight at the age of 55. Her MMSE and MoCA were all 0/30 with 12 years of education at the age of 55 (**Tables 2**, **3** and **Figure 1**).

Genotype-Phenotype Correlation and Protein–Protein Interaction Network

Initial symptoms of patients were with variants in *SORl1*, *PSEN2*, *NOTCH3*, *NOS3*, *MAPT*, *APT13A2*, and *ABCA7* genes (samples $n \geq 2$) (**Figure 2A**). Hypomnesia was the most common initial symptom among all patients, behavioral change was inclined to occur in *MAPT* and *ABCA7* variant carriers, and mood problems usually happened to the subjects with *SORL1*, *APT13A2*, and *ABCA7* variants. PPI networks showed that there were three clusters based on the K-means clustering algorithm among all proteins (**Figure 2B**). NOS3 and MPO proteins had no relationships with other proteins. Several important proteins such as APP, PSEN1, PSEN2, MAPT, C9orf72, and GRN, *etc*., had more interactions with other proteins.

DISCUSSION

This study aimed to find genetic variants contributing to clinical phenotypes of degenerative dementias. First, we identified five reported pathogenic/likely pathogenic variants in patients with AD or FTD. Second, we identified 1 novel pathogenic variant in *SQSTM1* genes, 3 novel likely pathogenic variants in *ABCA7*, *ATP13A2*, and *NOS3*, and 21 variants with uncertain significance in *PSEN2*, *C9orf72*, *NOTCH3*, *ABCA7*, *ERBB4*, *GRN*, *MPO*, *SETX*, *SORL1*, *NEFH*, *ADCM10*, and *SORL1*, *etc.,* in patients with AD or FTD.

The clinical symptoms of the *PSEN1* Y115C variant were rarely reported in the previous literature. Our case, with 13 years of the disease duration, was featured with early memory disturbance, visuospatial disorientation, behavioral and psychological symptoms, dysphasia, and motor disorder. The mean onset age of this variant reported was 42 years (range 39–49 years) (van Duijn et al., 1994), which was similar to our case (49 years). There was the observation of later occurrence of symptoms in variants after codon 200 in *PSEN*1 compared with those before it in EOAD (Ryan and Rossor, 2010). Furthermore, it was concluded that the patient with *PSEN1* missense variants located in hydrophobic regions had an earlier age onset than those with variants located in non-hydrophobic regions, and *PSEN1* Y115C just existed in the hydrophobic region. This phenomenon was that variants in hydrophobic regions affected the γ-secretase active site, promoting the aggregation of Aβ, and eventually inducing the onset of AD (Jia et al., 2020).

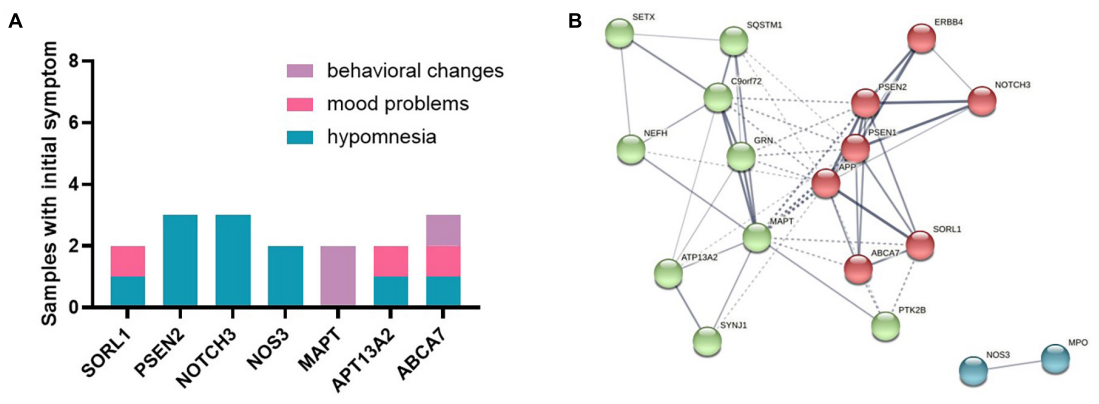

FIGURE 2 | (A) Initial symptoms of patients with variants in *SORl1*, *PSEN2*, *NOTCH3*, *NOS3*, *MAPT*, *APT13A2*, and *ABCA7* genes (samples $n \geq 2$). **(B)** The protein–protein interaction (PPI) network showed that there were three clusters among all proteins based on the K-means clustering algorithm.

APP V717I was identified in a previous pedigree (Murrell et al., 2000), which showed the clinical onset of disease in their mid-to-late 30s, with short-term memory deficit, gradually worsening the problems of visuospatial, executive, and expressive language functions. The disease duration is approximately 10 years based on the data of the family history. The pathological feature of this variant is the high production of Aβ fibril deposits (Murrell et al., 1991). No. 002 developed memory disturbance at the age of 56 and progressed that he got lost and incontinence in 6 years. His father also had the same symptoms at the age of 50+. Our investigations broadened the phenotype of AD with *APP* variants.

PSEN2 G34S variant was reported to relate to AD and mild cognitive impairment (MCI). This variant was identified in three pedigrees with the older mean age of onset (69, 52, 75, respectively) (Jia et al., 2020), which suggested relatively weak pathogenicity. No. 007 with *PSEN2* G34S developed memory disturbance at the age of 74 but progressed fast that he had to stay in bed in 4 years. Although there were fewer variants with late age onset in familial AD than variants with early age onset, they certainly existed. Furthermore, we explored two variants with uncertain significance in *PSEN2*, which may contribute to the disease occurrence. No. 006, carrying *PSEN2* T437C variant, developed memory disturbance at the age of 59 and showed an obvious change of personality and behavior in the next 5 years. The patient has identified the clinical diagnosis with uncertainty whether possible AD or FTD. No. 008, with *PSEN2* A785G variant, developed memory deficit at the age of 81, and the CSF examination identified the diagnosis of AD. These two variants were predicted to be damaging by three predictive algorithms, indicating the pathogenicity of these variants.

In this study, five missense variants were first identified to be related to FTD. Among these variants, two (i.e., *SQSTM1* C671A and *ERBB4* T2136G) were first reported by our team (Sun et al., 2018, 2020) and were confirmed function deficits of the target proteins. *MAPT* G389A was previously reported to affect a 17-year-old girl with the initial symptoms of atypical depression and emotional blunting and a 21-year-old woman with the initial symptom of postpartum depression. No. 003 also presented with the cognitive disorder at the age of 27 (Sun et al., 2017). It seemed that the early-onset cases of FTD were more likely to be found in tau G389R carriers. Tau G389R variant in the pseudo-repeat region (PRR) could increase microtubule dynamicity (Niewidok et al., 2016), and V248M variant impaired homeostatic control of spontaneous neuronal activity in response to depolarization (Sohn et al., 2019). Through our analysis, behavioral problem as the initial symptom was inclined to occur in *MAPT* variant carriers.

We explored three variants in *NOTCH3* with uncertain significance, which might contribute to the occurrence of AD. This gene encoding a single-pass transmembrane protein, predominantly expressed in vascular smooth muscle cells, and got involved in the mechanism of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Mašek and Andersson, 2017). Among these three missense variants, two were predicted to be damaging by at least two predictive algorithms (i.e., G4240A and G182T), and one was predicted as a polymorphism but did not find in dbSNP, HGMD, 1000g, and gnomAD database. A hypothesis of AD suggested that, in CADASIL, triggering events in the pathogenic cascade were not amyloid deposits but damaged blood vessels caused by inflammatory reactions that led to ischemia, amyloid accumulation, and axonal degeneration (Bonvicini et al., 2019). Variants in the *NOTCH3* gene were known to provoke inflammatory reactions (Marchesi, 2016). Furthermore, we explored the one variant in *NOS3* with likely pathogenicity in patients with AD. Nitric oxide performed vital physiological functions in the nervous and other systems under normal concentrations, but persistently high levels could create a toxic environment. *NOS3* was a strong candidate susceptibility gene for Parkinsonism syndromes (Hancock et al., 2008). Some studies genotyped EOAD and late-onset AD (LOAD) for *NOS3* polymorphism, which found the associate of *NOS3* gene with LOAD. This effect that is independent of *APOE* genotype concluded that *NOS3* may be a new genetic risk factor for LOAD (Styczyńska et al., 2008; Wang et al., 2008; Azizi et al., 2010). Our analysis also showed that NOS3 had no interaction with other proteins except MPO, which suggested that the pathway of the *NOS3* gene was different from other dementia-related genes. PPI network that demonstrated proteins such as PSEN1, PSEN2, APP, MAPT, and C9orf72 had abundant interactions with other proteins, which was consistent with the important roles of these dementia pathogenic genes.

Furthermore, our study reported that the *APOE*ε4 allele was not more common in the gene variant group. Of note, 27.60% of patients in the gene variant group and 50.90% of patients in the non-gene mutation group presented with one or two *APOE*ε4 alleles. Thus, it was possible that *APOE*ε4 did not act as a promoter of the clustering of AD with gene variants. Other unidentified genetic variants or environmental factors, acting independently or in concert, were involved in the development of dementia (Jia et al., 2020).

Our study had several limitations. The progression history of the disease was collected based on the recall of relatives with normal cognition, but still, there was a recall inaccuracy. In most cases of patients with AD, neither amyloid PET nor CSF Aβ biomarkers were included to corroborate the diagnosis. There was a limited ability to assess causality when screening individuals without affected and unaffected family members. The pathogenicity of variants with uncertain significance should be verified by large-scale studies and functional experiments. All patients involved in our analysis were severe, but the severity of patients was not strictly correlated with the findings of the pathogenic variants. Due to its incapability of detecting large segment INDEL, there were still many patients without detectable variants through exome sequencing, which could fail to explore the genetic factors in a subset of these patients with dementia. Genetic screening could not determine all the causes of dementia.

CONCLUSION

The WES techniques had the potential to give a genetic diagnosis for some intractable dementia associated with the

heterogeneity of clinical manifestations. This study also expanded the clinical spectrums of AD and FTD. The pathogenicity of the mentioned known genetic variants had been most appreciated in the European population, and this study showed the novelty of genetic variations in degenerative dementia in the Asian population.

DATA AVAILABILITY STATEMENT

According to the national legislation/guidelines, specifically the Administrative Regulations of the People's Republic of China on Human Genetic Resources (http://www.gov.cn/zhengce/content/2019-06/10/content_5398829.htm, http://english.www.gov.cn/policies/latest_releases/2019/06/10/content_281476708945462.htm), no additional raw data are available at this time. Data of this project can be accessed after an approval application to the China National Genebank (CNGB, https://db.cngb.org/cnsa/). Please refer to https://db.cngb.org/, or email: CNGBdb@cngb.org for detailed application guidance. The accession code CNP0002218 should be included in the application.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Shanghai Mental Health Center Ethical Standards Committee on human experimentation. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LS analyzed the data, wrote and revised the manuscript. NS and JZ collected the clinical data of patients. SZ, FY, and XinL assessed the cognitive function of patients. WL analyzed the gene data. SX and XiaL designed and supervised the experiment. All authors contributed to the article and approved the submitted version.

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frontiers in Aging Neuroscience

REVIEW
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Replenishing the Aged Brains: Targeting Oligodendrocytes and Myelination?

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Aging affects almost all the aspects of brain functions, but the mechanisms remain largely undefined. Increasing number of literatures have manifested the important role of glial cells in regulating the aging process. Oligodendroglial lineage cell is a major type of glia in central nervous system (CNS), composed of mature oligodendrocytes (OLs), and oligodendroglia precursor cells (OPCs). OLs produce myelin sheaths that insulate axons and provide metabolic support to meet the energy demand. OPCs maintain the population throughout lifetime with the abilities to proliferate and differentiate into OLs. Increasing evidence has shown that oligodendroglial cells display active dynamics in adult and aging CNS, which is extensively involved in age-related brain function decline in the elderly. In this review, we summarized present knowledge about dynamic changes of oligodendroglial lineage cells during normal aging and discussed their potential roles in age-related functional decline. Especially, focused on declined myelinogenesis during aging and underlying mechanisms. Clarifying those oligodendroglial changes and their effects on neurofunctional decline may provide new insights in understanding aging associated brain function declines.

Keywords: oligodendrocyte, OPC, myelinogenesis, aging, neurofunctional decline

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INTRODUCTION

Brain is sensitive to age with increasing neurofunction deficits including cognitive decline, motor and sensory abnormalities during aging (Sousounis et al., 2014; Damoiseaux, 2017; Jeromin and Bowser, 2017). Age-related impairments in cognition and memory lower the life quality of the elderly population and burden the society economically (Gazzaley et al., 2005). The neuron-loss hypothesis has been extensively tested; however, the neuron loss is obviously not the sole contributor for the severity of age-related functional decline (Morrison and Hof, 1997; Pakkenberg and Gundersen, 1997; Ihara et al., 2018). Noticeably, natural aging led to a reduction in white matter volume by as much as 28% (Liu et al., 2017). In addition, white matter abnormalities were identified and increased with age starting from middle age in humans (Kohama et al., 2012). White matter is mainly composed of bundled myelinated (87%) and unmyelinated axons and glia cells (Wang et al., 2008; Kohama et al., 2012). As the myelinating cells in the central nervous system (CNS), oligodendrocytes (OLs) are the most abundant glial cell type in white matter and also in some gray matter regions. For example, it was reported that OLs occupy about 75% of all glial cells in the neocortex of human brain (von Bartheld et al., 2016). More importantly, oligodendroglia lineage cells are undergoing dynamic changes during aging and that have been extensively reported recently (Stadelmann et al., 2019; Chapman and Hill, 2020; Sams, 2021).

Oligodendrocytes are exclusively derived from the differentiation of oligodendroglia precursor cells (OPCs), so does remyelination when demyelination occurs (Baumann and Pham-Dinh, 2001; Emery, 2010). OPCs distribute ubiquitously in the whole brain and have the capacities to proliferate and differentiate throughout lifetime. Each OL projects multiple processes to wrap the axons, forming the segmental, multiple-layered myelin sheaths that insulate axons. The denuded axon segment between two neighboring myelin sheaths is known as node of Ranvier, enriched with a number of ion channels. Action potential could travel along the axons quickly by jumping from one node of Ranvier to next one, so myelin could accelerate conduction velocity (Baumann and Pham-Dinh, 2001; Elbaz and Popko, 2019; Stadelmann et al., 2019). In addition, it is not hard to realize that through wrapping around the axon, myelin could protect the axon from damage. OLs may provide energy substance to axons via monocarboxylate transporter 1 (MCT1), and support axon survival (Fünfschilling et al., 2012; Lee et al., 2012; Morrison et al., 2013). Recent study suggested that myelin formation could regulate synaptic development in neonatal mouse brains (Wang et al., 2018).

Increasing evidence have demonstrated that myelinogenesis is continuously occurring in adult CNS and required for a number of neuro-functions in the adults, including memory function, motor coordination and motor skill learning (Young et al., 2013; McKenzie et al., 2014; Pan et al., 2020; Steadman et al., 2020; Wang et al., 2020; Chen L. et al., 2021). Because of the extremely high density of myelin sheaths in brains, dissecting the changes of oligodendroglial lineage cells and associated myelin during aging necessitates approaches to distinguish newly-formed myelin and pre-existing myelin. Recent advances by using cell-lineage labeling mouse lines and two-photon confocal imaging, allowed scientists to track the fate of oligodendroglial lineage cells and associated myelin during aging (Young et al., 2013; Baxi et al., 2017; Tripathi et al., 2017; Hill et al., 2018; Wang et al., 2020). This review aims to summarize recent evidence of dynamic changes of oligodendroglia lineage cells during aging and potential mechanisms of aging-related myelinogenesis decline. To that purpose, we first cover the development of oligodendroglia lineage cells and dynamic changes during aging. Next, we dissected the underlying mechanisms of inhibited myelination during aging. The pro-OPCs differentiation methods were also discussed as a potential therapy to improve age-related functional deficits.

OLIGODENDROCYTE MYELINATION IN ADULT CENTRAL NERVOUS SYSTEM

Oligodendroglia precursor cells distribute into the whole brain and have the capacities to proliferate and differentiate throughout lifetime. Upon differentiation or apoptosis in either physiological or pathological conditions, the neighboring OPCs could proliferate rapidly and maintain the stable OPC density (Chang et al., 2000; Hughes et al., 2013; Sun et al., 2018; Bottes and Jessberger, 2021). Myelination is initiated after birth in rodents and reaches to a peak from 2 weeks to a month postnatally. The process is orchestrated by a large amount of intrinsic and extrinsic factors (Baumann and Pham-Dinh, 2001; Young et al., 2013; Elbaz and Popko, 2019). Notably, the time and extent of myelin formation is variable in different brain regions, which may be relative to the development of neuronal functions. For example, the lateral olfactory tract is myelinated the earliest in mouse brains, starting from postnatal day 4, while the axons in optic nerve start to be myelinated about 8 days postnatally. The superficial layer of cortex is the last region to be myelinated, where new myelin sheaths are continuously added into adulthood (Purger et al., 2016; Hill et al., 2018). Though it was proposed that programmed cell death of a subset of pre-myelinating OLs and excess myelin sheaths elimination by microglia are involved (Sun et al., 2018; Hughes and Appel, 2020), the exact mechanisms that regulate the temporal and spatial process are still unknown yet.

More and more evidence has demonstrated the generation of new OLs in adult and aging brains. EdU or BrdU incorporation assay showed that there were a number of EdU or BrdU positive mature OLs in the adult brains (Lasiene et al., 2009; Young et al., 2013; Gibson et al., 2014). Advances in cell-lineage labeling mouse line and two-photon confocal imaging contribute to observing new myelin generation in adults directly. Tau-mGFP is a transgenic reporter mouse line to label newly-formed myelin. After crossed with an OPC-specific Cre mouse line and tamoxifen induction, the mGFP is only highly expressed in OLs and associated myelin sheaths (Young et al., 2013; McKenzie et al., 2014; Wang et al., 2018). Numerous mGFP positive new myelin sheaths were observed in the motor cortex, corpus callosum, sensory cortex, and hippocampus of 10-months old brains in NG2-CreErt; Tau-mGFP mice after induction at the age of 7 months (Chen L. et al., 2021). New OLs and associated myelin were also observed in superficial cortex of adult and aged brains (Hill et al., 2018; Hughes and Orthmann-Murphy, 2018).

Several studies have demonstrated that active myelinogenesis in adults plays an important role in neurological function, including motor coordination, motor skill learning and memory function, as a concrete form of neuro-function plasticity (McKenzie et al., 2014; Xiao et al., 2016; Pan et al., 2020; Steadman et al., 2020; Wang et al., 2020; Chen L. et al., 2021). Inhibition of new myelin generation directly disrupts neuro-functions in adults. Transcriptional factors Olig2 and myelin regulating factor (Myrf) are known as positive factors to promote OPCs differentiation (Emery et al., 2009; Mei et al., 2013; Yu et al., 2013; Elbaz and Popko, 2019). Newly-formed myelin was significantly decreased in Olig2 or Myrf conditionally knockout mice (McKenzie et al., 2014; Xiao et al., 2016; Pan et al., 2020; Steadman et al., 2020; Wang et al., 2020; Chen L. et al., 2021). Importantly, neuro-function deficits were detected by different types of behavioral tests. For instance, the Olig2 knockout mice had more foot slips in a modified beam-walking test and less crossings in the rehearsal phase of water maze test (Wang et al., 2020; Chen L. et al., 2021). Water maze test and conditional contextual fear test indicated that adult Myrf knockout mice showed deficits in spatial memory consolidation and recalling of remote fear memory (Pan et al., 2020; Steadman et al., 2020). Conditionally deleting Myrf could impair motor skill learning function in adults (McKenzie et al., 2014; Xiao et al.,

2016). In addition, recent studies revealed there was a new oligodendrogenic niche in the adult mouse median eminence, where the OLs differentiation was crucial for perineuronal net remodeling, which is also Myrf dependent (Zilkha-Falb et al., 2020; Kohnke et al., 2021). We speculated that losing the ability to accelerate action potential conduction along corresponding neuronal circuit may play a crucial role, and the underlying mechanisms of myelination in neuro-function plasticity needs further exploration.

AGE-RELATED CHANGES IN WHITE MATTER AND OLIGODENDROGLIAL CELLS

It is widely recognized that white matter alteration in rodents, monkeys and humans was greatly relevant to age-related neuro-functional decline. Critically, myelin pathology even emerges before neuronal change in normal aging animals (Pini et al., 2016; Hase et al., 2018; Nasrabady et al., 2018). Presently, there are large amount of imaging studies in both human and non-human primates showing white matter loss during aging (Tang et al., 1997; Kohama et al., 2012; Liu et al., 2017). Increasing histological studies further revealed the ultra-structural changes of myelin, while at the molecular level, the evidence about oligodendroglial change during aging is still limited.

Age-Related White Matter Changes

Advances in neuroimaging contributes to explore macroscopical and microstructural changes in white matter (Ding et al., 2021; Kolb et al., 2021). Taking advantage of MRI imaging and diffusion tensor imaging (DTI), a variety of age-related white matter changes, including reduced white matter volume, white matter lesions, disrupted white matter integrity, and subsequent cortical disconnection have all been observed in normal aging brains (Caligiuri et al., 2015). It is reported that in humans, white matter volume gradually increases in the first 40 years of life, peaks at around 50 years of age, and then decreases rapidly from 60 years of age onward (Bennett and Madden, 2014; Liu et al., 2016). Even with healthy aging, white matter lesions (also known as leukoaraiosis) are evident as hyperintensities in white matter, which increase with age. The location of white matter lesions in different brain regions is in accordance with distinct types of functional decline. For example, white matter lesions in the frontal lobe, which is believed to be especially vulnerable to age-related white matter changes, are responsible for cognitive impairments. Subcortical white matter lesions are mainly correlated with depression in the elderly whereas periventricular white matter lesions are mainly related to cognitive decline (Bartrés-Faz et al., 2001; Barrick et al., 2010; Bennett and Madden, 2014). Moreover, the severity of white matter hyperintensities is correlated with the cognitive decline extent (Barrick et al., 2010; Cox et al., 2016; Bells et al., 2019). Similarly, degradation of white matter integrity and subsequent cortical disconnection revealed by DTI studies were reported in aging brain and are significantly associated with reduced cognitive function, including memory, executive function and general cognition (Bennett and Madden, 2014; Coelho et al., 2021).

Age-Related Changes of Oligodendrocytes and Associated Myelin

As imaging results normally show a gross alteration of white matter, more and more histological studies are carried out to further confirm the underlying change of OLs and associated myelin sheaths. Both longitudinal live imaging and immunostaining give direct evidence that OL density, myelin segment length and myelin density undergo a steady increase but followed by a gradually obvious decrease (Young et al., 2013; Tripathi et al., 2017; Hill et al., 2018). A recent work in our lab also showed that in layers I–III of the motor cortex, myelin basic protein (MBP) intensity and OL number were significantly increased from 4 months to 13 months, though the pre-existing myelin was decreased by 10%, detected by a transgenic mouse line (PLP-CreErt; mT/mG). But the MBP intensity and OL number decreased steeply at 18 months, while OPCs number was unaltered (Wang et al., 2020). On the other hand, the number of myelin internodes maintained by individual cortical OLs is stable for at least 8 months but declines 12% in the following year (Tripathi et al., 2017). The average length of internodes also decreases with aging (Mukoyama, 1973; Lasiene et al., 2009; Hill et al., 2018).

More importantly, besides the quantity alteration, the structure of myelin also appears abnormal with aging. In aged rats, there is increased splitting of the myelin sheath, myelin balloon formation, and separation from the axon (Sugiyama et al., 2002; Attia et al., 2019). Aged CNS exhibit paranodal pilling that result in reorganization of the cluster of ion channels at the nodes of Ranvier, which would be detrimental to action potential conduction (Hinman et al., 2006; Shepherd et al., 2012). Meanwhile, ultra-structure of myelin also changes during aging. EM studies showed declined myelin thickness, myelin density and myelin fraction in aged marmosets (Phillips et al., 2019). In cingulate bundle and corpus callosum of rhesus monkeys, it was demonstrated that myelin sheath exhibited an increasing frequency of degenerative changes such as dense sheaths, myelin balloons and redundant sheaths during normal aging. Critically, the percentage of degenerative myelin was negatively associated with cognitive performance (Bowley et al., 2010). Although mature OLs and myelin sheath they formed are supposed to be stable, it is unavoidable that myelin debris will present in aging mice (up to 24 months), as electron microscopy images of white matter showed multilamellar myelin fragments in the extracellular space or inside of cells, immunohistochemistry further confirmed those myelin debris located in microglial cells (Safaiyan et al., 2016; Cantuti-Castelvetri and Fitzner, 2018). In live aged mice (910-day-old), formation of myelin spheroids, myelin debris and myelin loss were longitudinally recorded. It turned out that myelin spheroids occurred slowly over weeks, and once formed, some remained for at least a month (Hill et al., 2018).

The above morphological changes all lead to a fact that at least some of the mature OLs in the aging brain is experiencing degeneration. There should be extensive alteration in those OLs, such as their functional protein expression, their metabolism state and their interaction with other glial cells and neurons (Sugiyama et al., 2002; Wang et al., 2014; Tse and Herrup, 2017). However, related evidence is limited. In agree with our perspective, a recent work using transgenic mice revealed that spinal cord myelin MCT1 protein expression had declined by 35% by the age of P360, when mice are considered middle age, corresponding to about 42-year-old in humans (Philips et al., 2021). It is accepted that in later stage of life, the ability of OLs to supply energy to axon will drop intensely, which may participate in broad neuronal functional deficits. As is reported that age-related decreases in lactic acid emerges in the hippocampus in the senescence-accelerated mouse, which might be linked with cognitive impairments (Wang et al., 2014). Meanwhile, the increase in oligodendroglial NMDA receptors and decrease in glial glutamate uptake transporter GLAST1 is found in aging white matter, which is believed to be detrimental to myelin and OLs (Rivera et al., 2016). In addition, the expression of several myelin proteins was shown to change with aging. For example, the absence of the 21.5-kDa isoform of MBP in aged mice or age-related dysregulation of cyclic nucleotide phosphodiesterase (Sugiyama et al., 2002; Sloane et al., 2003; Hinman et al., 2008). The two proteins are vital elements for myelin maintenance. It should also be noted that myelin is composed of ∼70% lipid and ∼30% protein. The decrease in lipid component (especially cholesterol) rather than protein content during aging was believed to be account for the loss of myelin in old monkeys (Baumann and Pham-Dinh, 2001; Sloane et al., 2003; Summarized in **Figure 1**).

Changes in OLs and associated myelin with aging will undoubtedly affect the neuronal transmission efficiency, the energy supply of axons and the vulnerability of axons. Meanwhile, myelin dysfunction during aging will impact on microglial functions and subsequently the micro-environment, which has been recently proved to be an upstream AD risk factor (Depp et al., 2021). Unfortunately, there was no effective strategy to inhibit or delay age-related myelin loss or degeneration up to now.

Age-Related Changes of Oligodendroglia Precursor Cells

Increasing studies reveal that myelinogenesis decreases with normative aging. In the NG2-CreErt; Tau-mGFP mouse line, which could label newly formed myelin, there were abundant

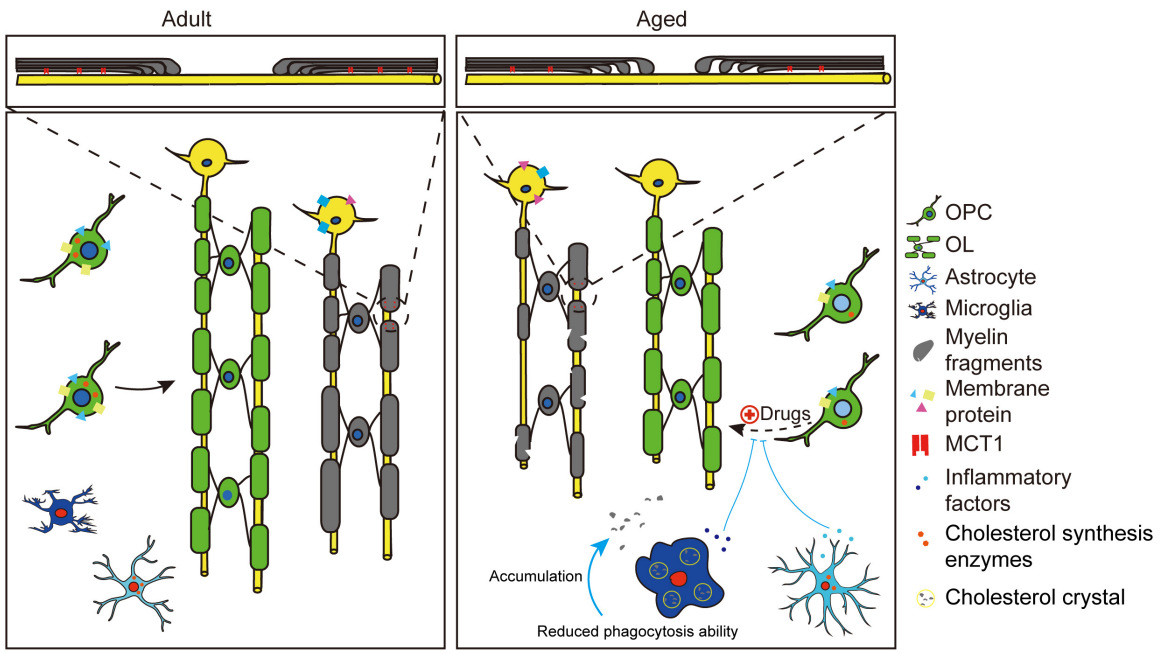

FIGURE 1 | Schematic showing changes of OPCs and OLs during aging. Compared to adults, aged brains showed myelin degeneration and inhibited myelinogenesis. The pre-existing myelin (gray) showed quantitative and qualitative changes during aging. Quantitative alterations include decreased OLs number and internodes, thinner myelin sheaths and shortened internodes. Qualitative changes include myelin debris formation, microstructural changes like paranodal pilling (zoomed area) and altered membrane protein expression (decreased MCT1, decreased GLAST1, and increased NMDA). Inhibited myelinogenesis (green) may be due to the age-related intracellular and extracellular changes of OPCs. Intracellular changes include varied epigenetic regulation (light blue nucleus), decreased cholesterol synthesis enzymes and decreased receptor expression (GPR17, APJ). Extracellular changes include stiffness of extracellular matrix (ECM) and age-related activation of astrocytes and microglia cells. Proinflammatory factors (cytokines, chemokines) released by activated glial cells will inhibit differentiation of adult OPCs. Activated microglia cells are associated with cholesterol crystal formation (see yellow circles) and reduced phagocytic ability, resulting in myelin debris accumulation. Activated astrocytes show decreased cholesterol synthesis enzymes. Note that the inhibited differentiation of OPCs in aged brain could be rejuvenated by drugs like clemastine.

mGFP positive new OLs and myelin sheath in the cortex, corpus callosum and hippocampus of 6 or 8-months old brains. Correspondingly, new OLs and associated myelin was steeply decreased in the 18- or 22-months old brains (Wang et al., 2020). Considering functional importance of myelinogenesis in adults, it was accepted that decreased myelinogenesis played important role in age-related memory function deficit. For instance, the above mentioned 13-months old mice showed spatial learning and memory function decline. Inspiringly, genetical and pharmaceutical interventions are proved to be helpful. Muscarinic receptor 1 (M1R) was a negative regulator for OPCs differentiation, and clemastine was proved to promote myelin development and remyelination through M1R (Mei et al., 2016; Wang et al., 2018). Specially deleting M1R in aging OPCs or clemastine treatment could increase mGFP positive OLs and associated myelin in cortex, corpus callosum and hippocampus in the aged brains. Amazingly, aged M1R knockout and clemastine treated mice showed improved spatial memory function. It seemed that memory function recovery was associated with reversed synaptic loss in hippocampus (Wang et al., 2020). These indicated that promoting myelinogenesis may be a potential strategy to recover age-related functional decline.

It is generally acknowledged that decreased myelinogenesis distributes to inhibited OPCs differentiation or decreased progenitor cells. Recent evidence showed that the total number of OPCs remained stable in the aged brains. For instance, the number of OPCs in 10-year-old human corpus callosum is about $2\sim3 \times 10^8$ and it remains stable even up to 90 years (Yeung et al., 2014). Consistent with this, the number of OPCs didn't have significant change in 18-month-old mouse cortex, comparing to the 4-month-old or 13-month-old mice (Wang et al., 2020). EdU or BrdU incorporation assays showed that NG2 or PDGFαR positive signal was co-expressed with EdU or BrdU during aging, suggesting that OPCs have reserved the ability to proliferate (Lasiene et al., 2009; Young et al., 2013). However, gene expression profile analysis found down-regulation of cell proliferation associated genes in OPCs during aging, accompanied with increased cell cycle time. Results from transgenic reporter mice showed proliferative OPCs in G2/M phase were obviously decreased in the aged brains (Spitzer et al., 2019). Though the proliferation ability of OPCs declined slightly with aging, total number of OPCs were stable. In a word, declined myelinogenesis is not likely caused by decreased OPCs.

Thus, it is widely accepted that decreased myelinogenesis during aging is attributed to declined differentiation capacity of the OPCs (Rivera et al., 2016; Hill et al., 2018; Wang et al., 2020). For instance, OPCs from the aged brains (18 months) differentiate slowly and have slower reaction to pro-differentiation compounds, compared to adult OPCs (2–3 months). Sequencing studies showed that aged OPCs have reduced OPC-specific gene expression and more markers of aging, including but not limited to mitochondrial dysfunction, unfolded protein response (UPR) and autophagy (Neumann et al., 2019a). Evidence from *in vitro* experiments also suggested inhibited differentiation of old OPCs (Neumann et al., 2019a). Moreover, the expression of several receptors which play essential roles in OPC differentiation and myelination are shown to change remarkably during aging, indicating the inhibited maturation ability of adult OPCs (Young et al., 2013; Spitzer et al., 2019). This will be further discussed later.

When demyelination occurred, remyelination contributed to myelin and neuro-function recovery. It was accepted that adult OPCs could migrate from adjacent or subventricular zone, proliferate and differentiate into mature OLs to form myelin (Smith et al., 1981; Deshmukh et al., 2013). Though surviving OLs were found to have the ability for myelin regeneration, it was demonstrated that newly-differentiated OPCs exhibited a much greater capacity for myelin regeneration after demyelination in zebrafish (Neely et al., 2020). In age -related neurodegenerative disease mouse model, including Alzheimer disease (AD) and Huntington's disease, new OLs and associated myelin were remarkably increased in early stages likely to compensate myelin loss in lesions (Jin et al., 2015; Chen J. F. et al., 2021). Intriguingly, newly-formed myelin in AD mice was proven to increase progressively and contributed to functional recovery (Chen J. F. et al., 2021), suggesting that newly-formed myelin was quite stable in neurodegenerative diseases. However, myelin repair is usually not efficient in the site of injury. Especially, increasing evidence showed declined remyelination ability during aging (Sim et al., 2002; Goldschmidt et al., 2009). Multiple sclerosis (MS) is known as a neuroinflammatory and demyelination disease that is characterized by auto-immune mediated demyelination in the CNS, accompanied with secondary axon injury and neuro-function deficits (Franklin and Ffrench-Constant, 2008; Reich et al., 2018). OPCs are present within and around the demyelination lesions in the aged MS model mice, but fail to differentiate and form new myelin, suggesting that in those pathological context, age-related differentiation arrest of OPCs may also be an important cause of remyelination failure (Gilson and Blakemore, 1993; Sim et al., 2002; Goldschmidt et al., 2009; Ruckh et al., 2012). Interestingly, Metformin or LY294002 treatment, or environmental modulation by replacing young macrophages could recover the capacity of OPCs differentiation and remyelination in the aged mice (Ruckh et al., 2012; Neumann et al., 2019a; Rivera et al., 2021).

MECHANISMS OF INHIBITED MYELINOGENESIS DURING AGING

It is reported that myelin showed remarkable homeostatic resilience in adult mice. Recent works also pointed out that new myelin generation is highly active in adult mice, reflecting the strong differentiation ability of OPCs. Why the myelinogenesis ability dropped during aging? Here we summarized possible intrinsic and extrinsic mechanisms.

Intrinsic Factors

Epigenetic Regulation

Increasing evidence suggested that the epigenetic regulators, including histone modifications and DNA methylation, played important roles in myelin development (Shen et al., 2005; Egawa et al., 2019). Histone deacetylases (HDACs) could remove acetyl

groups from histone tails to regulate myelination associated genes expression (Liu et al., 2009; Conway et al., 2012). It was demonstrated that HDAC recruitment was inefficient in old brains in a cuprizone induced demyelination mouse model, compared to the adults, resulting in remyelination failure. Age-dependent HDAC1 recruitment to repressive complexes Hes5 and Sox2 promoters may be the main cause (Shen et al., 2008). Besides, DNA methylation analysis showed that OPCs from 16-months old brains were characterized by global hypomethylation and declined DNA methyltransferases activity, compared to adults (Zhou et al., 2019). These suggested decreased myelinogenesis or failure of remyelination was relative to changes of epigenetic regulation during aging.

Age-Related Protein Change in Oligodendroglia Precursor Cells

A recent study using proteomic analysis of OPCs isolated from the brains of neonatal, young and aged rat revealed that the amount of proteins associated with oxidative phosphorylation, inflammatory responses and actin cytoskeletal organization increased with age, whereas cholesterol-biosynthesis, transcription factors and cell cycle proteins decreased (de la Fuente et al., 2020). Besides, the receptors or ion channel alterations in OPCs in aged brains will directly affect the function of OPCs and their differentiation ability. For instance, genetic-fate mapping showed gradual decrease of GPR17 expression in OPCs in aging cerebrum. Moreover, in this study, GPR17 was believed to be a major factor affected during OL degeneration in the aging brain (Rivera et al., 2021). Apelin receptor (APJ) is a newly identified G-protein-coupled receptor, which could regulate OPCs differentiation through Myrf signal. The expression of APJ was decreased during aging, and level of apelin, the ligand of APJ, was also reduced significantly in the plasma of aged mice. More importantly, APJ activation could promote remyelination in aged mice (Ito et al., 2021). NMDAR-mediated signal play essential roles in myelination. Consistent with declined myelinogenesis, electrophysiological recordings showed remarkable decrease in NMDAR density in OPCs from 300-day old mice, compared to OPCs derived from adults or neonates (Spitzer et al., 2019). Although recent genomic analysis provided plenty of altered molecules in adult OPCs, the associated functional changes during natural aging should be further explored.

Extrinsic Factors

Extracellular Signals

The elements and characters of microenvironment where OPCs lived changed with aging and the environmental changes may played an important role in age-related declined myelinogenesis. Firstly, the characters of microenvironment could regulate OPCs function. Atomic force microscopy showed that the prefrontal cortex progressively stiffened with aging. Seeding the adult OPCs in the aged decellularized brain extracellular matrix led to declined capacity of proliferate and differentiate, and the aged OPCs seeding in the adult brains could partially differentiate into mature OLs, suggesting that character of aged ECM impaired the OPCs function. Piezo1 is known as mechanosensitive ion channel, which could regulate cell density and stem cell activity. Knockdown of Piezo1 could recover the impaired OPCs differentiation in the aged brains (Neumann et al., 2019b; Segel et al., 2019). Secondly, molecules in extracellular matrix, such as hyaluronan and chondroitin sulfate proteoglycans, were negative regulators for effective remyelination in MS patients or EAE brains (Keough et al., 2016; Stephenson et al., 2019). These elements in ECM accumulated with aging, which may play an important role in the declined capacity of differentiation (Richard et al., 2018; Macke et al., 2020). It should be noted that neural stem cells in the subventricular zone (SVZ) also participate in myelin repair (Menn et al., 2006), while the output ability of those stem cells is similarly affected with the aging of SVZ niche (Luo et al., 2006; Bouab et al., 2011).

Age-Related Changes in Microglia and Astrocytes

Other glial cells, astrocytes and microglia, were known as regulators in myelination (Stadelmann et al., 2019). Age-related changes in astrocytes and microglia may play crucial roles in declined myelination/remyelination in the aged.

In the aging brain and in pathological conditions, microglia played an important role in clearing myelin pieces (Cignarella et al., 2020). In addition, microglia-derived transglutaminase-2 signals to GPR56 on OPCs could promote remyelination in murine models of demyelination (Giera et al., 2018). A recent study found that sterol synthesis in microglia/macrophages could resolve inflammation, which is essential for myelin repair (Berghoff et al., 2021). In aged mice, total number and density of microglia increased in various regions of the brain (Poliani et al., 2015). Myelin fragmentation increased with age and led to the formation of insoluble inclusions in microglia (Streit et al., 2004; Safaiyan et al., 2016; Rawji et al., 2018), accumulation of myelin debris in aged phagocytes led to cholesterol clearance deficits (Cantuti-Castelvetri and Fitzner, 2018). Moreover, those lipid droplet-accumulating microglia in the aging brain will produce high levels of reactive oxygen species, and secrete pro-inflammatory cytokines (Marschallinger et al., 2019). Single cell sequencing studies found age-associated microglial cells were characterized by activation of genes implicated in phagocytic activity and lipid metabolism in mice or genes involved in cell adhesion in humans (Galatro et al., 2017; Safaiyan et al., 2021). Those above changes of microglia during aging were demonstrated to play an important role in age-related declined myelin regeneration. More importantly, rejuvenating microglia in aged brains helps to myelin regeneration in injured brains in the aged mice (Ruckh et al., 2012; Cantuti-Castelvetri and Fitzner, 2018).

Astrocytes could secret many factors such as PDGF-A and FGF2 to facilitate OPCs proliferation and differentiation during myelin development (Rawji et al., 2020). In demyelinating diseases, astrocytes respond quickly by upregulating several proinflammatory cytokines, chemokines, as well as remyelination-signaling molecules (Williams et al., 2007). A recent study revealed that reactive astrocytes could be induced by inflammatory microglia cells and cause the death of neurons and OLs through producing saturated lipids (Liddelow et al., 2017; Guttenplan et al., 2021). In addition, astrocytes may

also play a role in recruiting phagocytic microglia in areas of demyelination (Skripuletz et al., 2013). Aging astrocytes appear more reactive, displaying an upregulation in cytoskeletal proteins and hypertrophic cell bodies with shorter processes (Cerbai et al., 2012; Jyothi et al., 2015; Robillard et al., 2016). Consistent with morphological changes, RNA sequencing reveals that aging astrocytes also showed upregulation of reaction related genes, turning into an inflammatory state. Moreover, aging astrocytes were found to have a decrease in transcripts encoding cholesterol synthesis enzymes, which may induce a deficiency for myelin synthesis substrate (Boisvert et al., 2018; Clarke et al., 2018). These changes may be detrimental to OPC differentiation and myelin formation in the aged brain. Besides, other factors, including oxidative stress induced by vascular changes during aging and deregulation of glutamate neurotransmission in the aged brains may also play roles in age-related myelinogenesis decline (Rivera et al., 2016; Bagi et al., 2018; Bors et al., 2018; Sams, 2021; Summarized in **Figure 1**).

Perspectives

Age-related neurofunction decline may negatively impact the daily life for the elderly, and no effective strategies are available so far in clinic. As mentioned above, this present review mainly focuses on myelin degeneration, decreased myelinogenesis during aging and the possible mechanisms. Admittedly, there are a lot of questions remain unanswered. For instance, whether there is spatial- or temporal-differences in the degeneration process in the CNS? What is the deciding point for one OL or one myelin segment to initiate degenerate and could we inhibit this bad process through modulating one key factor? Whether the newly generated myelin is more stable compared to pre-existed myelin in aged brain and if this is the case, we may find some clues about repressing myelin degeneration in the aged. The decreased myelinogenesis during aging is likely a result of arrested OPCs differentiation, thus it is plausible that promoting adult OPCs maturation may be a feasible and realistic approach to improve age-related neuronal function decline for the elderly. Meanwhile, rejuvenating the SVZ stem cells may also help with myelinogenesis ability in the aged. More efforts are needed to further confirm those effects in human.

Moreover, oligodendroglial lineage cells display more than differentiation and forming new myelin sheaths. For example, OPCs may form synaptic connection with neighboring neurons, and that regulates neuronal signal in CNS. In addition, the expression of connexin channel proteins in oligodendroglial lineage cells is an intriguing feature and the connexins could function either as hemichannels or gap junctions. The gap junction enables OLs to be connected as a glial network with astrocytes, allowing transporting small molecules such as calcium and energy metabolites, which may be important for the homeostasis of the CNS. Recent studies even showed that OPCs could exert immunomodulatory functions, which are particularly relevant in the context of neurodegeneration and demyelinating diseases. Besides, OLs are found to be heterogenetic in the mouse juvenile and adult CNS (Marques et al., 2016; Chamling et al., 2021), the response of different subtypes to aging remains unknown. It is not clear whether the functions mentioned above and their correspondent molecules are altered during aging. Future works are needed to give us a more comprehensive understanding of the role oligodendroglial lineage cells played in aged brains, which could shed light on the clinical therapeutic strategies considering age-related neuronal functional diseases.

AUTHOR CONTRIBUTIONS

XZ prepared the manuscript. NH prepared the figure and revised the manuscript for grammatic typos. LX provided views and revised the manuscript. FW and TL designed the framework of the manuscript and prepared and finalized the manuscript. All authors agreed to approved the final manuscript.

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Molecular Insight Into the Therapeutic Potential of Long Non-coding RNA-Associated Competing Endogenous RNA Axes in Alzheimer's Disease: A Systematic Scoping Review

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Alzheimer's disease (AD) is a heterogeneous degenerative brain disorder with a rising prevalence worldwide. The two hallmarks that characterize the AD pathophysiology are amyloid plaques, generated via aggregated amyloid β, and neurofibrillary tangle, generated via accumulated phosphorylated tau. At the post-transcriptional and transcriptional levels, the regulatory functions of non-coding RNAs, in particular long non-coding RNAs (lncRNAs), have been ascertained in gene expressions. It is noteworthy that a number of lncRNAs feature a prevalent role in their potential of regulating gene expression through modulation of microRNAs via a process called the mechanism of competing endogenous RNA (ceRNA). Given the multifactorial nature of ceRNA interaction networks, they might be advantageous in complex disorders (e.g., AD) investigations at the therapeutic targets level. We carried out scoping review in this research to analyze validated loops of ceRNA in AD and focus on ceRNA axes associated with lncRNA. This scoping review was performed according to a six-stage methodology structure and PRISMA guideline. A systematic search of seven databases was conducted to find eligible articles prior to July 2021. Two reviewers independently performed publications screening and data extraction, and quantitative and qualitative analyses were conducted. Fourteen articles were identified that fulfill the inclusion criteria. Studies with different designs reported nine lncRNAs that were experimentally validated to act as ceRNA in AD in human-related studies, including *BACE1-AS*, *SNHG1*, *RPPH1*, *NEAT1*, *LINC00094*, *SOX21-AS1*, *LINC00507*, *MAGI2-AS3*, and *LINC01311*. The *BACE1-AS/BACE1* was the most frequent ceRNA

pair. Among miRNAs, *miR-107* played a key role by regulating three different loops. Understanding the various aspects of this regulatory mechanism can help elucidate the unknown etiology of AD and provide new molecular targets for use in therapeutic and clinical applications.

Keywords: Alzheimer's disease, antisense oligonucleotides, competing endogenous RNA, long non-coding RNA, miRNA sponge

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder (NDD) and is a form of dementia that triggers difficulties with memory, thinking, and behavior (Kang et al., 2020). Based on the Alzheimer's Association, AD makes up about approximately 60–80% of dementia cases. Currently, worldwide, 50 million people are living with AD and other dementias. AD incidence doubles every 5 years after age 65 (Fan et al., 2020). Symptoms generally develop slowly and aggravate with time. From a hereditary point of view, AD is a heterogeneous polygenic condition. The condition has been categorized into two groups according to age-onset: early-onset AD (EOAD) and late-onset AD (LOAD). LOAD also called sporadic AD (SAD), is the most common type of dementia. AD is a multifactorial condition as a result of interactions between the susceptible genes and environmental factors (Rezazadeh et al., 2019). Genes play an important role in AD. The heritability of LOAD is 58–79%, while it is more than 90% in EOAD. The genetic association has helped us to understand the etiology of AD. Over 50 loci are currently associated with AD. These findings highly suggest that AD is a complex disease (Sims et al., 2020).

Alzheimer's Disease Pathogenesis

The pathophysiology of AD is defined by the accumulation of β-amyloid peptide (Aβ) in the brain, as well as hyperphosphorylated and cleaved structures of the microtubule-associated protein tau. It is known that metabolic dysfunction of Aβ precursor protein (APP) and abnormal tau protein phosphorylation (Kang et al., 2020) or maybe their interaction with each other (Busche and Hyman, 2020) results in senile plaques and neurofibrillary tangles (NFTs) formation. According to biochemical, behavioral, and genetic research, the pathologic development of the neurotoxic Aβ peptide resulting from serial APP proteolysis is a critical step in AD development. Moreover, APP is metabolized rapidly and in a highly complicated manner by groups of sequential secretases, including β-site APP-cleaving enzyme 1 (BACE1), γ-secretase, and the ADAM family as α-secretases. Regarding tau proteolysis, this process is crucial in neurodegeneration and the tau aggregation process. Tau is a microtubule-associated protein that is predominantly produced in neurons and is encoded by the Microtubule-Associated Protein Tau (*MAPT*) gene. Intracellular tau is sometimes hyperphosphorylated, resulting in hazardous oligomers and aggregates visible as NFTs (Kang et al., 2020). Aside from the tau and amyloid theory about AD, several additional ideas have been proposed, namely inflammatory reactions, oxidative stress, mitochondrial failure, and cholinergic hypothesis (Li et al., 2021). Although there are numerous hypotheses about AD pathogenesis, the actual triggers and ideal therapy strategies are still elusive.

Antisense Therapeutics

For therapeutic development, RNA is a novel target with numerous advantages, including: (Kang et al., 2020) it is applicable to a majority of RNAs in the cells, such as non-coding RNAs (ncRNAs), (Fan et al., 2020) genetic discoveries can directly be translated to drug discovery, and drug discovery would be more rapid and efficient. Whereas other promising progress has been made in the discovery of small-molecule medicines that affect RNA activity, antisense oligonucleotides (ASOs) provide a far straightforward technique (Bennett et al., 2019). ASOs are synthetic oligonucleotides or oligonucleotide analogs with lengths ranging from 12 to 30 nucleotides (nt) and are engineered for binding to RNA through Watson-Crick base pairing. ASOs can be constructed to bind to both protein-coding RNAs (mRNAs) and ncRNAs, including microRNAs (miRNAs) or long non-coding RNAs (lncRNAs). After ASOs bind with the RNAs of interest, antisense medicine can alter the activity of the RNAs in different ways (Bennett and Swayze, 2010; Crooke et al., 2018).

Long Non-coding RNAs

NcRNAs are classified into two types based on their lengths: short ncRNAs (less than 200 nt) and long ncRNAs (more than 200 nt). lncRNAs range in size from 200 nt to more than 100 kb and often lack a clear open reading frame. LncRNAs, resembling protein-coding genes, are widely transcribed by RNA polymerase II and are frequently post-transcriptionally changed by 5′ capping, 3′ polyadenylation, and RNA splicing processes. However, lncRNAs differ from protein-coding genes in that they possess shorter lengths and exhibit poorer sequence conservation among species (Quinn and Chang, 2016). Many biological functions rely on lncRNAs, including RNA transcription, translation, chromatin and DNA modifications, mRNA stability, and pre-RNA splicing (Li et al., 2021).

Long Non-coding RNAs and mRNA Stability

The rate of mRNA synthesis and degradation determines its steady-state level; hence, the degradation of mRNA is an important factor in controlling gene expression. Previous research has shown that instability of mRNAs that encode

synaptic transmission proteins leads to synaptic function loss in AD pathogenesis (Alkallas et al., 2017). Furthermore, in AD patients, the mRNA degradation rate of AD risk genes is abnormal (Beyer et al., 2009). It has been shown that lncRNAs have a role in AD pathogenesis via regulating mRNA stability (Li et al., 2021).

Competing Endogenous RNA Hypothesis

A new mechanism of interaction between RNAs, called ceRNA, is proposed by Pier Paolo Pandolfi's group in 2011. This hypothesis suggests that cross-talk between RNAs, both coding RNAs and ncRNAs (such as lncRNAs, circRNAs: circular RNAs, and pseudogenes) through miRNA complementary sequences called miRNA response elements (MREs) builds a large-scale regulatory network throughout the transcriptome. Based on the ceRNA hypothesis, if two RNA transcripts regulate each other via a ceRNA mediated mechanism, the expression levels of these two RNA transcripts would be negatively correlated with the levels of target miRNAs, and the expression levels of these two RNA transcripts would be positively associated with each other (Salmena et al., 2011). **Figure 1** demonstrates the most simplified ceRNA model. Studies of ceRNA interactions are according to the prediction of target RNA transcripts using multiple software programs (Shuwen et al., 2018) such as StarBase (Li et al., 2014), TargetScan (Agarwal et al., 2015), PicTar (Krek et al., 2005), and StarScan (Liu et al., 2015). These predictions are based on identifying the same MRE within multiple RNA sequences. As the precision of prediction programs is unclear (Amirkhah et al., 2015) due to insufficient raw data for algorithms to reference, the prediction results must still be validated (Shuwen et al., 2018).

Over the last few years, several studies have verified the ceRNA theory. It is well known that the disruption of the equilibrium of ceRNA cross-talk can play a role in various diseases (Sen et al., 2014). So far, the ceRNA mechanisms have been further studied in the field of cancer (Yang et al., 2016). During the last 4 years, the study in the field of NDDs has begun systematically, and significant improvements have been produced. Among NDDs, the ceRNA interactions in AD have been studied to a greater extent (Cai and Wan, 2018). Since 2017, there have been increasing studies to identify the genome-wide ceRNA networks in AD using bioinformatics prediction (Wang et al., 2017; Zhang et al., 2017; Sekar et al., 2018; Wang Z. et al., 2018; Yu et al., 2018; Ma N. et al., 2019; Zhang Y. et al., 2019; Zhou et al., 2019; Ma et al., 2020; Xu and Jia, 2020). Since interaction networks of ceRNAs are multifactorial, they might be advantageous in investigations of complicated diseases, like AD, in terms of therapeutic targets only by targeting one of them, the levels of several disease-related RNAs change at once (Moreno-García et al., 2020).

Aim of Study

In this study, we performed a scoping review to analyze validated ceRNA loops in AD. Our focus was on lncRNA-associated ceRNA axes that underlie AD pathophysiology and could potentially be therapeutic targets.

METHODS

The strategy for this review was according to the scoping review structure recommended by Arksey and O'Malley (2005) and later improved by Levac et al. (2010). This consists of five distinct steps: (1) identifying the research question, (2) identifying relevant studies, (3) study selection, (4) charting the data, and (5) collating, summarizing, and reporting results. An optional sixth step in the scoping review, consultation, was not used in our study. This review was also well guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) Checklist (Tricco et al., 2018).

Identifying the Research Question

This study focused on mapping the current literature on lncRNA-associated ceRNA loops in AD. To address this aim, we sought to answer the following question: Precisely what is known from existing literature about lncRNA-associated ceRNA regulatory axes in AD?

Identifying Relevant Studies

An initial limited search of PubMed and Embase was performed, and then the keywords in the title and abstract were assessed, as well as the index terms that were utilized in the articles. A second search was performed across PubMed, Embase, Scopus, Web of Science, and Cochrane databases according to specific search tips of each database without any restriction, using keywords, MeSH, or Emtree terms recognized from the primary search. The search strategies for PubMed and Embase are shown in Appendix I. Additionally, searches were also carried out in two gray (i.e., difficult to locate or unpublished) literature databases: Google Scholar and ProQuest. The last search was performed on July 10, 2021. We also examined the reference lists of the relevant literature and review articles for additional sources.

Selecting Studies

The included studies fulfilled the following criteria: (1) explicitly discussing the lncRNA-associated ceRNA axes in AD, (2) written in English, and (3) be original research. The exclusion criteria were (1) studies of non-AD or unspecified dementia, (2) studies that did not use human specimens or cell lines, and (3) studies that did not use molecular techniques to validate the components of the ceRNA loop. The title and abstract of articles were first independently screened by three reviewers (HS, NA) for eligibility according to the above criteria. The full texts of the remaining articles were evaluated, and articles going to fulfill the eligibility criteria were included in the final data analysis. Any disagreements were solved through discussion or with a third reviewer (MR) if required.

Charting the Data

Three reviewers (HS and NA) independently extracted data into a predesigned charting form in Microsoft Excel. It provided details about the first author, year of publication, origin, type of study, cell line(s), human samples, methods, ceRNAs, shared miRNA(s), and key findings.

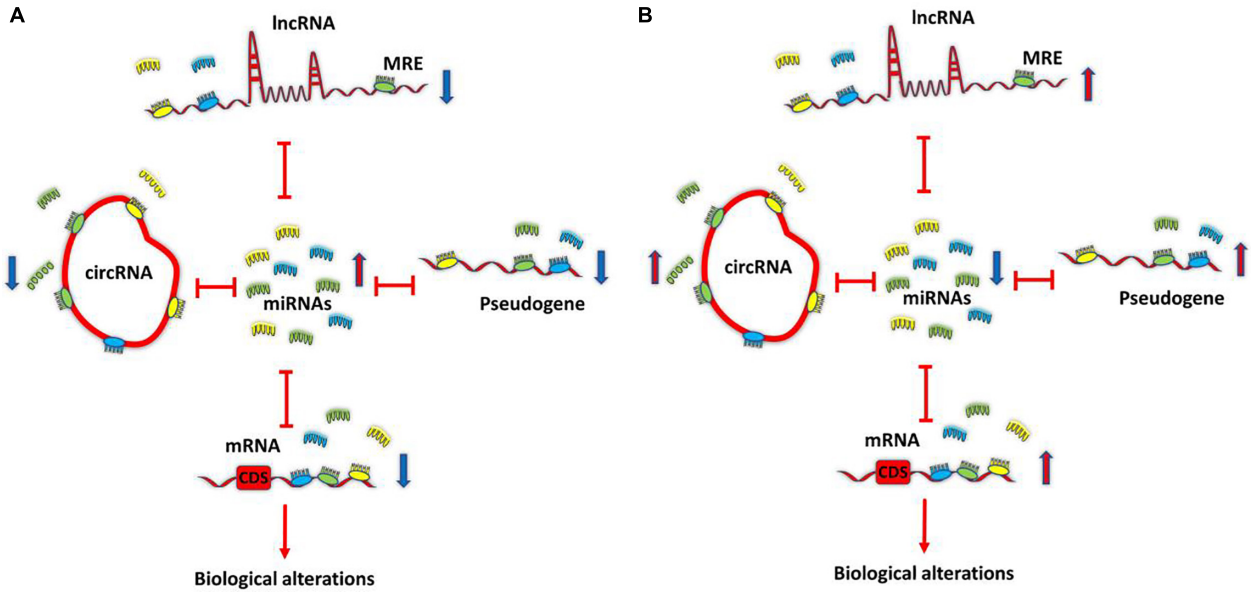

FIGURE 1 | Competing endogenous RNA (ceRNA) model. All transcriptome components, such as long non-coding RNAs (lncRNAs), circRNAs, pseudogenes, and mRNAs that share common MRE, can function as ceRNAs and can co-regulate each other by sponging shared miRNAs. Differentially expressed transcripts can lead to ceRNA dysregulation and biological alterations. ceRNA. **(A)** Down-regulation of ceRNAs increases the amounts of free miRNAs, thereby repressing target expression. **(B)** Conversely, up-regulation of ceRNAs reduces free miRNAs abundance, thereby derepressing target expression. Competing endogenous RNA; circRNA, circular RNA; lncRNA, long non-coding RNA; miRNA, microRNA; MRE, miRNA response element.

Collating, Summarizing, and Reporting the Results

We performed quantitative and qualitative analyses. For the quantitative part, we provided a descriptive numerical summary of the characteristics of the included articles. For the qualitative analysis, we provided a narrative review of the existing information addressing our earlier mentioned research question, focusing on the importance of results in the broader framework as suggested by Levac et al. (2010).

RESULTS

Search Results

The different steps of finding eligible studies are shown in the flow chart in **Figure 2**. A total of 395 articles were identified from different sources, of which 208 were duplicates. One hundred and fifty-three articles were excluded for irrelevance. The full texts of the remaining 34 articles were evaluated, and 20 more articles were also excluded because they did not validate the components of the ceRNA loop using molecular techniques (Wang et al., 2017; Yu et al., 2018; Zhou et al., 2019; Ma et al., 2020; Xu and Jia, 2020; Huaying et al., 2021; Lu et al., 2021; Ou et al., 2021; Tang et al., 2021; Zhang et al., 2021) and did not use human specimens or cell lines (Jiang et al., 2016; Cai et al., 2017; Wang J. et al., 2018; Wang X. et al., 2018; Ma P. et al., 2019; Zhao et al., 2019; Li L. et al., 2020; Li X. et al., 2020; Yue et al., 2020; Zhou et al., 2020). Lastly, a total of 14 eligible articles remained (Faghihi et al., 2010; Ke et al., 2019; Wang et al., 2019; Zeng et al., 2019; Zhu et al., 2019; Gao et al., 2020; Ge et al., 2020; Gu et al., 2020, 2021; He W. et al., 2020; Yan et al., 2020; Yue et al., 2020; Fan et al., 2021; Zhang and Wang, 2021).

Study Characteristics

The characteristics of the included studies are summarized in **Table 1**. Almost all studies have been published since 2019. All but one study (Faghihi et al., 2010) was conducted in China. Eleven studies examined the ceRNA regulatory loop on human cell lines (Ke et al., 2019; Wang et al., 2019; Zeng et al., 2019; Zhu et al., 2019; Gao et al., 2020; Ge et al., 2020; Gu et al., 2020, 2021; Yan et al., 2020; Yue et al., 2020; Fan et al., 2021) and three on both human specimens and cell lines (Faghihi et al., 2010; He W. et al., 2020; Zhang and Wang, 2021). All studies with human specimens had a case-control design. One study used brain samples (Faghihi et al., 2010), one used plasma samples (He W. et al., 2020), and one used serum samples (Zhang and Wang, 2021).

Bioinformatics analysis was used to identify the potential ceRNA interactions. Also, different molecular techniques were utilized to validate the components of the ceRNA loop and investigate their involvement in AD pathogenesis. The reported loops are shown in **Figure 3**. The lncRNA BACE1-antisense (*BACE1-AS*) was reported in four studies (Faghihi et al., 2010; Zeng et al., 2019; Ge et al., 2020; He W. et al., 2020), two of which had *BACE1-AS*/*miR-485-5p*/*BACE1* regulatory axis (Faghihi et al., 2010; Zeng et al., 2019), as well as *BACE1* was a target mRNA in three studies (Faghihi et al., 2010; Zeng et al., 2019; Zhang and Wang, 2021). The lncRNA small nucleolar RNA host gene 1 (*SNHG1*) (Wang et al., 2019; Gao et al., 2020) and the

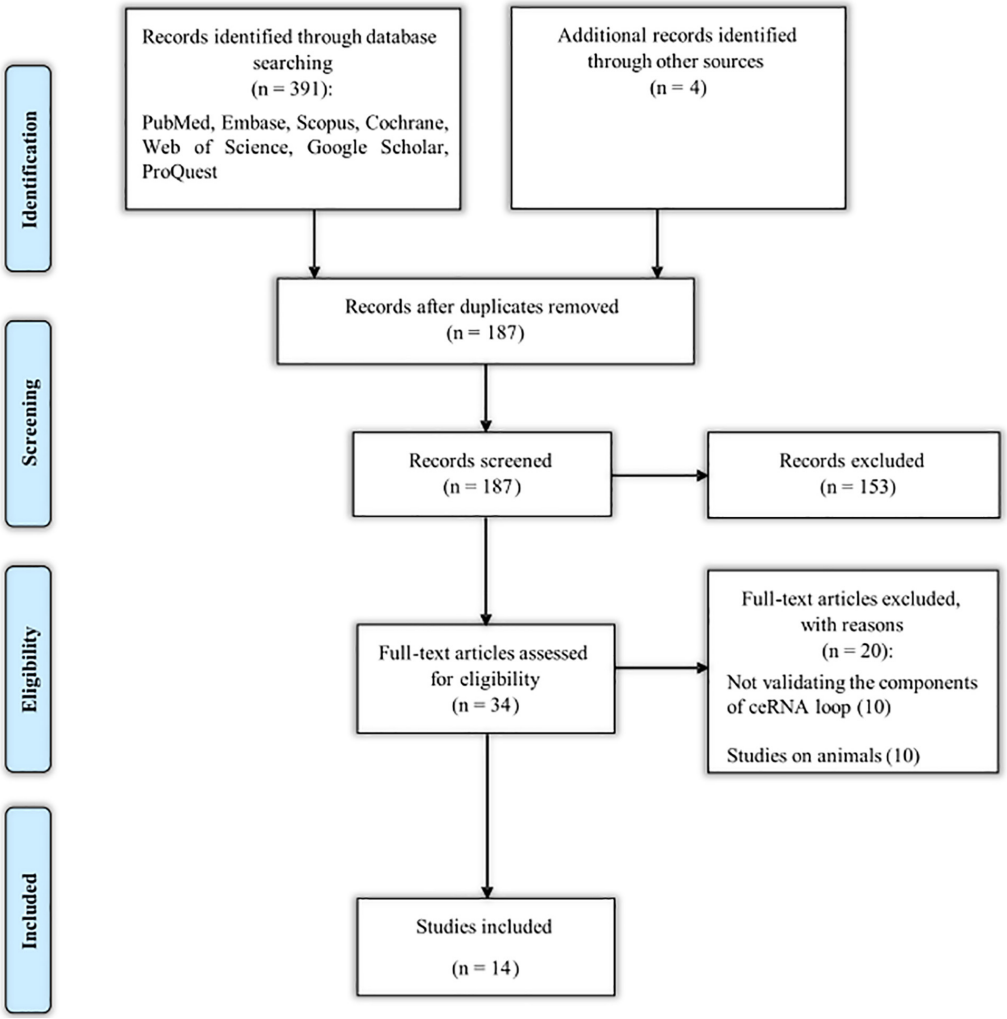

FIGURE 2 | Search strategy flow chart based on the PRISMA flow diagram.

lncRNA ribonuclease P RNA component H1 (*RPPH1*) (Gu et al., 2020, 2021) were each reported in two articles. The remaining ceRNAs were reported once each in six studies (Ke et al., 2019; Zhu et al., 2019; Yan et al., 2020; Yue et al., 2020; Fan et al., 2021; Zhang and Wang, 2021). Among the identified miRNAs, *miR-107* was reported in three regulatory axes in three different studies (Ke et al., 2019; Zeng et al., 2019; Yue et al., 2020). According to these results, we found that three independent ceRNA interaction loops (loop A, loop B, and loop C) are regulated by *miR-107*. Loop A includes nuclear enriched abundant transcript 1 (*NEAT1*) and targets of *miR-107*, loop B includes *SOX21* antisense RNA1 (*SOX21-AS1*) and targets of *miR-107*, loop C includes *BACE1-AS* and *BACE1* (**Figure 4**).

DISCUSSION

The most contentious issue with ceRNA regulation is whether or not it is effective in physiological contexts. Most studies questioning ceRNA functions reach the conclusion that regulation of ceRNA is highly improbable to yield biologically major impacts at physiological RNA concentrations; however, these experiments cannot rule out the potential of potent miRNA sponges or the marked downregulation or upregulation of ceRNAs and miRNAs at particular developmental stages or subcellular sites (Denzler et al., 2014; Cai and Wan, 2018). There are many reports indicating that ceRNA machinery operates in numerous diseases and that ceRNA is expressed differently with various tissues, cells, and subcellular situations. The literature represents novel visions by which designing ceRNA-mechanism-based therapeutical utilization can be facilitated for the manipulation of special developmental phases and disease pathogenicity with the use of synthesized oligonucleotides specific to sequences (Khvorova and Wolfson, 2012). Over the last decade, significant effort has been put toward the clinical use of RNA-based therapies, mainly using antisense oligonucleotides as well as small interfering RNAs, with many obtaining Food and Drug Administration (FDA)

TABLE 1 | Characteristics of studies included in the scoping review.

First author	Year of publication	Origin	Type of study	Cell line(s)	Human samples	Methods	ceRNAs	Shared miRNA(s)	Key findings	References
Faghihi et al.	2010	United States	Case-control, cell culture	HEK293T	Brain	Bioinformatics analysis, RT-PCR, high-throughput sequencing, enzyme complementation assay, luciferase activity assay	*BACE1-AS* and *BACE1*	*miR-485-5p*	*BACE1-AS* and to a lower level *BACE1* were up-regulated, and the expression of *miR-485-5p* was decreased in AD patients	Faghihi et al., 2010
Ke et al.	2019	China	Cell culture	SH-SY5Y, SK-N-SH, HEK293T	–	Bioinformatics analysis, qRT-PCR, cell viability assay, immunocytochemistry, cell apoptosis assay, western blot, luciferase activity assay, RIP assay	*NEAT1* and targets of *miR-107*	*miR-107*	*NEAT1* had been up-regulated in Aβ-treated cell lines. It was pointed out as a sponge of *miR-107*. Knockdown of *NEAT1* attenuated Aβ induced inhibition of viability and promotion of apoptosis and p-tau levels. *miR-107* was down-regulated, and it reversed Aβ-induced injury when overexpressed in Aβ-treated cells.	Ke et al., 2019
Wang et al.	2019	China	Cell culture	SH-SY5Y, HEK293T	–	Bioinformatics analysis, RNA interference, qRT-PCR, western blot, MTT assay, flow cytometry, MMP assay, caspase-3 activity assay, luciferase reporter assay	*SNHG1* and *KREMEN1*	*miR-137*	Neuronal cell damage caused by Aβ increased the expression of *SNHG1*. *SNHG1* acted as a sponge for *miR-137*, and the knockdown of *SNHG1* applied its neuronal protective effects through inhibiting *KREMEN1*.	Wang et al., 2019
Zeng et al.	2019	China	Cell culture	HEK293 T, SH-SY5Y, U251	–	Bioinformatics analysis, RIP assay, western blot, real-time PCR, RNA interference, dual-luciferase assay	*BACE1-AS* and *BACE1*	*miR-29b-3p/miR-107/miR-124-3p/miR-485-5p/miR-761*	The overexpression of *BACE1-AS* repressed the miRNAs that target *BACE1* and increased Aβ levels. Knockdown of *BACE1-AS* increased the expressions of these miRNAs and reduced the expression of *BACE1*.	Zeng et al., 2019
Zhu et al.	2019	China	Cell culture	hCMEC/D3, HEK293T	–	Bioinformatics analysis, real-time PCR, microarrays, TEER assays, western blot, immunofluorescence assays, luciferase reporter assay, RIP assay	*LINC00094* and *SH3GL2*	*miR-224-5p/miR-497-5p*	*LINC00094* was dramatically increased in the Aβ-incubated BBB model. *LINC00094* reduction inhibited the expression of *SH3GL2* through up-regulation of *miR-224-5p/miR-497-5p* and finally resulted in alleviated permeability of BBB in the AD microenvironment.	Zhu et al., 2019
Gao et al.	2020	China	Cell culture	SK-N-SH, CHP 212	–	Bioinformatics analysis, CCK8 assay, qRT-PCR, flow cytometry, western blot, ELISA, RNA interference, dual-luciferase reporter assay, RIP assay	*SNHG1* and *ZNF217*	*miR-361-3p*	*SNHG1* expression was positively regulated by Aβ and negatively regulated by resveratrol. It sponged *miR-361-3p* and promoted cell injury via *SNHG1/miR-361-3p/ZNF217* axis. *SNHG1* knockdown could reverse the promotion effect of Aβ on cell injury.	Gao et al., 2020
He et al.	2020	China	Case-control, cell culture	SK-N-SH, SK-N-AS	Plasma	Bioinformatics analysis, RNA interference, qRT-PCR, cell proliferation assay, flow cytometry, western blot, dual-luciferase reporter assay	*BACE1-AS* and targets of *miR-214-3p*	*miR-214-3p*	Up-regulation of *BACE1-AS* and down-regulation of *miR-214-3p* was found in the cell models treated with Aβ and isoflurane, as well as plasma samples of AD patients. *BACE1-AS* sponged *miR-214-3p* and exacerbated isoflurane-induced neurotoxicity. Both BACE1-AS reduction and miR-214-3p up-regulation reversed the suppression of proliferation and the facilitation of apoptosis and autophagy of Aβ-treated induced by isoflurane.	He W. et al., 2020

(Continued)

TABLE 1 | (Continued)

First author	Year of publication	Origin	Type of study	Cell line(s)	Human samples	Methods	ceRNAs	Shared miRNA(s)	Key findings	References
Xu et al.	2020	China	Cell culture	SH-SY5Y, SK-N-SH	–	Bioinformatics analysis, RNA interference, qRT-PCR, cell viability assay, flow cytometry, western blot, dual-luciferase reporter assay, RIP assay	*SOX21-AS1* and targets of *miR-107*	*miR-107*	Up-regulation of *SOX21-AS1* and down-regulation of *miR-107* were seen in Aβ-treated cell models. *SOX21-AS1* sponged *miR-107*, and its silencing reduced Aβ-induced neuronal damage.	Yue et al., 2020
Yan et al.	2020	China	Cell culture	SH-SY5Y	–	Bioinformatics analysis, RNA interference, qRT-PCR, western blot, FISH, luciferase reporter assay	*LINC00507* and *MAPT/TTBK1*	*miR-181c-5p*	The Up-regulation of *LINC00507* was seen in the AD model. *LINC00507* sponged *miR-330-5p* and caused the up-regulation of *MAPT/TTBK1*. This axis regulates tau hyperphosphorylation via P25/P35/GSK3β signaling pathway.	Yan et al., 2020
Gu et al.	2020	China	Cell culture	SK-N-SH	–	MTT assay, flow cytometry, caspase-3 activity, qRT-PCR, western blot, dual-luciferase reporter assay	*RPPH1* and *WNT1*	*miR-122*	Over-expressed *RPPH1* activated Wnt/β-catenin signaling to ameliorate amyloid-β induced neuronal apoptosis through direct miR-122.	Gu et al., 2020
Ge et al.	2020	China	Cell culture	HPN, SK-N-SH, HEK297T	–	Bioinformatics analysis, MTT assay, cytotoxicity assay, apoptosis assay, western blot, qRT-PCR, RNA interference, dual-luciferase reporter assay	*BACE1-AS* and targets of *miR-132-3p*	*miR-132-3p*	The combined therapy of Berberine treatment with *BACE1-AS* depletion protected neuronal cells against Aβ25–35 through the *BACE1-AS/miR-132-3p* axis.	Ge et al., 2020
Gu et al.	2021	China	Cell culture	SH-SY5Y	–	MTT assay, qRT-PCR, fluo-4 NW calcium assay, apoptosis assay, western blot, dual-luciferase reporter assay	*RPPH1* and *PKM2*	*miR-326*	*RPPH1* directly targeted *miR-326*. Thereby its inhibitory impact on the expression of *PKM2* was counteracted, helping to attenuate endoplasmic reticulum stress and apoptosis caused by Ab25-35.	Gu et al., 2021
Zhang and Wang	2021	China	Case-control, cell culture	SH-SY5Y, BV2, HEK293	Serum	RNA interference, dual-luciferase reporter assay, qRT-PCR, MTT assay, ELISA	*MAGI2-AS3* and *BACE1*	*miR-374b-5p*	The *MAGI2-AS3* expression was increased, and *miR-374b-5p* expression was declined in cell models exposed to Aβ25–35. The *miR-374b-5p* and *MAGI2-AS3* serum levels in patients with AD showed a negative correlation; however, they were correlated with the severity of the disorder. *MAGI2-AS3* reduction enhanced neuronal viability and attenuated neuroinflammation in AD. The neurotoxicity induced by Aβ is regulated by *MAGI2-AS3/miR-374b-5p* axis.	Zhang and Wang, 2021
Fan et al.	2021	China	Cell culture	SH-SY5Y	–	Bioinformatics analysis, qRT-PCR, RNA interference, apoptosis assay, proliferation assay, autophagy assay, endogenous APP assay, dual-luciferase reporter assay	*LINC01311* and targets of *miR-146a-5p*	*miR-146a-5p*	*LINC01311* was downregulated, whereas miR-146a-5p was up-regulated in the AD cell model. *LINC01311* up-regulation and *hsa-miR-146a-5p* downregulation led to the protection of the apoptosis induced by AB1-42, autophagy, decelerated proliferation, and accumulation of APP in cell models of AD.	Fan et al., 2021

Aβ, amyloid-beta; BBB, blood–brain barrier; AD, Alzheimer's disease; CCK8, cell counting kit 8; ceRNA, competing endogenous RNA; circRNA, circular RNA; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence in situ hybridization; lncRNA, long non-coding RNA; miRNA, microRNA; MMP, mitochondrial membrane potential; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RIP, RNA immunoprecipitation; RT-PCR, reverse transcription-polymerase chain reaction.

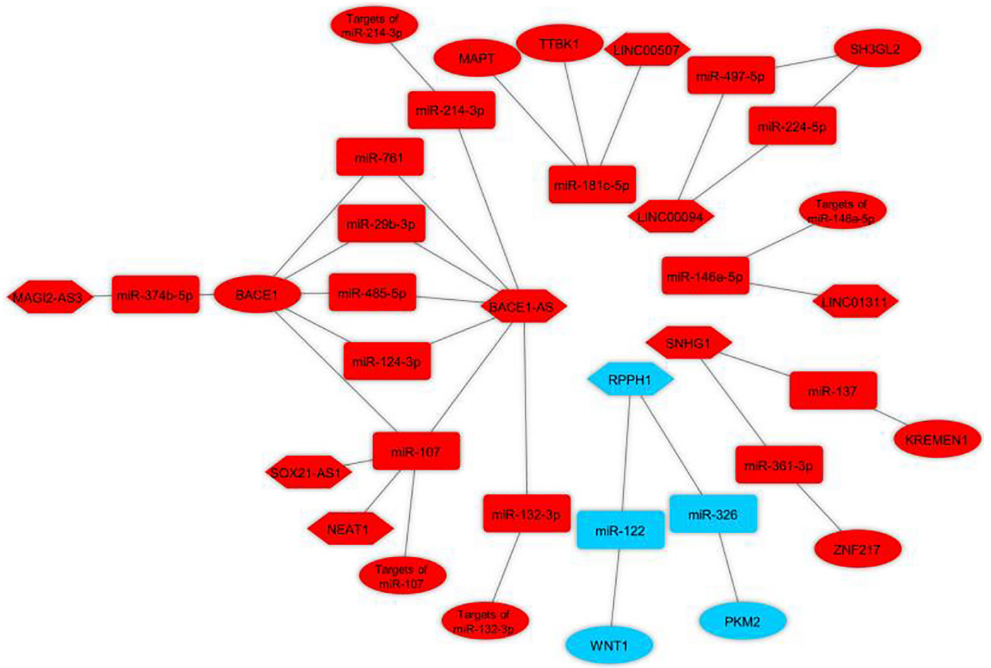

FIGURE 3 | Validated lncRNA-associated ceRNA axes in Alzheimer's disease. The loops were visualized using Cytoscape v3.8.0 software (Shannon et al., 2003) based on lncRNA-miRNA-mRNA interactions. Red represents the damaging role, and blue represents the protective role of competing endogenous RNA axes. LncRNAs, miRNAs, and mRNAs are represented by hexagon, round rectangle, and ellipse, respectively. LncRNA, long non-coding RNA; miRNA, microRNA.

approval (Winkle et al., 2021). However, trial findings have been mixed, with some research claiming strong benefits and others showing minimal effectiveness or toxicity. Alternate entities, including lncRNA-based therapies, are gaining interest (Winkle et al., 2021). The lncRNAs' wide function offers many possibilities for clinical application, the methods of which must be adapted to the lncRNA's mode of action (Arun et al., 2018). Investigating natural antisense transcripts (NATs): lncRNAs that are transcribed from opposite strands of adjacent genes and negatively regulate them in cis is an intriguing discovery. AntagoNATs are ASOs that target NATs and have demonstrated extremely promising preclinical findings for gene reactivation in the central nervous system (CNS) (Winkle et al., 2021). AntagoNATs effectively increased the expression of brain-derived neurotrophic factor (BDNF), a protein important in memory development (Modarresi et al., 2012), and the healthy allele of *SCN1A*, the haploinsufficiency of which triggers Dravet syndrome (Hsiao et al., 2016). Remarkably, *BDNF-AS*-targeting antagoNATs were effectively delivered across the blood–brain barrier in a murine model utilizing a minimally invasive nasal depot (MIND). MIND is a medication delivery system that directs drug administration to the olfactory submucosal region and has achieved about 40% effectiveness compared to riskier invasive delivery methods (Padmakumar et al., 2021). Such encouraging findings imply that lncRNA-based therapies will soon enter clinical testing. Moreover, the therapeutical impact of ASO-based lncRNA knockdown has shown promise in patients with Angelman syndrome, a single-gene illness represented by intellectual disability (Bird, 2014; Meng et al., 2015). Despite no development of ASOs that target lncRNA in the therapy of NDDs, ASOs that target mRNAs have received approval by the FDA for the therapy of Duchenne muscular dystrophy and spinal muscular atrophy (Wurster and Ludolph, 2018). Furthermore, ASOs have presented potential effectiveness in the therapy of Huntington's disease (HD) by affecting the huntingtin gene (*HTT*) as the target, in the therapy of amyotrophic lateral sclerosis by influencing *SOD1* and *C9ORF72* as the targets, and in the therapy of AD by affecting *MAPT* (tau) as the target (Scoles et al., 2019). Hence, lncRNAs and lncRNA-associated ceRNA axes can also serve as a new therapeutical target for AD therapy. In this study, we performed a scoping review and identified nine lncRNAs that were experimentally validated to act as ceRNA in AD in human studies. These lncRNAs are discussed below.

BASE1-AS

As mentioned above, in AD patients, the extracellularly deposited plaque of the Aβ peptide is one of the representative pathologic signs. To produce Aβ, APP needs to be sequentially cleaved proteolytically with β-secretase and γ-secretase. BACE1 is a critical enzyme in AD pathophysiology responsible for Aβ plaque formation through the cleavage of APP in collaboration with γ-secretase. Thus, a misregulated BACE1 can have a major contribution to this pathologic event. Both miRNAs and lncRNAs have been involved in regulating *BACE1* post-transcriptionally, with which lncRNA *BACE1-AS* has the utmost relevancy (Zeng et al., 2019). The lncRNA *BACE1-AS* was the first validated component of a ceRNA loop described in AD pathogenesis. Both *BACE1-AS* and *BACE1* mRNA transcripts

originate from a similar locus in chromosome 11 in humans; the transcription of *BACE1* mRNA and *BACE1-AS* is triggered from the sense and antisense strands, respectively. An RNA duplex is formed by pairing *BACE1-AS* to *BACE1*, leading to a structurally changed *BACE1* and improved stableness of mRNA. Consequently, *BACE1-AS* has a role in increasing both the mRNA and protein concentrations of *BACE1* (Faghihi et al., 2008, 2010). This ceRNA pair had been reported in two studies. The first loop was *BACE1-AS*/*miR-485-5p*/*BACE1*, which was shown to be involved in AD pathogenesis through *BACE1* post-transcriptional regulation (Faghihi et al., 2010). It was also shown that *BACE1-AS* and to a lower level *BACE1* were up-regulated, and the expression of *miR-485-5p* was decreased in the brain of AD patients (Faghihi et al., 2010). The second study showed that *BACE1-AS* prevented *BACE1* mRNA degradation by sponging *miR-29b-3p*/*miR-107*/*miR-124-3p*/*miR-485-5p*/*miR-761* in the pathophysiology of AD. In addition to the mentioned ceRNA pair, it was shown that *BACE1-AS* exacerbated isoflurane (anesthetic)-induced neurotoxicity by *BACE1-AS*/*miR-214-3p* axis in AD (He W. et al., 2020). This drug increases the risk of AD by increasing Aβ production and its oligomerization, as well as neuronal apoptosis (Xie et al., 2006, 2008; Xie and Xu, 2013). It is noteworthy that increased expression of *BACE1-AS* and down-regulation of *miR-214-3p* in the plasma samples of AD patients was also reported. In addition, *BACE1-AS* is a ceRNA for miR-132-3p. This axis was involved in the berberine-mediated neuroprotective effect in AD (Ge et al., 2020). *Berberine* is an isoquinoline alkaloid found in many medicinally important plants (Kumar et al., 2015). Berberine has been reported to play a crucial role in AD treatment (Yuan et al., 2019).

MAGI2 Antisense RNA 3 (*MAGI2-AS3*)

Alongside *BACE1-AS*, *MAGI2-AS3* (via *miR-374b-5p*) could reportedly regulate *BACE1* mRNA levels, Aβ-induced neurotoxicity, and neuroinflammation in AD (Zhang and Wang, 2021). This underlines that the *BACE1* is complexly regulated in AD by the mediation of multiple ceRNA networks. It was also shown that serum *MAGI2-AS3* and *miR-374b-5p* expression was significantly up-regulated and down-regulated in AD patients compared with healthy controls, respectively (Zhang and Wang, 2021). LncRNA *MAGI2-AS3* is reportedly a sponger of *miR-374b-5p* in ovarian carcinoma and hepatocellular carcinoma (Gokulnath et al., 2019; Yin Z. et al., 2019). It has been found to be involved in regulating cell survivability in a variety of diseases (Cao et al., 2020; He J. et al., 2020). There are also reports on the association between *MAGI2-AS3* and chronic inflammatory illnesses (Liao et al., 2020).

SNHG1

In AD pathology, *SNHG1* involves in Aβ-induced neuronal injury via two different ceRNA regulatory axes, *SNHG1*/*miR-137*/kringle containing transmembrane protein 1 (*KREMEN1*) (Wang et al., 2019) and *SNHG1*/*miR-361-3p*/zinc finger gene 217 (*ZNF217*) (Gao et al., 2020). *SNHG1* has been well-studied in different types of cancers due to its oncogenic role (Huang et al., 2018). It is shown that it can also promote neuronal autophagy and neuroinflammation in Parkinson's disease (PD) (Cao et al., 2018; Chen et al., 2018b). *KREMEN1* encodes a receptor for Dickkopf (DKK) proteins that functionally cooperates with DKK1/2 to inhibit wingless (WNT)/beta-catenin signaling (Mulvaney et al., 2016; Stelzer et al., 2016). It exerts its pro-apoptotic activity in a Wnt-independent pathway (Causeret et al., 2016). It has been shown that the silencing of *KREMEN1* prevents Aβ-mediated synapse loss in AD (Ross et al., 2018). Besides, the oncogenic role of *ZNF217* has been shown in many cancers (Quinlan et al., 2007). In AD studies, the down-regulation of *ZNF217* could relieve Aβ-induced neurotoxicity (Wang J. et al., 2018). It had also been shown that the neuroprotective effect of resveratrol could occur through *SNHG1*/*miR-361-3p*/*ZNF217* axis (Gao et al., 2020). Resveratrol is a polyphenolic compound that its high concentrations are found in red grapes, blueberries, and peanuts (Evans et al., 2017). Various studies have proved its antioxidant and anti-inflammatory effects on AD (Gomes et al., 2018; Corpas et al., 2019; Kong et al., 2019).

NEAT1

NEAT1 aggravated Aβ-induced neuronal injury via acting as a sponge for *miR-107* (Ke et al., 2019). Knockdown of NEAT1 attenuated Aβ induced inhibition of viability and promotion of apoptosis and p-tau levels. MiR-107 was down-regulated, and it reversed Aβ-induced injury when overexpressed in Aβ-treated cells (Ke et al., 2019). *NEAT1* is frequently overexpressed in human tumors and proposed as a novel target for human cancer therapy and diagnosis by sponging miRNAs (Dong et al., 2018). According to previous studies, it also has a crucial role in HD and PD (Chanda et al., 2018; Liu and Lu, 2018). In AD studies, its increased expression levels were shown in brain tissues of AD patients, compared to controls (Spreafico et al., 2018).

SOX21-AS1

SOX21-AS1, similar to *NEAT1*, exacerbated Aβ-induced neuronal injury via sequestering *miR-107* (Yue et al., 2020). *SOX21-AS1* is a recently discovered lncRNA that can suppress neuronal apoptosis of hippocampal cells and mitigate oxidative stress in AD, hence, being involved as an agent in AD pathogenicity (Zhang L. et al., 2019).

It is noteworthy that, as mentioned above, the present study identified that *miR-107* was at the center of three different loops, as shown in **Figure 4**, which suggests it is a key miRNA for both biological researches and ceRNA-based therapeutic purposes in AD studies. The *miR-107* family is a group of evolutionarily conserved miRNAs that show high expression in the human cerebral cortex. Numerous studies have shown misregulation of this miRNA in AD brains (Wang et al., 2008; Gupta et al., 2017; Moncini et al., 2017). It has been reported that it may have a protective role in AD by preventing Aβ-induced blood–brain barrier (BBB) disruption, endothelial cell dysfunction (Liu et al., 2016), and Aβ-induced neuronal damage (Jiao et al., 2016; Shu et al., 2018; Chen et al., 2020). *miR-107* shows a certain potential to be used as a biomarker in AD (Herrera-Espejo et al., 2019; Prendecki et al., 2019; Swarbrick et al., 2019). The ceRNA interactions are mediated by miRNAs, and altered miRNAs expression result in dysregulation of its competitive interactors. On the other hand, it is hypothesized that ceRNAs

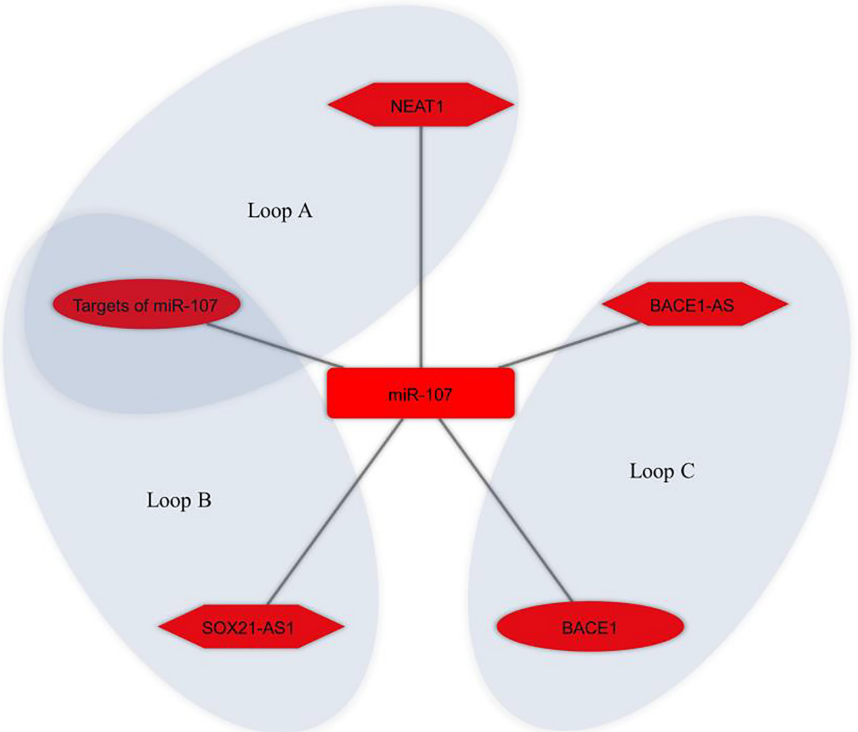

FIGURE 4 | *miR-107* regulates different competing endogenous RNA (ceRNA) loops. Loop A (*NEAT1* and targets of *miR-107*), loop B (*SOX21-AS1* and targets of *miR-107*), and loop C (*BACE1-AS* and *BACE1*) were all regulated by *miR-107*. Red represents the damaging role of ceRNA axes. Lon non-coding RNAs, microRNAs, and mRNAs are represented by hexagon, round rectangle, and ellipse, respectively.

in the modules play a role in disease progression as a whole instead of acting individually (Chen et al., 2018a). Thus, these identified ceRNA modules comprising *NEAT1*, *SOX21-AS1*, *BACE1-AS*, *miR-107*, targets of *miR-107*, and *BACE1* can be further assessed as potential ceRNA modules for ceRNA-based therapeutic purposes in AD.

Long Intergenic Non-protein Coding RNA 507 (*LINC00507*)

It was reported that *LINC00507*/*miR-181c-5p*/tau-tubulin kinase-1 (*TTBK1*)/*MAPT* axis regulated tau hyperphosphorylation via P25/P35/GSK3β signaling pathway (Yan et al., 2020). LincRNAs are a subclass of lncRNAs. *LINC00507* has an age-dependent expression pattern and is specifically expressed in the primate cortex (Mills et al., 2016). *MAPT* encodes the tau protein, and TTBK1 is a CNS-specific protein kinase that involves in tau hyper-phosphorylation and deposition in AD (Ikezu and Ikezu, 2014). It also has been shown that P25/P35/GSK3β signaling pathway deteriorates tauopathy (Noble et al., 2003).

Long Intergenic Non-protein Coding RNA 94 (*LINC00094*)

As indicated in previous reports, LncRNA *LINC00094* (called *BRD3OS* as well) is involved in regulating BBB penetrability in the AD microenvironment by sponging *miR-224-5p* and *miR-497-5p*, and *SH3GL2* mRNA is targeted by both of them (Zhu et al., 2019). *LINC00094* may reportedly act as a prognostic biomarker of lung cancer (Li et al., 2017). Additionally, microarray examination revealed the *LINC00094* down-regulation in Memantine-incubated cells. As an *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine has received wide applications for AD treatment (Zhu et al., 2019). *SH3GL2* encodes Endophilin-1, an endocytosis protein that has a marked increase in the AD brain and is responsible for Aβ-induced postsynaptic dysfunction (Yin Y. et al., 2019).

Long Intergenic Non-protein Coding RNA 1311 (*LINC01311*)

It was reported that the *LINC01311*/*hsa-miR-146a-5p* axis could operate as a functional regulator in AB1-42-stimulated apoptosis, proliferation slowdown, autophagy, and accumulated APP in human-lineage neurons (Fan et al., 2021). As a new lncRNA, *LINC01311* was discovered throughout human genome-wide screening, which has an aberrant expression in human liver and prostate cancers (Zhu et al., 2016; Imada et al., 2020). However, the functional activity of *LINC01311* has never been clarified in other human disorders.

RPPH1

Unlike the lncRNAs discussed above, *RPPH1* apparently exerts a neuroprotective compensatory mode of action in AD pathology via two varying ceRNA axes: *RPPH1*/*miR-122*/*WNT1*

(Gu et al., 2020) and *RPPH1*/*miR-326*/pyruvate kinase M2 (*PKM2*) (Gu et al., 2021). Specifically, evidence indicates that it can mitigate Aβ25–35-stimulated neuronal damage, apoptosis, and endoplasmic reticulum stress (Gu et al., 2020, 2021). *RPPH1* is the RNA component of RNase P, which plays a role in tRNA maturation in Archaea, Bacteria, and Eukarya (Evans et al., 2006). Wnt/β-catenin signaling has a confirmed essential function in developing AD. Huperzine A, which reversibly and selectively inhibits acetylcholinesterase and is utilized for AD treatment, has been reported to have a neuroprotecting impact by activating Wnt/β-catenin signaling in AD (Wang et al., 2011). As shown previously, the BBB failure in AD is caused by an impaired Wnt/β-catenin signaling (Liu et al., 2014). Besides, research indicates that activating the Wnt/β-catenin signaling is capable of protecting neuronal cells by regulating survival and c-myc, as well as apoptosis-linked proteins Bcl-2 and Bax (Jeong et al., 2014). As a glycolytic sensor, PKM2 has a crucial contribution to the dephosphorylation of phosphoenolpyruvate to pyruvate and catalysis of the final stage of glycolysis (Nakatsu et al., 2015). The emergence of recent documentation has highlighted that *PKM2* is involved in AD. According to reports, oxidatively inactivated PKM2 had an association with the progress of AD from mild cognitive impairment (Butterfield et al., 2006). The poly(ADP-ribose)polymerase 1 could also modulate PKM2, indicating that the PKM2-linked glycolytic pathway has a contribution to AD (Martire et al., 2016).

Limitations of Scoping Review

A scoping review is different from a systematic review. It systematically studies the literature, quantitatively synthesizes the accomplishments and sums up the gaps in a particular field's literature rather than evaluating it to offer a solution to a particular question. The approach is not substantially different from a systematic review, but an evaluation of methodological limitations or risk of bias of the evidence contained in a scoping review is not usually undertaken. A scoping review is often conducted before a systematic review to examine the literature and determine pertinent research topics to be addressed by a subsequent systematic review. Additionally, a scoping review does not seek to aggregate results via meta-analysis but rather maps the literature to determine themes, gaps, and patterns (Arksey and O'Malley, 2005; Levac et al., 2010). Because ceRNAs and AD is relatively new, a scoping review was conducted to evaluate the extent, scope, and type of research effort in this field to bring attention to topics where further study is required. Our results, however, should be interpreted with caution because we decided to perform a scoping review, which omits quality evaluations of the articles. The most significant limitation of this study is most likely the dearth of evidence available for review.

CONCLUSION

LncRNA-associated ceRNA regulation produces biologically significant effects in various diseases so it can elucidate the pathogenic procedures and offer options for new therapies. Thus, our efforts to understand different aspects of ceRNA regulatory mechanisms in AD pathogenesis provide new insights into the potential molecular targets, discover ceRNA-based biomarkers, and design ceRNA-based therapeutic applications.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

MT, MR, and HS wrote the draft and revised it. MA, YD, OR, AJ, and NA collected the data and designed the tables and figures. All authors read the draft and approved the submitted version.

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Does Chronic Sleep Fragmentation Lead to Alzheimer's Disease in Young Wild-Type Mice?

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Chronic sleep insufficiency is becoming a common issue in the young population nowadays, mostly due to life habits and work stress. Studies in animal models of neurological diseases reported that it would accelerate neurodegeneration progression and exacerbate interstitial metabolic waste accumulation in the brain. In this paper, we study whether chronic sleep insufficiency leads to neurodegenerative diseases in young wild-type animals without a genetic pre-disposition. To this aim, we modeled chronic sleep fragmentation (SF) in young wild-type mice. We detected pathological hyperphosphorylated-tau (Ser396/Tau5) and gliosis in the SF hippocampus. ^{18}F-labeled fluorodeoxyglucose positron emission tomography scan (^{18}F-FDG-PET) further revealed a significant increase in brain glucose metabolism, especially in the hypothalamus, hippocampus and amygdala. Hippocampal RNAseq indicated that immunological and inflammatory pathways were significantly altered in 1.5-month SF mice. More interestingly, differential expression gene lists from stress mouse models showed differential expression patterns between 1.5-month SF and control mice, while Alzheimer's disease, normal aging, and APOEε4 mutation mouse models did not exhibit any significant pattern. In summary, 1.5-month sleep fragmentation could generate AD-like pathological changes including tauopathy and gliosis, mainly linked to stress, as the incremented glucose metabolism observed with PET imaging suggested. Further investigation will show whether SF could eventually lead to chronic neurodegeneration if the stress condition is prolonged in time.

Keywords: sleep fragmentation, Alzheimer's disease, stress, F-18-fluorodeoxyglucose-positron emission tomography (^{18}F-FDG-PET), neuroinflammation, tau, amyloid-β

INTRODUCTION

Sleep is a highly conserved physiological phenomenon among mammals and important for multiple physiological processes, including cognitive function, immune function, and hormone release (Irwin, 2015; Krause et al., 2017). During a normal night sleep, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep alternately occur for 5–6 episodes in humans.

Both sleep stages are important for learning and memory consolidation. It has been shown that the risk of developing Alzheimer's disease and the prevalence of all-cause dementia increases with sleep disorders (Shi et al., 2018). In clinical observation, sleep disturbance is often present years before the symptomatic stages of neurodegenerative diseases and becomes more severe along with the disease progression (Guarnieri et al., 2012; Irwin and Vitiello, 2019). In the APP/PS1 animal model of Alzheimer's disease, partial sleep deprivation could accelerate Aβ plaques depositions and cognition impairment (Wang et al., 2021). All these important pieces of evidence pointed to the hypothesis that, sleep disturbance might be a key etiology of neurodegenerative diseases, especially AD. This hypothesis was mechanism-wise strongly supported by the recent discovery of a glia-based system, called the Glymphatic System, which manages the convection flows through brain parenchymal and drains the neurotoxic substances out of the brain (Iliff et al., 2012). In young wild-type mice, much higher efficiency of cerebrospinal fluid (CSF) -interstitial fluid (ISF) exchange and drainage was found occurring in the sleep phase *in vivo* (Iliff et al., 2012; Xie et al., 2013). Several follow-up studies reported the functional failure of the Glymphatic System was evident in neurodegenerative disease animal models, including i.e., Alzheimer's disease (AD) and Parkinson's disease (PD) (Rasmussen et al., 2018; Nedergaard and Goldman, 2020). These studies proposed that sleep disturbance could dampen interstitial space substance clearance, resulting in neurodegeneration due to excessive neurotoxic protein accumulation (Ross and Poirier, 2004). Neuronal metabolic waste, such as soluble Aβ and adenosine, exhibited circadian rhythms and accumulated in brain interstitial space after acute sleep deprivation (Kang et al., 2009; Roh et al., 2012; Wu et al., 2016; Peng et al., 2020). Based on these results, we were wondering if a young and healthy brain could be turned into a neurodegenerative brain under continuous sleep disturbance. It is a frightening hypothesis especially for people who have long-term inevitable night shifts and sleep insufficiency. It is a serious issue if we consider that chronic sleep insufficiency is becoming a common issue in young populations nowadays, mostly due to life habits and work stress. A study conducted in the population of university students reported that 40% of students with an average age of around 20-year-old had smartphone addiction and these students exhibited significantly poorer subjective sleep quality and more severe daytime dysfunction than the ones without smartphone addiction (Lane et al., 2021). In young to middle-aged medical workers, more than 50% had abnormal daytime sleepiness and poor sleep quality (Carvalho et al., 2021).

So far, there is no direct answer to the key scientific question of whether chronic sleep insufficiency could lead to neurodegeneration even in the absence of factors involving genetic pre-disposition and senescence. In our previous study, we reported chronic sleep fragmentation (SF) interventions induced AD-like pathology in young wild-type C57BL/6 mice. We found that 1.5-month SF treatment resulted in cognitive impairment, intracellular $A\beta_{1-42}$ accumulation, gliosis, and dysfunction of the endosomal-autophagosome-lysosomal (EAL) pathway(Xie et al., 2020a). Takahashi et al. observed in human autopsy samples that intracellular $A\beta_{1-42}$ accumulation was only seen at pre-symptomatic or early stage of AD but was absent at symptomatic stage (Takahashi et al., 2017). It has not been tested yet if pathological tau-aggregation, another even more important hallmark of AD pathogenesis (Wang and Mandelkow, 2016), occurs in chronic SF brain. Meanwhile, it has been reported glucose metabolic disorder appears before symptomatic AD in APP/PS1 mice. ^{18}F-labeled fluorodeoxyglucose positron emission tomography scan (^{18}F-FDG-PET) scan revealed that glucose utilization increases in multiple brain regions of APP/PS1 Tg mice at 2 and 3.5 months (pre-symptomatic stage) (Li et al., 2016). So far, no study has tested glucose metabolism in chronic sleep fragmentation brain in young wild-type mice. Based on our scientific question and current evidence, we hypothesized that chronic sleep fragmentation could probably initiate preceding pathological processes for neurodegeneration even in a young healthy brain.

In the current study, we further detected the intracellular deposition of pathological hyperphosphorylated tau, gliosis with immunohistochemistry, and western blot in 1.5-month chronic SF mice in comparison with normal young wild-type mice. We also evaluated the brain glucose metabolism with ^{18}F-FDG-PET and conducted hippocampal transcriptome mapping with RNA sequencing to better understand the pathological processes induced by chronic sleep fragmentation.

MATERIALS AND METHODS

Animals

The study involved 2–3-month-old wild-type male C57BL/6J mice obtained from the Hubei Research Center for Laboratory Animals (Hubei, China) which were used for experiments. All animal procedures were approved by the Institutional Animal Care and Use Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Chronic Sleep Fragmentation Modeling

Animals were randomly divided into a chronic SF group and a normal sleep (NS) group with 11–12 mice in each group. Both populations were kept in a 12-h light-dark cycle (8:00 a.m.−8:00 p.m. light-ON, 8:00 p.m.−8:00 a.m. light-OFF) with free access to food and water. Following the procedure described previously (Xie et al., 2020a), chronic SF model cages were secured on an orbital rotor, vibrating at 110 rpm and with a repetitive cycle of 10 s-on, 110 s-off, during the light-ON phase (**Figure 1A**). This chronic SF procedure was performed continuously for 1.5 months (45 days). And the NS group cages were placed in the same room as the SF cages, to keep the surrounding environment and labor effects identical (Xie et al., 2020b).

^{18}F-FDG-PET Scan

Both SF and NS mice were kept fasting for 24 h prior to the scanning. On the experimentation day, mice were anesthetized by inhalation of 2% isoflurane. Approximately 7 μCi/g 2-[^{18}F]FDG diluted in a 0.1 ml solution were injected intraperitoneally. The average total dose per mouse was ∼200 μCi. Then, 60 min after injection, a 10-min-long PET/CT scan (D180,

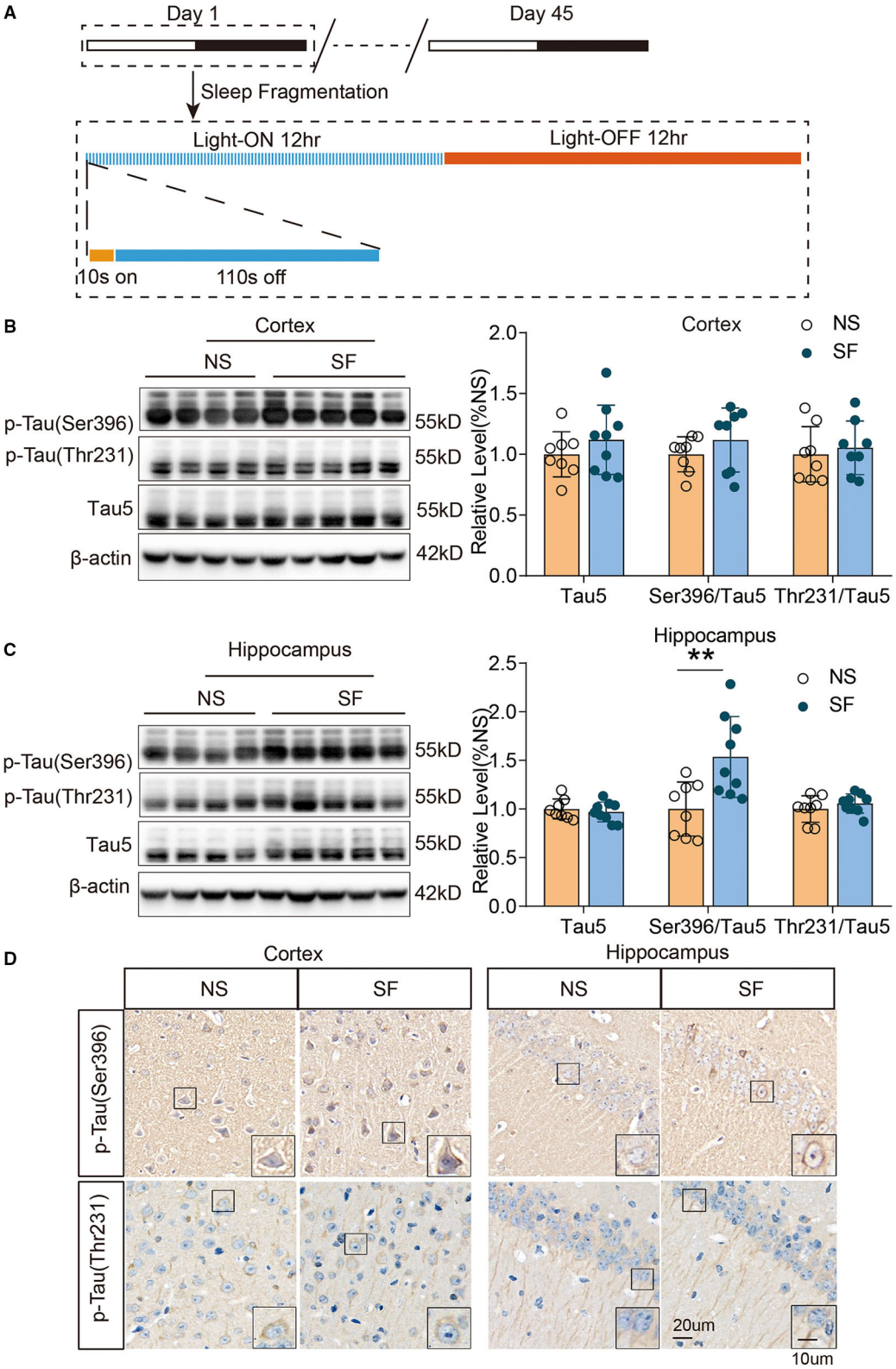

FIGURE 1 | Chronic SF increases pathologically phosphorylated tau (Ser396) in young wild-type mice hippocampus. **(A)** The schematic figure of the experimental design procedure, indicating the timing of the SF model. **(B,C)** Western blotting and quantitative density of expression of p-tau Ser396, p-tau Thr231, and tau5 in the cortex **(B)** and hippocampus **(C)** show an increase of p-tau Ser396 in SF hippocampus. β-actin was used as loading control. **(D)** Representative immunohistochemistry images of phosphorylated tau (p-tau Ser396 and p-tau Thr231) in the cortex and hippocampus of SF and NS group. Scale Bar = 20 μm. Local enlarged images were presented in the boxes. Scale Bar = 10 μm. $n = 8$ for NS and $n = 9$ for SF group. $^{**}P < 0.01$.

TABLE 1 | ^{18}F-FDG uptake per brain region in mice.

Brain region	Average SUV in NS (g/cc)	Average SUV in SF (g/cc)	Percentage difference in SF (SF-NS)/NS,%	*P*-value
Whole brain	1.47 ± 0.17	2.12 ± 0.44	44%	0.0154*
RSTR	1.77 ± 0.25	2.23 ± 0.40	26%	0.0627
LSTR	1.84 ± 0.26	2.35 ± 0.54	28%	0.0890
CTX	1.55 ± 0.14	2.05 ± 0.22	32%	0.0025*
RHIP	1.45 ± 0.18	2.27 ± 0.61	57%	0.0381*
LHIP	1.44 ± 0.20	2.27 ± 0.74	58%	0.0642
THA	1.46 ± 0.21	2.25 ± 0.77	54%	0.0824
CB	1.49 ± 0.20	2.36 ± 0.70	58%	0.0502
BFS	1.45 ± 0.25	1.79 ± 0.36	23%	0.1205
HYP	1.04 ± 0.13	1.57 ± 0.38	51%	0.0182*
RAMY	1.16 ± 0.14	1.63 ± 0.20	41%	0.0023*
LAMY	1.23 ± 0.18	1.97 ± 0.42	60%	0.0063*
BS	1.21 ± 0.21	2.01 ± 0.59	66%	0.0204*
CG	1.65 ± 0.25	2.61 ± 0.98	58%	0.0925
SC	1.62 ± 0.22	2.47 ± 0.83	52%	0.0829
OLF	1.39 ± 0.24	1.88 ± 0.47	35%	0.0662
RMID	1.37 ± 0.24	2.27 ± 0.84	66%	0.0742
LMID	1.42 ± 0.28	2.48 ± 0.97	75%	0.0695
LIC	1.68 ± 0.27	2.70± 0.95	61%	0.0735
RIC	1.63 ± 0.26	2.58 ± 1.00	58%	0.1023

*SUV, standard uptake value; NS, normal sleep; SF, sleep fragmentation; RSTR, right striatum; LSTR, left striatum; CTX, cortex; RHIP, right hippocampus; LHIP, left hippocampus; THA, thalamus; CB, cerebellum; BFS, basal forebrain/septum; HYP, hypothalamus; RAMY, right amygdala; LAMY, left amygdala; BS, brain stem; CG, central gray; SC, superior colliculi; OLF, olfactory bulb; RMID, right midbrain; LMID, left midbrain; LIC, left inferior colliculus; RIC, right inferior colliculus; *P < 0.05.*

RAYCAN, China) was performed. An OSEM-3D-PSF algorithm including random and attenuation corrections was adopted for image reconstruction. The CT scan consisted of 400 angular projections. CT data were reconstructed using a cone-beam algorithm for visualizing the skull structure. The voxels of the reconstructed PET and CT images had a size of 0.5 × 0.5 × 0.5 mm and 0.156 × 0.156 × 0.088 mm, respectively. The PET and CT images were co-registered with a mutual information method. Finally, the brain of the mouse was segmented with a threshold-based algorithm by using the standardized uptake value (SUV) of the PET image, followed by a topological closure. The segmented brain images were co-registered with a mouse brain atlas (Ma et al., 2005; D'Ascenzo et al., 2020) by using a mutual information method, and 19 brain regions were identified, as reported in **Table 1**. Finally, the SUV of each mouse was calculated both in the entire brain and in each of the segmented brain regions separately.

Tissue Preparation

The mice ($n = 8$ for NS and $n = 9$ for SF group) were sacrificed by decapitation and the brains were extracted. Brains were quickly divided into left and right hemispheres by sagittal incision. The left hemispheres were fixed in 4% paraformaldehyde (PFA) for the preparation of paraffin sections and subsequent immunohistochemical staining. Cortex and hippocampus tissues of the right hemispheres were dissected on ice, and immediately frozen in liquid nitrogen, and stored at −80°C for protein extraction and western blotting detection. A separate group pair of SF and NS mice ($n = 3$ for each group) were prepared for hippocampus dissection from fresh brain tissue and sent for RNA extraction and sequencing.

Immunohistochemistry and Silver Staining

Immunohistochemistry was performed on 4 μm coronal paraffin-embedded sections. The sampled slices were deparaffinized and rehydrated in xylene and graded ethanol. Then, slices were placed in citrate antigen retrieval solution (PH 6) at 96°C for 20 min for heat-induced antigen retrieval. Slices were incubated with 3% H_2O_2 for 25 min to block endogenous peroxidase. Non-specific binding sites were blocked by 10% donkey serum for 1 h at room temperature. The slices were incubated with the following primary antibodies: mouse anti-Tau5 (dilution 1:400, ab80579, Abcam, UK), rabbit anti-p-Tau (Ser396) (dilution 1:200, ab109390, Abcam), rabbit anti-p-Tau (Thr231) (dilution 1:400, ab151559, Abcam), mouse anti-p-Tau (Ser202/Thr205) (AT8, dilution 1:200, MN1020, Invitrogen, USA), rabbit anti-Iba-1 (dilution 1:500, 019–19741, Wako, Japan), and mouse anti-GFAP (dilution 1:50, 3670, CST) overnight at 4°C. In order to detect the specific binding of primary antibodies, the horseradish peroxidase-conjugated appropriate secondary antibodies (Servicebio Inc., China) were applied for 1 h at room temperature. Antibody binding was visualized by reaction with diaminobenzidine. The slices with hematoxylin were counterstained and then dehydrated.

The micrographs were quantified by a blinded investigator using the Image-Pro Plus software (MediaCybernetics, USA). Then, after deparaffinization and rehydration, slices were also conducted silver staining by using a silver staining kit (Servicebio Inc., China).

Western Blotting

The cortex and hippocampus of the mice were homogenized in RIPA lysis buffer (Beyotime, China) with protease inhibitor cocktail and phosphatase inhibitor (Roche, Switzerland). Protein concentration was determined by using a BCA Protein Assay Kit (Beyotime, China). A total of 30 μg of protein per well was loaded on SDS–PAGE gels. Separated proteins on the gel were then transferred onto the PVDF membrane (0.22 μm, Millipore, USA) after electrophoresis. The PVDF membranes were blocked by 5% non-fat milk for 1 h at room temperature and then incubated with primary antibodies on a shaker overnight at 4°C. The following primary antibodies were used for Western blotting: mouse anti-Tau5 (dilution 1:1,000, ab80579, Abcam), rabbit anti-p-Tau (Ser396) (ab109390, Abcam), rabbit anti-p-Tau (Thr231) (dilution 1:1,000, ab151559, Abcam), mouse anti-p-Tau (Ser202/Thr205) (AT8, dilution 1:500, MN1020, Invitrogen), mouse anti-GFAP (dilution 1:1,000, 3670, CST), and mouse anti-β-actin (1:5,000, A5316, Sigma-Aldrich, USA). The membranes were incubated with appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch, USA) for 1 h at room temperature. The bands were visualized by using enhanced chemiluminescence kits (Advansta, USA) *via* a Bio-Rad ChemiDoc XRS+ imaging system (USA). The gray values of the bands were analyzed by using ImageJ software.

RNA Extraction and Sequencing

The total RNA of the hippocampus was extracted from NS and SF mice using Trizol (Invitrogen). The hippocampus was grounded into powder using liquid nitrogen, the appropriate volume of Trizol was added and homogenized for 2 min. Then, the content was rested for 5 min and centrifuged at 12,000 g for 5 min at 4°C. The supernatant was moved into a new EP tube, the appropriate volume of chloroform was added, and the tubes were shaked for 15 s, then the samples were left at room temperature for 2 min. The tubes were centrifugated for 15 min at 12,000 g, 4°C. After centrifugation, the upper aqueous phase containing RNA was moved into a new EP tube and isopropyl alcohol was added, the mixture was incubated at room temperature for 10 min. The contents were centrifuged at 12,000 g for 10 min at 4°C, then the supernatant was poured off and the pellet was kept. The pellet was washed with 75% ethanol and centrifuged again at 12,000 g for 5 min at 4°C. The supernatant was decanted into tubes and air-dried at room temperature for 5 min. The quality of these samples was verified, and mRNA library construction was performed by using Illumina Novaseq 6,000 by Shanghai Majorbio Bio-pharm Tech Co. Ltd. (China).

Differential Expression Gene Analysis and Functional Annotations

Due to the limited sample size ($n = 3$ for each group), every sample of the SF group was paired with the 3 samples in the NS group to sort out the differential expression gene (DEG) lists. It gave rise to 9 pairs of comparisons. DEG analysis was performed by using the edgeR software (p-adjust < 0.05 and $|$ log2Fold Change $| \geq 1$). The DEGs present in more than 3 comparisons as the DEGs between NS and chronic SF group were listed. The functional annotation of DEGs by classification and enrichment analysis of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) was conducted.

Comparisons of SF-Induced Hippocampal Transcriptome Characteristics With Other Disease Models

Through works of literature and GEO database search, the DEGs lists were extracted from the published data sources of mouse hippocampal RNAseq, including GSE168137 for Alzheimer's disease (5xFAD, congenic C57BL/6J background), GSE 61915 for normal aging (29 month old, C57BL/6J background), GSE140205 for APOEε4 mutation (humanized APOEε4 targeted replacement homozygous mice, C57BL/6J background), as well as the acute stress model (wild-type C57BL/6J background) (Pulga et al., 2016) and the stress model induced by hypergravity interventions (wild-type C57BL/6J background) (Pulga et al., 2016). The DEseq2 Software was used to sort out DEGs (p-adjust < 0.05; $|$ log2FoldChange $| \geq 1$). Five DEGs lists were generated in correspondence to the 5 models. Based on these DEGs lists, the expression levels (quantified as Fragments Per Kilobase per Million, FPKM) were extracted from the current hippocampal transcriptome data. Cluster analysis was performed on the 5 gene lists and the FPKM readouts of the data, respectively, and the relative expression levels of genes were displayed with heatmaps.

Statistical Analysis

All quantities were characterized with mean and standard deviation calculated in the NS and SF groups. The comparison between these two classes was performed with a standard two-tailed t-test if the data qualified for normal distribution and homogeneity of variance, otherwise, with a Mann Whitney-test. Data were analyzed using GraphPad Prism 6. Differences were considered significant if $P < 0.05$.

RESULTS

Chronic SF Increased Pathologically Phosphorylated Tau (Ser396) in Young Wild-Type Mice Brain

To investigate another set of important pathological proteins in AD, total tau, and phosphorylated tau(p-tau) at residues Thr231, Ser396, and Ser202/Thr205, we quantified tauopathy in the cortex and hippocampus of SF and NS mice. Western blot identified that the ratio of p-tau (Ser396)/total Tau5 was significantly higher in chronic SF hippocampus (SF vs. NS: 1.5 ± 0.42 vs. 1 ± 0.28, $n = 9$ for SF and $n = 8$ for NS, Unpaired t-test, $P < 0.01$). Although the ratio of p-tau (Ser396)/total Tau5 was higher in the SF cortex, it was not significant. And the ratio of p-tau (Thr231)/total Tau5 was also comparable between chronic SF and control group. Total Tau5 protein levels did not show a significant difference between

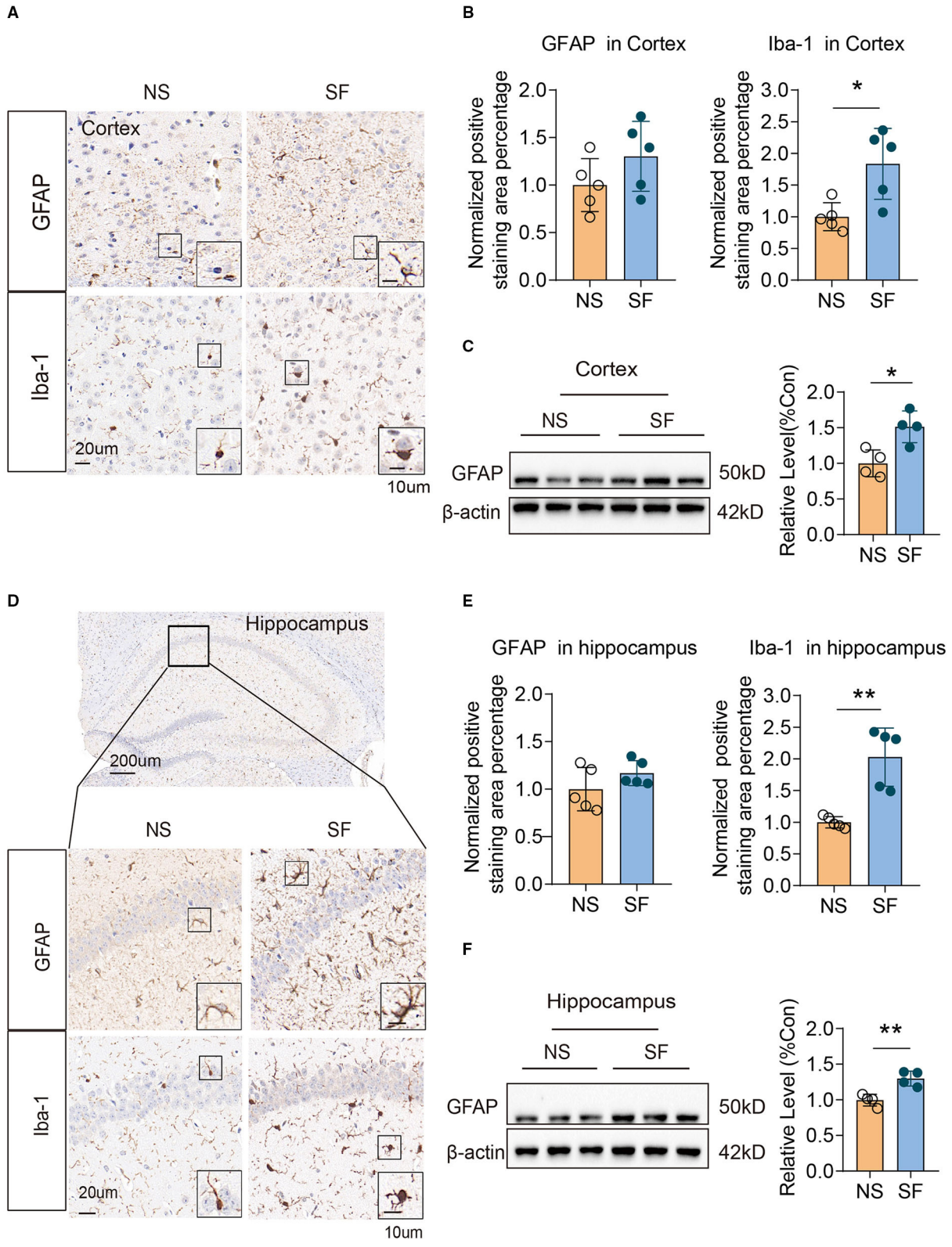

FIGURE 2 | Chronic SF induces gliosis in the mouse cortex and hippocampus. **(A–C)** Activation of astrocyte and microglia in SF cortex. **(A)** Representative immunohistochemistry images of GAFP and Iba1 staining in the cortex of NS and SF mice. Scale Bar = 20 μm. Astrocytes labeled by GFAP, and microglia labeled by Iba1 were shown in the boxes. Scale Bar = 10 μm. **(B)** Quantitative analysis of positive staining of GFAP and Iba1 in the cortex of NS and SF mice.

(Continued)

FIGURE 2 | (C) Western blotting and quantitative analysis of GFAP in NS and SF cortex. **(D–F)** Activation of astrocyte and microglia in SF hippocampus. **(D)** Representative immunohistochemistry images of GFAP and Iba-1 staining in the hippocampus of NS and SF mice. A representative image of the mouse hippocampus was shown. Scale Bar = 200 μm. GFAP and Iba1 staining in the hippocampus CA1 region were shown in the enlarged images. Scale Bar = 20 μm. Astrocytes labeled by GFAP, and microglia labeled by Iba1 were shown in the boxes. Scale Bar = 10 μm. **(E)** Quantitative analysis of positive staining of GFAP and Iba1 in the hippocampus of NS and SF mice. **(F)** Western blotting and quantitative analysis of GFAP in NS and SF hippocampus. For immunohistochemistry, $n = 5$; for western blotting $n = 4$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$.

both groups (**Figures 1B,C**). Immunohistochemistry staining was consistent with the results of western blot. Phosphorylated tau and p-tau (Ser396) exhibited a more condensed deposition in the cytoplasm of both cortex and hippocampus in the chronic SF group, while p-tau (Thr231) staining was similar between the SF group and control group (**Figure 1D**). However, phosphor-tau (Ser202/Thr205) was comparable between NS and SF groups. We further confirmed its expression by western blotting using AT8 antibody and found AT8 was almost absent in cortex samples of SF mice while it showed strong protein expression in the cortex of 12-month-old APP/PS1 mice (**Supplementary Figure 1**). Therefore, the chronic SF in young wild-type mice could increase certain pathological p-tau expressions in the hippocampus.

Chronic SF Induced Gliosis in Both Cortex and Hippocampus

Gliosis was evident in a variety of pathological conditions, which links tightly with injury repair, neuroinflammation. The positive staining area of microglial marker (Ionized calcium-binding adapter molecule 1, Iba-1) Iba-1, were both significantly higher in chronic SF group vs. control (cortex: 1.84 ± 0.56 vs. 1 ± 0.22, $n = 5$, Unpaired t-test, $P < 0.05$; hippocampus: 2.03 ± 0.46 vs. 1 ± 0.09, $n = 5$, Unpaired t-test, $P < 0.01$) (**Figures 2A,B,D,E**). As for quantification of the astrocytic marker (glial fibrillary acidic protein, GFAP) expression, western blot of GFAP protein was significantly higher in the chronic SF group in both cortex (SF vs. NS: 1.51 ± 0.22 vs. 1 ± 0.19, $n = 4$, Unpaired t-test, $P < 0.05$) (**Figure 2C**) and hippocampus (SF vs. NS: 1.3 ± 0.1 vs. 1 ± 0.08, $n = 4$, Unpaired t-test, $P < 0.01$) (**Figure 2F**), while the positive area of GFAP labeled by immunochemistry staining did not reach a statistically significant difference (**Figures 2A,B,D,E**). These results were consistent with our previous data (Xie et al., 2020a) and characterized the activation of gliosis in both cortex and hippocampus in the chronic SF group.

Chronic SF Enhanced Glucose Metabolism in Multiple Brain Regions

An example of the ^{18}F-FDG PET image of an SF and NS mouse is shown in **Figure 3A**. For a quantitative interpretation of the PET measurement, we summarized the SUV of each brain region, as well as the total SUV for both groups in **Table 1**. The chronic SF group exhibited a total SUV in the entire brain ∼44% higher with respect to the NS group (**Figure 3B**) (Unpaired t-test, $P < 0.05$). More interestingly, the average SUV in all the 19 brain regions separately was higher in the SF group than in the NS group (**Figure 3C**). In particular, the increased glucose metabolism of the SF group was statistically significant in the cortex (+32%, Unpaired t-test, $P < 0.01$), right hippocampal region (+57%, Unpaired t-test, $P < 0.05$), Hypothalamus (+51%, Unpaired t-test, $P < 0.05$), right amygdala (+41%, Unpaired t-test, $P < 0.01$), left amygdala (+60%, Unpaired t-test, $P < 0.01$) and brain stem (+66%, Unpaired t-test, $P < 0.05$).

Hippocampal RNAseq Revealed SF-Induced Alterations in a Broad Range of Pathways

Hippocampus is the key region involved in learning and memory. To explore the molecular alterations induced by chronic SF, we compared the RNA sequencing in hippocampal tissue between the two groups (**Figure 4A**). We sorted out a list of genes as DEGs following bioinformatics analysis flow and we found 98 DEGs genes (see **Supplementary Table 1**). We further conducted GO, KEGG biological pathway classification, and enrichment analysis. GO terms with enriched gene numbers are shown in **Figure 4B**, the highlighted biological processes included metabolic process, response to stimulus, immune system process, locomotion, etc. KEGG biological pathway classification is shown in **Figure 4C**, where several pathways involving inflammation, immune system, metabolic functions, aging, cancer, are visible. Enriched KEGG pathways are listed in **Figure 4D**. They involve infectious diseases, autoimmune diseases, allergic diseases, transcriptional misregulation in cancer, and calcium signaling pathway, NF-κB signaling pathway. These results found a broad range of biological processes involved in the pathogenesis in the hippocampus induced by chronic SF, mostly linking with immune system dysfunction, inflammation, metabolic dysregulation, and molecular transcription misregulation.

SF-Induced Transcriptome Alterations Were Similar With Stress Models But Not With Neurodegeneration or Aging Models

In order to better understand the pathological processes up- or down-regulated by chronic SF, we plotted the expression patterns of the DEGs extracted from hippocampal RNAseq experiments of several disease models with cluster analysis. First, we found the DEG lists from the published hippocampal RNA sequencing data in mouse models of Alzheimer's disease model (5xFAD on congenic C57BL/6J background, GSE168137), normal aging model (29-month-old C57BL/6J mice, GSE 61915), APOEε4 mutation model (humanized APOEε4 targeted replacement homozygous mice on C57BL/6J background, GSE140205), as well as two types of stress models (wild-type C57BL/6J background) (Pulga et al., 2016). Five DEG lists were generated corresponding to these 5 disease models. Based on these 5 gene lists, the expression levels (FPKM) were extracted from our current hippocampal transcriptome

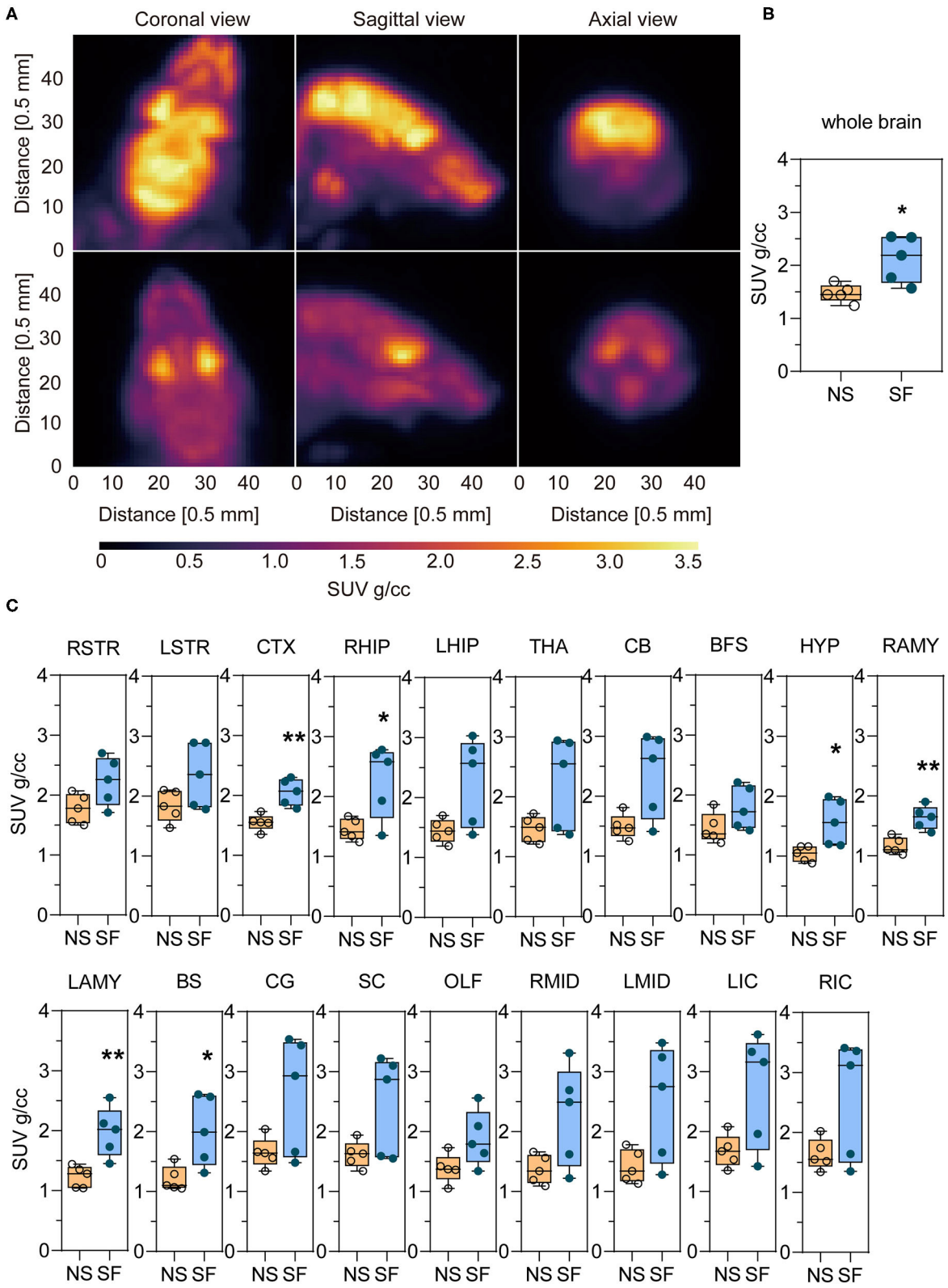

FIGURE 3 | Chronic SF enhances the glucose uptake in the brain monitored by ^{18}F-FDG-PET/CT. **(A)** Coronal, sagittal, and axial view of the brain images of an SF (top) and NS (bottom) mouse. **(B)** Statistical analysis of whole-brain SUV in NS and SF group. $n = 5$ per group. $^{*}P < 0.05$. **(C)** ^{18}F-FDG uptake in NS and SF mice in different brain regions expressed as SUV. $n = 5$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$. See **Table 1** for the nomenclature of the brain regions.

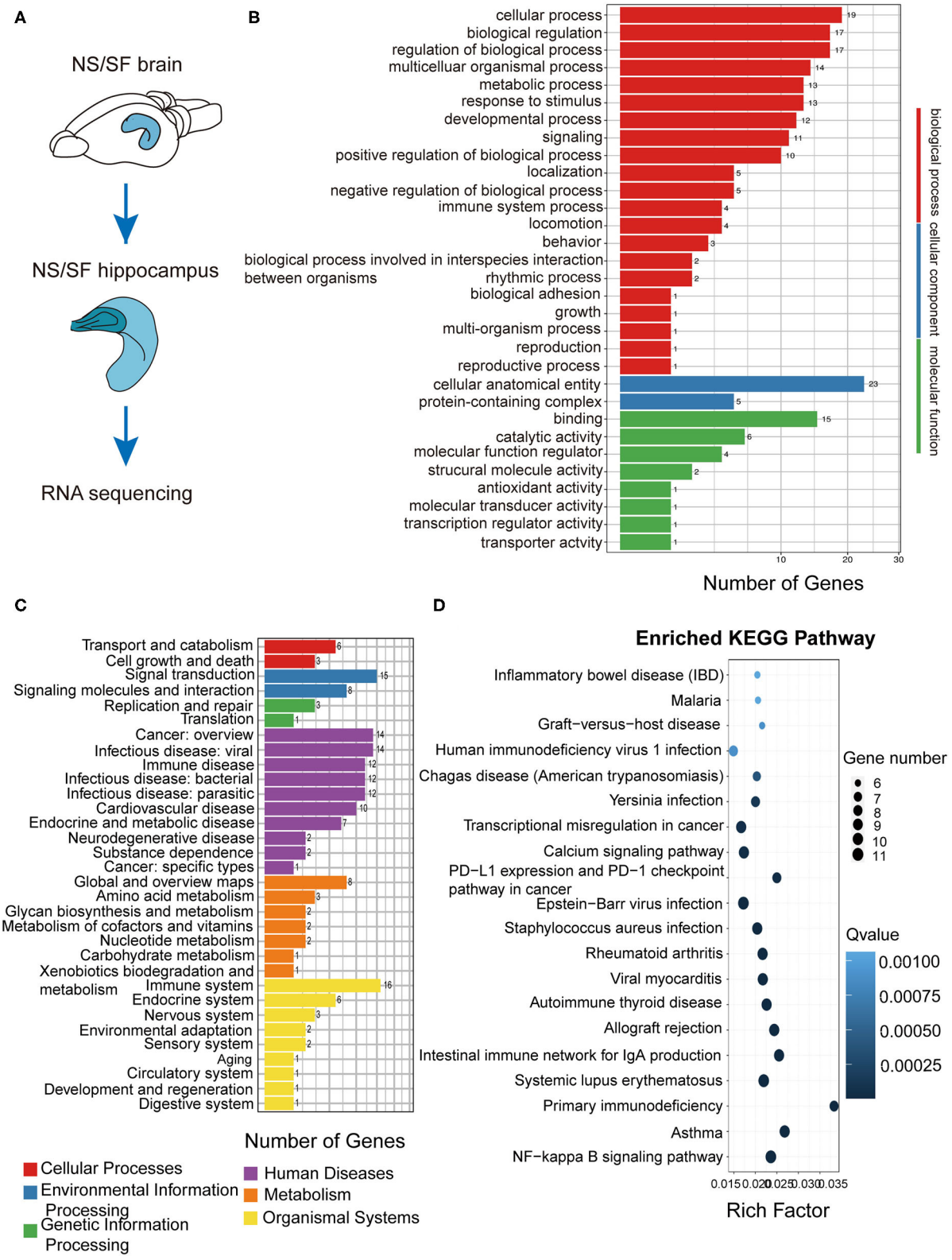

FIGURE 4 | Functional annotation of DEGs from the hippocampus in SF mice. **(A)** The schematic of hippocampus RNA sequencing. **(B)** The differential gene GO function classification map of the hippocampus. **(C)** KEGG classification on the DEGs from the SF hippocampus. **(D)** Statistics of KEGG pathway enrichment of DEGs map of SF hippocampus. The X-axis represents the enrichment factor, the Y-axis represents the pathway name, and color represents the Q value; the smaller the value, the more significant the enrichment result; the size of the point represents the number of DEGs. $n = 3$ per group.

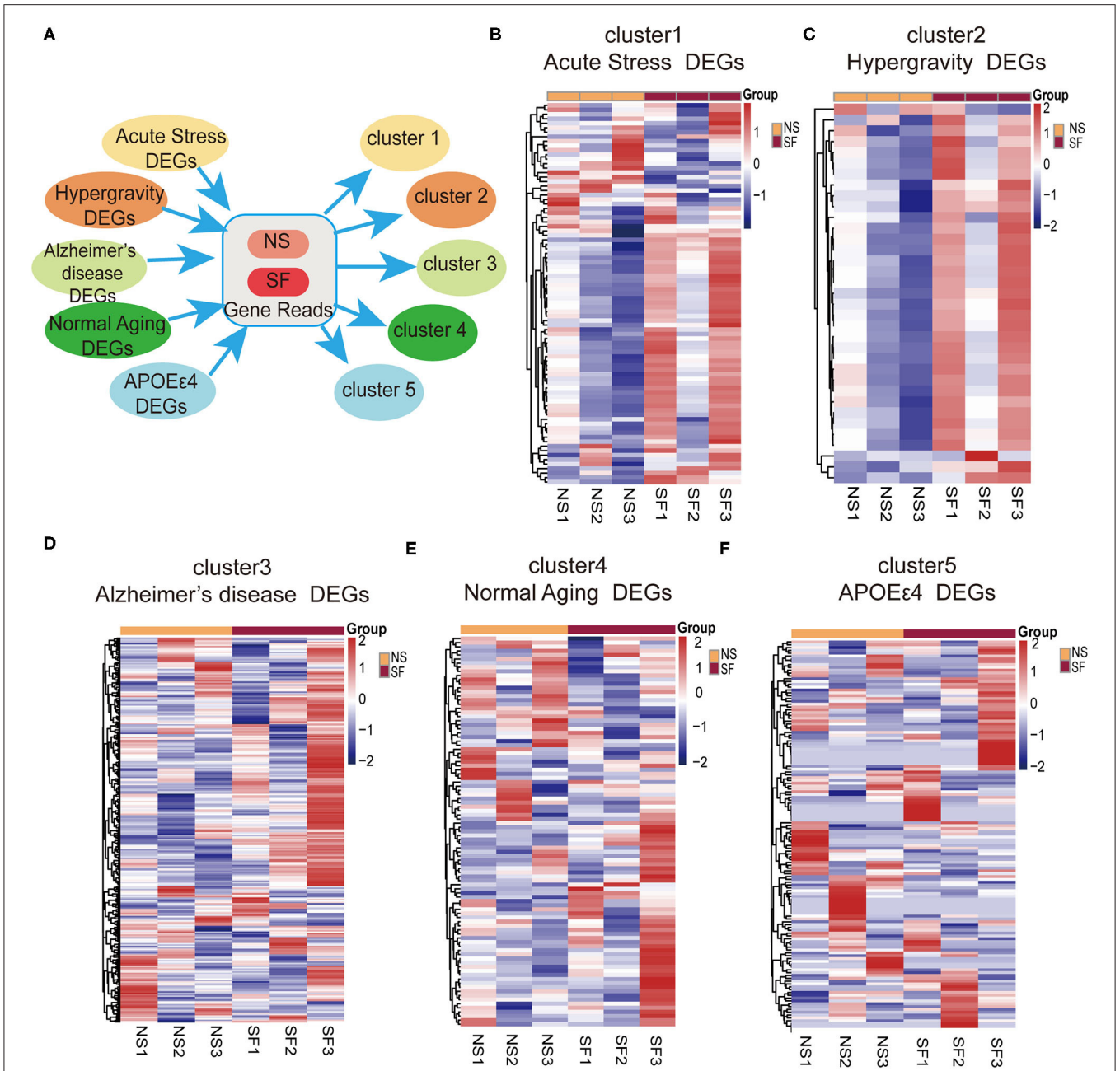

FIGURE 5 | Bioinformatic analysis revealed a link between chronic SF and stress. **(A)** Schematic instruction of cluster analysis of SF and related diseases or conditions. **(B–F)** Heat map showing relative gene expression in SF involved in different conditions [**(B)** acute stress, **(C)** hypergravity, **(D)** Alzheimer's disease, **(E)** normal aging, **(F)** APOEε4] $n = 3$ per group.

data. Cluster analysis was performed based on the 5 gene lists and the FPKM readouts, respectively (**Figure 5A**). The clustering heatmap demonstrates, that the gene lists of both stress models exhibit clear differential expression patterns between SF and NS mice (**Figures 5B,C**). One of the stress models was induced by intraperitoneal injection of 10 mg corticosterone, the other stress models were obtained by placing the mice under a hypergravity condition of 3G for 21 days (Pulga et al., 2016). As visible in the clustering heatmaps corresponding to the gene lists of Alzheimer's disease, normal aging, and APOEε4 mutation model, there are no significant differential expression patterns between SF and NS groups (**Figures 5D–F**). In other words, the major hippocampal transcriptome alterations induced by those 3 models were mostly not present in the chronic SF model. This analysis showed that SF-induced transcriptome alterations were similar with stress models but not with models of neurodegeneration or normal aging.

DISCUSSION

We have been interested in answering the scientific question: does chronic sleep fragmentation leads to Alzheimer's disease even in mice without genetic pre-disposition and senescence? Based on our previous study, we found intracellular $A\beta_{1-42}$ accumulation and protein degradation pathway dysfunction, as well as obvious cognitive decline in young wild-type mice after 1.5-month sleep fragmentation (Xie et al., 2020a). However, it was largely unknown what pathological processes were occurring in the chronic SF brain, and whether they provided the preceding conditions for developing neurodegeneration pathogenesis. Herein, as a follow-up study, we further found 1.5-month chronic SF could induce intracellular accumulation of pathological hyperphosphorylated tau (Ser396) and gliosis in young wild-type mice brains (**Figures 1**, **2**). ^{18}F-FDG PET scan identified a distinctive feature of chronic SF, namely the brain glucose utilization was upregulated, indicating an elevated metabolic activity throughout brain regions (**Figure 3**). Hippocampal RNA sequencing further showed that chronic SF induced alterations in a broad range of pathways, involving the immune system, inflammation, and metabolic dysregulation, transcriptional misregulation (**Figure 4**). In combination with 5 other models available hippocampal RNA seq data, we observed the stress models resulted in similar alterations of hippocampal transcriptome characteristics with chronic SF. To our surprise, the typical transcriptome changes in Alzheimer's disease, normal aging, and APOEε4 mutation were almost not present in chronic SF (**Figure 5**). Thus, 1.5-month SF in young wild-type mice induced pathological processes which were similar to stress models, but not with neurodegenerative disease and senescence models. However, current data indicated that, even without genetic background and senescence, the ongoing pathological processes in sleep fragmented brain provided the preceding basis for developing neurodegeneration if the stressor continuously exits. We will discuss it from several perspectives below.

Chronic Sleep Fragmentation Resulted in Neurotoxic Protein Accumulation

The two main pathological hallmarks of AD are senile plaques composed of amyloid-β (Aβ) and neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau. Clinical evidence found that cognitively normal individuals with amyloid-β deposition showed worse sleep quality compared with those without amyloid deposition, meanwhile, Aβ plaques could occur 10–15 years before cognitive impairment in AD (Ju et al., 2013). It matches our previous observation of intracellular $A\beta_{1-42}$ in sleep fragmented brain (Xie et al., 2020a). The "intracellular amyloid hypothesis" suggested that neuronal necrosis occurs in the ultra-early stage of Alzheimer's disease due to intracellular amyloid accumulation. Okazawa et al. explained that the early accumulation of intracellular amyloid deprives a critical effector molecule, Yes-associated protein (YAP) in the Hippo signaling pathway that is essential for cell survival (Tanaka et al., 2020). The initial neuronal necrosis releases high mobility group box 1 (HMGB1) into the interstitial space and induces a cluster of secondary necrosis in the surrounding area (Okazawa, 2021). Okazawa et al. also reported that inhibition of HMGB1 by anti-HMGB1 antibody prevents progression of neurodegeneration (Fujita et al., 2016). These studies supported the hypothesis that interstitial space Aβ deposition (senile plaques) doesn't represent the peak of pathological cascade since the pathological processes were initiated much earlier at the stage of intracellular amyloid accumulation. The significantly increased ratio of pathological hyperphosphorylated tau (Ser396)/total tau found in sleep fragmented brains could be partially explained by intracellular protein degradation dysregulation. We previously reported the aberrant expressions of critical molecules for endosome-autophagosome-lysosomal (EAL) pathways (Xie et al., 2020a). Therefore, intracellular AD-like neurotoxic accumulation could be initiated in young healthy brains due to chronic sleep fragmentation.

Disrupted Normal Circadian Rhythms Induced Neuroendocrinological Dysregulation and Emotional Stress

It has been proposed that emotional stress, mainly including anxiety and depression, links with cognitive decline and AD pathogenesis (Mendez, 2021). Furthermore, according to the observation, stress disorders, such as PTSD, were associated with the development of dementia (Bonanni et al., 2018). The aged primates having a bad early life experience showed more amyloid plaque deposition in the neocortex and a significant reduction in synaptophysin, suggesting stress increased the vulnerability to neurodegeneration (Merrill et al., 2011). We previously identified both cognitive decline and anxiety-like behaviors in 1.5-month SF mice (Xie et al., 2020a). The hypothalamus-pituitary-adrenal (HPA) axis responds to stress, inducing the adrenal cortex release of glucocorticoid hormones, cortisol, in humans. In patients with AD, high basal cortisol concentration is associated with smaller hippocampus volume and cognitive decline (Huang et al., 2009). Similarly, high cortisol levels in plasma and cerebrospinal fluid (CSF) are found related to the progression of cognition decline, which is useful in predicting clinical worsening from MCI to AD (Popp et al., 2015; Lehallier et al., 2016). Glucocorticoid was reported to increase the expression of amyloid precursor protein (APP) and β-site APP-cleaving enzyme1(BACE1) in astrocytes, and decrease some Aβ-degrading proteases, leading to Aβ deposition (Bai et al., 2011). Moreover, hippocampal corticosteroid exposure can promote tau phosphorylation *via* activating glycogen synthase kinase 3β(GSK3β) (Dey et al., 2017). In addition, glucocorticoids impair the endo-lysosomal and autophagic mechanisms of tau degradation, inducing accumulation of aggregated tau (Vaz-Silva et al., 2018; Silva et al., 2019).

Sleep has a suppressive effect on the HPA stress system. Sleep disturbance would maintain or further elevate the activity of the stress system, which could affect the circadian rhythm of stress system functional dynamics. Studies have shown that acute partial sleep loss can alter glucocorticoid regulation, increasing the cortisol level the next evening (Leproult et al., 1997). In the current study, we found out that differential expression gene lists from two stress mice models showed differential

expression patterns between 1.5-month SF and control mice, while Alzheimer's disease, normal aging, and APOEε4 mutation models did not exhibit any significant differential expression patterns (**Figure 5**). One of the stress models was induced by intraperitoneal injection of corticosteroid to mimic acute stress, while the other model was to give continuous hypergravity stress during certain time blocks for 21 days. The later model displayed the best clustering pattern (**Figure 5**), which also shared the most similarity with our chronic sleep fragmentation models. In summary, chronic sleep fragmentation in young wild-type mice induced neuroendocrinology system dysregulation and hippocampal transcriptome alterations similar to stress models. It provided supporting evidence for the tight connections observed clinical-wise between emotional stress and AD pathogenesis.

Chronic Sleep Fragmentation Induced Brain Glucose Metabolism Imbalance

In the central nervous system, glucose metabolic rate reflects neuronal activity, synaptic density, and neuroinflammation. ^{18}F-FDG, the most commonly used radiotracer analog of brain glucose, was used to capture the brain glucose metabolic changes using a pre-clinical PET system. A previous study identified in APP/PS1 mice that, Tg mice of 2-month-old (equivalent to pre-clinical) and 3.5-month-old (equivalent to sub-clinical) exhibited significant glucose utilization increase in multiple brain regions vs. normal controls. It was explained by a compensatory state due to neuronal hyperexcitability in pre- or early AD. In 2- and 3.5-month-old Tg mice, the hyperglycolytic brain regions were highlighted to be the entorhinal cortex, hippocampus, and frontal cortex (Li et al., 2016). It was proposed that hypermetabolism before the onset of clinical dementia could be an early biomarker for AD diagnosis in clinical patients (Herholz, 2010). So far as we know, we were the first to characterize brain glucose metabolism in a chronic sleep fragmentation model with ^{18}F-FDG-PET in young wild-type mice. We found out that the SUV values in brain regions of the cortex, hippocampus, hypothalamus, amygdala, brain stem were significantly enhanced in the SF group (**Figure 3**; **Table 1**). Hippocampal pathology in AD patients usually happens early and contributes to cognitive dysfunction (Wang, 2014). Similar to APP/PS1 mice at the age of 2–3.5 month-old, hippocampus glucose metabolism in the SF brain was also increased, it matched with the cognitive decline in behavioral tests reported previously (Xie et al., 2020a). Meanwhile, the hypothalamus, hippocampus, amygdala, and brain stem link with the HPA axis (Fries et al., 2009; Contoreggi, 2015). Hypothalamus is the center regulating the stress hormone released from the pituitary and control of the autonomic nervous system *via* releasing corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN). Amygdala has abundant CRH receptors, and it provides excitatory input to PVN and receives excitatory signals from PVN. Hippocampus also has high concentrations of corticosteroid receptors and has been implicated in negative feedback regulation of HPA axis activity. That explains why these regions were found closely linked with stress-related diseases, such as anxiety disorder, PTSD, autoimmune conditions, and metabolic syndrome (Contoreggi, 2015). It is consistent with the findings of anxiety-like behaviors alterations (Xie et al., 2020a) and stress-like hippocampal transcriptome changes in 1.5-month SF mice (**Figures 4**, **5**). Alternatively, studies showed that neuronal synaptic strength/connectivity is higher during wakefulness than during sleep (Vyazovskiy et al., 2008; Bero et al., 2011). The enhanced glucose metabolism could also possibly be due to the disrupted circadian rhythms in SF mice, and all the PET scans were performed during the light-ON phase which is sleep hours for the NS group. In summary, the enhanced brain glucose metabolism could be due to hyperexcitability of neurons and networks, very similarly with pre- and early stages of AD. This explains the cognitive and emotional behavioral outcomes of SF mice from the perspective of energy imbalance and matches with stress-like hippocampal transcriptome changes.

Chronic Sleep Fragmentation Induced Pathological Neuroinflammation in the Brain

Hippocampal RNAseq in 1.5-month SF and NS mice revealed that inflammatory and immunological pathways were activated by SF (**Figure 4**). Enrichment of the KEGG pathway showed that the signaling pathways involved in the DEGs were also mainly in infection (virus, malaria, and bacterial infection), immune-related pathways (IBD, rheumatoid arthritis, viral myocarditis, autoimmune thyroid disease, SLE, asthma, and NF-κB pathway) (**Figure 4**). Sleep deficiency can clearly induce systemic inflammation which links with the development of cardiovascular disease, autoimmune and neurodegenerative diseases (Besedovsky et al., 2019; Irwin and Vitiello, 2019). Inside the central nervous system, microglia function as macrophages in peripheral tissue, monitoring the pathogens and maintaining homeostasis in the brain. They are sensitive to pathological protein aggregates. Microgliosis has been widely observed in the pre- or early stages of neurodegenerative diseases. Their important contribution to neurodegeneration pathogenesis has been intensively studied (Thawkar and Kaur, 2019). We found that SF induced significant microgliosis both in the cortex and hippocampus (**Figure 2**). Interstitial fluid (ISF) Aβ and tau levels exhibit diurnal fluctuation, negatively linked with sleep time (Kang et al., 2009; Holth et al., 2019). Sleep deprivation increased overnight CSF $A\beta_{1-42}$ levels in healthy adults (Ooms et al., 2014; Lucey et al., 2018). Worse sleep quality and slow-wave sleep (SWS) disruption are significantly associated with higher CSF tau levels (Ju et al., 2017; Holth et al., 2019). Therefore, the interstitial space microenvironment in SF could be quite similar to pre- or early stages of neurodegenerative diseases. The possible explanation could be that microglia were activated by SF-induced excessive interstitial Aβ, p-tau, dysregulated glucocorticoids levels (Fonken et al., 2016), and stress-induced by disrupted circadian rhythms (Fonken et al., 2015, 2016). We are wondering if these activated microglia could initiate neurodegenerative pathogenesis in the SF brain. Besides secretion of inflammatory cytokines which could directly harm the neuronal cells, microglia was reported

to induce synapse elimination *via* C1q and C3 (Schafer et al., 2012), while C3 is upregulated after acute and chronic sleep deprivation (Bellesi et al., 2017). Meanwhile, activated microglia exhibiting amoeboid phenotype, termed as "primed" microglia, showed impaired phagocytic function and therefore dampened neurotoxins clearance (Norden et al., 2015). Thus, SF-activated microglia could probably undergo similar pathological processes as in neurodegeneration, since the stressors were quite similar in the pre- and early stages of neurodegenerative diseases, such as elevated neuroendocrine hormones, excessive metabolic waste, as well as cell debris generated from neuronal necrosis.

CONCLUSION

The current study reported 1.5-month SF in young wild-type mice could generate AD-like pathological changes. Based on the hippocampal transcriptome analysis and literature evidence, we proposed that 1.5-month SF induced pathological processes which were quite similar with pre- and early stages of neurodegenerative diseases, however not yet reaching the symptomatic stage. The mechanisms shared in common were probably neuroinflammation, neuroendocrinological dysregulation, energy metabolism imbalance. Avoiding long-term sleep disturbance or dealing with chronic insomnia-induced stress could be a preventative strategy for neurodegenerative diseases.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/sra/PRJNA757396.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

AUTHOR CONTRIBUTIONS

ND'A and FD designed the research and contributed to revisions and the final draft of the manuscript. LB and LH conducted the experiment, data analysis, and drafted the manuscript. YX contributed to brain sample preparations for RNA sequencing. ZHe participated in the bioinformatic analysis. SD contributed to setting up the chronic sleep fragmentation system. MZ, ZHu, and CY contributed to revisions. EA, WX, YL, and QX were responsible for the PET scans. All authors agree to be accountable for the content of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2021.759983/full#supplementary-material

Supplementary Figure 1 | Phosphor-tau (Ser202/Thr205) is comparable between NS and SF. **(A)** Immunohistochemistry of phosphor-tau (Ser202/Thr205) with AT8 antibody in cortex and hippocampus in slices of NS and SF groups. Scale bar = 20 μm. **(B)** Western blot of AT8 in SF cortex, using cortex of 12-month-old APP/PS1 mice as the positive control. **(C)** Silver staining of brain slices collected from cortex and hippocampus of mice in NS and SF groups as well as APP/PS1 mice. Scale bar = 20 μm.

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Associations of Alcohol Dehydrogenase and Aldehyde Dehydrogenase Polymorphism With Cognitive Impairment Among the Oldest-Old in China

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Recent literature suggested that *ALDH2* mutation is associated with alcohol metabolism, and ethanol intake might jointly increase the risk of Alzheimer's disease (AD) in mice. However, it is unclear whether this synergistic effect exists among humans. We examined the associations of four single nucleotide polymorphisms (SNPs) on aldehyde dehydrogenase (*ALDH*) and alcohol dehydrogenase (*ADH*) genes (i.e., *ALDH2* rs671, *ADH1B* rs1229984, *ADH1B* rs1042026, and *ADH1C* rs1693482) and cognitive impairment among the oldest-old. We also investigated whether this association was modified by ethanol intake from alcohol consumption. Data were from the Chinese Longitudinal Healthy Longevity Survey genetic sub-study, including 1,949 participants aged over 90 years. Participants with a Mini-Mental State Examination (MMSE) score of < 18 were considered cognitively impaired. Alcohol consumption was categorized as heavy, moderate, or never drinkers. With the dominant model, carrying A allele on rs671, C allele on rs1229984, and T allele on rs1042026 was associated with 33% (95% confidence interval [CI]: 5%, 69%), 33% (95% CI: 2%, 75%), and 29% (95% CI: 3%, 62%) higher odds of cognitive impairment in the multivariable-adjusted logistic model, respectively. We did not observe a significant interaction between those SNPs and alcohol consumption. Among the oldest-old, carrying *ALDH2* rs671 mutation was associated with higher odds of cognitive impairment independent of alcohol consumption.

Keywords: ethanol intake, Asian flush, alcohol metabolizing genes, Alzheimer's disease, cognitive impairment

INTRODUCTION

Aging is a major contributor to cognitive decline and dementia across the world, and it is becoming a major public health concern. Previous studies have discussed demographic or socioeconomic risk factors for dementia among old adults, with limited attention on genetic risk factors using large samples. Most of the genetic research on cognitive function focused on candidate genes which have been demonstrated to be associated with Alzheimer's disease (AD) or identified by genome-wide

association studies (GWAS). Those candidate genes include apolipoprotein E (APOE), catechol-*O*-methyltransferase (COMT) (Komulainen et al., 2008), brain-derived neurotrophic factor (BDNF) (Bray et al., 2005), and dystrobrevin-binding protein 1 (DTNBP1) (Wray et al., 2008), with the *APOE* ε4 allele being by far the strongest genetic risk factor and accounts for about 5% of the variance in lifetime cognitive change and 4% of the variance in AD. In addition to those genetic factors, some recent works have also indicated the key role of some alcohol metabolism-related genes as enzymes involved in the detoxification of the ethanol metabolism in the pathology of AD.

Alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) are the two major alcohol-metabolizing enzymes (Chen et al., 2014). The mutation of several single nucleotide polymorphisms (SNPs) on those two genes results in the change of enzymatic activity, such as rs671 in *ALDH*, rs1693482 in *ADH1C*, and rs1229984 and rs1042026 in *ADH1B* (Hurley and Edenberg, 2012). Among the Asian population, the prevalence of *ADH1B*, *ADH1C*, and *ALDH2* mutation is performed. It is estimated that nearly 30% of people in Asia (∼8% of the world population) carry the genetic variants of the *ALDH2* A allele by rs671 (Eng et al., 2007). Previous literature has indicated that a reduced activity of *ADH* and *ALDH* would lead to an excess of acetaldehyde and result in oxidative stress and mitochondrial dysfunctions which have been identified in both familial and sporadic AD (Zhang and Ren, 2011; Chen et al., 2012; Swerdlow, 2018). At the population level, *ADH* and *ALDH* genes have been shown to be associated with alcohol dependence (Macgregor et al., 2009)—a leading cause of dementia (Koch et al., 2019). At present, the associations between *ALDH2* and *ADH* genetic polymorphisms with cognitive function or AD were inconclusive. Some studies suggested that *ALDH2* (rs671 polymorphism) is a risk factor for AD in Japanese (Kamino et al., 2000), whereas others reported no association. A recent study including 339 AD patients and 168 healthy controls investigated the association of several SNPs on *ADH* and *ALDH* with AD and found a suggestive association between *ADH1C* rs2241894 and AD among women (Wu et al., 2021). However, some other genetic studies have found that the negative impact of genetic factors may be cumulative by age, such as *APOE* gene on cognitive function, and there is a fewer study of *ADH* and *ALDH* genes among the oldest-old population.

A recent experimental study suggested that chronic excessive ethanol intake and the *ALDH2* gene mutation might jointly increase the risk of AD in mice (Joshi et al., 2019). Alcohol consumption, as one of the major risk factors of brain damage, is associated with the incidence of dementia. There is a J-shaped association of alcohol consumption with dementia, where excessive alcohol intake or abstinence increased dementia risk, compared with consuming 9–112 g/week (Topiwala and Ebmeier, 2018). In a large cross-sectional study from Southern China, occasional rather than moderate alcohol use was found to be associated with better cognitive function (Au Yeung et al., 2011), suggesting that the observed effects could be driven by a complex interaction between alcohol and other factors. In systematic reviews, drinking patterns are associated with AD and also cognitive function. It is unclear whether *ALDH2* mutation is associated with cognitive function and whether this mutation and ethanol intake synergistically contribute to the development of AD among humans.

The aim of the present study was twofold. First, we investigated the associations of four SNPs (i.e., *ALDH2* rs671, *ADH1B* rs1229984, *ADH1B* rs1042026, and *ADH1C* rs1693482) and cognitive function among nearly 2,000 Chinese oldest-old from a population-based cohort study. Considering the high prevalence of *ADH* and *ALDH* gene mutation in the Chinese population and their unique characterization, we then investigated whether this association would be modified by the level of alcohol consumption.

MATERIALS AND METHODS

Participants

The present study used data from the CLHLS, which is an ongoing longitudinal study that began in 1998 with follow-up surveys for every 2–3 years. The CLHLS is a Chinese nationwide survey conducted in randomly selected counties and cities in 22 of 30 provinces covering 85% of the population of China. All centenarians from the selected areas who agreed to participate were included in the study. Based on sex and place of residence (i.e., living in the same street, village, city, or county) for a given centenarian, randomly selected octogenarians and non-agenarians were also sampled. More details about the sampling procedure and quality of data of this survey have been published elsewhere (Zeng et al., 2017). Ethical approval was obtained from the Research Ethics Committees of Peking University and Duke University (IRB00001052-13074). All participants or their legal representatives signed written consent forms in the baseline and follow-up surveys. In this cross-sectional study, we derived the data from the baseline survey of each participant. The analyses were based on the CLHLS genetic dataset, comprising 1,949 adults aged over 90 years.

Cognitive Function

The cognitive function of CLHLS participants was assessed by the Chinese version of the Mini-Mental State Examination (MMSE) through a home-based interview, which includes 24 items, covering 7 subscales including orientation (4 points for time orientation and 1 point for place orientation); naming foods (naming as many kinds of food as possible in 1 min, 7 points); registration of 3 words (3 points); attention and calculation (mentally subtracting 3 iteratively from 20, 5 points); copy a figure (1 point); recall (delayed recall of the 3 words mentioned above, 3 points); and language (2 points for naming objectives, 1 point for repeating a sentence, and 3 points for listening and following directions). The MMSE score ranges from 0 to 30. Higher scores represent a better cognitive function. The validity and reliability of this Chinese MMSE have been verified in several previous studies (Zhang, 2006; An and Liu, 2016). Consistent with previous studies (Zhang et al., 2019), because a high proportion of our participants did not have formal education (∼70%), cognitive impairment was defined as an MMSE score of < 18.

Alcohol Consumption and Other Covariates

During each interview, the interviewers measured a range of demographic, behavioral, and socioeconomic covariates. Alcohol consumption was self-reported and categorized as heavy, moderate, or never drinkers. Current alcohol users who consumed >25 g of alcohol per day (for men) and > 15 g (for women) were considered heavy drinkers. Current alcohol users who consumed ≤25 g (for men) and ≤15 g (for women) were considered moderate drinkers. Participants who reported they never drank were considered never drinkers. All variables were measured at baseline (indicate year). All self-reported information was collected through face-to-face home interview by trained research staff members with more than 12 years of education.

Following the previous studies, we included the following variables as confounders: age (years), sex, residence (rural vs. urban), education years, smoking (never, former, and current), regular physical activity (yes vs. no), dietary pattern, leisure activity score, and self-reported chronic diseases [e.g., hypertension, diabetes, heart disease, stroke, and chronic obstructive pulmonary disease (COPD)]. Dietary pattern was categorized as unfavorable, intermediate, or favorable by a simplified healthy eating index based on the intake frequency of nine food categories, including fruits, vegetables, fish, bean products, tea, garlic, eggs, sugar, and salt-preserved vegetables. A 3-point scale question was used to measure the current intake frequency of each food group: "always or almost every day," "sometimes or occasionally," or "rarely or never." Those 3 terms received the scores of 2, 1, or 0, respectively, with higher scores indicating a higher level of consumption. Two of those food groups, i.e., sugar and salt-preserved vegetables, were received the scores of 0, 1, and 2, respectively, for some evidence of the negative impact of the high consumption of those two food groups. The leisure activity score was summarized from the following eight activities: visiting neighbors, shopping, cooking, washing clothes, walking 1 km, lifting 5 kg, crouching and standing up three times, and taking public transportation. We scored each activity as 1 for "never," 2 for "sometimes," and 3 for "almost every day." The score ranged from 5 to 21 with a higher score, indicating more leisure activities. All self-reported information was collected through face-to-face home interview by trained research staff members at the baseline survey. Interviewees were encouraged to answer as many questions as possible. If they were unable to answer questions, a close family member or another proxy, such as a primary caregiver, provided answers (Zeng, 2012).

Genotyping

The CLHLS collected DNA samples from parts of participants in 1998, 2000, 2002, 2005, 2008–2009, and 2011–2012 waves of the survey. Genotyping of DNA samples was produced by the Beijing Genomics Institute (BGI), and the BGI genotyping quality control procedures of the CLHLS genetic study have been published elsewhere (Zeng et al., 2016). We extracted four SNPs associated with alcohol metabolism from the GWAS data (i.e., *ALDH2* rs671, *ADH1B* rs1229984, *ADH1B* rs1042026, and *ADH1C* rs1693482).

The genotypes were defined by following the additive and dominant models. In a dominant model, any genotype that contains one or two copies of the minor allele is coded as one; otherwise, the genotype that does not contain any copy of the minor allele is coded as zero. In an additive model, carrying two copies of the minor allele was coded as three. One copy of the minor allele with one copy of the major allele and two major alleles were coded as two and one, respectively. We mainly used the dominant models in the GxE analysis to define the genotype because it can distinguish the genotype carriers and non-carriers, but the additive model cannot. In addition, further grouping the samples in the additive model would result in many more GxE interaction terms in the regressions and would, in turn, negatively affect the estimates and complicate the discussions.

Statistical Analysis

The characteristics of participants were compared according to cognitive function (with vs. without impairment). Means and standard deviations (SDs) were calculated for continuous variables; counts and percentages were calculated for categorical variables. We used both logistic regression (binary outcome: cognitive impairment) and linear regression (continuous outcome: MMSE score) models to examine the unadjusted and adjusted associations between the four polymorphisms and cognitive impairment, respectively. We build two regression models, namely, partly adjusted: adjusted for age at baseline in years, sex, residency, and education years; and fully adjusted: additionally adjusted for smoking status, alcohol consumption, current physical activity, dietary pattern and leisure activity score, and five kinds of self-reported diseases on the basement of the partly adjusted model.

To examine whether the association between the four polymorphisms and cognitive impairment was modified by alcohol consumption, we first ran a logistic regression model with an interaction term between those four polymorphisms and alcohol consumption, respectively, and then conducted the regression analysis among persons with different levels of alcohol consumption (e.g., heavy, moderate, and never drinkers), separately.

All statistical analyses were conducted using STATA version 14.0 (Stata Corp., College Station, TX, United States).

RESULTS

Study Sample Characteristics

Demographic and the four polymorphisms information are detailed by cognitive function in **Table 1**. A total of 1,949 participants were included after excluding 101 participants who lacked cognitive assessment (n = 64) and aged below 90 years (n = 37). Of 1,949 participants aged over 90 years, the average age was 101.3 ± 3.3 years; 76.3% were women. Participants with cognitive impairment are more likely to be older, women, with lower education level, not smoking, with unfavorable

TABLE 1 | Selected characteristics of the participants by cognitive function.

Characteristics[a]		Cognitive impairment[a]		*P*-value
	Total *N* = 1,949	Without *N* = 805	With *N* = 1,144	
	Count (%) unless otherwise indicated			
Age, mean ± SD	101.3 (3.3)	100.9 (3.3)	101.5 (3.2)	<0.001
Sex				<0.001
Women	1487 (76.3)	540 (67.1)	947 (82.8)	
Men	462 (23.7)	265 (32.9)	197 (17.2)	
MMSE score, mean ± SD	14.2 (10.4)	24.9 (3.4)	6.76 (6.4)	<0.001
Residence				0.081
Urban	719 (36.9)	323 (40.1)	396 (34.6)	
Rural	1230 (63.1)	482 (59.9)	748 (65.4)	
Education years, mean ± SD	0.93 (2.5)	1.5 (3.2)	0.57 (1.8)	<0.001
Alcohol consumption				0.053
Never drink	1513 (77.6)	604 (75.0)	909 (79.5)	
Moderate drink	199 (10.2)	88 (10.9)	111 (9.7)	
Heavy drink	237 (12.2)	113 (14.0)	124 (10.8)	
Smoking				0.026
Never	1712 (90.6)	828 (89.0)	884 (92.2)	
Former	148 (7.8)	82 (8.8)	66 (6.9)	
Current	29 (1.5)	20 (2.2)	9 (0.9)	
Dietary pattern[b]				<0.001
Unfavorable	804 (41.3)	261 (32.4)	543 (47.5)	
Intermediate	634 (32.5)	273 (33.9)	361 (31.6)	
Favorable	511 (26.2)	271 (33.7)	240 (21.0)	
Regular physical activity				0.05
Yes	522 (27.8)	310 (33.4)	212 (22.2)	
No	1359 (72.2)	617 (66.6)	742 (77.8)	
Leisure activity score[b]	9.6 (2.4)	10.6 (2.5)	8.7 (1.9)	<0.001
***ALDH2*, rs671[c]**				0.083
GG	527 (65.5)	733 (64.1)	1260 (64.6)	
AG	241 (29.9)	359 (31.4)	600 (30.8)	
AA	37 (4.6)	52 (4.5)	89 (4.6)	
***ADH1B*, rs1229984**				0.315
TT	495 (61.5)	639 (55.9)	1134 (58.2)	
CT	254 (31.6)	431 (37.7)	685 (35.1)	
CC	56 (7.0)	74 (6.5)	130 (6.7)	
***ADH1B*, rs1042026**				0.328
CC	516 (64.1)	683 (59.7)	1199 (61.5)	
CT	244 (30.3)	398 (34.8)	642 (32.9)	
TT	45 (5.6)	63 (5.5)	108 (5.5)	
***ADH1C*, rs1693482**				0.323
CC	694 (86.2)	958 (83.7)	1652 (84.8)	
CT	103 (12.8)	177 (15.5)	280 (14.4)	
TT	8 (1.0)	9 (0.8)	17 (0.9)	

MMSE, Mini-Mental State Examination.

[a]Cognitive impairment: cognitive impairment was defined by an MMSE score of < 18.

[b]Dietary score: the score was calculated from the frequency for intake of nine foods: fruits, vegetables, fish, bean products, tea, garlic, eggs, sugar, and salt-preserved vegetables. For two of 9 variables, i.e., sugar and salt-preserved vegetables, the answer of "always or almost every day," "sometimes or occasionally," or "rarely or never" received the scores of 0, 1, or 2, respectively; for the other 7 variables, the same three responses received the scores of 2, 1, or 0, respectively. Scores for the 9 variables were then summed to obtain a scale ranging from 0 to 18 with higher scores indicating higher frequency for fruits, vegetables, fish, bean products, tea, garlic, and eggs, while higher scores meant lower frequencies for sugar and salt-preserved vegetables. The dietary pattern was defined by the trisection of the dietary pattern score (lowest trisection: unfavorable, intermediate: intermediate, highest: favorable).

[c]Leisure activity score: eight activities were assessed: visiting neighbors, shopping, cooking, washing clothes, walking 1 km, lifting 5 kg, crouching and standing up three times, and taking public transportation. We scored each activity as 1 for "never," 2 for "sometimes," and 3 for "almost every day." The score ranged from 5 to 21 with higher score indicating more leisure activities.

dietary pattern, and with lower leisure activities (**Table 1**, p-values < 0.05).

Association of Cognitive Function With *ADH* and *ALDH2* Genes

In the partially adjusted logistic regression model, carrying A allele on rs671, C allele on rs1229984, T allele on rs1042026, and T allele on rs1693482 was associated with higher odds of cognitive impairment [rs671: odds ratio (OR): 1.19, 95% CI: 0.97, 1.47; rs1229984: OR: 1.27, 95% CI: 1.00, 1.61; rs1042026: OR: 1.26, 95% CI: 1.04, 1.55; rs1693482: OR: 1.19, 95% CI: 0.91, 1.57; **Table 2**]. In the fully adjusted logistic regression model, the associations of rs671, rs1229984, and rs1042026 with cognitive impairment persisted to be significant. In the partially adjusted linear regression model, carrying T allele on rs1042026 and T allele on rs1693482 was significantly associated with a lower MMSE score of 0.90 (95% CI: 0.01, 1.80). In the fully adjusted linear regression model, carrying A on rs671 was associated with a lower MMSE score of 1.05 (95% CI: −2.03, −0.09).

In the additive model, the one major allele with one minor allele genotypes of rs671, rs1229984, and rs1042026 was significantly associated with lower MMSE scores and higher odds of cognitive impairment compared with the two major allele genotypes (**Table 2**). However, the two minor allele genotypes of those four SNPs were not significantly associated with MMSE score or cognitive impairment in the fully or partially adjusted model.

Association Between Alcohol Consumption and Cognitive Function

The association between alcohol consumption and cognitive impairment was analyzed in the partially adjusted model and fully adjusted model (**Supplementary Table 1**). In the fully adjusted linear regression model, moderate alcohol use and heavy alcohol use were significantly associated with a lower MMSE score (moderate: −2.52, 95% CI: −2.49, −2.55; heavy: −2.83, 95% CI: −2.36, −3.30) compared with participants who never consumed alcohol.

Subgroup Analyses by Alcohol Consumption

Table 3 presents the associations between four SNPs (those specified here) with the dominant model and cognitive impairment stratified by alcohol consumption. In the subgroup analyses, there is no evidence that the association of each of four SNPs with cognitive impairment differed by alcohol consumption (p-values for interactions > 0.05).

DISCUSSION

Using data from nearly 2,000 Chinese adults aged over 90 years, we found that the SNPs on *ADH* and *ALDH2* genes were associated with higher odds of cognitive impairment. In addition, the results showed that the associations between those polymorphisms and cognitive impairments were not modified by

TABLE 2 | Association of *ALDH2* rs671, *ADH1B* rs1229984, *ADH1B* rs1042026, and *ADH1C* rs1693482 polymorphisms with cognitive impairment.[a]

Independent variable	Logistic regression OR of cognitive impairment[a], (95% CI)		Linear regression Coefficient for MMSE score (95% CI)	
	Partially adjusted[b]	Fully adjusted[c]	Partially adjusted	Fully adjusted **
Additive model				
rs671 GG vs. AG	1.19 (1.00, 1.47) **	1.36 (1.07, 1.74) **	−0.61 (−1.62, 0.40)	−1.23 (−2.22, −0.26) **
rs671 GG vs. AA	1.02 (0.64, 1.64)	1.12 (0.66, 1.90)	1.25 (−0.99, 3.51)	0.84 (−1.32, 3.00)
rs1229984 TT vs. CT	1.30 (1.06, 1.60) **	1.32 (1.05, 1.67) **	−1.11 (−2.09, −0.13) **	−1.02 (−1.97, −0.08) **
rs1229984 TT vs. CC	1.25 (0.83, 1.88)	1.09 (0.70, 1.72)	−0.17 (−2.13, 1.78)	0.35 (−1.49, 2.21)
rs1042026 CC vs. CT	1.22 (1.00, 1.51) **	1.29 (1.03, 1.64) **	−0.82 (−1.82, 0.17) *	−0.92 (−1.87, −0.01) **
rs1042026 CC vs. TT	1.29 (0.83, 2.03)	1.05 (0.65, 1.72)	0.02 (−2.11, 2.16)	0.99 (−1.04, 3.04)
rs1693482 CC vs. CT	1.22 (0.93, 1.62) *	1.16 (0.84, 1.59)	−1.39 (−2.71, −0.08) **	−1.27 (−2.55, −0.004) **
rs1693482 CC vs. TT	0.88 (0.31, 2.56)	1.07 (0.34, 3.28)	2.54 (−2.58, 7.68)	1.39 (−3.50, 6.28)
Dominant model				
rs671 GG vs. AG/AA	1.19 (0.97, 1.47) *	1.33 (1.05, 1.69) **	−0.53 (−1.49, 0.42) *	−1.05 (−2.03, −0.09) **
rs1229984 TT vs. CT/CC	1.27 (1.00, 1.61) **	1.33 (1.02, 1.75) **	−0.90 (−1.80, −0.01) **	−0.93 (−2.00, 0.12) *
rs1042026 CC vs. CT/TT	1.26 (1.04, 1.55) **	1.29 (1.03, 1.62) **	−0.76 (−1.69, 0.17)	−0.70 (−1.63, 0.23)
rs1693482 CC vs. CT/TT	1.19 (0.91, 1.57) *	1.15 (0.84, 1.57)	−1.19 (−2.42, 0.05) *	−1.09 (−2.35, 0.15) *

*Significance: *p < 0.1, **p < 0.05.*

[a] *Defined as a Mini-Mental State Examination score of < 18.*

[b] *Model was adjusted for age at baseline in years, sex, residency, and education years.*

[c] *Model was additionally adjusted for smoking status, alcohol consumption, current physical activity, dietary index, and leisure activity score (continuous) and five kinds of self-reported diseases on the basement of partly adjusted model.*

alcohol consumption, which is different from the findings of a recent experimental study (Joshi et al., 2019).

The results indicated that carrying the *ALDH2* and *ADH* mutation was associated with higher odds of cognitive impairment among the oldest-old population. Its mutation results in a reduction of the *ALDH2* enzymatic activity, which is widely mutated in the Asian population (∼30%). One possible explanation was that those two mutations have a negative impact on mitochondrial functions and the metabolism of 4-hydroxy-2-nonenal (4-NHE). *ALDH2* and *ADH* are involved in the detoxification of the ethanol metabolism and other aldehydes, including 4-HNE. It has been shown that 4HNE concentration increased in the brain tissue of the *ALDH2*2* transgenic mice in an age-dependent manner. Such increases correlated with neurodegeneration memory loss and AD-like pathological changes in these *ALDH2*2* transgenic mice (Ohsawa et al., 2008). In addition, 4HNE levels are higher in the hippocampus of postmortem samples from patients with AD (Williams et al., 2006). Among the population level, previous studies reported the inconsistent results on this association. A cross-sectional case-control study has shown that *ALDH2* genotype is associated with cognitive function among 139 Chinese patients with Parkinson's disease (mean age: 63.0 years) (Yu et al., 2016), and another study has demonstrated its association among 411 Chinese with an average age of 77.4 years (Wang et al., 2008). However, in another study including 690 Korean community residents (mean age: 72.8 years) (Kim et al., 2004), the association between *ALDH2* genotype and cognitive functions was not significant. This paradox finding may be due to the mean age difference of study samples. It is plausible that the genetic risk of carrying the *ALDH2* mutation is cumulative (Licher et al., 2019). Researchers found that *APOE* as well as other common genetic variants could have a cumulative risk on the progression of dementia and AD as age advances (van der Lee et al., 2018). To be more specific, with the negative influence of those mutations on neuronal functioning, the carriers may have a higher speed of neuronal cell loss which is irreversible. Accordingly, during the early life stage, the difference in cognitive function between the carriers and non-carriers may not be significant, while as people age, the harmful effects of genetic variants may build up, and the carriers may have a lower average cognitive function compared with non-carriers of the same age due to the cumulative damage on neurons (Bai and Mei, 2011). In addition to rs671 on *ALDH2*, we also found that rs1229984 and rs1042026 on *ADH1B* gene were associated with the cognitive function among the oldest-old

TABLE 3 | The association of *ALDH2* rs671 polymorphism with cognitive impairment stratified by alcohol consumption and fresh fruit consumption.[a]

	Participants (N)	Odds ratio (95% Confidence Interval)[b]	*P* for interaction
Alcohol consumption			
Never			
rs671 GG vs. AG/AA	912/601	1.25 (0.97, 1.62)	
rs1229984 TT vs. CT/CC	889/624	1.36 (1.05, 1.75)	
rs1042026 CC vs. CT/TT	937/576	1.38 (1.07, 1.79)	
rs1693482 CC vs. CT/TT	1295/218	1.39 (0.95, 2.03)	
Moderate drink			
rs671 GG vs. AG/AA	146/53	1.45 (0.63, 3.34)	0.58
rs1229984 TT vs. CT/CC	121/78	0.97 (0.47, 2.02)	0.44
rs1042026 CC vs. CT/TT	131/68	0.77 (0.36, 1.63)	0.15
rs1693482 CC vs. CT/TT	167/32	0.47 (0.19, 1.23)	0.079
Heavy drink			
rs671 GG vs. AG/AA	203/34	2.14 (0.84, 5.50)	0.26
rs1229984 TT vs. CT/CC	124/113	1.13 (0.63, 2.13)	0.63
rs1042026 CC vs. CT/TT	131/106	1.01 (0.54, 1.90)	0.51
rs1693482 CC vs. CT/TT	190/47	0.85 (0.39, 1.87)	0.54

[a] *Defined as a Mini-Mental State Examination score of < 18.*

[b] *From logistic regression with adjustments for age at baseline in years, sex, residency, education years, smoking status, alcohol consumption, current physical activity, dietary index, and leisure activity score (continuous) and five kinds of self-reported diseases on the basement of partly adjusted model.*

population. The *ADH1B* rs1229984 T allele encodes a super active enzyme subunit that could accelerate the conversion from ethanol into acetaldehyde (Lin et al., 2021). *ADH1C* rs1693482 is found to be associated with a twofold difference in *ADH* Vmax *in vitro* (Birley et al., 2009). One experimental study has found that in the animal model of AD, the overexpressing of *ADH1B* suppresses the β-amyloid-induced neuron apoptosis (Wang et al., 2019). Corresponding the results, it may indicate that the genes associated with alcohol metabolism might be important in the progress of cognitive impairment or AD. To note, these results also determined the potential role of mitochondrial dysfunctions in the pathology of AD.

We found that those associations were not modified by alcohol consumption. One experimental study found that the *ALDH2*2* mutation increases the damage by sustained ethanol exposure in mouse brain, and they found that *ALDH2*2* deficiency increases ethanol-induced neuroinflammation *in vivo* (Joshi et al., 2019). However, we found that on the population level, such interactive effect was not significant. It may indicate that the damage of alcohol to the brain is stable and consistent which may not be varied by genotype. In addition, another possible explanation was that the consumption of stored fruits and vegetables, as well as fruit juice, would bring ethanol into the human body and the total ethanol intake is more difficult to measure.

Methodological strengths of this study include a large sample size of the oldest old, and we have included multiple genes related to alcohol metabolism. This study also has several limitations: (1) it has a cross-sectional design and cannot evaluate changes in alcohol consumption or establish causality. However, the results were robust to adjustment of a number of indicators such as diseases. Nevertheless, prospective studies on the incidence of cognitive impairment are warranted; (2) the alcohol consumption was self-reported, thus non-specific (Muntner et al., 2014). This possibility of recall bias is a common concern in the longitudinal cohort studies, although CLHLS data have been validated as being reliable in previous studies (Zeng et al., 2008). In the CLHLS study, all self-reported information was collected through face-to-face home interview by trained research staff members. Interviewees were encouraged to answer as many questions as possible. If they were unable to answer questions, a close family member or another proxy, such as a primary caregiver, provided answers (Zeng et al., 2008). (3) We used the Chinese version of MMSE to measure cognition, which is not a clinical diagnosis for cognitive impairment (Zhang, 1993; Chou, 2003). However, it is a validated instrument in population-based studies. (4) As the study included only participants who aged over 90 years and the average lifespan of women is longer than men, a higher proportion of women were taken into the analysis sample which may lead to bias. (5) The percentage of drinker is 20% and the results need to be validated within a larger cohort.

CONCLUSION

We found that some SNPs associated with alcohol metabolism were associated with higher odds of cognitive impairment among the Chinese oldest-old and those associations were independent of alcohol consumption. Due to its mutation can be easily observed even without genotyping, it may have more public health implications especially for the Asian population on the AD risk stratification and alcohol control. Further studies on how to modify the genetic risk for cognitive impairment associated with gene mutation associated with alcohol metabolism are warranted.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://sites.duke.edu/centerforaging/programs/chinese-longitudinal-healthy-longevity-survey-clhls.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Biomedical Ethics Review Committee of Peking University (IRB00001052-11015). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2021.710966/full#supplementary-material

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REVIEW
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Alzheimer's Disease: An Update and Insights Into Pathophysiology

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Alzheimer's disease (AD) is an irreversible brain disorder associated with slow, progressive loss of brain functions mostly in older people. The disease processes start years before the symptoms are manifested at which point most therapies may not be as effective. In the hippocampus, the key proteins involved in the JAK2/STAT3 signaling pathway, such as p-JAK2-Tyr1007 and p-STAT3-Tyr705 were found to be elevated in various models of AD. In addition to neurons, glial cells such as astrocytes also play a crucial role in the progression of AD. Without having a significant effect on tau and amyloid pathologies, the JAK2/STAT3 pathway in reactive astrocytes exhibits a behavioral impact in the experimental models of AD. Cholinergic atrophy in AD has been traced to a trophic failure in the NGF metabolic pathway, which is essential for the survival and maintenance of basal forebrain cholinergic neurons (BFCN). In AD, there is an alteration in the conversion of the proNGF to mature NGF (mNGF), in addition to an increase in degradation of the biologically active mNGF. Thus, the application of exogenous mNGF in experimental studies was shown to improve the recovery of atrophic BFCN. Furthermore, it is now coming to light that the FGF7/FGFR2/PI3K/Akt signaling pathway mediated by microRNA-107 is also involved in AD pathogenesis. Vascular dysfunction has long been associated with cognitive decline and increased risk of AD. Vascular risk factors are associated with higher tau and cerebral beta-amyloid (Aβ) burden, while synergistically acting with Aβ to induce cognitive decline. The apolipoprotein E4 polymorphism is not just one of the vascular risk factors, but also the most prevalent genetic risk factor of AD. More recently, the research focus on AD shifted toward metabolisms of various neurotransmitters, major and minor nutrients, thus giving rise to metabolomics, the most important "omics" tool for the diagnosis and prognosis of neurodegenerative diseases based on an individual's metabolome. This review will therefore proffer a better understanding of novel signaling pathways associated with neural and glial mechanisms involved in AD, elaborate potential links between vascular dysfunction and AD, and recent developments in "omics"-based biomarkers in AD.

Keywords: Alzheimer, diagnose, therapeutic, JAK, NGF, omic, dementia, vascular

INTRODUCTION

Alzheimer's disease (AD) is a progressive brain disease that is attributed by the Alzheimer Association as the cause of 60–80% of dementia cases. Depending on the stage of the disease, it is characterized by factors that progress to hinder the performance of everyday activities, such as apathy, depression, impaired communication, disorientation, poor judgment, difficulty in swallowing and walking, and behavioral changes (Alzheimer's Association, 2021). The duration takes to develop a continuum of these symptoms is determined by factors such as age, genetics, and sex (Vermunt et al., 2019). Current statistics show that over six million Americans aged 65 and above are living with AD, with a projection of about 13.8 million by 2060, and death cases have increased by 16% during the COVID-19 pandemic (Alzheimer's Association, 2021). Aside from informal caregiving, the total cost of care payment for AD patients and related diseases was estimated at $355 billion in 2021.

The progressive cognitive decline in AD is associated with the accumulation of amyloid-beta (Aβ) and tau proteins (Selkoe and Hardy, 2016). Aβ is derived from the sequential cleavage of amyloid precursor protein (APP) by beta-secretase and gamma-secretase. The aggregation of Aβ thus forms oligomers that are toxic to the neurons (Haass and Selkoe, 2007). Tau, on the other hand, is derived from alternative splicing from the microtubule-associated protein tau (MAPT) gene to form soluble protein isoforms (Goedert et al., 1989). Several functional interactions have been revealed between Aβ and tau in the neural circuit damage and cognitive decline in AD (Tripathi and Kalita, 2019; Busche and Hyman, 2020). This is in a bid to encourage a broad approach in the design of a potential therapy.

Unfortunately, no treatment option is available to cure the disease to date (Husna Ibrahim et al., 2020). Recent approaches in the treatment of AD include exploring the potentials of some natural products with neuroprotective effects (Kamil et al., 2018, 2020; Prom-In et al., 2020; Kamal et al., 2021) and metabolites to modulate signaling pathways associated with neurovascular endothelium through multi-omic analyses (Corral-Jara et al., 2021). There have also been reports on cellular signaling-related sex-dependent effects under hyperglycemic and lipid stress (Nuthikattu et al., 2020, 2021). However, this review focuses on significant signaling pathways associated with the stages of AD, the potential links between vascular dysfunction and AD, as well as recent developments in "omics"-based approaches in AD. Without publication date restriction until May 2021, PubMed, ScienceDirect, and Google Scholar databases were searched for published articles containing the search terms related to "Alzheimer," "dementia," "signaling pathways," "vascular dysfunction," "cognitive impairment," and "omics." Relevant articles retrieved were thereafter included for synthesis.

SIGNALING PATHWAYS ASSOCIATED WITH ALZHEIMER'S DISEASE

From several studies, a group of researchers was able to develop a database containing a compilation of signaling pathways associated with AD, called AlzPathway[1] (Mizuno et al., 2012). There are several components of the AlzPathway, which include the Aβ cleavage and degradation, apolipoprotein E (ApoE)-cholesterol pathway and NFT accumulation, acetylcholine production, Wnt signaling pathway, Ubiquitin mediated proteolysis, apoptosis, calcium signaling pathway, Notch signaling pathway, MAPK signaling pathway, abnormal ceramide accumulation, reactive oxidation process, neurotrophin signaling pathway, cell cycle, mTOR signaling pathway, lipid pathway, insulin pathway, and inflammation pathway (Mizuno et al., 2012). Here, we discuss newly emerging pathways as potential diagnostic and therapeutic targets.

In the 2021 Alzheimer Drug Development Pipeline, agents that are currently in clinical trials were divided into 3 categories; agents that are disease-modifying biologic, disease-modifying small molecule, and symptom-reducing small molecule (Cummings et al., 2021). Twenty-nine percentage of the disease-modifying therapies (DMTs) that were successfully shifted to the 3rd phase trials are targeting the amyloid. Meanwhile, out of 50 drugs that were repurposed for AD treatments, 10 of the agents proceed to Phase 3 clinical trials. AD drug development has been very challenging due to its heterogeneity in AD pathogenesis, with a 0.4% success rate as memantine is the only drug that has significant effects and was approved by FDA out of 244 drugs investigated in AD clinical trials from the year 2002 to 2012 (Fish et al., 2019).

As microglia activation also plays a role in the pathogenesis of AD, minocycline, an anti-inflammatory tetracycline drug was investigated in AD clinical trials due to its ability to penetrate the blood-brain barrier (BBB) while hindering the proinflammatory microglia. Besides, minocycline attenuates the fibrillization of Aβ, hence, halting the deposition of Aβ plagues and neuronal death *in vitro* (Romero-Miguel et al., 2021). However, 24 months of 400 mg minocycline treatment in a randomized clinical trial failed to delay the cognitive impairments in mild AD patients (Howard et al., 2020). This finding is parallel to the previous trials on non-steroidal anti-inflammatory drugs (NSAIDs) which failed to exert positive effects on delaying AD developments. Thus, it can be deduced that maybe although neuroinflammation is one of many pathways involved in the heterogenic pathogenesis of AD, targeting neuroinflammation alone may not be enough to exert significant neuroprotective effects in AD.

Vitamin E is an antioxidant consisting of α-, β-, γ-, and δ-tocophenols and tocotrienols, that can neutralize free radicals and ROS which eventually protecting the cellular membrane from the destructive oxidative stress (Lloret et al., 2019). α-tocopherol is a critical antioxidant in the brain due to the abundance α-tocopherol transfer protein (α-TTP) transporter for the regulation of vitamin E distribution in other tissues. Besides, vitamin E is also involved in the protein kinase C (PKC) pathway and exerts anti-inflammatory effects. The first clinical trial of vitamin E in 1997 showed a decline in progression of AD which remarkably shifted the attention toward the advancement of vitamin E studies in the development of AD treatments. However, several failed clinical trials later left the efficacy of vitamin E questionable. The study results may have been influenced by the

[1] http://alzpathway.org/

discrepancies in the bioavailability of vitamin E such as the base nutritional status, intestinal differences, and rate of absorption for each cohort in the clinical trials, alongside the acclaimed heterogeneity of AD pathogenesis itself (**Table 1**).

The Neural Mechanisms Involved in Alzheimer's Disease

Currently, the major theories related to the mechanisms involved in the pathogenesis of AD are the neuronal extracellular deposition of Aβ peptides (senile/amyloid plaques), and neuronal intracellular accumulation of hyperphosphorylated tau protein to form neurofibrillary tangles (NFTs). However, the major underlying factor for the cognitive and behavioral dysfunction observed in AD is synaptic dysfunction. For instance, human neuronal dysfunction is associated with increased Aβ and phosphorylated tau reduces synaptic strength due to its aggregation in the dendritic spine and consequent internalization of N-methyl-d-aspartic acid receptors (NMDARs) (Sperling et al., 2009; Palop and Mucke, 2010; Wesson et al., 2011). Other factors such as oxidative stress, which is increased in the brain in aging, have been shown to precede the formation of senile plaques and deposition of NFTs (Huang et al., 2016; Singh et al., 2016; Uddin and Kabir, 2019). Moreover, increased levels of inflammatory cytokines and associated genes have also been implicated in the development of AD (Hollingworth et al., 2011; López González et al., 2016). Some emerging neural mechanistic signaling pathways that have been associated with the pathogenesis of AD are discussed below.

The Nerve Growth Factor Metabolic Pathway

Nerve growth factor (NGF) is a member of the neurotrophin family, which plays a critical role in the functions of the central and peripheral nervous systems (PNSs). NGF was earlier recognized for its notable role in the development of the embryo, the differentiation and survival of the PNS, as well as the differentiation and maintenance of the PNS in adulthood (Levi-Montalcini and Angeletti, 1968; Levi-Montalcini, 1987). The role of NGF in the central nervous system (CNS) was later recognized from the expression of NGF receptors in the basal forebrain cholinergic neurons (BFCN) (Schwab et al., 1979; Seiler and Schwab, 1984).

NGF is mainly released as a precursor protein (proNGF) into the extracellular space, which is then cleaved by the action of a serine protease, plasmin, to mature NGF (mNGF)

TABLE 1 | Agents in Phase 3 clinical trials from 2016 to 2021.

2016	2017	2018	2019	2020	2021
Albumin + IVIG CAD106 Gantenerumab Solanezumab Aducanumab	Albumin + IVIG CAD106 Gantenerumab Solanezumab Aducanumab Crenezumab	Albumin + IVIG CAD106 Gantenerumab Solanezumab Aducanumab Crenezumab	Plasma exchange with Albumin + IVIG CAD106 Gantenerumab Solanezumab Aducanumab Crenezumab	CAD106 Gantenerumab Solanezumab Aducanumab BAN2401	Gantenerumab Solanezumab Aducanumab Lecanemab
MK-8931 Pioglitazone CNP520 GV-971 ALZT-OP1a/b Nilvadipine Insulin AZD3293 TTP488 JNJ54861911 TRx0237 Masitinib	MK-8931 Pioglitazone CNP520 GV-971 ALZT-OP1a/b Nilvadipine Insulin AZD3293 JNJ54861911 TRx0237 Azeliragon E2609	MK-8931 CNP520 GV-971 ALZT-OP1a/b Insulin AZD3293 JNJ54861911 TRx0237 Azeliragon E2609 Icosapentethyl (IPE)	CNP520 ALZT-OP1a/b TRx0237 E2609 COR388 ANAVEX2-73 Icosapentethyl (IPE) Losartan + Amlodipine + Atorvastatin AGB101 BHV4157 Masitinib	ALZT-OP1a/b TRx0237 COR388 ANAVEX2-73 Icosapentethyl (IPE) Losartan + Amlodipine + Atorvastatin AGB101 BHV4157 Masitinib Azeliragon Metformin Tricaprilin	TRx0237 Icosapentethyl (IPE) Losartan + Amlodipine + Atorvastatin AGB101 Atuzaginstat Azeliragon Blarcamesine GV-971 Metformin NE3107 Tricaprilin Troriluzole Omega-3
AC-1204 Aripiprazole AVP-786 Brexpiprazole Lu AE58054 Nabilone RVT-101	AC-1204 Aripiprazole AVP-786 Brexpiprazole Nabilone RVT-101 ITI-007 Methylphenidate Idalopirdine Suvorexant	AVP-786 Nabilone ITI-007 Methylphenidate Suvorexant AXS-05 Escitalopram Octohydroaminoacridine succinate Zolpidem	AVP-786 Brexpiprazole Nabilone Methylphenidate AXS-05 Mirtazapine Escitalopram Octohydroaminoacridine succinate Guanfacine Zolpidem Ginkgo Biloba	AVP-786 Brexpiprazole Methylphenidate AXS-05 Mirtazapine Escitalopram Octohydroaminoacridine succinate Guanfacine Zolpidem Ginkgo Biloba BPDO-1603 Zoplicone	AVP-786 Brexpiprazole Nabilone Mirtazapine Escitalopram Octohydroaminoacridine succinate Guanfacine Ginkgo Biloba BPDO-1603 Caffeine Donepezil

Agents in Phase 3 since 2016–2021 (Cummings et al., 2016, 2017, 2018, 2019, 2020, 2021).

(Bruno and Cuello, 2006). Upon the release of proNGF by the postsynaptic neurons of the cortex and hippocampus, and its conversion to mNGF, the physiological roles of NGF are executed by binding to two specific cell-surface receptors, the tropomyosin-related kinase A (TrkA) receptor and p75 neurotrophin receptor (p75NTR). When bound to its receptor, predominantly TrkA, on pre-synaptic cholinergic neurons, NGF gets to the cell bodies of the innervating neurons, the BFCN, through a retrograde transport, to initiate, a signaling cascade for the release of acetylcholine. NGF maturation stimulated by plasmin is positively regulated by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), which convert plasminogen to plasmin, and are negatively regulated by plasminogen activator inhibitor 1 (PAI-1) and neuroserpin, inhibiting the activators. The mNGF degradation, on the other hand, is by activated matrix metalloproteinases (MMPs), for example, MMP-9 release upon neuronal stimulation, and regulated by the tissue inhibitor of metalloproteinases 1 (TIMP-1). Plasmin is as well responsible for the activation of proMMP-9 to MMP-9. In addition, it has also been shown that there is *in-vitro* and *in-vivo* degradation of mNGF by MMP-3, a protease produced in response to cholinergic stimulation in cortical cells. Although alteration in MMP-3 has been shown to be sex-specific, its level is associated with cognitive decline and AD pathology in humans in addition to its observed upregulation in the experimental model of AD (Pentz et al., 2021).

In AD, this coordinated array of activities is altered at different levels of the pathway. These include alteration in the conversion of proNGF to mNGF, downregulation of TrkA receptor, impaired retrograde signaling and transport, increased mNGF degradation, and inflammatory response due to reduced acetylcholine production and increased Aβ levels (Mitra et al., 2019). Therefore, the NGF metabolic pathways involve a coordinated activity of proteases responsible for NGF maturation (e.g., tPA, plasmin, and neuroserpin) as well as those involved in NGF degradation (e.g., MMP-3, MMP-9, and TIMP-1).

Owing to the promising effect of NGF in ameliorating pathologic conditions associated with AD, a number of approaches have been made and ongoing to therapeutically deliver NGF to the diseased brain (Mitra et al., 2019). Studies in experimental model organisms have shown that the t-PA/plasmin system is an important factor that hinders the pathogenesis of AD, through its involvement in the clearance of Aβ microaggregates as well as inhibition of Aβ-induced neurodegeneration (Melchor et al., 2003; Oh et al., 2014). However, human cohort studies showed no significant alteration in the net enzymatic activities of plasmin in AD, although there was an observed increase in the mRNA level of both activators (tPA and uPA) and inhibitors (PAI-1 and α2-antiplasmin) of the plasminogen system, with increases more at the late Braak stage (Barker et al., 2012). Studies have reported increased levels of MMPs such as MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, and MT1-MMP, in AD, as well as their neuroprotective effect and Aβ cleavage properties in the animal model of AD (Fragkouli et al., 2014; Kaminari et al., 2017). As beneficial as some of these proteins appear to be in AD, efforts to overexpress them have been reported to be detrimental (Wilcock et al., 2011; Terni and Ferrer, 2015), thus caution has to be taken in any chosen therapeutic approach.

The Janus Kinase/Signal Transducer and Activator of the Transcription Pathway

The Janus kinase/signal transducer and activator of the transcription (JAK/STAT) pathways ensure that an extracellular signal is translated into a transcriptional response through a direct mechanism.

The JAK/STAT signaling is a pathway responsible for the regulation of the cytokine responsive genes by transducing extracellular signals from ligands such as cytokines, hormones, and growth factors to the nucleus. This pathway is known to regulate processes such as cell growth and proliferation, differentiation, and apoptosis. The binding of these ligands to their receptors activates JAKs, which are of four family members: JAK1, JAK2, JAK3, and Tyk2 (tyrosine kinase 2). This will in turn phosphorylate the receptors as well as the JAKs. The STAT proteins, consisting of seven members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6, are therefore recruited to the sites formed by the phosphorylated JAKs. The STATs became phosphorylated and activated to form dimers. The dimerized STATs translocate to the nucleus to bind specific regulatory sequences and regulate gene expression (Kisseleva et al., 2002; Rawlings et al., 2004).

Evidence linking the involvement of oxidative stress to the pathogenesis of AD is buttressed by the role of reactive oxygen species in the activation of the JAK/STAT signaling pathway (Simon et al., 1998). Moreover, inhibition of the JAK/STAT pathway by various pharmacological agents has been shown to have a protective effect on AD, through the regulation of Nrf2 signaling (Sharma et al., 2020). In addition, the potential therapeutic role of receptor activation (e.g., TREM2) and folic acid supplementation has been associated with the modulation of the JAK/STAT signaling pathway (Li et al., 2016; Ruganzu et al., 2021). Micropeptides such as humanin and colivelin have also been shown to have neuroprotective effects in models of AD by activating the JAK2/STAT3 signaling pathway (Chiba et al., 2009). Moreover, a combinatorial study involving data from genetic, experimental, observational, and empirical analyses further emphasized the importance of the JAK-STAT signaling as a potential therapeutic target for AD (Nevado-Holgado et al., 2019).

Various experimental models have shown that phosphorylated STAT co-localizes with markers of astrocytes and microglia but not neurons. In addition, some genes such as Ntrk3, Cox17, and Grid2ip have been identified as the cell-specific target, because their predominant expression in astrocytes points to a specific role of STAT3 in AD pathology (Chintapaludi et al., 2020). One of the several functions of the astrocyte in the brain is the regulation of synaptic transmission, and protection and support for neurons, through the release of growth factors and cytokines (Murphy, 1993; Pascual et al., 2005). Astrocytes have been shown to play a crucial role in the degradation of amyloid plaques through the production of TGF-β to inhibit microglial activity, as well as the modulatory effect of ApoE, which is highly expressed in astrocytes (Vincent et al., 1997;

Wegiel et al., 2000; Wyss-Coray et al., 2003; Koistinaho et al., 2004). Therefore, loss of astrocyte function has been found central to the pathogenesis of AD (Acosta et al., 2017). In AD, there is astrocyte involvement in oxidative stress, suppression of innate immunity, and increased expression of pro-inflammatory cytokines, suggesting the relevance of astrocyte-targeted anti-inflammatory therapy (Furman et al., 2012; González-Reyes et al., 2017; Kamal et al., 2020).

Increased expression of proteins such as glial fibrillary acidic protein (GFAP) was recognized as a "gold standard" biomarker of reactive astrocyte, a responsive state of astrocyte to a pathology (Eng et al., 2000). GFAP expression was shown to increase in the Braak stage (Simpson et al., 2010). Other potential protein biomarkers not entirely astrocyte-specific but have been shown to be altered in AD-related astrocyte dysfunction include excitatory amino acid transporters (EAATs) (of which EAAT2 has been shown to decrease with an increase in Braak stage) (Simpson et al., 2010), astrocyte-derived S100B, CD44, glutamine synthetase, and aldehyde dehydrogenase 1 family, member L1, (ALDH1L1) (Garwood et al., 2017).

The FGF7/FRFR2/PI3K/AKT Pathway

Fibroblast growth factors (FGFs) FGF family of growth factors is a large family of proteins with 22 identified ligands. They bind to high-affinity tyrosine kinase receptors, the FGFRs, to exert their cellular and physiological effects, which include proliferation, angiogenesis, invasion, and migration. This binding activates the intracellular tyrosine kinase domain of FGFR by phosphorylation of specific tyrosine residues. Once activated, the FGFR couple downstream intracellular signaling pathways, one of which is the PI3K/AKT signaling. In the PI3K/AKT pathway, the protein GRB2 is recruited, which then recruits another protein GAB1 to activate PI3K. Activated PI3K thereafter phosphorylates AKT (Beenken and Mohammadi, 2009; Ornitz and Itoh, 2015).

FGF/FGFR signaling is involved in the development of many organs in the body, including the brain, and its deregulation is associated with a variety of disease conditions (Xie et al., 2020). Among the family of FGFs, the mRNA levels of FGF7 have been shown to be elevated in patients with AD and cells treated with β-amyloid peptides (Aβ). In corroboration, overexpression of FGF7 in Aβ-treated cells was also shown to be associated with a reduction in cell viability and proliferative rate. Studies have indicated altered expression of miRNAs in the pathogenesis of AD and in experimental models of AD (Samadian et al., 2021). There is reduced expression of miR-107 in the cell model of AD and in CSF samples from AD patients (Chen et al., 2020). Interestingly, the 3′UTR of FGF7 was identified to have its binding site on the miR-107. Overexpression of miR-107 in AD model cells treated with Aβ reduced mRNA and protein levels of FGF7, and as well reduced inflammation and apoptosis, and restored cell viability (Chen et al., 2020). Western blotting analysis showed detection of FGFR2, PI3K, Akt, and Akt phosphorylation as a downstream pathway of FGF7. Therefore, data from this study implicates the involvement of the FGF7/FGFR2/PI3K/Akt signaling pathway in the pathogenesis of AD and hence a potential for therapeutic intervention (**Figure 1**).

Some important proteins and their modulating factors associated with the above AD pathways are shown in **Table 2**.

VASCULAR DYSFUNCTION AND COGNITIVE IMPAIRMENT

Recently, there has been an increasing interest in the relationship between vascular dysfunction and dementia. Clinicopathological and epidemiological studies have shown that various forms of systemic vascular disorders and cerebrovascular disease contribute to cognitive impairment and dementia (He et al., 2020). Several modifiable vascular risk factors are linked to AD type of dementia. These include hypertension, diabetes mellitus, metabolic syndrome, hypercholesterolemia, obesity, and smoking (Akinyemi et al., 2013). Vascular risk factors are associated with higher tau burden (Vemuri et al., 2017), higher cerebral beta-amyloid (Aβ) deposition (Langbaum et al., 2012; Gottesman et al., 2017), and act with Aβ synergistically to stimulate cognitive decline (Rabin et al., 2018).

Vascular dysfunction is a significant early component of AD pathology (Sweeney et al., 2019). The brains of AD patients are usually marked by abnormal NFT and amyloid plaque deposition not just in and around the neurons, but also in the blood vessels and perivascular space as well (Merlini et al., 2016). This leads to morphological changes and dysfunction of the vascular wall components (Greenberg et al., 2004). The tau, amyloid, and vascular dysfunction collectively contributed toward a speedy cognitive decline (Merlini et al., 2016).

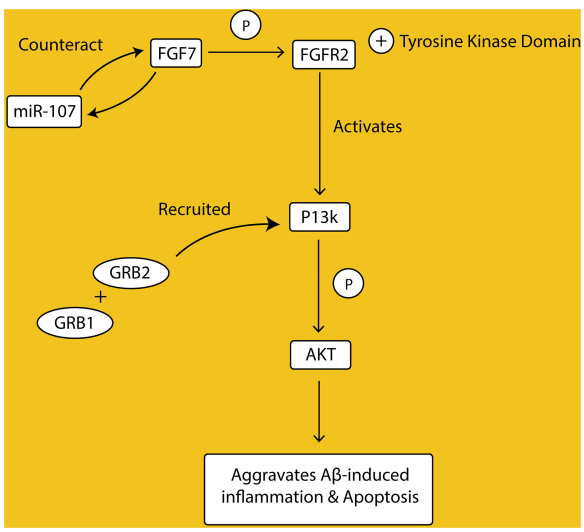

FIGURE 1 | Fibroblast growth factors (FGFs) bind to their receptors (FGFRs), activating their tyrosine kinase domain through phosphorylation. FGF/FGFR complex initiates the downstream signaling, by recruiting Growth factor receptor-bound protein 2 (GRB2) and Growth factor receptor-bound protein 1 (GRB1), which then activate Phosphoinositide 3-kinases (P13K) and phosphorylate Protein kinase B (AKT). Activation of P13K/AKT pathway exacerbates amyloid beta-induced inflammation and apoptosis. FGF7 has a binding site on MicroRna17 (miR-107) and overexpression of miR-107 reduces FGF7 levels.

TABLE 2 | Important proteins involved in the AD pathways.

Protein	Model	Relationship with AD pathology	Protein (or gene) changes in AD in pre-clinical and clinical stages	Modulating drug	Drug effect	Drug mechanism of action	References
NGF metabolic pathway							
tPA/plasmin system	APPswe/PS1 transgenic mice	Cerebral Aβ levels and cognitive function		Recombinant tPA (Activase rt-PA)	Reduced cerebral Aβ levels and improved cognitive function	Increased frequency of monocytes in circulation and brain microglia stimulation for neuroprotective phenotype	ElAli et al., 2016
				TM5275 (inhibitor of PAI-1)	Hippocampal and cortical reduction of Aβ load, and improvement of learning/memory function	Increased activities of tPA, uPA and plasmin, and LRP-1-mediated efflux of Aβ in the brain	Akhter et al., 2018
				Tert-butyl hydroquinone	Reduction of Aβ load and oxidative stress	Inhibition of PAI-1, stimulation of Aβ degradation, and increased antioxidant capacity.	(Akhter et al., 2011)
	Human	Activated by Aβ	Increase in the mRNA level of both activators (tPA and uPA) and inhibitors (PAI-1 and α2-antiplasmin) at late Braak stage				Barker et al., 2012
MMPs	Human	Microglial activation and inflammation	Increase in preclinical (e.g., MMP-3) and clinical (e.g., MMP-3) stages of AD				Hanzel et al., 2014; Pentz et al., 2021
JAK/STAT pathway							
p-JAK2-Tyr1007 and p-STAT3-Tyr705	APP/PS1 transgenic mice	Neuroinflammation	Increased level in AD mice	Suan-Zao-Ren Decoction	Anti-inflammatory action	Downregulation of hippocampal JAK2/STAT3-related signaling pathway.	Long et al., 2021
p-JAK2 and p-STAT3	Aβ1–42-induced AD mice model	Neuroinflammation	Downregulation in AD	Hydroxy-safflor yellow A	Inhibited inflammatory response	Up-regulated the JAK2/STAT3 pathway and inhibited the activation of NF-κB signaling pathways.	Zhang et al., 2014
p-JAK2-Tyr1007/1008 and p-STAT3-Tyr705	N2a Swe cells	Increased Aβ and C99 levels	Increased in activated N2a Swe cells	Taxifolin and cilostazol	Inhibited amyloidogenesis	Suppressed P-JAK2/STAT3/NF-κB-signaling *via* up-regulation of SIRT1	Park et al., 2016
FGF7/FGFR2 pathway							
FGF7	Aβ-induced SH-SY5Y cells	Upregulation promotes inflammation and apoptosis	Elevated in Aβ-treated cells	miRNA107	*In-vitro* mediation of cell viability, proliferation, inflammation, and apoptosis.	Negative regulation of FGF7/FGFR2/PI3K/Akt signal pathway	Chen et al., 2020

NGF, nerve growth factor; tPA, tissue plasminogen activator; Aβ, amyloid beta PAI-1, plasminogen activator inhibitor 1; uPA, urokinase plasminogen activator; LRP-1, Low density lipoprotein receptor-related protein 1; MMPs, Matrix metalloproteinases; JAK/STAT, Janus kinase/signal transducer and activator of transcription; p-JAK2, phosphorylated Janus kinase 2; p-STAT3, phosphorylated signal transducer and activator of transcription 3; NF-κB, nuclear factor kappa B; FGF7, Fibroblast growth factor7.

The dense network of macro- and microvasculature supplies the brain with a continuous but regulated cerebral blood flow (CBF). The neurovascular unit (NVU) describes a structural and functional relationship among the cerebral microvascular cells (endothelium, pericytes and adventitial cells), neurons, and glial cells (astrocytes, microglia and oligodendrocytes). The NVU ensures a coherent response to injury to any components of the unit that are vulnerable to amyloid (Iadecola, 2004). Cerebrovascular autoregulation and the BBB are essential to sustain the NVU's integrity (Bell and Zlokovic, 2009).

Cerebrovascular autoregulation maintains the CBF despite the changes in mean arterial pressure, while the BBB restricts the entry of toxic substances into the brain through its impermeable membrane (Bell and Zlokovic, 2009). Endothelial dysfunction associated with vascular risk factors such as hypertension and diabetes mellitus may cause the breakdown of these defense mechanisms, leading to BBB leakage, loss of autoregulation, cerebral hypoperfusion, and hypoxia (Kivipelto et al., 2002; Solomon et al., 2009). Additionally, Aβ accumulation within the microvessels itself further aggravates the endothelial damage (Jeynes and Provias, 2006; Burgmans et al., 2013). Brain magnetic resonance imaging of patients with clinically diagnosed AD often showed white matter changes demonstrated as white matter hyperintensities (WMH). WMH corresponds to areas of reduced CBF and hypoxia (Yamaji et al., 1997; Takahashi et al., 2000).

Neuroimaging has emerged as a revolutionary method to capture the alteration in CBF and its link to cognitive impairment and AD. Among the neuroimaging techniques to measure CBF include but are not restricted to functional MRI, single-photon emission computed tomography, arterial spin-labeling MRI, and dynamic susceptibility contrast MRI (de la Torre, 2018). A prospective study using cerebral arterial spin-labeling-MRI (ASL) in cognitively intact elderly subjects showed that compared to subjects who did not deteriorate to MCI, subjects who developed MCI 18 months later had a global reduction in CBF as captured during baseline ASL (Xekardaki et al., 2015). This study shows that ASL is a useful neuroimaging method to predict the onset of MCI in cognitively intact individuals whose brains showed abnormal CBF. Nevertheless, decreased CBF has been persistently demonstrated in various brain regions before and during cognitive impairment and AD. The most affected brain regions are the hippocampus, posterior cingulate cortex, medial temporal lobe, and precuneus (Dai et al., 2009; Schuff et al., 2009; Alexopoulos et al., 2012; Park et al., 2012; Xekardaki et al., 2015). Since MCI is a high-risk state to progress into dementia, including AD, CBF assessment combined with neurocognitive tests can be used to find out the susceptibility of patients with MCI to convert to AD, thus allowing physicians to decide which patients need more vigorous intervention and close follow-up. The Alzheimer's Disease Neuroimaging Initiative also recommended that neuroimaging for CBF should be appraised as the primary predictive biomarker for AD (Iturria-Medina et al., 2016).

Vascular disorders are strongly associated with disintegrated BBB and AD (Provias and Jeynes, 2014). The breach of the endothelial tight junctions that leads to intense vascular leakage at the BBB is a feature of the early stages of dementia and AD (Teri et al., 1997). The transport of Aβ across the BBB is typically regulated by two major transporter proteins, LDL-receptor-related protein-1 (LRP-1) and receptor for advanced glycation end products (RAGE). LRP-1 promotes the efflux of Aβ from the cerebral parenchyma to the vessels across the BBB (Deane et al., 2009). In contrast, RAGE facilitates Aβ influx from the circulation crossing the BBB into the cerebral parenchyma (Jeynes and Provias, 2008). An Aβ-RAGE interface enhances oxidative stress, neuroinflammation, and subsequent BBB disintegration, which further potentiates cerebral Aβ accumulation (Schmidt et al., 2009). Whereas P-glycoprotein (PGP) has been demonstrated to damper RAGE activity in brain capillary endothelial cells that compose the BBB (Candela et al., 2010). In short, an imbalance between LRP-1, RAGE, and PGP expression together with disintegrated BBB remarkably contributed to vascular dysfunction and AD.

Vasoactive regulatory proteins such as vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) also play a role in maintaining BBB integrity and therefore affect the cerebral Aβ transport (Farkas and Luiten, 2001; Jeynes and Provias, 2011). Patients with AD commonly have low serum VEGF levels (Mateo et al., 2007) and this is associated with progressive loss of cognitive function (Tang et al., 2013). Decreased expression of VEGF and eNOS within the cerebral microvasculature disrupts the normal angiogenesis and BBB integrity. This leads to less Aβ efflux across the BBB from the cerebral parenchyma into the circulation in the human brain with AD (Provias and Jeynes, 2014).

Genome-wide association studies have revealed a number of genetic associations with AD (Naj et al., 2017), of which the most important is the apolipoprotein E gene on chromosome 17 (APOE gene, apoE protein) (Apostolova et al., 2018). Other polymorphisms are associated with genes linked to lipid metabolism, inflammation, and intracellular trafficking, but none of them are as consistent as that of apoE (Bis et al., 2020). ApoE is a component of very-low-density lipoproteins that is involved in cholesterol transport among various cells in the body. ApoE4 allele frequency is higher in patients with vascular dementia, AD, and ischemic cerebrovascular disease (Ji et al., 1998). ApoE4 carriers with atherosclerosis commonly have co-morbid AD, cognitive impairment, and dementia (Slooter et al., 1999). AD patients who are ApoE4 carriers have a higher Aβ burden compared with non-carriers (Dorey et al., 2014). ApoE4 disrupts brain lipid metabolism, impedes CBF, and triggers cerebral amyloid angiopathy (Reinvang et al., 2013). The binding of Aβ to ApoE reduced LRP-1 functioning in mice (Bell et al., 2007). Furthermore, an *in vitro* BBB model comprising mouse brain capillary endothelial cells demonstrated that ApoE4 disrupts endothelial tight junction integrity and BBB permeability (Nishitsuji et al., 2011; **Figure 2**).

OMICS IN ALZHEIMER'S DISEASE

Fresh molecular and high-throughput methods are shedding new insight into the pathways and networks at the heart of this complicated disease. This is required in order to go forward with the precision medicine paradigm toward the discovery of novel molecular markers, systematic risk categorization, and translational targeted medicines (Kosik, 2015; Castrillo and Oliver, 2016; Kovacs, 2016).

The implementation of a complete precision medicine strategy, as well as the creation of relevant biomarkers, are likely to result in significant advancements in recognizing, treating, and preventing AD.

AD and many other genetically heterogeneous illnesses (e.g., autism, other NDs, and some malignancies) are far too varied

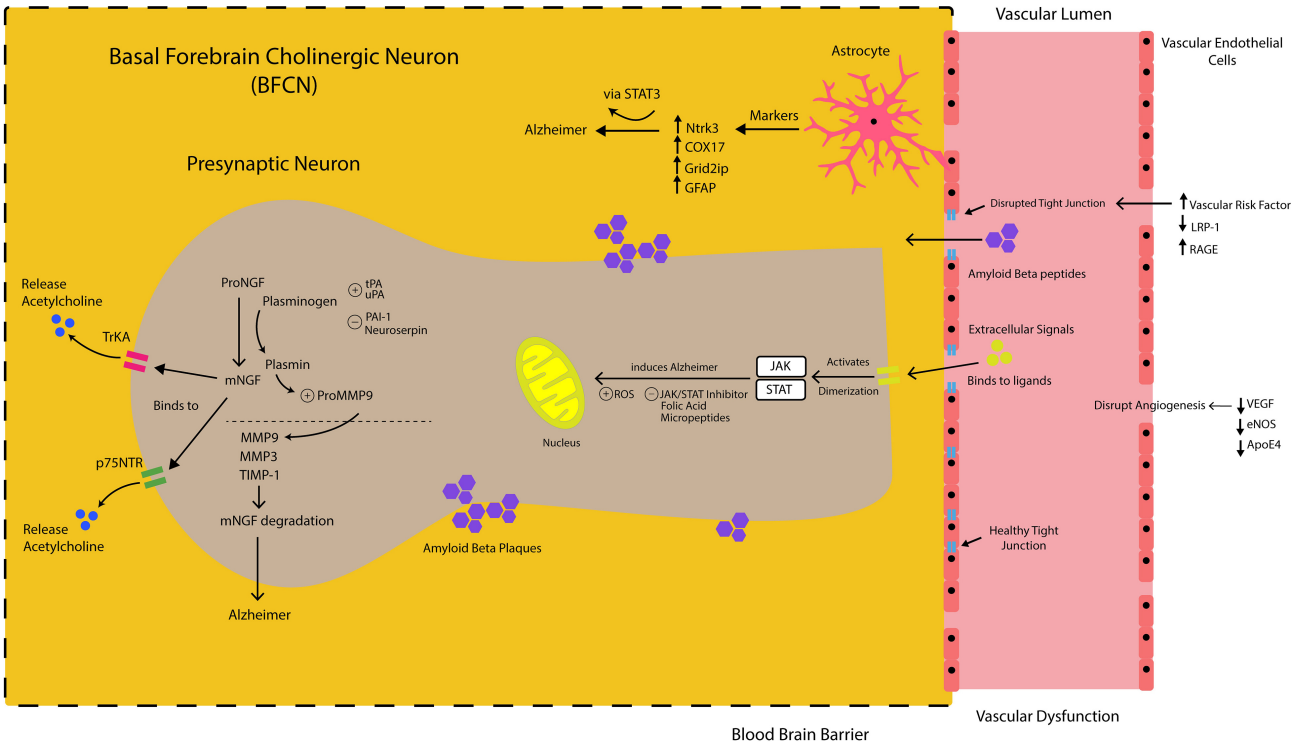

FIGURE 2 | Nerve growth factor (NGF) metabolic pathway in basal forebrain cholinergic neuron (BFCN) is mainly regulated by proteases involved in NGF maturation [tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), Plasminogen Activator Inhibitor 1 (PAI-1) and Neuroserpin)] and those involved in NGF degradation [matrix metalloproteinases (MMP9, MMP3) and tissue inhibitor of metalloproteinases 1 (TIMP-1)]. Plasmin is also responsible for proMMP-9 activation to MMP9. Extracellular signals (i.e., cytokines, hormones, and growth factor) bind to their ligands and activate Janus Kinase/ signal transducer and activator of transcription (JAK/STAT). This causes dimerization of the two and allows them to be translocated to the nucleus for transcriptional response. Reactive Oxygen Species (ROS) is involved in JAK/STAT-induced Alzheimer. JAK/STAT inhibitor, folic acid and micropeptides (i.e., humorin and colivelin) attenuate JAK/STAT-induced Alzheimer. Increased astrocytes biomarker Glial fibrillary acidic protein (GFAP) and Genes were predominantly expressed on Astrocytes- STAT3-induced Alzheimer's disease (Neurotrophic Receptor Tyrosine Kinase 3 (ntrk3), Cytochrome C Oxidase Copper Chaperone (*COX17), and* Grid2 Interacting Protein (Grid2ip). Decreased vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS) and apolipoprotein e4 (ApoE4) disrupt normal angiogenesis. Increased vascular risk factor, receptor for advanced glycation end products (RAGE) and reduced LDL-receptor related protein-1 (LRP-1) disrupt healthy tight junction. Disruption of normal angiogenesis and healthy tight junction allow Amyloid-Beta peptides efflux from vessels to brain parenchyma and promoting Amyloid-Beta plaques formation.

to be the sole cause or to be centered on genetic variants. Certain complicated disorders are distinguished by the following characteristics: (1) multifactorial in nature, integrating genetic, epigenomic, and interactomics factors; (2) primarily caused by "modified" networks that influence important modules and interactomes; and (3) deeply complicated, with a strong interplay between impacted and homeostatic defense systems. As a result, for many complex disorders, comprehensive system-level therapies are necessary (Castrillo and Oliver, 2016).

Advanced theoretical and statistical systems biology techniques are being applied to better understand the pathophysiology of multifactorial illnesses such as neurodegenerative illnesses. These methods include high-throughput omics techniques that investigate genome, transcriptome, proteome, and metabolome patterns and interactions, as well as integrative approaches (Castrillo and Oliver, 2016). Multi-omics trials are currently being utilized in integrated personal omics profiling (iPOP) longitudinal research to track panels of biomarkers and trends for diagnosis and customized therapy (Chen et al., 2012).

Among the most important systems-level techniques, including the use of omics technology to dissect AD (Tosto and Reitz, 2016), are 1. Transcriptomics: RNA-Seq to elucidate early patterns of dysregulation underlying ADe (Chen et al., 2016); experimental approaches applied to the analysis of RNA networks in AD (Pichler et al., 2017); RNA-Seq experiments demonstrating (Bai et al., 2014), 2. Proteomics investigations indicate that amyloid disrupts splicing pathways (Nuzzo et al., 2017), metalloproteomics and redox proteomics studies in AD (Hare et al., 2016), and plasma proteomics biomarkers in AD (Perneczky and Guo, 2016), 3. Metabolomics: AD-related changes in metabolic processes and networks (Toledo et al., 2017).

The human blood metabolome is made up of hundreds of tiny molecular species, most of which have a molecular weight of less than 1,500 Da (Daltons; 1.7 1027 kg) and are predominantly composed of monosaccharides, acylcarnitines, biogenic amines, amino acids, fatty acids, and complex lipids. The metabolome is regarded as a downstream linear mirror of the genome/epigenome, transcriptome, and proteome, sequentially

and in close proximity to the clinical phenotype, using typical reductionistic methodologies. Although the aforementioned may be accurate from a systems biology standpoint, intricate interrelationships occur between the many "omic" levels (Yugi et al., 2016), which, if correctly integrated, are likely to give a greater understanding of a complicated disease state or human health.

Recent reports of metabolomic biomarkers for AD have been developed using specimens from cross-sectional studies analyzed with MS platforms, typically comparing metabolite abundances between control subjects and individuals with either prodromal or manifest AD (Klavins et al., 2015).

Recent approaches in the treatment of AD include exploring the potentials of some metabolites to modulate signaling pathways associated with neurovascular endothelium through multi-omic analyses (Corral-Jara et al., 2021). There have also been reports on cellular signaling-related sex-dependent effects under hyperglycemic and lipid stress (Nuthikattu et al., 2020, 2021). However, this review focuses on significant signaling pathways associated with the stages of AD, the potential links between vascular dysfunction and AD, as well as recent developments in "omics"-based approaches in AD. Without publication date restriction, PubMed, ScienceDirect, and Google Scholar databases were searched for published articles containing the search terms related to "Alzheimer," "dementia," "signaling pathways," "vascular dysfunction," "cognitive impairment," and "omics."

SUMMARY AND FUTURE DIRECTIONS

In this review, we discussed many signaling pathways and postulated mechanisms associated with AD. Mild cognitive impairment (MCI) can be an early stage of the disease continuum for dementia, including AD. In line with this, the aforementioned signaling markers are being increasingly studied as potential early diagnostic or prognostic markers for cognitive decline-related pathologies. As for the NGF pathway, a meta-analysis and systematic review of 170 studies reported a significant increase in the peripheral levels of NGF along with other proinflammatory markers in MCI compared with controls (Shen et al., 2019). More recently, markers associated with the NGF pathway, such as proNGF, plasminogen, neuroserpin, and MMP9 was shown to be elevated, whereas mNGF was downregulated in the brain tissue of MCI and AD patients (Pentz et al., 2020). In line with this, Senescence-Associated Secretory Phenotype (SASP) biomarkers, which is upstream of JAK/STAT signaling at the cellular level, has been reported to be elevated in the plasma of older adults with MCI, MCI + Major Depressive Disorder (MDD), and MDD (Diniz et al., 2021). To date no reports have directly implicated FGF7/FGFR2 as early markers of cognitive impairment at the clinical level, hence more studies should look into this.

It is important to distinguish between brain aging and AD dementia. With age, our brains experience natural structural, chemical and functional degenarations, as well as significant cognitive decline, which is characterized by brain shrinkage, decreased blood flow, synapse degeneration, and neurochemical alternations (Damoiseaux, 2017). Most of the human studies, on the other hand, seldom exclude prevalent health disorders associated with aging, such as hypertension, which may have an impact on how accurate the conclusions are. The study of age-related brain alterations in non-human animal models is therefore essential for distinguishing between normal aging and pathological brain abnormalities in humans. Studying the basic processes of aging in animals will consequently provide an essential foundation for the development of innovative therapies for age-related brain problems in people (Alexander et al., 2020).

Normal aging has been shown to be associated with significant changes in the brain network and the processes that it controls (Huang et al., 2015; Bonte et al., 2017). The human brain's network has been reorganized as a result of aging-related changes in functional, metabolic, and structural connections, according to recent research. It has been discovered that functional connection reduces with age, both within and between numerous resting-state networks, including the default mode network, salience network, dorsal attention network, and sensorimotor network (Huang et al., 2015). The aged also have lower modularity and efficiency in their brain networks (Liu et al., 2014; Geerligs et al., 2015; Zhao et al., 2015), as well as a degeneration process in which the aging brain system transitions from a small-worldness network to a regular network in conjunction with normal aging. The findings of functional connectivity studies (Damoiseaux, 2017) show that age-related changes in structural network metrics are comparable to those seen in structural network metrics.

A good method for evaluating energy consumption in neurons is [18F] fluorodeoxyglucose positron emission tomography (18F-FDG PET), which is a reflection of neural transmission signals (Rocher et al., 2003). As a result, 18F-FDG PET is regarded as a functional neuroimaging tool for identifying changes in brain activity associated with advancing years of age. A large number of studies have been conducted using 18F-FDG PET to explore the changes in brain processes that occur as a result of normal aging throughout the past several decades. Overall, a considerable age-related reduction in glucose metabolism in the frontal lobe has been seen in most investigations (Pardo et al., 2007; Kalpouzos et al., 2009), which is consistent with other findings. Glucose uptake may be decreased as a result of tissue loss or shrinking (Bonte et al., 2017). As well as in metabolic brain networks, age-related alterations can be observed in association and paralimbic cortex areas, including increased clustering, decreased efficiency and robustness, as well as altered nodal centralities (Rocher et al., 2003; Liu et al., 2014). Whether the age-related changes in glucose metabolism are consistent between the rat brain and the human brain, particularly in terms of metabolic brain networks, has not been determined conclusively at this point. It is yet unknown if the metabolic network in the brains of old rats exhibits age-related alterations that are comparable to those observed in the brains of humans.

A recent study of single-cell transcriptome datasets from the human brain revealed a link between AD gene signals and microglia, as well as astrocytes and astrocyte-like cells. Studies of human microglia (MG) produced from embryonic

stem cells in the mouse brain have shown the cells are transcriptionally like human primary microglia ex vivo and express genes associated with Alzheimer's disease (AD). However, just one-third of the suspected Alzheimer's disease risk genes have appropriate mouse orthologs (Mancuso et al., 2019), which is concerning. Furthermore, oligomeric amyloid-beta causes a different response in human microglia compared to mice microglia (MG). As research advances, it is becoming increasingly clear that signals from the CNS microenvironment are required to maintain microglial specification, and that the absence of these cues results in a dramatic disruption of the microglia phenotype, driving them toward an activated state. Additionally, certain well-known disease-associated genes (for example, triggering receptor expressed on myeloid cells 2 (TREM2)–membrane phospholipids–apolipoprotein E (APOE), CD33–sialic acid) play a role in the cross-talk between microglia and other brain cells.

Soreq et al. (2017) recently discovered significant differences in glial gene expression as well as neuronal and oligodendrocyte (OLG) cell counts in the brains of elderly people as compared to young people. A total of 1,231 postmortem brain samples from 134 people (ages 16–104), profiled by exon microarrays (10 brain areas), and 480 samples from the North American brain bank were analyzed (Soreq et al., 2017; Elmore et al., 2018). The 3′ microarray data from microglia-depleted animals (including comparisons between young and elderly mice) was also used to analyze the mice. Authors discovered considerable alterations in gene expression. Similarly, significant changes in AD may be observed in the future.

A detailed analysis of RNA-Seq data can be performed using machine learning and statistics, including classification methods [for example, unsupervised hierarchical classification (HCL) and bioinformatic algorithms], correlation analyses, and the detection of gene expression changes in cell-specific markers, pro-inflammatory genes, and splicing factors, as well as genes associated with the cell cycle. Linear regression may also be used to identify genes that have been changed. Functional Gene Ontology (GO) analysis, which is non-parametric, may detect changes in molecular functions (MF) and biological processes (BP) (Luo and Chen, 2016). The genes that were discovered can also be examined in the human cell Atlas (Regev et al., 2017). Aside from that, developments in genetics and sequencing have uncovered an abundance of disease-associated genetic modifications; nonetheless, precise models for molecular dissection are still needed to fully understand these changes.

The precision of molecular medicine is significantly improved by using a multi-omics approach. When it comes to precisely understanding the impact of mutations or therapies, single-cell studies are crucial. Transcriptional networks may be constructed from single-cell data, if necessary. The information obtained may aid in gaining a better understanding of the impact of altered genes on disease pathophysiology. Cell lines or model organisms can be used to determine the underlying cause of disease, but other technologies are required to complete the task. The introduction of single-cell RNA-Seq had a significant influence on the field. It is possible to obtain accurate cell state comparisons by comparing healthy samples to sick samples in the same experiment. Major faults may be identified *via* cell harmony analysis, and genetic rescue of mutations is also conceivable after investigations of cell state models and more clinical Alzheimer's disease samples. Additionally, differentiating gene expression across distinct cell states may be investigated.

When analyzing data, clustering is a way of grouping closely related observational findings into groupings called "clusters" that are based on the results of numerous variables for each subject. Because it serves as a critical intermediatory tool in experimental investigations, it is incredibly significant in scientific research. However, it is particularly valuable in gene analysis because it contributes to the knowledge of specific cell processes. This easy and helpful adaptation to a variety of different scientific fields has not only enabled its widespread usage but has also facilitated the creation of a variety of distinct clustering methodologies. Because of this, these sophisticated approaches may be utilized to struttingly analyze data in order to give trustworthy gene analysis (Park et al., 2009; Habib et al., 2020). Other research has discovered that previously illness-associated astrocytes are changed in AD and aging using single-cell RNA-Seq, demonstrating that astrocytes are linked to hereditary and age-related disease factors (Habib et al., 2020).

CONCLUSION

The pathogenesis of AD is multifactorial. Continuous discovery of novel signaling pathways in AD reflects the complex nature of the disease. Such complexity should be addressed in patients' management for better treatment outcomes, and tools such as omics are better fit to be incorporated into treatment plans. Vascular dysfunction has a deleterious impact on the brain that builds up to AD pathogenesis. Since measures to tackle the vascular risk factors are available, the future prevalence of AD can be minimized by employing strategies that preserve cardiovascular health.

AUTHOR CONTRIBUTIONS

JK contributed to the conceptual framework and design of the manuscript. MA, KS, AU, WM, HK, NHI, and JK drafted the manuscript. HK contributed to the preparation of figures and figure legends. MA, KS, AU, WM, CK, and JK critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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GLOSARRY

AD, Alzheimer's disease; COVID-19, corona virus disease-2019; HIV, human immunodeficiency virus; APP, amyloid precursor protein; Aβ, amyloid beta; MAPT, microtubule-associated protein tau; ApoE, apolipoprotein E; NFT, neurofibrillary tangles; Wnt, Wingless-related integration site; MAPK, mitogen-activated protein kinase;NFTs, neurofibrillary tangles; NMDARs, *N*-methyl-d-aspartic acid receptors; NGF, Nerve growth factor; PNS, peripheral nervous system; CNS, central nervous system; BFCN, basal forebrain cholinergic neurons; proNGF, precursor nerve growth factor; mNGF, mature nerve growth factor; TrkA, tropomyosin-related kinase A; p75NTR, p75 neurotrophin receptor; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor 1; MMPs, matrix metalloproteinases; MMP-9, matrix metalloproteinases; TIMP-1, tissue inhibitor of metalloproteinases 1; proMMP-9, precursor matrix metalloproteinases-9; MMP-3, matrix metalloproteinases-3; MMP-1, matrix metalloproteinases-1; MMP-2, matrix metalloproteinases-2; MMP-13, matrix metalloproteinases-13; MT1-MMP, Membrane type 1 matrix metalloproteinase; JAK/STAT, Janus kinase/signal transducer and activator of the transcription; JAK1, Janus kinase 1; JAK2, Janus kinase 2; JAK3, Janus kinase 3; Tyk2, Tyrosine kinase 2; STAT1, signal transducer and activator of the transcription 1; STAT2, signal transducer and activator of the transcription 2; STAT3, signal transducer and activator of the transcription 3; STAT4, signal transducer and activator of the transcription 4; STAT5A, signal transducer and activator of the transcription5A; STAT5B, signal transducer and activator of the transcription 5B; and STAT6, signal transducer and activator of the transcription 6; Nrf2, nuclear erythroid 2-related factor 2; TREM2, triggering receptor expressed on myeloid cells 2; Ntrk3 neurotrophic tyrosine kinase receptor type 3; Cox17, Cytochrome C Oxidase Copper Chaperone; Grid2ip, Grid2 Interacting Protein; TGF-β, transforming growth factor-beta; GFP, glial fibrillary acidic protein; EAATs, excitatory amino acid transporters; ALDH1L1, aldehyde dehydrogenase 1 family member L1; FGFs, Fibroblast growth factors; FGFRs, Fibroblast growth factor receptors; AKT, Protein kinase B; P13K, phosphatidylinositol 3-kinases; GRB2, Growth factor receptor-bound protein 2; GAB1; GRB2 Associated Binding Protein 1; CSF, cerebrospinal fluid; FGF7, Fibroblast Growth Factor 7; mir-107, MicroRNA-107.

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