IMMUNOLOGICAL MECHANISMS, BIOMARKERS AND IMMUNOTHERAPIES OF ALZHEIMER'S DISEASE

EDITED BY: Yu-Hui Liu, Jun Tan and Yang Xiang
PUBLISHED IN: Frontiers in Aging Neuroscience

frontiers Research Topics

frontiers

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-88971-364-6
DOI 10.3389/978-2-88971-364-6

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

IMMUNOLOGICAL MECHANISMS, BIOMARKERS AND IMMUNOTHERAPIES OF ALZHEIMER'S DISEASE

Topic Editors:
Yu-Hui Liu, Third Military Medical University, China
Jun Tan, Guizhou Medical University, China
Yang Xiang, University of Electronic Science and Technology of China, China

Citation: Liu, Y.-H., Tan, J., Xiang, Y., eds. (2021). Immunological Mechanisms, Biomarkers and Immunotherapies of Alzheimer's Disease. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-364-6

Table of Contents

frontiers in Aging Neuroscience

EDITORIAL
published: 13 September 2021
doi: 10.3389/fnagi.2021.733282

Editorial: Immunological Mechanisms, Biomarkers and Immunotherapies of Alzheimer's Disease

Jia-yan Xin[1,2], *Xiao-yan Zhu*[3], *Xiao Huang*[1,2], *Yu-hui Liu*[4], *Jun Tan*[5] *and Yang Xiang*[6,7]*

[1] Department of Clinical Medicine, North Sichuan Medical College, Nanchong, China, [2] Department of Neurology, General Hospital of Western Theater Command, Chengdu, China, [3] Basic Medical Laboratory, General Hospital of Western Theater Command, Chengdu, China, [4] Department of Neurology and Centre for Clinical Neuroscience, Daping Hospital, Third Military Medical University, Chongqing, China, [5] Department of Obstetrics and Gynecology, Affiliated Hospital of Guizhou Medical University, Guiyang, China, [6] Department of Neurology, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China, [7] Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu, China

Keywords: immunological mechanism, biomarker, immunotherapy, Alzheimer's disease, peripheral system

Editorial on the Research Topic

Immunological Mechanisms, Biomarkers, and Immunotherapies of Alzheimer's Disease

Alzheimer's disease is the most common type of dementia characterized by neuropathological changes such as intracellular tau tangles and extracellular deposition of β-amyloid (Aβ) as plaques. At present, the study of pathogenesis, the search for biomarkers, and the development of treatment strategies of AD are all closely related to immunological theory and techniques. The main aim of the current Research Topic was to provide a current collection of immunological mechanisms, biomarkers, and therapeutic strategies in AD.

Growing evidence has proved that Alzheimer's disease (AD), as a typical degenerative disease of the central nervous system, has complex connections with the peripheral system (Wang et al., 2017), such as the peripheral clearance of pathogenic substances (Aβ and Tau) or the impact of gut microbiota on AD-like pathological characteristics, etc. Among them, the relationship between systemic autoimmune diseases and AD has been puzzling researchers for years. Culibrk and Hahn summarized the link of chronic inflammatory osteoarthritis disease with late-onset AD. In particular, they focused on the pathophysiological characteristics and immunotherapy of rheumatoid arthritis, osteoarthritis, and osteoporosis, as well as their implications on AD pathogenesis. They also summarized some other possible mechanisms, including the age-related cellular/immuno-senescence, the aberrant peripheral nervous system activity, dysregulated autophagic homeostasis, and the pathological microRNA profiles, which could attract more attention from the audience.

The relation between hereditary amyloidosis and AD is a topic rarely discussed before. Although the pathogenic substances of the two diseases are different, it is not an obstacle to investigating their association from the perspective of genetics. Jiang et al. reported two novel likely pathogenic frame-shift mutations of gelsolin in AD patients using the genes targeted sequencing (GTS) method. Intriguingly, both mutations seem to correlate with the age at which clinical symptoms of AD appeared, respectively, even though the gelsolin is a common pathogenic gene charged with hereditary amyloidosis.

The development of biomarkers is a spotlight in AD research over the past decade. The proposal of the NIA-AA research framework of AD in 2018 not only categorizes AD biomarkers as per the

OPEN ACCESS

Edited and reviewed by:
Thomas Wisniewski,
NYU Grossman School of Medicine, United States

***Correspondence:**
Yang Xiang
xiangyangttt@126.com

Received: *30 June 2021*
Accepted: *15 July 2021*
Published: *13 September 2021*

Citation:
Xin J-y, Zhu X-y, Huang X, Liu Y-h, Tan J and Xiang Y (2021) Editorial: Immunological Mechanisms, Biomarkers and Immunotherapies of Alzheimer's Disease. Front. Aging Neurosci. 13:733282. doi: 10.3389/fnagi.2021.733282

AT(N) system but also provides more objective and rigid biological criteria for the diagnosis of AD than ever before (Jack et al., 2018). Since the presence or absence of Aβ is the sole criterium for the etiologic diagnosis of AD in the AT(N) system, the emerging novel non-Aβ biomarkers are mostly classified in the (N) catalog. Based on the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, Pillai, Bebek et al. measured the levels of tumor necrosis factor receptor (TNFR) 2 in the cerebrospinal fluid (CSF) and investigated its correlations with CSF t-Tau, CSF p-Tau, the cognitive domains, the MRI measures, and the longitudinal cognitive changes, together indicating the potential value of TNFR2 in the diagnosis and treatment of AD.

The biomarker research has gradually shifted from CSF to blood due to the invasive operation, difficult acquisition, and patient unacceptability of CSF sampling (Zetterberg and Blennow, 2018). Wang J. et al. compared the naturally occurring antibody to α-synuclein (NAb-a-Syn) in the blood of patients with Parkinson's disease dementia, AD, vascular dementia, and normal controls, and further explored the correlations between NAb-a-Syn and the severity of cognitive impairment and PD, respectively. Of note, the naturally occurring antibodies also have a good performance in the immunotherapy of AD (Liu et al., 2021).

As a shining star of AD biomarkers in the past years, the value of neurofilament light chain (NfL) in AD diagnosis and prognosis is no longer needed to be described. Especially, the blood-NfL is consistent and comparable with CSF-NfL, which greatly increases the clinical application value of NfL (Fortea et al., 2018). Wang J-H. et al. evaluated the difference of blood-NfL in patients with post-stroke subjective cognitive decline (SCD). Although this study was a single-center prospective study with a small sample size, post-stroke SCD is a clinical condition that has received little attention before, showing the careful observation and thinking of the authors.

Likewise, neurogranin has emerged as an important biomarker for neurodegenerative diseases in recent years, although the current studies have not demonstrated that its clinical value is comparable to that of the NfL. Xiang et al. reviewed the associations between neurogranin and neurological and psychiatric diseases and further made the perspectives on the research and applications of neurogranin in the future.

Compared with body fluid-based biomarkers, image-based biomarkers have not developed rapidly over recent decades. The clinical applications of Aβ-positron emission tomography (PET) and tau-PET have promoted the early diagnosis and severity assessment of AD (Hansson, 2021); however, the high cost and low coverage of PET limit its clinical application. Many reports on functional magnetic resonance imaging (fMRI) emerged, but fMRI has not yet reached the same value as that of Aβ-PET and tau-PET in the diagnosis of AD. Based on ADNI, Yang et al. studied the dynamics and concordance changes in different brain regions in the patients with SCD using resting-state fMRI (RS-fMRI), adding the new evidence for the use of fMRI in AD.

Researchers have long been interested in acupuncture for the treatment of neurological diseases, even though its mechanism is not fully understood (Kaptchuk, 2002). Based on growing published data, Huang et al. analyzed the therapeutic effect of acupuncture on AD using a meta-analysis method. The authors proposed that high-quality studies with rigorous study designs and larger samples are required in the future, even though it is now generally believed that acupuncture is a promising complementary treatment for AD.

Over the past decades, a variety of hypotheses about the mechanism of AD have emerged. At present, even the amyloid cascade hypothesis either have to face updates or have to face challenges (Tolar et al., 2020). A growing body of evidence suggests that the mechanisms of AD beyond Aβ and Tau are possible in which the neurovascular unit, brain lymphatic system, gut microbiota, and neuroinflammation could be involved (Henstridge et al., 2019). Among them, the activation of the immune system is the key regulatory factor of AD pathology (Long and Holtzman, 2019). Moreover, almost all the clinical trials of anti-AD drugs targeting Aβ or Tau failed or have been inconclusive (Mullard, 2021), suggesting that the immunotherapy strategies may also need to concentrate on some other targets rather than just Aβ and Tau.

We have always regarded AD as a degenerative disease of the central nervous system. However, more and more evidence confirms that the peripheral system has a great influence on AD. As an essential part of the peripheral system, the role played by the immune system and tissues and organs rich in immune cells in the pathogenesis of AD have not been fully answered. The spleen, intestine, omentum, and blood, these seemingly distant and unrelated organs and tissues are likely to affect our brain structure and cognitive function *via* immunological regulation, fascinating scientific issues for further and future research.

AUTHOR CONTRIBUTIONS

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

FUNDING

This study was supported by the National Natural Science Foundation of China (81601112, 81801090), Top Project of the Youth Incubation Program of Military Medical Science and Technology (19QNP065), and the Sichuan Department of Science and Technology Fund (2018SZ0141, 2019YSF0213).

REFERENCES

Fortea, J., Carmona-Iragui, M., Benejam, B., Fernandez, S., Videla, L., Barroeta, I., et al. (2018). Plasma and CSF biomarkers for the diagnosis of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *Lancet Neurol.* 17, 860–869. doi: 10.1016/S1474-4422(18)30285-0

Hansson, O. (2021). Biomarkers for neurodegenerative diseases. *Nat. Med.* 27, 954–963. doi: 10.1038/s41591-021-01382-x

Henstridge, C. M., Hyman, B. T., and Spires-Jones, T. L. (2019). Beyond the neuron-cellular interactions early in Alzheimer disease pathogenesis. *Nat. Rev. Neurosci.* 20, 94–108. doi: 10.1038/s41583-018-0113-1

Jack, C. R. Jr., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., et al. (2018). NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 14, 535–562. doi: 10.1016/j.jalz.2018.02.018

Kaptchuk, T. J. (2002). Acupuncture: theory, efficacy, and practice. *Ann. Intern. Med.* 136, 374–383. doi: 10.7326/0003-4819-136-5-200203050-00010

Liu, Y. H., Wang, J., Li, Q. X., Fowler, C. J., Zeng, F., Deng, J., et al. (2021). Association of naturally occurring antibodies to beta-amyloid with cognitive decline and cerebral amyloidosis in Alzheimer's disease. *Sci. Adv.* 7:abb0457. doi: 10.1126/sciadv.abb0457

Long, J. M., and Holtzman, D. M. (2019). Alzheimer disease: an update on pathobiology and treatment strategies. *Cell* 179, 312–339. doi: 10.1016/j.cell.2019.09.001

Mullard, A. (2021). Landmark Alzheimer's drug approval confounds research community. *Nature* 594, 309–310. doi: 10.1038/d41586-021-01546-2

Tolar, M., Abushakra, S., and Sabbagh, M. (2020). The path forward in Alzheimer's disease therapeutics: reevaluating the amyloid cascade hypothesis. *Alzheimers Dement.* 16, 1553–1560. doi: 10.1016/j.jalz.2019.09.075

Wang, J., Gu, B. J., Masters, C. L., and Wang, Y. J. (2017). A systemic view of Alzheimer disease - insights from amyloid-beta metabolism beyond the brain. *Nat. Rev. Neurol.* 13, 612–623. doi: 10.1038/nrneurol.2017.111

Zetterberg, H., and Blennow, K. (2018). From cerebrospinal fluid to blood: the third wave of fluid biomarkers for Alzheimer's disease. *J. Alzheimers Dis.* 64, S271–S279. doi: 10.3233/JAD-179926

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Xin, Zhu, Huang, Liu, Tan and Xiang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 10 September 2020
doi: 10.3389/fnagi.2020.581524

Analyses Mutations in *GSN, CST3, TTR*, and *ITM2B* Genes in Chinese Patients With Alzheimer's Disease

Yaling Jiang[1], *Bin Jiao*[1,2,3], *Xinxin Liao*[1,2,3], *Xuewen Xiao*[1], *Xixi Liu*[1] and *Lu Shen*[1,2,3,4]*

[1]*Department of Neurology, Xiangya Hospital, Central South University, Changsha, China,* [2]*National Clinical Research Center for Geriatric Disorders, Central South University, Changsha, China,* [3]*Key Laboratory of Hunan Province in Neurodegenerative Disorders, Central South University, Changsha, China,* [4]*Key Laboratory of Organ Injury, Aging and Regenerative Medicine of Hunan Province, Changsha, China*

Amyloid protein deposition is a common mechanism of hereditary amyloidosis (HA) and Alzheimer's disease (AD). Mutations of g*elsolin* (*GSN*), *cystatin C* (*CST3*), *transthyretin* (*TTR*), and *integral membrane protein 2B* (*ITM2B*) genes can lead to HA. But the relationship is unclear between these genes and AD. Genes targeted sequencing (GTS), including *GSN, CST3, TTR*, and *ITM2B*, was performed in a total of 636 patients with clinical AD and 365 normal controls from China. As a result, according to American College of Medical Genetics and Genomics (ACMG) guidelines, two novel likely pathogenic frame-shift mutations (GSN:c.1036delA:p.K346fs and GSN:c.8_35del:p.P3fs) were detected in five patients with AD, whose initial symptom was memory decline, accompanied with psychological and behavioral abnormalities later. Interestingly, the patient with K346fs mutation, presented cerebral β-amyloid protein deposition, had an early onset (48 years) and experienced rapid progression, while the other four patients with P3fs mutation had a late onset [(Mean ± SD): 69.50 ± 5.20 years] and a long course of illness [(Mean ± SD): 9.24 ± 4.86 years]. Besides, we also discovered 17 variants of uncertain significance (VUS) in these four genes. To our knowledge, we are the first to report AD phenotype with *GSN* mutations in patients with AD in the Chinese cohort. Although mutations in the *GSN* gene are rare, it may explain a small portion of clinically diagnosed AD.

Keywords: Alzheimer's disease, hereditary amyloidosis, gelsolin, genetics, China

OPEN ACCESS

Edited by:
Yu-Hui Liu,
Third Military Medical University, China

Reviewed by:
Guohua Zhao,
Zhejiang University, China
Eva Bagyinszky,
Gachon University, South Korea

***Correspondence:**
Lu Shen
shenlu@csu.edu.cn

Received: *09 July 2020*
Accepted: *20 August 2020*
Published: *10 September 2020*

Citation:
Jiang Y, Jiao B, Liao X, Xiao X, Liu X and Shen L (2020) Analyses Mutations in GSN, CST3, TTR, and ITM2B Genes in Chinese Patients With Alzheimer's Disease. Front. Aging Neurosci. 12:581524. doi: 10.3389/fnagi.2020.581524

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the most common form of dementia in the elderly, which mainly characterized by the progressive decline in memory and cognitive function. Epidemiological data showed that there were 50 million AD patients worldwide in 2018, and it was expected to grow to 152 million by 2050 (International AsD, 2019). Although the vast majority of AD occur on a sporadic basis, mutations in three genes [*amyloid precursor protein* (*APP*), *presenilin 1* (*PSEN1*), and *presenilin 2* (*PSEN2*)] could lead to rare familial AD (FAD; <0.5%), whose symptoms occur earlier than sporadic AD, usually between 30 and 50 years of age, also named as early-onset AD. "Typical" late-onset AD may be motivated by a complex interaction between genetic and environmental factors, usually more than 65 years of age. It is currently believed that about 70% of AD risk

can be attributed to genetic factors (Bateman et al., 2011; Lane et al., 2018). The prevalent theory of AD pathogenesis is the amyloid hypothesis, suggesting that accumulation of pathological forms of β-amyloid protein (Aβ) is the primary pathological process (Lane et al., 2018).

Hereditary amyloidosis (HA) represents a series of single-gene diseases that caused by amyloidogenic precursor protein genes mutations (Chyra Kufova et al., 2018). There are four genes, which were *gelsolin* (*GSN*), *cystatin C* (*CST3*), *transthyretin* (*TTR*), and *integral membrane protein 2B* (*ITM2B*), whose mutations can lead to autosomal dominant HA, while playing an important role in the pathogenesis of AD (Ray et al., 2000; Sastre et al., 2004; Hirko et al., 2007; Mi et al., 2007; Buxbaum et al., 2008; Buxbaum and Johansson, 2017; Tamayev et al., 2011; Matsuda and Senda, 2019). Established associations between these genes and HA include *GSN* and familial amyloidosis of the Finnish type (FAF; Nikoskinen et al., 2015), *TTR* and transthyretin-related amyloidosis (AMYL-TTR; Sekijima, 2015), *ITM2B* and familial British dementia or familial Danish dementia (Del Campo and Teunissen, 2014), as well as *CST3* and cerebral amyloid angiopathy (Abrahamson et al., 1989). The *GSN* gene encodes gelsolin, which is a calcium-regulated actin regulatory protein that involved in inflammation, cell movement, apoptosis, and cancer development. The gelsolin protein could also inhibit the fibrillization of Aβ, and defibrillize its preformed fibrils (Ray et al., 2000; Hirko et al., 2007). The cystatin C protein is an inhibitor of cysteine proteinases, which could inhibit amyloid fibril formation and Aβ deposition (Sastre et al., 2004; Mi et al., 2007). The transthyretin protein, a thyroid hormone-binding protein, contains a BRICHOS domain, which could serve as the efficient inhibitor of Aβ fibril formation and toxicity (Buxbaum et al., 2008; Buxbaum and Johansson, 2017). Then integral membrane protein 2B, a type II transmembrane protein, could bind APP and inhibit all alpha, beta, and gamma pathways of APP proteolysis (Tamayev et al., 2011; Matsuda and Senda, 2019). In summary, they all could play as physiological inhibitors of Aβ under specific conditions, which might be associated with AD.

Although there are few reports of *CST3* (Hua et al., 2012; Paz-Y-Miño et al., 2015) and *TTR* (Sassi et al., 2016; Xiang et al., 2017) genes in patients with AD, there is no report of *GSN* and *ITM2B* genes. Our study is the first to screen mutations in *GSN*, *CST3*, *TTR*, and *ITM2B* genes in patients with AD by genes targeted sequencing (GTS).

MATERIALS AND METHODS

Subjects

The study included 636 AD patients in China [female 59.3%, onset age (Mean ± SD): 66.17 ± 11.18 years]. Patients were diagnosed by at least two experienced doctors of Xiangya Hospital according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Diseases and Related Disorders Associations (NINCDS–ADRDA). A total of 365 cognitive normal individuals (MMSE ≥ 27) were recruited from a physical examination center of Xiangya hospital [female 52.1%, age (Mean ± SD): 70.65 ± 5.35 years]. All subjects signed informed consent. Patients carrying with pathogenic genes of AD (*APP, PSEN1, PSEN2*) and vascular cognitive impairment [*notch receptor 3* (*NOTCH3*), *HtrA serine peptidase 1* (*HTRA1*), *collagen type IV alpha 1 chain* (*COL4A1*), *three prime repair exonuclease 1* (*TREX1*), *galactosidase alpha* (*GLA*)] were excluded.

Genes Targeted Sequencing and Data Analysis

GTS, including *GSN* (*NM_000177.4*), *CST3* (*NM001288614.1*), *TTR* (*NM000371.3*), and *ITM2B* (*NM_021999.4*), was performed in all subjects. Genomic DNA of all samples was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). Then the genomic DNA was fragmented by Covaris LE220 (MA, USA) to generate paired-end library (200–250 bp) and constructed into the libraries. The baits, a pool of 423 individually synthesized 5′-biotinylated 120 bp RNA oligonucleotides, cover four genes related with Aβ protein processing in HA. The targeted regions were captured with the baits as described below. DNA libraries (1 μg each) were mixed with the adaptor blockers and 5 μg of Cot-I DNA. The DNA mixture was denatured at 95°C for 5 min and then snap cooled on ice immediately. Next, the denatured DNA mixture and baits were transferred into hybridization solution (6× SSC, 1% SDS, 5× Denhardt's Solution). Hybridization was performed at 65°C for 4 h. After hybridization, the capture chip was washed with 2× SSC and 0.1% SDS for 5 min and 0.2× SSC and 0.1% SDS for 2 × 5 min at 55°C. The captured DNAs were eluted with 100 μl of TE at 95°C for 10 min and purified by using a PCR clean-up kit. The eluted DNAs were subjected to 15 cycles of PCR amplification using the Illumina P5 and P7 primers and subjected to another round of hybridization capture with the same conditions. The products were then subjected to Agilent 2100 Bioanalyzer and ABI StepOne to estimate the magnitude of enrichment. After quality control, captured library sequencing was carried out on Illumina HiSeq X Ten Analyzers (Illumina, San Diego, CA, USA). Following the manufacturer's standard sequencing protocols for 150 cycles per read to generate paired-end reads. Image analysis, error estimation, and base calling were performed using Illumina Pipeline software to generate raw data.

Then, we performed bioinformatics processing and data analysis to detect the potential variants. We using AfterQC to generate "clean reads" for further analysis. The "clean reads" (with a length of 150 bp) derived from targeted sequencing and filtering were then aligned to the human genome reference (hg19) using the BWA (Burrows Wheeler Aligner) software. After alignment, the output files were used to perform sequencing coverage and depth. We used GATK (Genome Analysis Toolkit) software[1] to detect SNVs and indels. All SNVs and indels were filtered and estimated *via* multiple databases, including Genome AD (Genome Aggregation Database dataset) and ExAC (The Exome Aggregation Consortium dataset). We used dbNSFP (Liu et al., 2016) to predict the effect of missense variants. Pathogenic variants were assessed by the American

[1]https://software.broadinstitute.org/gatk

College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015).

Sanger Sequencing

All likely pathogenic variants were screened using sanger sequencing. The sanger sequencing was amplified using identical forward and reverse primers (GSN-K346fs-F: 5′-CTTCCCAT GTGCAGTTTGTGTT-3′, GSN-K346fs-R: 5′-AGCCCAAGAC TTCTGATTTCCA-3′; GSN-P3fs-F: 5′-GCCTCGGTGAAAAG CTTTCAAA-3′, GSN-P3fs-R: 5′-TTTCCTAGCGCTGTATCT GCAA-3′). All PCR products were sequenced with Big Dye terminator v3.1 sequencing chemistry on an ABI 3730xl DNA analyzer (Applied Biosystems). DNA sequences were analyzed using sequencing software of Mutation Surveyor (Softgenetics).

Multiple Sequence Alignment and Structure Modeling

To evaluate the effect of the novel frame-shift mutations on structure and function of proteins, multiple sequence alignment was analyzed by T-Coffee[2], and three-dimensional (3D) models of the mutant protein structures were built by Discovery Studio software. We used homology models of gelsolin in the Protein Data Bank (PDB) to construct the 3D structure of the mutant proteins by Discovery Studio software.

RESULTS

This study included 636 patients with clinical AD and 365 cognitive normal controls from China, and the basic information of patients and controls was shown in **Table 1**. According to the ACMG guidelines, we identified two novel "likely pathogenic" mutations and 11 variants of uncertain significance (VUS) in the *GSN* gene, two VUS in the *CST3* gene, two VUS in the *ITM2B* gene, and two VUS in the *TTR* gene in patients with AD in the Chinese cohort (**Table 2**). The two novel "likely pathogenic" mutations were not detected in normal controls. The first "likely pathogenic" mutation (PVS1 + PM2) in the *GSN* gene was c.1036delA:p.K346fs, whose frequency in all databases was not available (NA), such as East Asian population of Genome Aggregation Database dataset (gnomAD_genome_EAS), All population of Genome Aggregation Database dataset (gnomAD_genome_ALL), and East Asia population of the Exome Aggregation Consortium (ExAC_EAS). The second "likely pathogenic" mutation (PVS1 + PM2) in the GSN gene was c.8_35del:p.P3fs, whose frequency in gnomAD_genome_EAS was 0.0049, in gnomAD_genome_ALL was 0.0003, and in ExAC_EAS was 0, suggesting that the P3fs mutation was only detected in East Asian populations. The multiple sequence alignment and 3D models of the mutant protein structures were shown in **Figure 3**.

The K346fs mutation was detected in a sporadic female patient whose onset age was 48 years old (case 1; **Figure 1A**). The patient first presented memory decline, manifested as forgetting things just done. Simultaneously, she became apathetic and did not communicate with others. About 1 year later, she could not recognize her relatives or take care of herself in daily life such as wearing clothes and bathing. She also developed psychiatric symptoms about the same time, which were emotionally violent, hitting people and crying for no reasons. Cognitive assessments: (1) mini-mental state examination (MMSE): 9/30; (2) montreal cognitive assessment scale (MoCA): 2/30; (3) neuropsychiatric inventory (NPI): 6; (4) daily living ability scale (ADL): 37; and (5) clinical dementia rating scale (CDR): 2. Electroencephalogram (EEG) showed moderate abnormal EEG (frontal and temporal regions were paroxysmal slow waves). Brain magnetic resonance imaging (MRI) showed mild leukoencephalopathy and brain atrophy (**Figure 1G**). Positron emission tomography-computed tomography (PET-CT) revealed: (1) fluorodeoxyglucose (FDG) metabolism decreased in bilateral frontal and parietal lobes; (2) diffuse Aβ protein deposition in bilateral cerebral cortex and subcortical nuclei; (3) lacunar infarction in brain stem; and (4) brain atrophy (**Figure 1G**). In addition, ophthalmologic symptoms are common in FAF patients. The ophthalmologic examinations of the case 1 patient revealed that the cornea was normal, but bilateral optic nerves were atrophied. Unfortunately, the patient refused to do the skin biopsy.

The P3fs mutation was detected in four sporadic patients (case 2–5; **Table 3**), whose onset age was older than 65 years [onset age (Mean ± SD): 69.50 ± 5.20 years; **Figures 1B–E**). The initial symptom was memory decline and the symptom progressed slowly in all patients. Among them, case 2, case 3, and case 4 patients experienced cognitive disturbance (spatial disorientation and count disturbance) and behavior change (became irritable and prone to temper) later. The case 2 patient developed behavioral and psychological symptoms in the third year after onset. The case 5 patient, who had a shorter course, did not suffer above symptoms, and her clinical manifestations were milder than other patients. Case 2 and case 3 patients cannot be traced at present. The case 4 patients were seriously ill and stayed in bed all day during our follow-up, so he could not cooperate with our study. The symptom of case 5 patient has not changed obviously so far, and she did more

TABLE 1 | Basic information of patients and controls.

	AD patients	Cognitive normal controls
Numbers	636	365
Age of onset (years)	66.17 ± 11.18	70.65 ± 5.35
Gender		
Male	259	175
Female	377	190
Race		
Han nationality	630	365
Others	6	0
APOE		
ε2/ε2	2	2
ε2/ε3	50	54
ε2/ε4	12	6
ε3/ε3	321	237
ε3/ε4	207	63
ε4/ε4	44	3

[2]https://www.ebi.ac.uk/Tools/msa/tcoffee/

TABLE 2 | Variants in genes of *gelsolin* (*GSN*), *cystatin C* (*CST3*), *transthyretin* (*TTR*), and *integral membrane protein 2B* (*ITM2B*).

Gene name	Mutation name	ACMG	Patients	Normal controls	Mutation mode	HET/HOM	Risk dbSNP	gnomAD_genome_EAS	ExAC_EAS	Polyphen2	MutTaster	PROVEAN
GSN	c.8_35del:p.P3fs	Likely pathogenic (PVS1 + PM2)	**4**	**0**	Frameshift deletion	HET	rs764841269	4.90E-03	0	NA	NA	NA
GSN	c.1036delA:p.K346fs	Likely pathogenic (PVS1 + PM2)	**1**	**0**	Frameshift deletion	HET	NA	NA	NA	NA	NA	NA
GSN	c.425G>A:p.R142Q	Uncertain significance (N)	**1**	**0**	Nonsynonymous SNV	HET	rs138153246	0	0	B	D	N
GSN	c.613G>A:p.V205M	Uncertain significance (PM2)	**1**	**0**	Nonsynonymous SNV	HET	NA	NA	NA	D	D	N
GSN	c.863C >T:p.A288V	Uncertain significance (N)	**3**	**0**	Nonsynonymous SNV	HET	rs780252276	4.06E-04	0.0006	B	N	N
GSN	c.902C >T:p.Y301C	Uncertain significance (PM2)	**2**	**0**	Nonsynonymous SNV	HET	rs758752620	5.80E-05	0.0001	D	D	D
GSN	c.958C >T:p.P320S	Uncertain significance (N)	**1**	**0**	Nonsynonymous SNV	HET	rs768184900	0	0	D	D	D
GSN	c.1055C >T:p.T352M	Uncertain significance (PM2 + BP4)	**1**	**0**	Nonsynonymous SNV	HET	NA	NA	NA	B	N	N
GSN	c.1406C >T:p.Y469C	Uncertain significance (PM2)	**1**	**1**	Nonsynonymous SNV	HET	rs375227932	4.06E-04	0.0003	D	D	D
GSN	c.1655dupC:p.S522fs	Uncertain significance (PM4)	**1**	**0**	Frameshift insertion	HET	rs769989772	1.76E-04	0.0003	NA	NA	NA
GSN	c.1730G >T:p.R577L	Uncertain significance (PM2)	**1**	**0**	Nonsynonymous SNV	HET	rs528604896	1.11E-03	0.001	D	D	D
GSN	c.1793C >T:p.T598I	Uncertain significance (N)	**2**	**1**	Nonsynonymous SNV	HET	rs376326631	1.16E-04	0.0001	D	D	D
GSN	c.2198C >T:p.T733M	Uncertain significance (PM2)	**1**	**0**	Nonsynonymous SNV	HET	rs142854368	0	0	D	D	D
CST3	c.236G >T:p.R79L	Uncertain significance (PM2 + BP4)	1	0	Nonsynonymous SNV	HET	NA	NA	NA	P	N	D
CST3	c.371C >T:p.S124F	Uncertain significance (PM2)	2	0	Nonsynonymous SNV	HET	rs754306266	0	0	D	D	D
TTR	c.62G>C:p.G21A	Uncertain significance (PM2)	1	0	Nonsynonymous SNV	HET	NA	NA	NA	B	N	N
TTR	c.370C >T:p.R124C	Uncertain significance (PM2 + PP3)	1	1	Nonsynonymous SNV	HET	rs745834030	4.64E-04	0.0001	P	N	N
ITM2B	c.20C >T:p.N7S	Uncertain significance (N)	1	2	Nonsynonymous SNV	HET	rs779234032	0	0	B	D	N
ITM2B	c.325G >T:p.A109S	Uncertain significance (N)	2	2	Nonsynonymous SNV	HET	rs748146945	5.22E-04	0.0003	B	D	N

PVS1: predicted null variant in a gene where loss of function (LOF) is a known mechanism of disease. PM2: absent in population databases. PM4: protein length changing variant. PP3: multiple lines of computational evidence support a deleterious effect on the gene/gene product. BP4: multiple lines of computational evidence suggest no impact on gene/gene product. Uncertain significance (N): does not meet any ACMG standards. Polyphen2 (D: probably damaging, P: possibly damaging; B: benign). MutTaster (D: disease causing; N: polymorphism). Provean (D: deleterious SNV; N: neutral). gnomAD_genomeEAS: frequency in the East Asian population of Genome Aggregation Database dataset; ExACEAS: frequency in East Asia population of the Exome Aggregation Consortium (ExAC) database; HET: heterozygous; HOM: homozygous; NA: not available; SNV: single nucleotide variant.

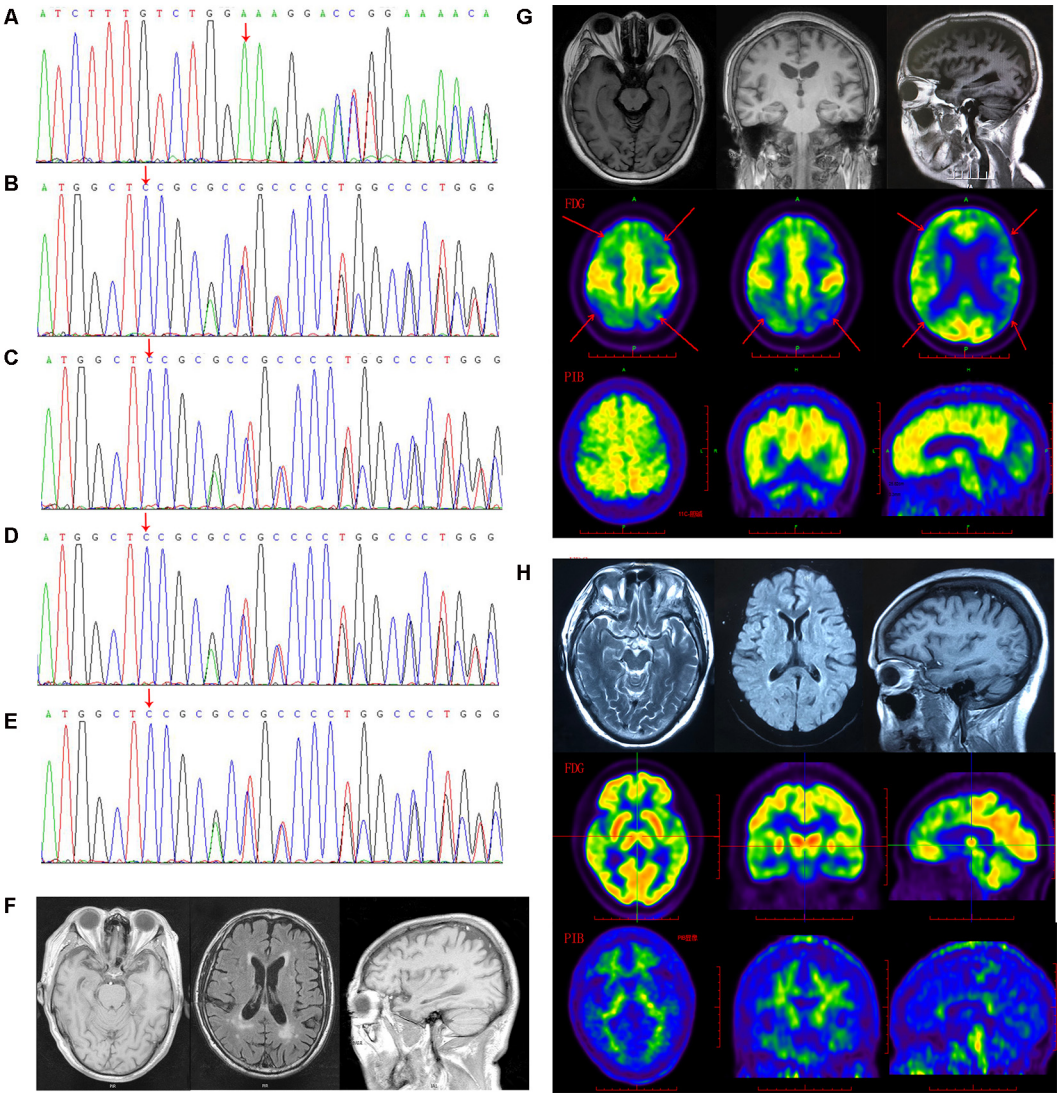

FIGURE 1 | The *gelsolin* (*GSN*) gene mutation sequencing diagram and patients' imaging. **(A)** The sequencing diagram of case 1 (GSN:c.1036delA:p.K346). **(B)** The sequencing diagram of case 2 (GSN:c.8_35del:p.P3fs). **(C)** The sequencing diagram of case 3 (GSN:c.8_35del:p.P3fs). **(D)** The sequencing diagram of case 4 (GSN:c.8_35del:p.P3fs). **(E)** The sequencing diagram of case 5 (GSN:c.8_35del:p.P3fs). **(F)** The magnetic resonance imaging (MRI) of case 4 with P3fs mutation. **(G)** The MRI, FDG-PET and PIB-PET of case 1 with K346fs mutation. **(H)** The MRI, FDG-PET and PIB-PET of case 5 with P3fs mutation).

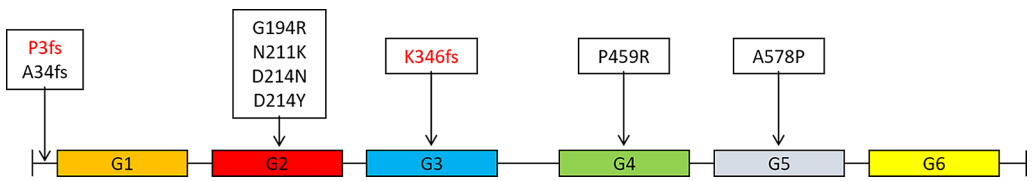

FIGURE 2 | Distribution of mutations in the *GSN* gene in different domains of gelsolin protein (Gelsolin protein has six domains, named G1 to G6).

examinations during the follow-up. PET-CT revealed: (1) FDG imaging showed no abnormal increase or decrease in glucose metabolism in the brain; (2) PIB imaging showed no abnormality of imaging agent uptake in cerebral cortex, and suggested no obvious Aβ protein deposition in the cerebral cortex; and (3) mild brain atrophy (**Figure 1H**). As the case 5 patient had a history of numbness of the limbs, an extra electromyogram (EMG) was performed. Nerve conduction velocity (NCV) was

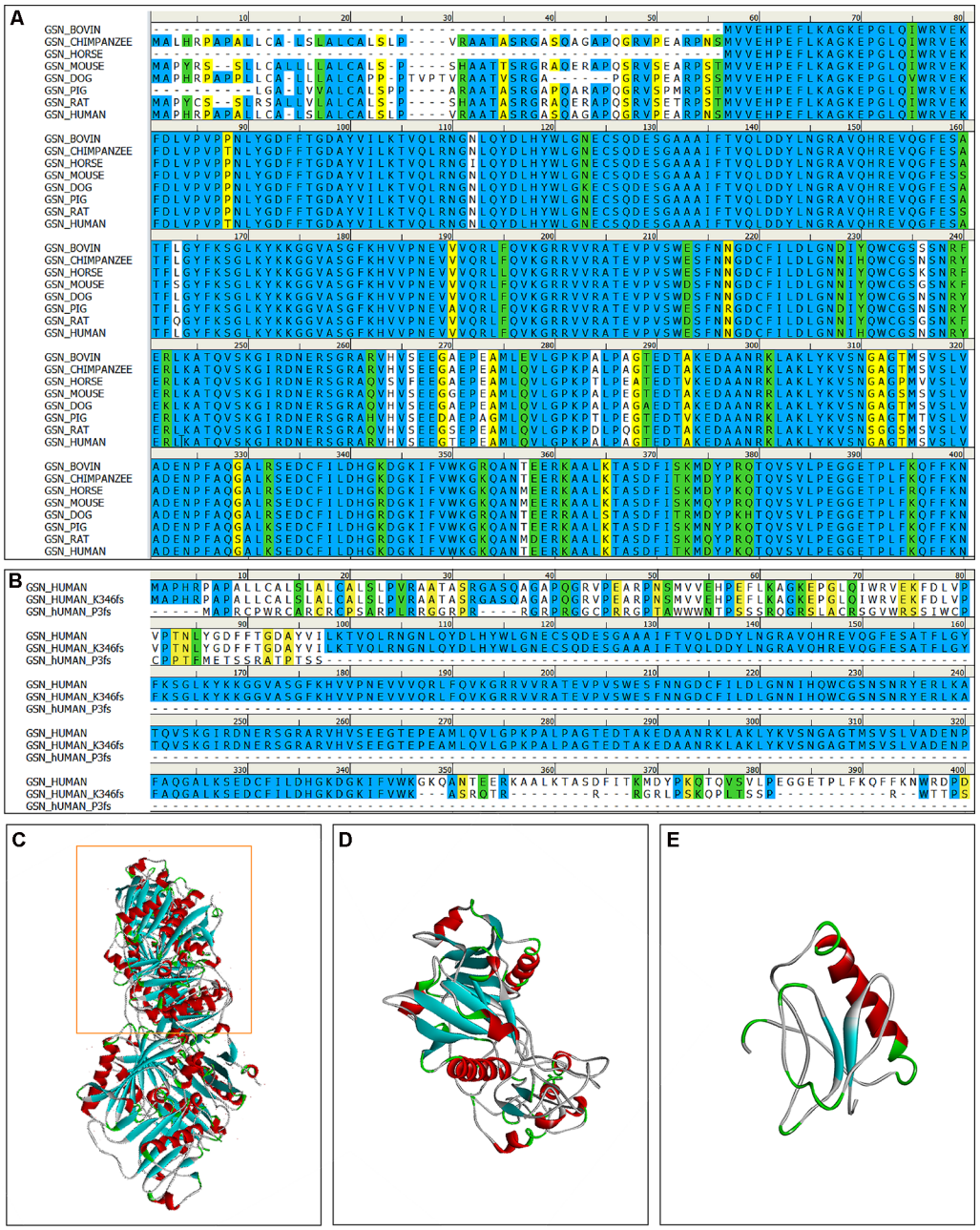

FIGURE 3 | The multiple sequence alignment and 3D models of the mutant protein structures. **(A)** The multiple sequence alignment of the gelsolin protein among vertebrates (amino acid residues 1–400); The degree of conservation between sequences was showed in different colors (identical: bule, strong: green, weak: yellow, non-matching: white). **(B)** The multiple sequence alignment of the gelsolin protein, K346fs and P3fs mutant gelsolin protein (amino acid residues 1–400). **(C)** 3D model of the wild-type gelsolin. **(D)** 3D model of the mutant protein with K346fs mutation, which is similar to part of the wild-type gelsolin (yellow box in **C**). **(E)** 3D model of the mutant protein with P3fs mutation.

normal in bilateral median nerve, ulnar nerve, tibial nerve, common peroneal nerve, and sural nerve. EMG showed no significant changes were observed in the limb muscles and no obvious abnormality in the skin sympathetic response of the extremities. Ophthalmic examination showed that the cornea was normal. Unfortunately, the patient refused to do the skin biopsy.

DISCUSSION

In our study, we screened mutations of *GSN, CST3, TTR*, and *ITM2B* genes by GTS in patients with AD in China, and identified two novel "likely pathogenic" mutations K346fs and P3fs in the *GSN* gene, suggesting that *GSN* gene may explain a small portion of AD.

TABLE 3 | Information of five patients with mutations in the *GSN* gene.

	Case 1	Case 2	Case 3	Case 4	Case 5
Mutation	K346fs	P3fs	P3fs	P3fs	P3fs
Gender	Female	Female	Male	Male	Female
Onset age	48	77	69	66	66
Visiting ages	50	80	75	68	67
Course (years)	4	12	14	8	3
First symptoms	Memory decline	Memory decline	Memory decline	Memory decline	Memory decline
Additional symptoms	Behavioral and Psychological symptoms	Behavioral and Psychological symptoms	Behavior change	Behavior change	No
Past medical history		Coronary heart disease	Cerebral infarction, hypertension, rhinitis, left inguinal hernia	Hypertension, hyperlipidemia, diabetes	Headache, numbness of the limbs, patent foramen ovale
Family history	No	No	No	No	No
APOE	ε3/ε4	ε3/ε3	ε3/ε3	ε3/ε3	ε3/ε4
Cognitive Assessment					
MMSE	9/30	9/30	0/30	18/30	23/30
MoCA	2/30	8/30	0/30	14/30	15/30
ADL	37	61	-	-	23
NPI	6	-	-	-	7
CDR	2	-	-	-	0.5
MRI	Mild leukoencephalopathy and brain atrophy (**Figure 1G**)	-	-	Multiple lacunar infarction in the brain, leukoencephalopathy, brain atrophy (**Figure 1F**)	Mild brain atrophy and mild leukoencephalopathy (**Figure 1H**)

The *GSN* gene is located on the chromosome 9q33.2 and is inherited by dominance. Up to now, seven pathogenic mutations in the *GSN* gene have been reported in worldwide, namely A34fs, G194R, N211K, D214N, D214Y, P459R, and A578P (**Figure 2** and **Table 4**, Hiltunen et al., 1991; Stewart et al., 2000; Conceição et al., 2003; Chastan et al., 2006; Ardalan et al., 2007; Huerva et al., 2007; Carrwik and Stenevi, 2009; Luttmann et al., 2010; Makioka et al., 2010; Asahina et al., 2011; Solari et al., 2011; Taira et al., 2012; Sethi et al., 2013; Efebera et al., 2014; Park et al., 2016; Caress et al., 2017; de Souza et al., 2017; Feng et al., 2018; Mustonen et al., 2018; Oregel et al., 2018; Sridharan et al., 2018). The D214N/Y mutation is the most common mutation and could cause the disease of FAF, which mainly manifested as corneal lattice dystrophy, cranial neuropathy, peripheral neuropathy, and cutis laxa (Nikoskinen et al., 2015). FAF has also been reported in other areas besides Finland. Due to differences in regions and races, it could be seen that, in East Asia, the clinical manifestations of FAF were mainly neurological symptoms (Taira et al., 2012; Park et al., 2016; Feng et al., 2018). Followed by G194R and N211K mutations, whose clinical phenotype is different from D214N/Y, mainly gelsolin-related renal amyloidosis (Sethi et al., 2013; Efebera et al., 2014). A34fs, P459R, and A578P mutations were reported recently, corresponding totally different manifestations from mutations that we mentioned before (Feng et al., 2018; Oregel et al., 2018; Sridharan et al., 2018; **Table 4**). Patients with A34fs mutation presented with seizures and brain lesions, without skin and eye symptoms. The patient with P459R mutation manifested as cranial nerve palsy (facial nerve) and proximal muscle weakness, then dead due to unexplained dyspnea and severe sepsis. The patient with A578P mutation combination with V122I mutation in the TTR gene (mainly related to cardiac involvement), characterized by progressive dyspnea, without cranial nerve, eye, and skin symptoms. That is to say, the *GSN* gene has a heterogeneity between genetic phenotype and clinical phenotype, different mutations lead to different locations of the lesions, resulting in different clinical manifestations. But there was no report of the AD phenotype, and we are the first to report that K346fs and P3fs mutations in the *GSN* gene may lead to AD.

Gelsolin protein consists six domains, named G1 to G6. Most mutations currently found (D214N/Y, G194R, and N211K) were located in the G2 domain (**Figure 2**), affecting the stability of the G2 domain and leading to disease (Bonì et al., 2016, 2018; Giorgino et al., 2019). Recently, Zorgati et al. (2019) proposed a new hypothesis that the D214N/Y mutation affected the stability of the G2 domain by affecting the interactions between G2–G3 domains. They validated this hypothesis by making G3 domain non-FAF mutations (K341M, L388D, and Q391L), confirming that these mutations disrupted the interactions of G2 and G3 domains, making the cleavage site more susceptible to exposure; and they also predicted that mutations in the G3 domain will also lead to disease. Our newly identified K346fs mutation was located in the G3 domain, which was near the sites that we mentioned above, and might promote the occurrence of disease in the similar way. Moreover, the case 1 patient carried K346fs mutation started disease early, and her PIB-PET showed Aβ deposition. Therefore, we considered that the K346fs mutation is most likely to be pathogenic.

Both the P3fs mutation, which was our newly identified, and the A34fs mutation had a frame shift at the start site. Due to the frame shift at the start site, some scholars believed that it might not translate the functional domain of gelsolin. Therefore, the amyloid protein formed by the A34fs mutation might have different composition relative to other FAF fibrils

TABLE 4 | Pathogenic mutations in the *GSN* gene in worldwide.

Mutation	Area	Disease	Pathogenic protein deposition	Clinical manifestations
P3fs*	China	AD	Not known, maybe brain	Cognitive dysfunction, mild peripheral neurological symptoms, no eye or skin symptoms.
A34fs	China (Feng et al., 2018)	Atypical FAF	Not known, maybe brain and cerebral vessels.	Seizures and brain lesions. no skin, or eye symptoms.
G194R	USA (Sethi et al., 2013)	Gelsolin-related renal amyloidosis	Kidney	Chronic kidney disease and anemia.
N211K	USA (Efebera et al., 2014)	Gelsolin-related renal amyloidosis	Kidney	Nephrotic range proteinuria of 13.2 g/day as the only presenting symptom.
D214N/Y	Finland (Hiltunen et al., 1991; Mustonen et al., 2018), USA (Caress et al., 2017), Japan (Makioka et al., 2010; Asahina et al., 2011; Taira et al., 2012), Spain (Huerva et al., 2007), France (Chastan et al., 2006), Portugal (Conceição et al., 2003), England (Stewart et al., 2000), Iran (Ardalan et al., 2007), Brazil (Solari et al., 2011; de Souza et al., 2017), Sweden (Carrwik and Stenevi, 2009), Germany (Luttmann et al., 2010), Korea (Park et al., 2016)	FAF	Eye, nerve, and skin	The main clinical manifestations are corneal lattice dystrophy, cranial neuropathy, peripheral neuropathy and cutis laxa. in East Asia (Japan and Korea), the clinical manifestations of FAF were mainly neurological symptoms.
K346fs*	China	AD	Not known, maybe brain	Cognitive dysfunction, personality changes, psychiatric symptoms, symptoms in multiple systems of the body (eyes, skin and thyroid).
P459R	USA (African descent; Oregel et al., 2018)	Atypical FAF	Muscle tissue	Cranial nerve palsy (facial nerve) and proximal muscle weakness, then dead due to unexplained dyspnea and severe sepsis. The MRI of the head and spinal cord was normal. Biopsy of left quadriceps femoris biopsy showed focal myopathy and denervation atrophy (severe, type II).
A578P	USA (Sridharan et al., 2018)	ATTR (transthyretin amyloidosis)	Myocardium (amyloid deposition); abdominal fat and rectum mucosa (gelsolin deposition).	Combination with V122I mutation of the *TTR* gene (mainly related to cardiac involvement), characterized by progressive dyspnea, no cranial nerve, eye or skin symptoms.

**Novel mutation.*

(Feng et al., 2018; Zorgati et al., 2019). Both patients with A34fs and P3fs mutations mainly presented with central nervous symptoms, without skin, eyes, and peripheral nervous symptoms. All patients (case 2–5) with P3fs mutation had a late onset of disease, and the PIB-PET showed no Aβ deposition in the case 5 patient. But the patient with A34fs mutation reported recently did not have a biopsy of skin or other sites. It was uncertain whether the patient had the deposition of gelsolin protein. Although the P3fs mutation was assessed as "likely pathogenic" by ACMG, we considered that it needed more studies to verify.

There was also a close relationship between AD and gelsolin protein. The level of gelsolin changed as AD progressed (Antequera et al., 2009; Guntert et al., 2010; Peng et al., 2015; Yao et al., 2018). Mechanism studies found that gelsolin contained two Aβ binding sites (Chauhan et al., 1999), through binding to Aβ protein, gelsolin could inhibit Aβ-induced toxicity (Harms et al., 2004; Qiao et al., 2005), inhibit Aβ fibrosis and degrade fibers that already formed (Ray et al., 2000; Hirko et al., 2007). Studies also showed that injection or over expression of gelsolin resulted in a significant reduction in amyloid loads and a decrease in Aβ levels in AD transgenic mice (Hirko et al., 2007; Antequera et al., 2009; Yang et al., 2014). In general, gelsolin acted as an anti-amyloid-forming protein and had neuroprotective effects in AD patients. When the *GSN* gene mutated in AD patients, its protective effect on the nerve might be decreased, thus promoting the occurrence of AD.

Some variants in genes of *CST3*, *IMT2B*, and *TTR* were detected in our study, but they were not pathogenic. Although some evidence suggested that the cystatin C could affect the Aβ protein processing (Kaur and Levy, 2012), there were no pathogenic mutations found in the *CST3* gene in Chinese patients with AD (Hua et al., 2012; Paz-Y-Miño et al., 2015), which was similar to our results. As for the *TTR* gene, some potential pathogenic mutations were reported in patients with AD (Sassi et al., 2016; Xiang et al., 2017), which was different to our results. The reasons for the difference might be our smaller sample size and different races. The *ITM2B* gene was similar to *CST3* (Fotinopoulou et al., 2005; Matsuda et al., 2005), and no related pathogenic mutations were reported. Therefore, we suspected that these three genes may not be closely related to AD.

In summary, we are the first to report AD phenotype with *GSN* mutation in patients with AD in Chinese cohort, expanding

the *GSN* gene mutation spectrum and its corresponding clinical phenotype spectrum. Although mutations in the *GSN* gene are rare, it may explain a small portion of AD.

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: BioProject NCBI, accession no.: PRJNA656640 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA656640).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of National Center for Geriatrics Clinical Medical Research, 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

LS designed the experiment. YJ performed the experiment. YJ, XX and XLiu processed the data. YJ wrote the article. BJ and XLiao modified the article.

FUNDING

This study was supported by the National Natural Science Foundation of China (No. 81671075 to LS, No. 81971029 to LS, No. 81701134 to BJ, No. 81901171 to XLiao), the National Key R&D Program of China (Nos. 2017YFC0840100 and 2017YFC0840104 to LS), the Provincial Key Plan for Research and Development of Hunan (No. 2017SK2031 to LS), the Provincial Technology Innovation Guidance Plan Project of Hunan (No. 2018SK52601 to BJ), and the Youth Science Foundation of Xiangya Hospital (No. 2018Q020).

REFERENCES

Abrahamson, M., Islam, M. Q., Szpirer, J., Szpirer, C., and Levan, G. (1989). The human cystatin C gene (CST3), mutated in hereditary cystatin C amyloid angiopathy, is located on chromosome 20. *Hum. Genet.* 82, 223–226. doi: 10.1007/BF00291159

Antequera, D., Vargas, T., Ugalde, C., Spuch, C., Molina, J. A., Ferrer, I., et al. (2009). Cytoplasmic gelsolin increases mitochondrial activity and reduces Aβ burden in a mouse model of Alzheimer's disease. *Neurobiol. Dis.* 36, 42–50. doi: 10.1016/j.nbd.2009.06.018

Ardalan, M. R., Shoja, M. M., and Kiuru-Enari, S. (2007). Amyloidosis-related nephrotic syndrome due to a G654A gelsolin mutation: the first report from the Middle East. *Nephrol. Dial. Transplant.* 22, 272–275. doi: 10.1093/ndt/gfl548

Asahina, A., Yokoyama, T., Ueda, M., Ando, Y., Ohshima, N., Saito, I., et al. (2011). Hereditary gelsolin amyloidosis: a new Japanese case with cutis laxa as a diagnostic clue. *Acta Derm. Venereol.* 91, 201–203. doi: 10.2340/00015555-1011

Bateman, R. J., Aisen, P. S., De Strooper, B., Fox, N. C., Lemere, C. A., Ringman, J. M., et al. (2011). Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. *Alzheimers Res. Ther.* 3:1. doi: 10.1186/alzrt59

Bonì, F., Milani, M., Barbiroli, A., Diomede, L., Mastrangelo, E., and de Rosa, M. (2018). Gelsolin pathogenic Gly167Arg mutation promotes domain-swap dimerization of the protein. *Hum. Mol. Genet.* 27, 53–65. doi: 10.1093/hmg/ddx383

Bonì, F., Milani, M., Porcari, R., Barbiroli, A., Ricagno, S., and de Rosa, M. (2016). Molecular basis of a novel renal amyloidosis due to N184K gelsolin variant. *Sci. Rep.* 6:33463. doi: 10.1038/srep33463

Buxbaum, J. N., and Johansson, J. (2017). Transthyretin and BRICHOS: the paradox of amyloidogenic proteins with anti-amyloidogenic activity for Aβ in the central nervous system. *Front. Neurosci.* 11:119. doi: 10.3389/fnins.2017.00119

Buxbaum, J. N., Ye, Z., Reixach, N., Friske, L., Levy, C., Das, P., et al. (2008). Transthyretin protects Alzheimer's mice from the behavioral and biochemical effects of Aβ toxicity. *Proc. Natl. Acad. Sci. U S A* 105, 2681–2686. doi: 10.1073/pnas.0712197105

Caress, J. B., Johnson, J. O., Abramzon, Y. A., Hawkins, G. A., Gibbs, J. R., Sullivan, E. A., et al. (2017). Exome sequencing establishes a gelsolin mutation as the cause of inherited bulbar-onset neuropathy. *Muscle Nerve* 56, 1001–1005. doi: 10.1002/mus.25550

Carrwik, C., and Stenevi, U. (2009). Lattice corneal dystrophy, gelsolin type (Meretoja's syndrome). *Acta Ophthalmol.* 87, 813–819. doi: 10.1111/j.1755-3768.2009.01686.x

Chastan, N., Baert-Desurmont, S., Saugier-Veber, P., Derumeaux, G., Cabot, A., Frebourg, T., et al. (2006). Cardiac conduction alterations in a French family with amyloidosis of the Finnish type with the p.Asp187Tyr mutation in the GSN gene. *Muscle Nerve* 33, 113–119. doi: 10.1002/mus.20448

Chauhan, V. P., Ray, I., Chauhan, A., and Wisniewski, H. M. (1999). Binding of gelsolin, a secretory protein, to amyloid β-protein. *Biochem. Biophys. Res. Commun.* 258, 241–246. doi: 10.1006/bbrc.1999.0623

Chyra Kufova, Z., Sevcikova, T., Januska, J., Vojta, P., Boday, A., Vanickova, P., et al. (2018). Newly designed 11-gene panel reveals first case of hereditary amyloidosis captured by massive parallel sequencing. *J. Clin. Pathol.* 71, 687–694. doi: 10.1136/jclinpath-2017-204978

Conceição, I., Sales-Luis, M. L., De Carvalho, M., Evangelista, T., Fernandes, R., Paunio, T., et al. (2003). Gelsolin-related familial amyloidosis, Finnish type, in a Portuguese family: clinical and neurophysiological studies. *Muscle Nerve* 28, 715–721. doi: 10.1002/mus.10474

de Souza, P. V. S., Bortholin, T., Naylor, F. G. M., Dias, R. B., Pinto, W., and Oliveira, A. S. B. (2017). Familial progressive bilateral facial paralysis in Finnish type hereditary amyloidosis. *Pract. Neurol.* 17, 408–409. doi: 10.1136/practneurol-2017-001690

Del Campo, M., and Teunissen, C. E. (2014). Role of BRI2 in dementia. *J. Alzheimers Dis.* 40, 481–494. doi: 10.3233/jad-131364

Efebera, Y. A., Sturm, A., Baack, E. C., Hofmeister, C. C., Satoskar, A., Nadasdy, T., et al. (2014). Novel gelsolin variant as the cause of nephrotic syndrome and renal amyloidosis in a large kindred. *Amyloid* 21, 110–112. doi: 10.3109/13506129.2014.891502

Feng, X., Zhu, H., Zhao, T., Hou, Y., and Liu, J. (2018). A new heterozygous G duplicate in exon1 (c.100dupG) of gelsolin gene causes Finnish gelsolin amyloidosis in a Chinese family. *Brain Behav.* 8:e01151. doi: 10.1002/brb3.1151

Fotinopoulou, A., Tsachaki, M., Vlavaki, M., Poulopoulos, A., Rostagno, A., Frangione, B., et al. (2005). BRI2 interacts with amyloid precursor protein (APP) and regulates amyloidβ (Aβ) production. *J. Biol. Chem.* 280, 30768–30772. doi: 10.1074/jbc.C500231200

Giorgino, T., Mattioni, D., Hassan, A., Milani, M., Mastrangelo, E., Barbiroli, A., et al. (2019). Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant. *Biochim. Biophys. Acta Mol. Basis Dis.* 1865, 648–660. doi: 10.1016/j.bbadis.2019.01.010

Guntert, A., Campbell, J., Saleem, M., O'Brien, D. P., Thompson, A. J., Byers, H. L., et al. (2010). Plasma gelsolin is decreased and correlates with rate of decline in Alzheimer's disease. *J. Alzheimers Dis.* 21, 585–596. doi: 10.3233/jad-2010-100279

Harms, C., Bösel, J., Lautenschlager, M., Harms, U., Braun, J. S., Hortnagl, H., et al. (2004). Neuronal gelsolin prevents apoptosis by enhancing actin

depolymerization. *Mol. Cell. Neurosci.* 25, 69–82. doi: 10.1016/j.mcn.2003.09.012

Hiltunen, T., Kiuru, S., Hongell, V., Helio, T., Palo, J., and Peltonen, L. (1991). Finnish type of familial amyloidosis: cosegregation of Asp187—Asn mutation of gelsolin with the disease in three large families. *Am. J. Hum. Genet.* 49, 522–528.

Hirko, A. C., Meyer, E. M., King, M. A., and Hughes, J. A. (2007). Peripheral transgene expression of plasma gelsolin reduces amyloid in transgenic mouse models of Alzheimer's disease. *Mol. Ther.* 15, 1623–1629. doi: 10.1038/sj.mt.6300253

Hua, Y., Zhao, H., Lu, X., Kong, Y., and Jin, H. (2012). Meta-analysis of the cystatin C(CST3) gene G73A polymorphism and susceptibility to Alzheimer's disease. *Int. J. Neurosci.* 122, 431–438. doi: 10.3109/00207454.2012.672502

Huerva, V., Velasco, A., Sánchez, M. C., Mateo, A. J., and Matías-Guiu, X. (2007). Lattice corneal dystrophy type II: clinical, pathologic, and molecular study in a Spanish family. *Eur. J. Ophthalmol.* 17, 424–429. doi: 10.1177/112067210701700326

International AsD. (2019). *World Alzheimer's Report 2018.* Alzheimer's Disease International (ADI), London.

Kaur, G., and Levy, E. (2012). Cystatin C in Alzheimer's disease. *Front. Mol. Neurosci.* 5:79. doi: 10.3389/fnmol.2012.00079

Lane, C. A., Hardy, J., and Schott, J. M. (2018). Alzheimer's disease. *Eur. J. Neurol.* 25, 59–70. doi: 10.1111/ene.13439

Liu, X., Wu, C., Li, C., and Boerwinkle, E. (2016). dbNSFP v3.0: a one-stop database of functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Hum. Mutat.* 37, 235–241. doi: 10.1002/humu.22932

Luttmann, R. J., Teismann, I., Husstedt, I. W., Ringelstein, E. B., and Kuhlenbaumer, G. (2010). Hereditary amyloidosis of the Finnish type in a German family: clinical and electrophysiological presentation. *Muscle Nerve* 41, 679–684. doi: 10.1002/mus.21534

Makioka, K., Ikeda, M., Ikeda, Y., Nakasone, A., Osawa, T., Sasaki, A., et al. (2010). Familial amyloid polyneuropathy (Finnish type) presenting multiple cranial nerve deficits with carpal tunnel syndrome and orthostatic hypotension. *Neurol. Res.* 32, 472–475. doi: 10.1179/174313209x409007

Matsuda, S., Giliberto, L., Matsuda, Y., Davies, P., McGowan, E., Pickford, F., et al. (2005). The familial dementia BRI2 gene binds the Alzheimer gene amyloid-β precursor protein and inhibits amyloid-β production. *J. Biol. Chem.* 280, 28912–28916. doi: 10.1074/jbc.c500217200

Matsuda, S., and Senda, T. (2019). BRI2 as an anti-Alzheimer gene. *Med. Mol. Morphol.* 52, 1–7. doi: 10.1007/s00795-018-0191-1

Mi, W., Pawlik, M., Sastre, M., Jung, S. S., Radvinsky, D. S., Klein, A. M., et al. (2007). Cystatin C inhibits amyloid-β deposition in Alzheimer's disease mouse models. *Nat. Genet.* 39, 1440–1442. doi: 10.1038/ng.2007.29

Mustonen, T., Schmidt, E. K., Valori, M., Tienari, P. J., Atula, S., and Kiuru-Enari, S. (2018). Common origin of the gelsolin gene variant in 62 Finnish AGel amyloidosis families. *Eur. J. Hum. Genet.* 26, 117–123. doi: 10.1038/s41431-017-0026-x

Nikoskinen, T., Schmidt, E. K., Strbian, D., Kiuru-Enari, S., and Atula, S. (2015). Natural course of Finnish gelsolin amyloidosis. *Ann. Med.* 47, 506–511. doi: 10.3109/07853890.2015.1075063

Oregel, K. Z., Shouse, G. P., Oster, C., Martinez, F., Wang, J., Rosenzweig, M., et al. (2018). Atypical presentation of gelsolin amyloidosis in a man of african descent with a novel mutation in the gelsolin gene. *Am. J. Case Rep.* 19, 374–381. doi: 10.12659/ajcr.907550

Park, K. J., Park, J. H., Park, J. H., Cho, E. B., Kim, B. J., and Kim, J. W. (2016). The first korean family with hereditary gelsolin amyloidosis caused by p.D214Y mutation in the GSN gene. *Ann. Lab. Med.* 36, 259–262. doi: 10.3343/alm.2016.36.3.259

Paz-Y-Miño, C. A., Garcia-Cardenas, J. M., Lopez-Cortes, A., Salazar, C., Serrano, M., and Leone, P. E. (2015). Positive association of the cathepsin D Ala224Val gene polymorphism with the risk of Alzheimer's disease. *Am. J. Med. Sci.* 350, 296–301. doi: 10.1097/MAJ.0000000000000555

Peng, M., Jia, J., and Qin, W. (2015). Plasma gelsolin and matrix metalloproteinase 3 as potential biomarkers for Alzheimer disease. *Neurosci. Lett.* 595, 116–121. doi: 10.1016/j.neulet.2015.04.014

Qiao, H., Koya, R. C., Nakagawa, K., Tanaka, H., Fujita, H., Takimoto, M., et al. (2005). Inhibition of Alzheimer's amyloid-β peptide-induced reduction of mitochondrial membrane potential and neurotoxicity by gelsolin. *Neurobiol. Aging* 26, 849–855. doi: 10.1016/j.neurobiolaging.2004.08.003

Ray, I., Chauhan, A., Wegiel, J., and Chauhan, V. P. (2000). Gelsolin inhibits the fibrillization of amyloid β-protein and also defibrillizes its preformed fibrils. *Brain Res.* 853, 344–351. doi: 10.1016/s0006-8993(99)02315-x

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the association for molecular pathology. *Genet. Med.* 17, 405–424. doi: 10.1038/gim.2015.30

Sassi, C., Ridge, P. G., Nalls, M. A., Gibbs, R., Ding, J., Lupton, M. K., et al. (2016). Influence of coding variability in APP-Aβ metabolism genes in sporadic Alzheimer's disease. *PLoS One* 11:e0150079. doi: 10.1371/journal.pone.0150079

Sastre, M., Calero, M., Pawlik, M., Mathews, P. M., Kumar, A., Danilov, V., et al. (2004). Binding of cystatin C to Alzheimer's amyloid β inhibits *in vitro* amyloid fibril formation. *Neurobiol. Aging* 25, 1033–1043. doi: 10.1016/j.neurobiolaging.2003.11.006

Sekijima, Y. (2015). Transthyretin (ATTR) amyloidosis: clinical spectrum, molecular pathogenesis and disease-modifying treatments. *J. Neurol. Neurosurg. Psychiatry* 86, 1036–1043. doi: 10.1136/jnnp-2014-308724

Sethi, S., Theis, J. D., Quint, P., Maierhofer, W., Kurtin, P. J., Dogan, A., et al. (2013). Renal amyloidosis associated with a novel sequence variant of gelsolin. *Am. J. Kidney Dis.* 61, 161–166. doi: 10.1053/j.ajkd.2012.07.016

Solari, H. P., Ventura, M. P., Antecka, E., Belfort Junior, R., and Burnier, M. N. Jr. (2011). Danish type gelsolin-related amyloidosis in a Brazilian family: case reports. *Arq. Bras. Oftalmol.* 74, 286–288. doi: 10.1590/s0004-27492011000400012

Sridharan, M., Highsmith, W. E., Kurtin, P. J., Zimmermann, M. T., Theis, J. D., Dasari, S., et al. (2018). A patient with hereditary ATTR and a novel AGel p.Ala578Pro amyloidosis. *Mayo Clin. Proc.* 93, 1678–1682. doi: 10.1016/j.mayocp.2018.06.016

Stewart, H. S., Parveen, R., Ridgway, A. E., Bonshek, R., and Black, G. C. (2000). Late onset lattice corneal dystrophy with systemic familial amyloidosis, amyloidosis V, in an English family. *Br. J. Ophthalmol.* 84, 390–394. doi: 10.1136/bjo.84.4.390

Taira, M., Ishiura, H., Mitsui, J., Takahashi, Y., Hayashi, T., Shimizu, J., et al. (2012). Clinical features and haplotype analysis of newly identified Japanese patients with gelsolin-related familial amyloidosis of Finnish type. *Neurogenetics* 13, 237–243. doi: 10.1007/s10048-012-0330-0

Tamayev, R., Matsuda, S., Giliberto, L., Arancio, O., and D'Adamio, L. (2011). APP heterozygosity averts memory deficit in knockin mice expressing the Danish dementia BRI2 mutant. *EMBO J.* 30, 2501–2509. doi: 10.1038/emboj.2011.161

Xiang, Q., Bi, R., Xu, M., Zhang, D. F., Tan, L., Zhang, C., et al. (2017). Rare genetic variants of the transthyretin gene are associated with Alzheimer's disease in han chinese. *Mol. Neurobiol.* 54, 5192–5200. doi: 10.1007/s12035-016-0065-2

Yang, W., Chauhan, A., Mehta, S., Mehta, P., Gu, F., and Chauhan, V. (2014). Trichostatin A increases the levels of plasma gelsolin and amyloid β-protein in a transgenic mouse model of Alzheimer's disease. *Life Sci.* 99, 31–36. doi: 10.1016/j.lfs.2014.01.064

Yao, F., Zhang, K., Zhang, Y., Guo, Y., Li, A., Xiao, S., et al. (2018). Identification of blood biomarkers for Alzheimer's disease through computational prediction and experimental validation. *Front. Neurol.* 9:1158. doi: 10.3389/fneur.2018.01158

Zorgati, H., Larsson, M., Ren, W., Sim, A. Y. L., Gettemans, J., Grimes, J. M., et al. (2019). The role of gelsolin domain 3 in familial amyloidosis (Finnish type). *Proc. Natl. Acad. Sci. U S A* 116, 13958–13963. doi: 10.1073/pnas.1902189116

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.

Copyright © 2020 Jiang, Jiao, Liao, Xiao, Liu and Shen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 25 September 2020
doi: 10.3389/fnagi.2020.571437

Differential Circulating Levels of Naturally Occurring Antibody to α-Synuclein in Parkinson's Disease Dementia, Alzheimer's Disease, and Vascular Dementia

*Jian Wang[1], Bo Zheng[1], Shu Yang[2], Mei Hu[3] and Jian-Hong Wang[2]**

[1]Department of Neurology, Yaan People's Hospital, Yaan, China, [2]Department of Neurology, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China, [3]Department of Imaging, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China

Background: Aggregation of alpha-synuclein (α-Syn) is considered to be a significant pathological hallmark and a driving force of Parkinson's disease (PD). PD dementia (PDD) occurs in a substantial number of PD patients. Naturally occurring antibody against α-Syn (NAb-α-Syn) exists ubiquitously in human blood and is reported to be altered in PD. However, it is not clear yet whether PDD had similar changes of circulating NAb-α-Syn.

Methods: In this study, we recruited 61 PDD patients, 52 patients with Alzheimer's disease (AD), 51 patients with vascular dementia (VaD), and 50 normal controls (NCs). ELISA was used to examine NAb-α-Syn levels in serum.

Results: In comparison with NCs, serum levels of NAb-α-Syn were significantly lower in patients with PDD. However, serum levels of NAb-α-Syn were comparable among AD, VaD, and NC groups. Serum levels of NAb-α-Syn were positively correlated with the cognitive function, as reflected by Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). Serum levels of NAb-α-Syn were negatively correlated with the severity of PD [as reflected by the Unified Parkinson Disease Rating Scale (UPDRS)] and the duration of PD and PDD. Serum NAb-α-Syn can differentiate PDD patients from AD and VaD patients.

Conclusion: These results suggest that circulating NAb-α-Syn might be a potential biomarker of PDD.

Keywords: α-Synuclein, dementia, naturally occurring antibody, biomarker, circulating

OPEN ACCESS

Edited by:
Yu-Hui Liu,
Third Military Medical University, China

Reviewed by:
Yang Yang,
Third Military Medical University, China
Shu Yu,
Army Medical University, China

****Correspondence:***
Jian-Hong Wang
jhwangneurology@yeah.net

Received: *10 June 2020*
Accepted: *17 August 2020*
Published: *25 September 2020*

Citation:
Wang J, Zheng B, Yang S, Hu M and Wang J-H (2020) Differential Circulating Levels of Naturally Occurring Antibody to α-Synuclein in Parkinson's Disease Dementia, Alzheimer's Disease, and Vascular Dementia. Front. Aging Neurosci. 12:571437. doi: 10.3389/fnagi.2020.571437

INTRODUCTION

Neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), and Creutzfeldt–Jakob disease (CJD) are characterized by the deposition and aggregation of misfolded proteins in the central nervous system (CNS; Skovronsky et al., 2006). The characteristic pathological changes in PD consist of dopaminergic neuronal loss, gliosis, and intraneuronal Lewy body formation in the substantia nigra pars compacta and striatum. Alpha-Synuclein (α-Syn) was found to be the predominant protein in Lewy body inclusions (Tofaris and Spillantini, 2007).

Although PD is considered to be a motor disorder, non-motor symptoms may occur from early stages, even before the manifestation of motor symptoms. Dementia can occur in a substantial number of PD patients with a prevalence of about 30% (Hanagasi et al., 2017). PD dementia (PDD) is characterized by a dysexecutive syndrome with early and prominent impairment of attention and visuospatial functions, moderately impaired episodic memory, and relatively preserved core language functions (Hanagasi et al., 2017). Although the symptoms and clinical courses vary in PDD and other types of dementia, there is some overlap of clinical characteristics among different dementias. Currently, blood-based biomarkers are limited to distinguish them. After damage of neurons or axons, soluble α-Syn is released into the cerebral spinal fluid and efflux to the circulation in PD patients and in healthy individuals (El-Agnaf et al., 2006). This event may stimulate an induction of autoantibody formation against α-Syn [naturally occurring antibody against α-Syn (NAb-α-Syn); Papachroni et al., 2007]. The levels of circulating NAb-α-Syn have been reported to be altered in PD patients (Besong-Agbo et al., 2013). However, studies investigating the levels of NAb-α-Syn in PDD patients are limited. In this study, we investigated whether circulating NAb-α-Syn was capable of distinguishing PDD from AD and vascular dementia (VaD).

MATERIALS AND METHODS

Participants

A total number of 61 PDD, 52 AD, and 51 VaD patients were consecutively recruited from the Department of Neurology, Sichuan Provincial People's Hospital. Fifty normal controls (NCs) without cognitive dysfunction were recruited from the health examination center of Sichuan Provincial People's Hospital. PDD was diagnosed according to criteria proposed by the Movement Disorder Society Task Force (Emre et al., 2007). Patients who developed dementia within 1 year after PD onset were excluded. AD diagnoses were based on the clinical criteria for probable AD as described by the National Institute on Aging-Alzheimer's Association (McKhann et al., 2011). The diagnosis of VaD was based on the Diagnostic and Statistical Manual of Mental Disorders–fourth Edition (DSM-IV) and also fulfilled the imaging criteria for VaD (Erkinjuntti et al., 2000). Patients with cognitive and behavioral symptoms presenting as a result of other conditions, such as acute confusion due to systemic disease, abnormality or drug intoxication, depression, normal pressure hydrocephalus, progressive supranuclear palsy, or history of significant brain trauma followed by persistent neurologic deficit or known structural brain abnormality, were excluded. All participants received cognitive assessment using Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). Severity of PD was assessed by the Unified Parkinson Disease Rating Scale (UPDRS). Informed consent was obtained from all patients and their legal relatives. This research project was approved by the institutional review boards at Sichuan Provincial People's Hospital.

Isolation of Naturally Occurring Antibody Against Alpha-Synuclein

NAb-α-Syn isolated from human intravenous immunoglobulin G (IVIg; Yuanda, Sichuan, China) was used as a standard. NAb-α-Syn was extracted using affinity chromatography. Briefly, a column was packed with 2 ml resin (PIERCE Biotechnology, Rockford, IL, USA), labeled with 1 mg recombinant α-Syn (rPeptide, Bogart, GA, USA), equilibrated, and washed with phosphate-buffered saline (PBS, pH 7.4). After passing purified IVIg through the column, fractions were eluted with glycine buffer, pH 2.8. The main fractions containing the highest amount of NAb-α-Syn were pooled, and their concentration was determined using the NanoDrop spectrometer (Nanodrop1000, PeqLab, Erlangen, Germany). Pooled NAb-α-Syn was stored at −20°C until use.

Naturally Occurring Antibody Against Alpha-Synuclein ELISA

High-bind 96-well ELISA plates (Nunc, Denmark) were coated overnight with recombinant α-Syn 50 μg/ml (100 ml/well) in phosphate-coating buffer (1.7 mM sodium dihydrogen phosphate, 98 mM disodium hydrogen phosphate, 0.05% sodium azide; pH 7.6) at 4°C. Wells were then blocked with 5% bovine serum albumin (BSA; Sigma, St. Louis, MO, USA) in PBS for 1 h at 37°C. Standards were prepared by diluting purified NAb-α-Syn from IVIg in dilution buffer (5% BSA). Sera were diluted 1:100. Plates were washed four times with 300 ml washing buffer (PBS with 0.05% Tween-20) and incubated with standards and samples (100 ml/well) for 1 h at room temperature (RT). After washing, plates were incubated with 100 ml/well of detection antibody, a 1:5,000 dilution of peroxidase-labeled goat antihuman IgG antibody (Sigma, St. Louis, MO, USA) in dilution buffer, at RT for 1 h. After a final wash, the assay was developed using 100 ml/well TMB (Sigma, St. Louis, MO, USA) for 15 min. The reaction was stopped with 30 ml 2 N sulfuric acid (Roth, Karlsruhe, Germany) and read at 450 nm. The difference in signal between coated and uncoated wells was considered to be solely due to α-Syn-nAbs binding to α-Syn and was used for further calculations.

Statistics

Statistical analysis was performed using SPSS (version 19.0, SPSS, Chicago, IL, USA). The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to check for normal distribution. To compare demographic, clinical, and serum data between groups, parametric and nonparametric tests such as one-way analysis of variance were used. Kruskal–Wallis test was used to compare multiple groups (PDD, AD, VaD, and NC), followed by *post hoc* pairwise comparisons using Dunn multiple comparison procedures. In order to test for significant correlations between clinical and demographic characteristics of patients and the serum data, Spearman rank correlation was used. Optimal sensitivity and specificity were determined *via* the receiver operating characteristic (ROC) curve analysis to determine the capacity of NAb-α-Syn in differentiating different types of dementia. A $p < 0.05$ was regarded as significant.

TABLE 1 | Demographic data of subjects.

	NC (n = 50)	PDD (n = 61)	AD (n = 52)	VaD (n = 51)	p
Age, years	66.68 ± 8.63	69.07 ± 8.25	66.90 ± 9.58	65.00 ± 8.99	0.115
Female, n (%)	25 (50.00)	31 (50.82)	25 (48.08)	24 (47.06)	0.978
ApoE ε4, n (%)	10 (20.00)	12 (19.67)	24 (46.15)	10 (19.61)	0.003
Education, years	8.84 ± 4.49	8.92 ± 4.55	8.08 ± 4.27	8.47 ± 4.58	0.752
MMSE	27.96 ± 1.88	14.52 ± 6.62	14.87 ± 6.21	15.27 ± 5.37	<0.001
MoCA	28.12 ± 1.76	12.90 ± 6.24	13.19 ± 5.15	12.10 ± 4.61	<0.001
UPDRS	NA	29.70 ± 1.75	NA	NA	NA
Duration of PD	NA	7.26 ± 3.83	NA	NA	NA
Duration of dementia	NA	2.57 ± 2.51	5.73 ± 2.96	6.04 ± 3.38	<0.001

Age, education, MMSE, MoCA, and UPDRS were expressed as means ± SD. Female and ApoE ε4 carriers were expressed as number (frequencies). NA, not applicable; NC, normal control; PDD, Parkinson's disease dementia; AD, Alzheimer's disease; VaD, vascular dementia; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; UPDRS, Unified Parkinson Disease Rating Scale.

RESULTS

Demographics

The present study recruited 61 PDD patients, 52 AD patients, 51 VaD patients, and 50 NCs. The average age was not significantly different among groups (NC: 66.68 ± 8.63 years, PDD: 69.07 ± 8.25 years, AD: 66.90 ± 9.58 years, VaD: 65.00 ± 8.99 years; p = 0.115). The percentages of females were similar among groups (NC: 50.00%, PDD: 50.82%, AD: 48.08%, VaD: 47.06%; p = 0.978). However, the percentage of ApoE ε4 carriers was significantly higher in the AD group (NC: 20.00%, PDD: 19.67%, AD: 46.15%, VaD: 19.61%; p = 0.003). The average education level was also not significantly different among groups (NC: 8.84 ± 4.49 years, PDD: 8.92 ± 4.55 years, AD: 8.08 ± 4.27 years, VaD: 8.47 ± 4.58 years; p = 0.752). The cognitive status, as reflected by MMSE (NC: 27.96 ± 1.88, PDD: 14.52 ± 6.62, AD: 14.87 ± 6.21, VaD: 15.27 ± 5.37; $p < 0.001$) and MoCA (NC: 28.12 ± 1.76, PDD: 12.90 ± 6.24, AD: 13.19 ± 5.15, VaD: 12.10 ± 4.61; $p < 0.001$), was significantly different among groups (**Table 1**).

Serum Naturally Occurring Antibody Against Alpha-Synuclein Levels in Different Types of Dementia

We first investigated serum levels of NAb-α-Syn in different types of dementia. We found that serum levels NAb-α-Syn were significantly higher in the PDD group (86.2 ± 53.6 pg/ml) in comparison with those in the NC (156.3 ± 79.4 pg/ml; $p < 0.001$), AD (136.9 ± 48.2 pg/ml; $p < 0.001$), and VaD (139.7 ± 56.6 pg/ml; $p < 0.001$) groups. However, no significant difference was found among the NC, AD, and VaD groups (**Figure 1**). These findings indicated that the alteration of serum NAb-α-Syn levels was relatively specific in PDD.

Correlations of Serum Naturally Occurring Antibody Against Alpha-Synuclein Levels With Cognitive Function

We next investigated the correlations of serum NAb-α-Syn levels with the cognitive functions. We found that serum NAb-α-Syn levels were significantly correlated with MMSE in the overall group (γ = 0.288, $p < 0.001$). Subgroup analysis indicated that serum NAb-α-Syn levels were significantly correlated with MMSE in the PDD group (γ = 0.342, p = 0.007), but not in the NC (γ = 0.079, p = 0.585), AD (γ = 0.086, p = 0.544), or VaD (γ = 0.138, p = 0.334) group (**Figure 2**). Similarly, serum NAb-α-Syn levels were significantly correlated with MoCA in the overall group (γ = 0.260, $p < 0.001$) and the PDD subgroup (γ = 0.300, p = 0.019), but not in the NC (γ = −0.027, p = 0.852), AD (γ = 0.013, p = 0.929), or VaD group (γ = 0.157, p = 0.273; **Figure 3**).

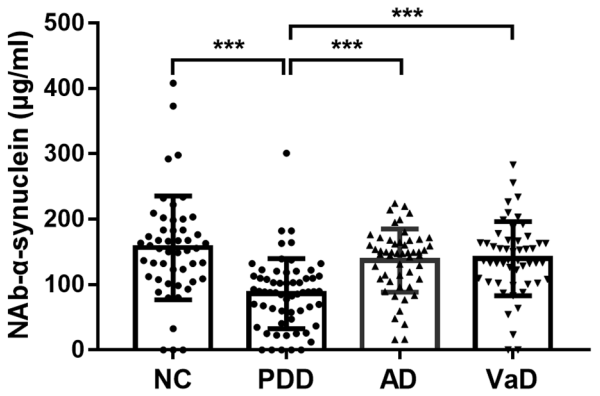

FIGURE 1 | Serum levels of naturally occurring antibody against alpha-synuclein (NAb-α-Syn) in different types of dementia. Serum levels of NAb-α-Syn were significantly higher in the Parkinson's disease dementia (PDD) group in comparison with those in the normal control (NC), Alzheimer's disease (AD), and vascular dementia (VaD) groups. ***$p < 0.001$. One-way ANOVA.

Correlations of Serum Naturally Occurring Antibody Against Alpha-Synuclein Levels With Disease Severity and Duration

We next investigated the correlations between serum NAb-α-Syn levels and UPDRS in the PDD group. We found that serum NAb-α-Syn levels were negatively correlated with UPDRS (γ = −0.531, $p < 0.001$; **Figure 4A**). However, serum NAb-α-Syn levels were not significantly correlated with the age of PDD patients (γ = −0.096, p = 0.463; **Figure 4B**). Serum NAb-α-Syn levels had a positive correlation with both the duration of PD (γ = −0.374, p = 0.003) and dementia (γ = −0.498, $p < 0.001$)

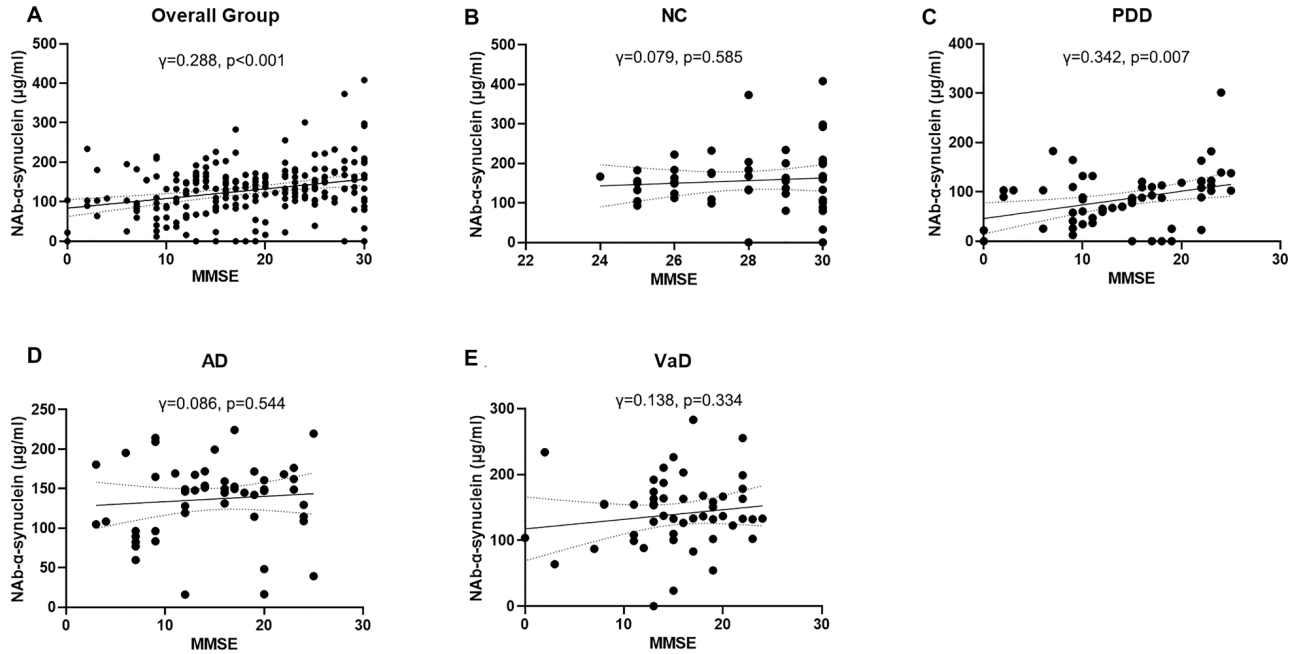

FIGURE 2 | Correlations between serum levels of naturally occurring antibody against alpha-synuclein (NAb-α-Syn) and Mini-Mental State Examination (MMSE). Correlations between serum levels of NAb-α-Syn in the **(A)** overall group, **(B)** normal control (NC) group, **(C)** PDD group, **(D)** AD group, and **(E)** VaD group. Spearman correlation analysis.

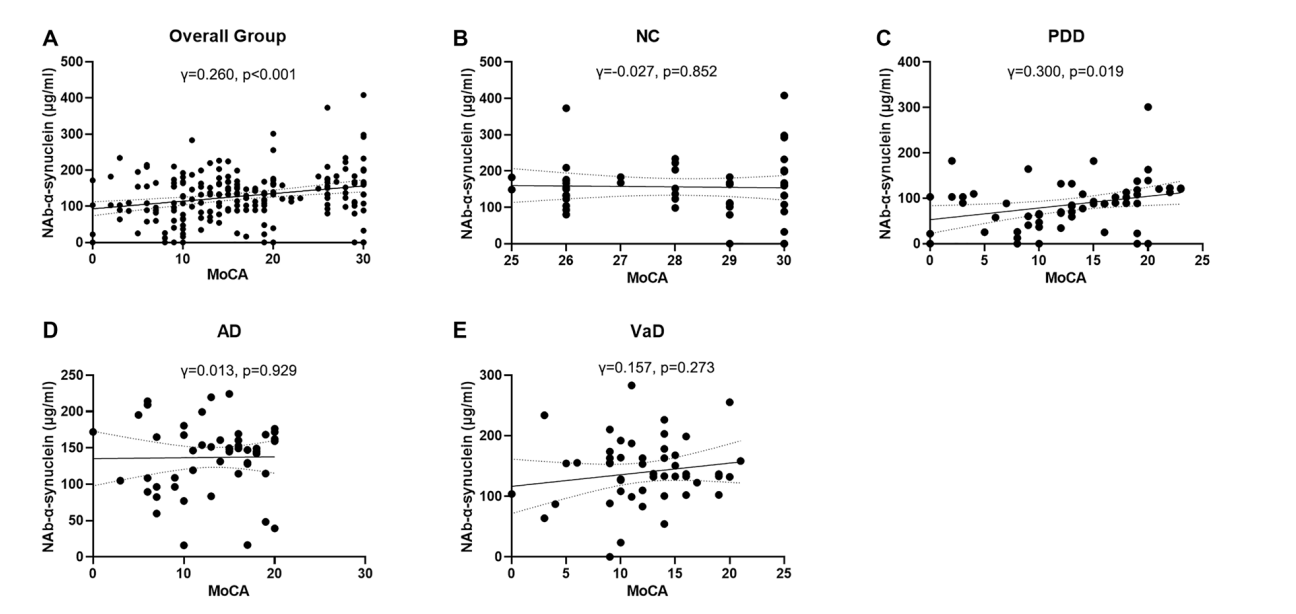

FIGURE 3 | Correlations between serum levels of naturally occurring antibody against alpha-synuclein (NAb-α-Syn) and Montreal Cognitive Assessment (MoCA). Correlations between serum levels of NAb-α-Syn in the **(A)** overall group, **(B)** normal control (NC) group, **(C)** PDD group, **(D)** AD group, and **(E)** VaD group. Spearman correlation analysis.

in the PDD group (**Figures 4C,D**). However, serum NAb-α-Syn levels were not correlated with the duration of dementia in the AD ($\gamma = 0.051$, $p = 0.718$; **Figure 4E**) or VaD group ($\gamma = -0.167$, $p = 0.242$; **Figure 4F**).

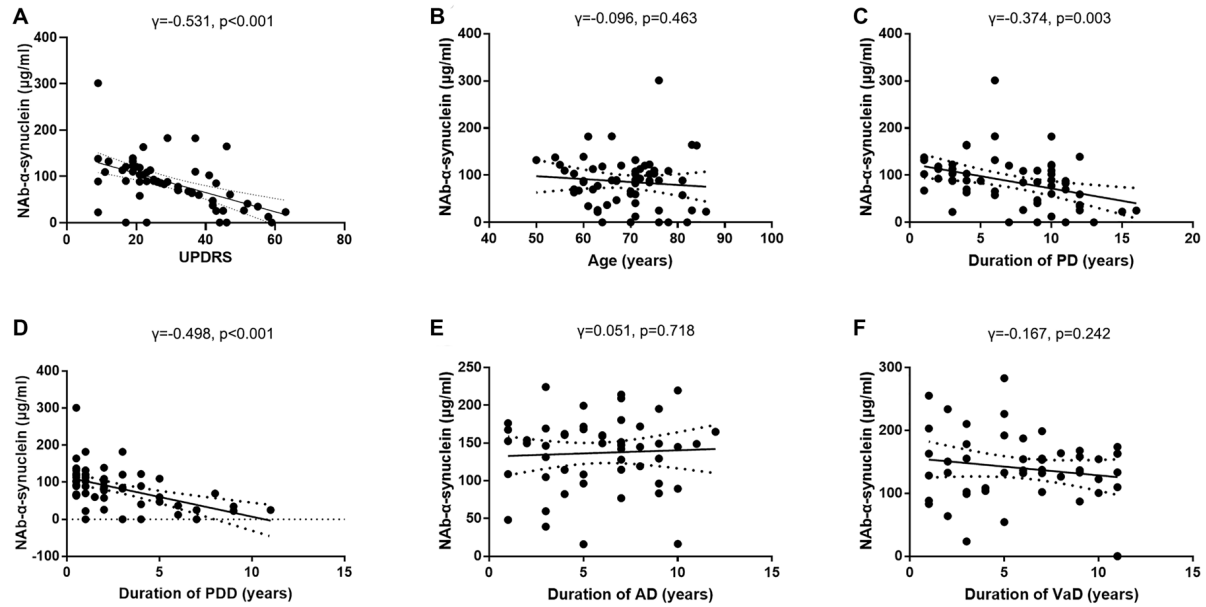

FIGURE 4 | Correlations of serum levels of naturally occurring antibody against alpha-synuclein (NAb-α-Syn) with disease severity and duration. **(A)** Correlation between serum levels of NAb-α-Syn and Unified Parkinson Disease Rating Scale (UPDRS) of PDD. **(B)** Correlation between serum levels of NAb-α-Syn and age of PDD. **(C)** Correlation between serum levels of NAb-α-Syn and duration of Parkinson's disease (PD). **(D)** Correlation between serum levels of NAb-α-Syn and duration of PDD. **(E)** Correlation between serum levels of NAb-α-Syn and duration of AD. **(F)** Correlation between serum levels of NAb-α-Syn and duration of VaD. Spearman correlation analysis.

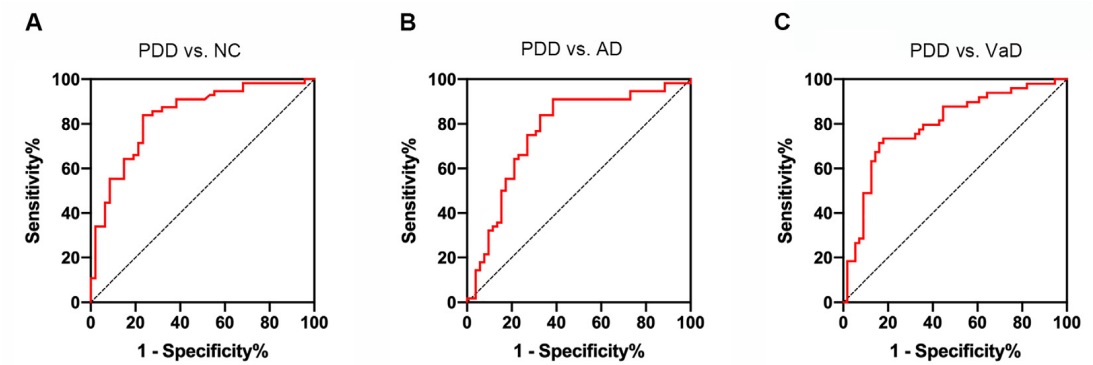

FIGURE 5 | Receiver operating characteristic (ROC) curves of serum naturally occurring antibody against alpha-synuclein (NAb-α-Syn) in differentiating PDD from normal control (NC) and other types of dementia. **(A)** The ROC curve of serum NAb-α-Syn in differentiating PDD from NC. **(B)** The ROC curve of serum NAb-α-Syn in differentiating PDD from AD. **(C)** The ROC curve of serum NAb-α-Syn in differentiating PDD from VaD.

The Capacity of Serum Naturally Occurring Antibody Against Alpha-Synuclein in Differentiating Different Types of Dementia

Using the ROC curve analysis, we found that the area under the ROC curve (AUC) of serum NAb-α-Syn in PDD vs. NC was 0.837 ($p < 0.001$, 95% confidence interval = 0.758–0.915). When applying the optimal cut-off value of 123.0 pg ml^{-1} calculated using the Youden index, the overall sensitivity and specificity of serum NAb-α-Syn for distinguishing PDD from NC was 83.9% and 76.6%, respectively (**Figure 5A**). The AUC of serum NAb-α-Syn in PDD vs. AD was 0.712 ($p < 0.001$, 95% confidence interval = 0.672–0.859). When applying the optimal cut-off value of 140.7 pg ml^{-1}, the overall sensitivity and specificity of serum NAb-α-Syn for distinguishing PDD from AD were 91.1% and 61.5%, respectively (**Figure 5B**). The AUC of serum NAb-α-Syn in PDD vs. VaD was 0.794 ($p < 0.001$, 95% confidence interval = 0.706–0.882). When applying the optimal cut-off value of 140.7 pg ml^{-1}, the overall sensitivity and specificity of serum NAb-α-Syn for distinguishing PDD from AD were 73.5% and 82.1%, respectively (**Figure 5C**).

DISCUSSION

In the present study, we found differential serum levels of NAb-α-Syn among PDD, AD, and VaD patients. Serum levels of NAb-α-Syn were correlated with the cognitive function and disease severity of PDD. Moreover, serum levels of NAb-α-Syn is a potential biomarker for differentiating PDD from AD and VaD.

α-Syn exists in its soluble form and can efflux from the brain into the periphery, which may stimulate the production of NAb-α-Syn. The levels of NAb-α-Syn in PD were controversial in previous studies (Besong-Agbo et al., 2013; Folke et al., 2019). Currently, few studies have investigated the circulating levels of NAb-α-Syn in PDD. We found in this study that circulating NAb-α-Syn was decreased in PDD patients. However, AD and VaD patients had comparable circulating NAb-α-Syn levels. These findings imply that the pattern of circulating NAb-α-Syn may have disease specificity among neurodegenerative diseases. The mechanism underlying the decrease of NAb-α-Syn is not clear yet. We found that serum NAb-α-Syn levels were correlated with the duration of PD and PDD, but not with the age of PDD patients, indicating that the alteration of NAb-α-Syn levels are not consequent to aging, but to the α-Syn pathogenesis. It is possible that NAb-α-Syn is consumed by increased circulating α-Syn.

The decrease of NAb-α-Syn in PDD may have some clinical and pathological relevance. We found that circulating levels of NAb-α-Syn were positively correlated with MMSE and MoCA scores in the overall group and PDD subgroup. Furthermore, circulating levels of NAb-α-Syn were also negatively correlated with UPDRS in the PDD group. These findings suggest an association between NAb-α-Syn and the severity of neurodegeneration in PDD. While the etiology of PDD is multifactorial, α-Syn is suggested to be a central component to the pathogenesis of the disease (Rocha et al., 2018). NAb-α-Syn may aid in the clearance of α-Syn, resulting in the alleviation of neuronal injury by α-Syn. This hypothesis is supported by previous studies, which suggests that NAb-α-Syn rescues memory and motor functions and attenuates α-Syn pathologies in animal models of PD (Huang et al., 2019). In this regard, NAb-α-Syn might be a physiological protective factor against α-Syn pathologies. The decrease of NAb-α-Syn in PDD might contribute to α-Syn-induced memory loss and motor dysfunctions.

Currently, the differential diagnosis of dementia is largely based on the symptom spectrum of patients. Recent progress in molecular imaging (Janelidze et al., 2020; Kantarci et al., 2020; Kozlova et al., 2020) and cerebrospinal fluid (CSF) biomarkers (Altomare et al., 2019; Llibre-Guerra et al., 2019) improved the diagnostic accuracy of dementia. However, due to the high cost and invasiveness of these diagnostic strategies, blood-based biomarkers are in urgent need for the diagnosis and differential diagnosis of dementia. Our ROC curve analysis found that circulating NAb-α-Syn has relatively high accuracy to differentiate PDD from AD and VaD. As there is some overlap of clinical manifestations among different types of dementia, NAb-α-Syn might be a potential differential diagnostic biomarker of PDD.

This study is limited by its cross-sectional nature and small sample size. Besides, this study is a pure correlative study that investigated the association between NAb-α-Syn and PDD. However, we found a disease-specific change of circulating NAb-α-Syn in PDD. This study also proposed a possible role of NAb-α-Syn in the pathogenesis of PDD from a clinical aspect.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Sichuan Provincial Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-HW and MH designed the study and drafted the manuscript. JW and BZ conducted the experiments, collected and analyzed the data, and drafted the manuscript. SY and MH were responsible for clinical assessment of the subjects.

FUNDING

This study was supported by major scientific research project of Sichuan Science and Technology Agency (No. 2019ZYZF0063).

REFERENCES

Altomare, D., De Wilde, A., Ossenkoppele, R., Pelkmans, W., Bouwman, F., Groot, C., et al. (2019). Applying the ATN scheme in a memory clinic population: the ABIDE project. *Neurology* 93, e1635–e1646. doi: 10.1212/WNL.0000000000008361

Besong-Agbo, D., Wolf, E., Jessen, F., Oechsner, M., Hametner, E., Poewe, W., et al. (2013). Naturally occurring α-Synuclein autoantibody levels are lower in patients with Parkinson disease. *Neurology* 80, 169–175.doi: 10.1212/WNL.0b013e31827b90d1

El-Agnaf, O. M., Salem, S. A., Paleologou, K. E., Curran, M. D., Gibson, M. J., Court, J. A., et al. (2006). Detection of oligomeric forms of α-Synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB J.* 20, 419–425. doi: 10.1096/fj.03-1449com

Emre, M., Aarsland, D., Brown, R., Burn, D. J., Duyckaerts, C., Mizuno, Y., et al. (2007). Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov. Disord.* 22, 1689–1707; quiz 1837. doi: 10.1002/mds.21507

Erkinjuntti, T., Inzitari, D., Pantoni, L., Wallin, A., Scheltens, P., Rockwood, K., et al. (2000). Research criteria for subcortical vascular dementia in clinical trials. *J. Neural Transm. Suppl.* 59, 23–30. doi: 10.1007/978-3-7091-6781-6_4

Folke, J., Rydbirk, R., Lokkegaard, A., Salvesen, L., Hejl, A. M., Starhof, C., et al. (2019). Distinct autoimmune anti-α-Synuclein antibody patterns in multiple system atrophy and Parkinson's disease. *Front. Immunol.* 10:2253. doi: 10.3389/fimmu.2019.02253

Hanagasi, H. A., Tufekcioglu, Z., and Emre, M. (2017). Dementia in Parkinson's disease. *J. Neurol. Sci.* 374, 26–31. doi: 10.1016/j.jns.2017.01.012

Huang, Y. R., Xie, X. X., Ji, M., Yu, X. L., Zhu, J., Zhang, L. X., et al. (2019). Naturally occurring autoantibodies against α-Synuclein rescues memory and motor deficits and attenuates α-Synuclein pathology in mouse model of Parkinson's disease. *Neurobiol. Dis.* 124, 202–217. doi: 10.1016/j.nbd.2018.11.024

Janelidze, S., Mattsson, N., Palmqvist, S., Smith, R., Beach, T. G., Serrano, G. E., et al. (2020). Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat. Med.* 26, 379–386. doi: 10.1038/s41591-020-0755-1

Kantarci, K., Lowe, V. J., Chen, Q., Przybelski, S. A., Lesnick, T. G., Schwarz, C. G., et al. (2020). β-Amyloid PET and neuropathology in dementia with Lewy bodies. *Neurology* 94, e282–e291. doi: 10.1212/WNL.0000000000008818

Kozlova, I., Parra, M. A., Titova, N., Gantman, M., and Sala, S. D. (2020). Alzheimer's disease and parkinson dementia distinguished by cognitive marker. *Arch. Clin. Neuropsychol.* doi: 10.1093/arclin/acz082 [Epub ahead of print].

Llibre-Guerra, J. J., Li, Y., Schindler, S. E., Gordon, B. A., Fagan, A. M., Morris, J. C., et al. (2019). Association of longitudinal changes in cerebrospinal fluid total tau and phosphorylated tau 181 and brain atrophy with disease progression in patients with Alzheimer disease. *JAMA Netw. Open* 2:e1917126. doi: 10.1001/jamanetworkopen.2019.17126

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R. Jr., Kawas, C. H., et al. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 263–269. doi: 10.1016/j.jalz.2011.03.005

Papachroni, K. K., Ninkina, N., Papapanagiotou, A., Hadjigeorgiou, G. M., Xiromerisiou, G., Papadimitriou, A., et al. (2007). Autoantibodies to α-Synuclein in inherited Parkinson's disease. *J. Neurochem.* 101, 749–756. doi: 10.1111/j.1471-4159.2006.04365.x

Rocha, E. M., De Miranda, B., and Sanders, L. H. (2018). α-Synuclein: pathology, mitochondrial dysfunction and neuroinflammation in Parkinson's disease. *Neurobiol. Dis.* 109, 249–257. doi: 10.1016/j.nbd.2017.04.004

Skovronsky, D. M., Lee, V. M., and Trojanowski, J. Q. (2006). Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. *Annu. Rev. Pathol.* 1, 151–170. doi: 10.1146/annurev.pathol.1.110304.100113

Tofaris, G. K., and Spillantini, M. G. (2007). Physiological and pathological properties of α-Synuclein. *Cell. Mol. Life Sci.* 64, 2194–2201. doi: 10.1007/s00018-007-7217-5

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.

Copyright © 2020 Wang, Zheng, Yang, Hu and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

REVIEW
published: 06 October 2020
doi: 10.3389/fnagi.2020.584743

Neurogranin: A Potential Biomarker of Neurological and Mental Diseases

Yang Xiang[1,2†], Jiayan Xin[3,2†], Weidong Le[1] and Yongjian Yang[4]*

[1] Institute of Neuroscience, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, School of Clinical Medicine, University of Electronic Science and Technology of China, Chengdu, China, [2] Department of Neurology, General Hospital of Western Theater Command, Chengdu, China, [3] North Sichuan Medical College, Nanchong, China, [4] Department of Cardiovasology, General Hospital of Western Theater Command, Chengdu, China

Neurogranin (Ng) is a small protein usually expressed in granule-like structures in pyramidal cells of the hippocampus and cortex. However, its clinical value is not fully clear so far. Currently, Ng is proved to be involved in synaptic plasticity, synaptic regeneration, and long-term potentiation mediated by the calcium- and calmodulin-signaling pathways. Due to both the synaptic integrity and function as the growing concerns in the pathogenesis of a wide variety of neurological and mental diseases, a series of researches published focused on the associations between Ng and these kinds of diseases in the past decade. Therefore, in this review, we highlight several diseases, which include, but are not limited to, Alzheimer's disease, Parkinson disease, Creutzfeldt–Jakob disease, neuro-HIV, neurosyphilis, schizophrenia, depression, traumatic brain injury, and acute ischemic stroke, and summarize the associations between cerebrospinal fluid or blood-derived Ng with these diseases. We propose that Ng is a potential and promising biomarker to improve the diagnosis, prognosis, and severity evaluation of these diseases in the future.

Keywords: neurodegenerative disorder, mental disorder, biomarker, cerebrospinal fluid, neurogranin

OPEN ACCESS

Edited by:
Mark Stecker,
Independent Practitioner, Fresno, United States

Reviewed by:
Guangming Zhu,
Stanford University, United States
Yanxing Chen,
Zhejiang University, China

****Correspondence:***
Yongjian Yang
yangyj86570999@sina.com

† These authors have contributed equally to this work

Received: *18 July 2020*
Accepted: *02 September 2020*
Published: *06 October 2020*

Citation:
Xiang Y, Xin J, Le W and Yang Y (2020) Neurogranin: A Potential Biomarker of Neurological and Mental Diseases. Front. Aging Neurosci. 12:584743. doi: 10.3389/fnagi.2020.584743

INTRODUCTION

Neurogranin (Ng, also called RC3, p17, and BICKS) is a protein with a molecular weight of 7.5 kD and composed of 78 amino acids (Watson et al., 1990). It is often found in granule-like structures in pyramidal cells of the hippocampus and cortex, which gives rise to its name of "neurogranin" (Represa et al., 1990). Ng was discovered in 1990; however, its clinical value is not fully clear so far.

The mammalian Ng gene NRGN spans around 12.5 kbp and contains four exons and three introns (Martinez de Arrieta et al., 1997). The human Ng sequence predicts five amino acids encoded by exon 1 and 73 amino acids encoded by exon 2 (Martinez de Arrieta et al., 1997). However, the other two exons contain untranslated sequences (Martinez de Arrieta et al., 1997). The coding sequence homology of NRGN between humans and rats is 90% at the nucleic acid level and 96% at the protein level (Martinez de Arrieta et al., 1997). In early studies, Ng was found principally as a neuronal postsynaptic protein in the telencephalon of the adult rat, specifically

Abbreviations: AD, Alzheimer's disease; AIS, acute ischemic stroke; Aβ, amyloid β; BACE1, precursor protein cleaving enzyme; CCI, controlled cortical impact; CJD, Creutzfeldt–Jakob disease; CNS, central nervous system; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; GPI, general paresis of the insane; HD, Huntington disease; MCI, mild cognitive impairment; NDEs, neuronal-derived exosomes; NDs, neurodegenerative disorders; neuro-HIV, neuro-human immunodeficiency virus; NfL, neurofilament light; Ng, neurogranin; NRGN, neurogranin gene; NS, neurosyphilis; PD, Parkinson disease; PDD, PD with dementia; p-tau, phosphorylated tau; ROC, receiver operating characteristic; TBI, traumatic brain injury; t-tau, total tau.

located in the cell bodies and dendrites of neurons in the cerebral cortex, hippocampus, and striatum (Represa et al., 1990) (**Figure 1**). Thereafter, Ng was detected successively in the lung, spleen, and bone marrow with a low expression level (Diez-Guerra, 2010). Moreover, high and moderate levels of Ng were found in platelets and B type lymphocytes, respectively (Glynne et al., 2000; Gnatenko et al., 2003). A recent study first identified Ng expression in both human and mouse endothelia (Cheriyan et al., 2020).

The present data strongly point that Ng is involved in the plasticity and regeneration of synapse mediated by the calcium- and calmodulin-signaling pathways. For instance, Zhong et al. (2009) found that Ng enhances the postsynaptic sensitivity an elevates the synaptic strength in an activity- and NMDAR-dependent manner (Zhong et al., 2009). Besides, the potentiation of synaptic transmission modulated by Ng mimics and occludes the long-term potentiation (Zhong et al., 2009). A recent study revealed that long-term blockade of NMDAR significantly decreases Ng expression (Garrido-Garcia et al., 2019). Moreover, the long-term bicuculline administration facilitates synaptic activity and increases Ng expression (Garrido-Garcia et al., 2019). Lentiviral expression of Ng results in the elevated density of both excitatory and inhibitory synapses (Garrido-Garcia et al., 2019). In addition, Ng is involved in a variety of biochemical processes and molecular interactions (**Figure 2**).

Of note, synaptic integrity and function are both the growing concerns in the pathogenesis of a wide variety of neurological and mental diseases (Fyfe, 2015; Hellwig et al., 2015; Yang et al., 2015; Zetterberg and Blennow, 2015; Bereczki et al., 2017; De Vos et al., 2017; Guha et al., 2018; Blennow et al., 2019). In animal experiments, it showed that mice lacking NRGN show a remarkable decline in hippocampus-dependent spatial memory and deficits in hippocampal long-term potentiation (Pak et al., 2000). Aging is associated with the cognitive decline as well as the decreased Ng levels in pyramidal neurons (Mons et al., 2001). Ng reduction and cognitive deficit detected in 5XFAD mice are restored after the intra-hippocampal injection with an Ng-expressing lentiviral vector (Jeon et al., 2018). On the basis of these basic studies, a series of researches published in the past decade focused on the associations between Ng and these kinds of diseases, which include, but are not limited to, AD, PD, CJD, HIV, infection (neuro-HIV), NS, TBI, AIS, and schizophrenia.

Therefore, in this review, we highlight and summarize several neurological and mental diseases associated with Ng and propose that Ng is a potential and promising biomarker to improve the diagnosis, prognosis, and severity evaluation of these diseases in the future.

ASSOCIATIONS BETWEEN NEUROGRANIN AND NEUROLOGICAL AND MENTAL DISEASES

Using the keywords, including "neurogranin," "Ng," "RC3," and "BICKS," we searched the clinical research articles involved in Ng via PUBMED and categorized them as per the kind of disease. An overview of the major clinical researches involved in Ng was currently published (**Table 1**).

Cerebrospinal Fluid Neurogranin in Neurodegenerative Disorders

Neurodegenerative disorders (NDs) are characterized by progressive dysfunction of neurons, glias, synapses, as well as the neural networks (Kovacs, 2016, 2017). A critical feature of NDs is the aggregation and deposition of variants of physiological proteins in the CNS (Kovacs, 2016, 2017). Both neurons and glias have the capacity to accumulate these pathological variants (Kovacs, 2016, 2017). NDs can be broadly classified by their clinical presentations, most of which are the disorders of movement, cognition, mentation, or behavior. A small portion of patients develop pure syndromes, but most patients show mixed clinical features (Dugger and Dickson, 2017). AD and PD are the two kinds of the most common NDs. The occurrence of these types of NDs is usually in middle or old age, and the incidence is elevated with an increasing life expectancy of the population. In general, the diagnostic gold criteria of diverse NDs are neuropathological evaluation at autopsy. In comparison, the detectable biomarkers *in vivo* are supposed to improve the diagnosis, stratification, and prognosis of patients (Kovacs, 2016).

Cerebrospinal Fluid/Plasma Neurogranin in Alzheimer's Disease

As the most common kind of dementing disease, AD is a relentlessly progressive and fatal disorder of CNS, which begins approximately 10–15 years before the clinical manifestations (Rafii, 2016). Pathologically, AD is characterized by both certain hallmarks in the brain, including the extracellular plaques composed of Aβ peptide and the intracellular neurofibrillary tangles composed of the hyperphosphorylated tau protein (Blennow et al., 2006). Undoubtedly, the core CSF biomarkers of Aβ reflecting brain amyloidosis, t-tau reflecting neurodegeneration intensity, and p-tau that is related to tau pathology, have good diagnostic accuracy in clinical practice. However, in view of the multifactorial pathogenesis of AD and the overlapping pathology with other kinds of dementia, it is necessary to integrate the core CSF biomarkers with other novel biomarkers that are capable of reflecting different aspects of neuropathology. Synaptic degeneration is an essential component of AD pathophysiology, which is present in early disease stages (Masliah et al., 2001; Scheff et al., 2007). An increasing amount of data suggests that synaptic dysfunction is associated with cognitive decline and ahead of neuronal degeneration (DeKosky and Scheff, 1990). Thus, the biomarkers reflecting the integrity and plasticity of synapses may be useful for the early diagnosis and prognosis of AD.

Many of clinical studies support the findings that the levels of CSF Ng are higher in AD or MCI patients than those in healthy controls (HCs) or non-AD dementia patients (Fyfe, 2015; Hellwig et al., 2015; Kester et al., 2015; Portelius et al., 2015). Higher levels of CSF Ng are positively correlated to higher scores of Aβ neuritic plaques and tau tangles pathology (Portelius et al., 2018). High levels of CSF Ng in AD and prodromal AD have

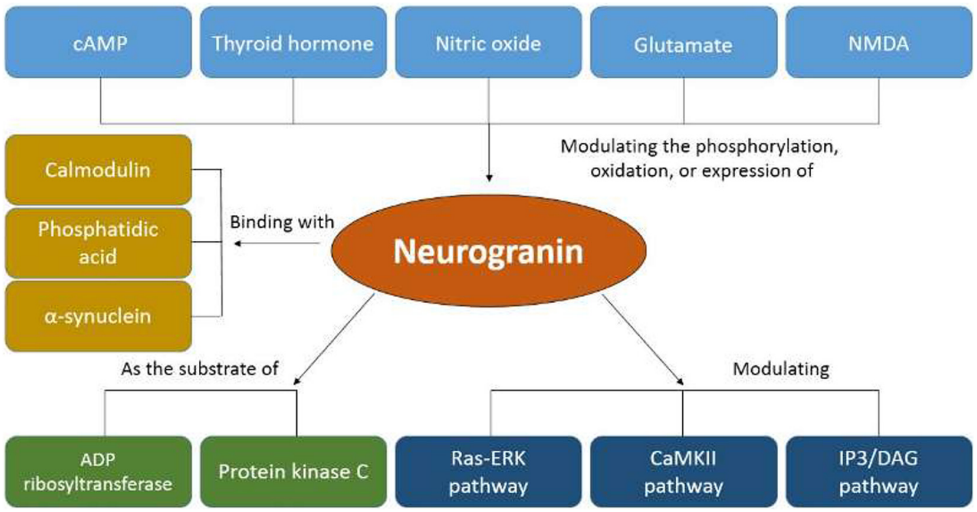

FIGURE 1 | Cellular and regional distribution of neurogranin in the adult rat brain.

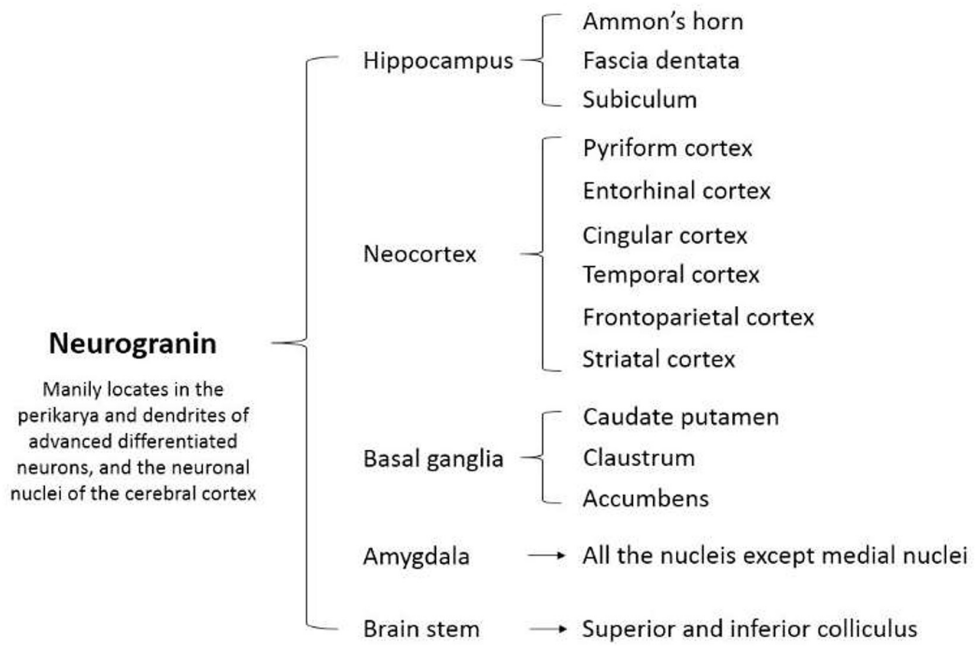

FIGURE 2 | A diagram of the molecular and signaling pathways involved in neurogranin. cAMP, cyclic adenosine monophosphate; NMDA, *N*-methyl-D-aspartate; ADP, adenosine diphosphate; ERK, extracellular signal-regulated kinase; CaMK II, calmodulin-dependent protein kinase II; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol.

been verified in several subsequent studies (Hellwig et al., 2015; Kvartsberg et al., 2015a).

A study explored the correlations between baseline CSF Ng levels with baseline and longitudinal cognitive decline, brain atrophy, and glucose metabolism (Portelius et al., 2015). They found that high baseline levels of CSF Ng in the MCI patients are associated with the longitudinal decline of hippocampal volume and cortical glucose metabolism at clinical follow-up (Portelius et al., 2015). Further, within the progressive MCI group, elevated CSF Ng levels correlate with accelerated deterioration in Alzheimer's disease Assessment Scale—cognitive subscale (Portelius et al., 2015). In a recent meta-analysis, it revealed that the CSF Ng level is significantly higher in MCI patients progressed to AD than that in stable MCI patients (Mavroudis et al., 2019).

A cross-sectional and longitudinal observational study of cognitive decline between the symptomatic AD patients and cognitively normal controls proved that the CSF levels of Ng can

TABLE 1 | An overview of the major clinical researches involved in Ng published currently.

	Participants	Measures	Outcomes	Correlations	Study design	References
AD	ADD ($n = 39$) MCI-AD ($n = 13$) MCI-o ($n = 29$) Non-ADD ($n = 14$)	CSF Ng	MCI-AD ↑ vs. MCI-o ADD ↑ vs. MCI-o	Positively correlated with CSF tau and p-tau.	Case–control study	Hellwig et al., 2015; Kvartsberg et al., 2015b
	AD ($n = 65$) MCI ($n = 61$) CTRL ($n = 37$)	CSF Ng	Baseline CSF levels of Ng: AD ↑ vs. CTRL MCI-AD ↑ vs. sMCI Predicting progression from MCI to AD	Positively correlated with CSF T-tau and P-181 tau, but not with Aβ42.	Longitudinal study	Kester et al., 2015; Zetterberg and Blennow, 2015
	ADD ($n = 95$) MCI ($n = 173$) CTRL ($n = 110$)	CSF Ng	ADD ↑ vs. CTRL MCI ↑ vs. CTRL sMCI ↑ vs. CTRL pMCI ↑ vs. CTRL pMCI ↑ vs. sMCI	High baseline CSF Ng predicting cognitive decline as reflected by decreased MMSE.	Case–control study	Portelius et al., 2015
	ADD ($n = 397$) MCI ($n = 114$) FTD ($n = 96$) PDD ($n = 29$) DLB ($n = 33$) CTRL ($n = 75$)	CSF Ng and autopsy for the neuropathology	ADD ↑ vs. CTRL ADD ↑ vs. MCI AD biomarker-positive CTRL subjects ↑ vs. AD biomarker negative CTRL group ADD vs. PD ↓/PD MCI ↓/PDD ↓ ADD ↑ vs. FTD/ALS	Positively associated with: Aβ neuritic plaque and tau tangle pathology scores.	Prospective study	Portelius et al., 2018
	ADD ($n = 100$) MCI ($n = 40$) CTRL ($n = 80$)	CSF Ng	Both AD and MCI-AD: markedly decrease; The highest level in ADD; High CSF Ng levels at the MCI stage; Predicting progression to ADD.	Positively correlated with t-tau and p-tau, but no correlations with Aβ 1-42.	Case–control study	Kvartsberg et al., 2015a
	AD ($n = 95$) CTRL ($n = 207$)	CSF Ng	The mean (SE) AUC was 0.73 (0.04) for Ng to differentiate patients with early symptomatic AD from CTRL; Predicting future cognitive impairment (adjusted hazard ratio, 1.89).	CSF Ng level correlates with a whole brain and regional atrophy in AD, and the amyloid load in preclinical AD.	Cross-sectional and longitudinal observational study	Tarawneh et al., 2016
	AD ($n = 10$) MCI ($n = 20$) MCI-AD ($n = 20$) CTRL ($n = 10$)	Plasma NDEs levels of: PT-181-tau PS-396-tau Aβ 1-42 Ng	AD/MCI vs. CTRL: Plasma NDE levels of PT-181-tau PS-396-tau, and Abeta 1-42 ↑ Plasma NDE levels of Ng ↓	N/A	Case–control study	Winston et al., 2016
	Discovery stage: AD ($n = 28$) aMCI ($n = 25$) CTRL ($n = 29$) Validation stage: AD ($n = 73$) aMCI ($n = 71$) CTRL ($n = 72$) pre-AD ($n = 160$) CTRL ($n = 160$)	Blood nero-exosomal: GAP43 Ng SNAP25 Synaptotagmin 1	Discovery stage: AD ↓ vs. CTRL aMCI ↓ vs. CTRL aMCI ↑ vs. AD Validation stage: same with discovery stage The combination of exosomal biomarkers detected AD 5 to 7 years before cognitive impairment (AUC = 0.87–0.89)	Exosomal biomarker levels were correlated with those in CSF ($R^2 = 0.54$–0.70).	Longitudinal and retrospectively study	Antonell et al., 2020; Jia et al., 2020
	MCI or ADD (in total $n = 59$) CTRL ($n = 29$)	Paired CSF/plasma samples	CSF: MCI ↑ vs. CTRL AD ↑ vs. CTRL plasma: AD vs. CTRL: no change	Positively correlated with CSF tau; Negatively correlated with CSFAβ 1-42/Aβ 1-40; No correlation between CSF and plasma Ng.	Case–control study	De Vos et al., 2015; Kvartsberg et al., 2015a

(Continued)

TABLE 1 | Continued

	Participants	Measures	Outcomes	Correlations	Study design	References
PD	PD ($n = 52$) PD Drug naïve ($n = 30$) CTRL ($n = 87$)	CSF Ng: Motor disease stage (Hoehn and Yahr scale) Cognitive performance (MoCA).	PD ↑ vs. CTRL	Associated with reduced cognition and higher motor disease stage.	Case–control study	Bereczki et al., 2017
	PD ($n = 30$) CTRL ($n = 26$)	CSF Aβ; a-synuclein; Ng; Cortical glucose metabolism.	PD ↓ vs. CTRL	Lower CSF Ng concentrations were found with more severe reductions on FDG-PET.	Longitudinal study	Selnes et al., 2017
	CTRL ($n = 47$) PD ($n = 157$) PDD ($n = 29$) AD ($n = 124$) MSA ($n = 26$)	CSF Ng	PD ↓/PDD ↓/ MSA ↓ /PSP ↓ vs. CTRL PD ↓/PDD ↓/ MSA ↓ /PSP ↓ vs. AD	No significant associations between Ng and clinical progression.	Case–control study	Hall et al., 2020
HD	CTRL ($n = 12$) HD gene expansion carriers ($n = 20$)	CSF Ng	HD vs. CTRL: No change	N/A	Case–control study	Byrne et al., 2018
CJD	AD ($n = 46$) CJD ($n = 81$) CTRL ($n = 64$)	CSF Ng T-tau NfL 14-3-3 protein postmortem brain tissue	CSF: CJD ↑ (4.75 times of CTRL) vs. CTRL AD ↑ (1.94 times of CTRL) vs. CTRL Differentiating CJD from AD (AUC = 0.85) Brain tissue: Ng reduced in AD, and more significantly in CJD	Showed a good correlation with tau, but did not correlate with NfL.	Case–control study	Blennow et al., 2019
	CJD ($n = 38$) Pre-AD ($n = 21$) MCI-AD ($n = 56$) ADD ($n = 108$) FTD ($n = 34$) CTR ($n = 50$)	CSF Ng	FTD ↓<CTRL < ADD ↑ <CJD ↑ Applying the AT(N) system, 62% of subjects were positive for neurodegeneration if Ng was used.	N/A	Unicentric cohort study	Antonell et al., 2020; Jia et al., 2020
Neuro-HIV	HIV-1-positive ($n = 8$) HIV-1-negative ($n = 4$)	The expression of Ng in FC tissues	HIV-1-positive ↓ vs. HIV-1-negative	Associated with a decreased level of CaMKII	Case-control study	Guha et al., 2018
	HIV-infected ($n = 138$) CTRL ($n = 13$)	CSF Ng	HIV-infected individuals vs. CTRL: No change	N/A	Cross-sectional study	Sinharay and Hammoud, 2019; Yilmaz et al., 2019
Neurosyphilis	NS ($n = 13$) GPI ($n = 55$) AD ($n = 23$)	CSF/plasma: Ng, Aβ, BACE1	CSF Ng, BACE1, and tau, as well as plasma BACE1 levels, were significantly different among groups.	CSF tau and plasma Ng correlated with cognitive scale scores	Case–control study	Zhang et al., 2020
Schizophrenia	Schizophrenia ($n = 7$) CTRL ($n = 7$)	Prefrontal cortex expression of Ng in pyramidal cells in layers III and V in area 9 and 32.	A marked decrease in Ng immunostaining in both areas 9 and 32 of the prefrontal cortex.	N/A	Case–control study	Broadbelt et al., 2006
Depression	Major depression ($n = 12$)	Whether the ECT will change CSF Ng	CSF Ng concentrations do not change before and after a course of ECT	Baseline Ng levels were positively correlated with the therapeutic response.	Prospective study	Kranaster et al., 2017

(Continued)

TABLE 1 | Continued

	Participants	Measures	Outcomes	Correlations	Study design	References
FEP	FEP patients ($n = 40$) CTRL ($n = 20$)	CSF Ng	FEP patients ↓ vs. CTRL	N/A	Longitudinal study	Santillo et al., 2019
TBI	TBI patients ($n = 76$) CTRL ($n = 150$)	Serum Ng	TBI patients ↑ vs. CTRL with an ROC for diagnosing TBI of 0.72	N/A	Case–control study	Yang et al., 2015
	CTRL ($n = 328$) mTBI ($n = 179$)	Serum Ng	mTBI patients ↑ vs. CTRL	N/A	Prospective observational study	Peacock et al., 2017
AIS	AIS ($n = 50$)	Paired CSF/plasma Ng	Ng was elevated in both CSF and plasma.	Positively correlated with infarct volume	Prospective study	De Vos et al., 2017

MCI-AD, MCI due to AD; MCI-o, MCI not due to AD; aMCI, amnestic MCI; CTRL, control; sMCI, stable MCI; pMCI, progressive MCI; Ng, neurogranin; AD, Alzheimer's disease; PD, Parkinson disease; CJD, Creutzfeldt–Jakob disease; TBI, traumatic brain injury; mTBI, mild TBI; AIS, acute ischemic stroke; CSF, cerebrospinal fluid; Aβ, amyloid β; t-tau, total tau; p-tau, phosphorylated tau; MCI, mild cognitive impairment; HD, Huntington disease; FTD, frontotemporal dementia; DLB, dementia with Lewy bodies; PDD, PD with dementia; NfL, neurofilament light; neuro-HIV, neuro-human immunodeficiency virus; FC, frontal cortex; MMSE, Mini–Mental State Examination; ECT, electroconvulsive therapy; MoCA, the Montreal Cognitive Assessment scores; FEP, first episode psychosis; ROC, receiver operating characteristic; NDEs, neuronal-derived exosomes; NS, neurosyphilis; GPI, general paresis of the insane; BACE1, precursor protein cleaving enzyme; N/A, not applicable due to lack of evidence; ↑, increased level; ↓, decreased level.

develop the diagnosis and prognosis for early symptomatic AD that is comparable with other CSF biomarkers of AD (Tarawneh et al., 2016). Importantly, CSF Ng enhances the comprehensive capacity of these biomarkers to predict future cognitive decline in the cognitively normal controls (Tarawneh et al., 2016).

Additionally, the data of Ng expression in postmortem brain tissues of AD demonstrated that the elevated CSF Ng levels are in accordance with the decreased Ng levels in the cerebral cortex and hippocampus (Blennow et al., 2019). Ng levels in brain tissues of AD do not differ between early and late Braak stages, indicating that synaptic loss is not only a late-stage pathological feature (Blennow et al., 2019). Therefore, CSF Ng is a promising biomarker for early diagnosis and progression prediction of AD, which could be a useful complement to the panel of AD biomarkers currently.

For clinical applications, sample collection needs to be as accessible and reproducible as possible. However, no significant differences were found in plasma levels of Ng between AD patients and controls (De Vos et al., 2015), indicating the necessity of developing other kinds of Ng-related biomarkers from the blood. In that regard, a pilot study investigated the blood-derived Ng and revealed that the concentrations of Ng in the plasmatic NDEs are significantly lower in AD compared with the controls and correlate with the progression from MCI to AD (Winston et al., 2016). A recent meta-analysis uncovered that compared with the cognitively normal controls, the levels of plasmatic NDEs Ng in AD patients have an obvious decrease (Liu et al., 2020). Moreover, a recent study confirmed the difference in plasmatic NDEs Ng between AD patients and controls (Jia et al., 2020). Furthermore, the plasmatic exosomal levels of Ng are found to be correlated with CSF Ng levels. Also, the plasmatic exosomal Ng distinguishes AD with amnestic MCI and controls with the highest accuracy among all the plasmatic exosomal synaptic protein candidates, including growth-associated protein 43, Ng, synaptosome-associated protein 25, and synaptotagmin 1 (Jia et al., 2020).

The NIA-AA Research Framework, published in 2018, emphasized the necessity of a biological definition of AD and established the A/T/(N) biomarker classification system (Jack et al., 2018). In the framework, "A," "T" and "(N)" stand for Aβ, tau, and neurodegeneration, respectively. It is generally recognized that "N" includes the cellular injury, regional volume loss of the brain, and the destruction of system-level circuits (Jack et al., 2018). Taken together, as a postsynaptic protein, the current evidence suggests that Ng is a promising biomarker reflecting synaptic dysfunction in AD. The value of CSF Ng in the diagnosis and prediction of AD has been clarified, but the relationship between blood-derived Ng and AD still needs further study.

Cerebrospinal Fluid Neurogranin in Parkinson Disease

Characterized by the loss of nigrostriatal dopaminergic neurons, PD is the second most common primary ND of the CNS, whose major clinical manifestation is the development of movement disorder (Barker and Transeuro consortium, 2019). Synaptic dysfunction is an early change in PD, which has been shown in a previous animal study (Yarnall et al., 2013). It proved that the neurons expressing Ng in the cortex degenerate in the late stage of PD (Yarnall et al., 2013). Besides, the levels of phosphorylated Ng are also lower in the superior temporal cortex in PD patients (Koob et al., 2014).

A study enrolled 52 PD patients and 87 HCs, measured the CSF concentrations of Ng, and explored the associations between Ng with motor symptoms (evaluated by Hoehn and Yahr scale) as well as cognitive symptoms (evaluated by the Montreal Cognitive Assessment scores) (Bereczki et al., 2017). It showed significant associations between increased concentrations of CSF Ng and cognitive impairment in the PD group, and CSF Ng is increased in PD patients in a disease-specific manner and associated with the severity of cognitive decline and motor disorder (Bereczki et al., 2017). Confusingly, a subsequent study showed the inconsistent results that enrolled 30 patients with mild-to-moderate PD and 26 HCs and tested the correlation between hypometabolism, CSF Aβ, CSF Ng, and CSF α-synuclein (Selnes et al., 2017). It showed that the CSF Ng levels are significantly lower in mild-to-moderate PD than those in controls

and associated with CSF Aβ levels, CSF α-synuclein levels, and motor stage (Selnes et al., 2017). A prospective study showed that the Ng levels are significantly lower in PD, PD with MCI, and PDD) relative to AD dementia (Portelius et al., 2018). A recent study tested the CSF Ng in patients with PD, PDD, AD, and HCs and investigated the possible correlations between CSF Ng with cognitive and motor impairment (Hall et al., 2020). They found that Ng is decreased in patients with PD and PDD compared with the HCs and AD patients, respectively (Hall et al., 2020). Nevertheless, they did not find that Ng correlates with a motor disorder, cognitive impairment, longitudinal cognitive decline, or the progression to dementia in PD (Hall et al., 2020).

To sum up, the research on PD and Ng is booming currently, but the diagnostic value of Ng in CSF still needs to be further explored. More importantly, the correlation between blood Ng and PD remains unclear.

Cerebrospinal Fluid Neurogranin in Huntington Disease

As an autosomal dominant inheritance disease, HD is devastating to patients and their families, which is caused by an expanded trinucleotide repeat of CAG in the gene of huntingtin (Bates et al., 2015). There is evidence that synaptic dysfunction is a critical feature in HD pathogenesis (Smith et al., 2005; Sepers and Raymond, 2014). The whole-brain gene expression study in postmortem HD patient brains proved that the NRGN is one of the most robustly downregulated genes in HD caudate compared with the controls (Hodges et al., 2006; Runne et al., 2007). However, Byrne et al. (2018) quantified Ng and triggering receptor expressed on myeloid cells-2 in CSF samples from HD mutation carriers and controls and found that CSF Ng levels do not significantly differ between HD and HCs (Byrne et al., 2018). In addition, it did not find the significant associations between CSF Ng levels and the disease burden score, total functional capacity, or motor score (Byrne et al., 2018).

In a word, Ng-related research in HD is still far behind that of AD and PD. This may attribute to the type and characteristics of the disease or the limitation of the current testing methods of Ng.

Cerebrospinal Fluid Neurogranin in Creutzfeldt–Jakob Disease

Creutzfeldt–Jakob disease is a rapidly progressive and fatal neurodegenerative disease that is caused by misfolded, transmissible proteinaceous infectious particles (Uttley et al., 2020). One fundamental characteristic of CJD is synaptic degeneration and disorganization, resulting in neuronal loss and spongiform changes (Blennow et al., 2019). Actually, over a 30% reduction of the certain synaptic index in the brain has been found in prion disease compared with the controls (Clinton et al., 1993).

Blennow et al. (2019) investigated CSF Ng, t-tau, neurofilament light, and 14-3-3 protein in CJD ($n = 81$), AD ($n = 46$), and neurological controls (NCs, $n = 64$). The accuracy of Ng that differentiates the three groups and Ng expression in postmortem brain tissue was evaluated. They found that CJD has the highest levels of CSF Ng, which is helpful in the prediction of prognosis of CJD, is not influenced by age or sex, and is dependent on disease subtype (Blennow et al., 2019). In detail, CSF Ng is elevated in MM1/MV1 molecular subtypes compared with the VV2 subtype, which is in line with the severity of cortical pathological affectation (Blennow et al., 2019). However, the authors did not consider CSF Ng as a specific marker of synaptic degeneration but rather a marker of neuronal damage (Blennow et al., 2019). A recent study had a unicentric cohort of 353 participants, including HC subjects, AD, frontotemporal dementia (FTD), and CJD (Antonell et al., 2020). They analyzed and compared the diagnostic accuracy and differentiating capacity of four noncore biomarkers, which stand for the distinct aspects of the neurodegeneration process (Antonell et al., 2020). The rank of CSF Ng concentrations from lower to higher is FTD < HC < AD < CJD, which is in concordance with previously published data. Comparing their capacity in differentiating among neurodegenerative dementias, CSF Ng shows the significant differences across all three groups (AD, FTD, and CJD) (Antonell et al., 2020).

At present, the relation of Ng and CJD is still scatteredly reported, in which CSF Ng has been evaluated for diagnosis and differential diagnosis, and the results are relatively consistent, suggesting that CSF Ng has the potential to be a CJD biomarker.

Cerebrospinal Fluid Neurogranin in Other Neurodegenerative Disorders

In addition to the AD, PD, and HD, some scattered studies about CSF Ng in other NDs, including FTD, DLB, progressive supranuclear palsy (PSP), and multiple system atrophy (MSA), have been published so far (Wellington et al., 2016; Portelius et al., 2018). An optimized immunoassay was introduced to analyze CSF Ng in a retrospective cohort, which showed FTD does not have significantly elevated CSF Ng concentrations compared with controls (Wellington et al., 2016). Of note, CSF Ng concentrations are slightly higher in speech variant frontotemporal dementia compared with behavioral variant frontotemporal dementia (Wellington et al., 2016). A study investigated CSF levels of Ng and other two synaptic proteins in FTD. CSF samples were analyzed in 66 patients in the FTD spectrum and 19 HCs. Patients were stratified as per their tau-to-Aβ42 ratio (tau/Aβ42) (Clarke et al., 2019). In detail, patients with a ratio of >1 were considered as undergoing the likely AD pathology ("AD biomarker" group [$n = 18$]), and patients with a ratio <1 were considered as undergoing the likely FTD pathology ("FTD biomarker" group [$n = 48$]) (Clarke et al., 2019). However, no CSF synaptic proteins showed a pathological abnormality in the "FTD biomarker" group, and the higher CSF concentrations of Ng appear to be more related to AD pathology (Clarke et al., 2019).

In a study in which a total of 129 postmortem human brain samples were analyzed in brain regional-specific manner, it found that Ng levels are reduced across the brain regions in all the three dementia groups (DLB, PDD, and AD) compared with the controls (Bereczki et al., 2016). The most significant changes reflecting synaptic dysfunction were found in DLB patients, followed by patients with PDD and AD (Bereczki et al., 2016). The authors suggested that the proposition that synaptic biomarkers predicting cognitive decline in AD is supposed

to be extended to DLB (Bereczki et al., 2016). In contrast, another retrospective cohort study did not show the significant differences in CSF Ng concentrations between DLB and controls (Wellington et al., 2016).

Cerebrospinal Fluid Neurogranin in Infectious Diseases of the Central Nervous System

Infectious diseases of the CNS have a sizable effect on local health-care systems and economies (Vora et al., 2014). The change in mental status induced by the inflammation is a hallmark of neurotropic pathogen infections of the CNS (Klein et al., 2017). Pathogens, including bacteria, viruses, fungi, and parasites, can invade the brain parenchyma and give rise to the inflammation and/or the infection of both meningeal and parenchymal compartments, which lead to the dysfunction of neurons, glia cells, and the neural networks (Cain et al., 2019). On the basis of the published studies so far, we mainly summarize the relationship between Ng and the following two infectious diseases of the nervous system.

Cerebrospinal Fluid Neurogranin in Neuro-Human Immunodeficiency Virus Infection

Soon after transmission, HIV can be detected in the CSF in most patients (Valcour et al., 2012). The antiretroviral therapy has decreased the rates of mortality and morbidity in HIV-positive (HIV^+) patients and has decreased the incidence of HIV-associated dementia, which is the most severe stage of neuro-HIV (Sinharay and Hammoud, 2019). Synaptic disruption is crucial in the mechanisms of cognitive impairment in HIV-1-infected patients (Everall et al., 1999; Green et al., 2019). Compared with neuronal apoptosis and HIV-encephalitis, the dendritic injury due to HIV-1 infection is more closely related to cognitive impairments among HIV-associated neurocognitive disorder (HAND) patients (Everall et al., 1999). Guha et al. (2018) compared the expression of Ng in the frontal cortex (FC) between HIV-1-positive subjects with and without HAND and the controls (Guha et al., 2018). The study found that the expression levels of Ng are reduced significantly in FC of HAND-positive patients in contrast with the uninfected individuals. Yet, a recent cross-sectional study showed that CSF Ng concentrations are in the same range for all the groups of HIV-infected patients and uninfected controls (Yilmaz et al., 2019).

Cerebrospinal Fluid Neurogranin in Neurosyphilis

NS, the clinical outcomes of nervous system infection of *Treponema pallidum*, can occur at any stage of syphilis (Ropper, 2019). NS is very insidious in the early stage, while its clinical manifestation in the late stage is very serious, which includes the general paresis and tabes dorsalis. Thus the early diagnosis and differential diagnosis are critical.

Intriguingly, patients with NS at a later stage general paresis of the insane (GPI) are found to have the brain pathology features of AD (Zhang et al., 2020). In a recent study, the levels of Ng and amyloid precursor protein cleaving enzyme (BACE1) in CSF and plasma, together with Aβ40, Aβ42, and t-tau in the CSF of AD patients ($n = 23$), GPI patients ($n = 55$), and NS patients ($n = 13$) were tested (Zhang et al., 2020). It found that the CSF concentrations of Ng, BACE1, and tau and the plasma BACE1 levels significantly differ among all the groups (Zhang et al., 2020). Pooling data from GPI and NS patients, both CSF tau and plasma Ng levels, are associated with cognitive scale score. These findings indicate the potential of diagnosis, differential diagnosis, and assessment of the severity of NS (Zhang et al., 2020). However, there are a few other reports, and further research is needed.

Cerebrospinal Fluid Neurogranin in Mental Disorders

Millions of people experience mental disorders, such as schizophrenia and depression. These mental diseases are characterized by a combination of abnormal thoughts, emotions, behaviors, and perceptions (Quintero et al., 2019). Given the multifactorial complexity of these disorders, the biomarkers are supposed to assist in the early diagnosis, monitoring, and treatment selection. As a severe and complex mental disorder, schizophrenia has a lifetime prevalence of ∼1%, constituting ∼1% of the global burden of the disease (Lora et al., 2012). A genome-wide association study identified a relationship between schizophrenia and the single nucleotide polymorphism of rs12807809 in the NRGN (Stefansson et al., 2009). In recent years, several reports attempted to reveal the association between rs12807809 polymorphism and schizophrenia among different populations, but the results were controversial (Li et al., 2010; Sudesh et al., 2017). A recent meta-analysis aiming to integrate the present studies on the 12807809 polymorphism showed a statistically significant association between schizophrenia and rs12807809 polymorphism in the overall population in the allelic model (odds ratio = 1.10, 95% confidence interval 1.04–1.17). Nevertheless, the subgroup analysis revealed that a similar association only exists in Caucasians but not in Asians (Jin et al., 2019). A study of postmortem brain tissues showed a significant decrease in Ng immunostaining in both areas 9 and 32 of the prefrontal cortex (PFC) in schizophrenia compared with controls (Broadbelt et al., 2006).

In an animal study, transgenic mice overexpressing Ng in the PFC show the enhanced local plasticity and increased rate of extinction learning among different behavioral tasks, suggesting that Ng signaling in the PFC may be a specific therapeutic target for the treatment of disorders that are characterized by impaired extinction of fearful stimuli, e.g., post-traumatic stress disorder, or of reward-associated stimuli, e.g., drug addiction (Zhong et al., 2015). Electroconvulsive therapy (ECT) is a wildly used treatment for severe depression, which is considered to facilitate the neurogenesis and neural plasticity (Rotheneichner et al., 2014). A study investigated the changes of CSF Ng in response to ECT treatment in patients with depression and found that the mean CSF Ng levels do not alter within a course of ECT, but the low baseline Ng levels in the patients with major depression are positively associated with the degree of therapeutic response (Kranaster et al., 2017).

Further, a study examined CSF Ng in patients with first-episode psychosis (FEP) and HCs. It showed that CSF Ng is

lower in FEP patients compared with the controls, although it is not statistically significant. In the FEP group, the significant effects of antipsychotic treatment, which is correlated to the lower levels of CSF Ng, suggest that CSF Ng is probably changed as a consequence of minimal exposure to the antipsychotic treatment (Santillo et al., 2019).

In fact, studies on the association between Ng genotypes and psychiatric disorders, particularly schizophrenia, have been carried out for many years. However, the research on the relationship between body fluids-based Ng and mental disorders is still at an early stage and is worth further exploration.

Serum Neurogranin in Traumatic Brain Injury

Traumatic brain injury is a significant medical problem worldwide, which may cause short- or long-term synaptic changes in the CNS, resulting in an increased risk for cognitive impairment later in life (Svirsky et al., 2020). Animal studies showed that TBI could cause significant changes in axonal structure, synaptic structure, dendritic morphology, and spine density as a result of diffuse axonal injury and synaptic loss (Gao et al., 2011; Park and Biederer, 2013). A study developed a sensitive Ng sandwich ELISA to measure Ng quantitatively in serum samples from both cohorts of acute TBI patients and non-TBI controls. It found that serum Ng levels in acute TBI patients are significantly higher than those in non-TBI controls, with a ROC of 0.72 for diagnosing TBI (Yang et al., 2015). An observational emergency department study of head-injured and control patients also reached the consistent conclusions that Ng is elevated within 2–6 h after injury (Peacock et al., 2017). A recent study aimed to explore the effect of TBI on Ng by detecting the protein expression at different time points after injury (Svirsky et al., 2020). Adult male rats were subjected to either CCI group or sham group, and the expression of Ng and postsynaptic density (PSD) 95 was measured by Western blotting in the cortex and hippocampus at 1, 7, 14, and 28 days after injury. It found that the contralateral and ipsilateral hippocampus have a significant reduction in Ng levels at 1 day after CCI injury. Besides, the levels of Ng in the ipsilateral hippocampus are still significantly decreased at 7 and 14 days after CCI injury, whereas they recover to sham levels by 28 days. These results indicated that CCI lowers Ng expression in a temporal and regional specificity manner (Svirsky et al., 2020).

As a disease entity, TBI is also a risk factor for a variety of neurological diseases. Current studies suggest that Ng has potential in TBI diagnosis and disease progression. However, the lack of clinical research has limited the further transformation and application of Ng.

Cerebrospinal Fluid and Plasma Neurogranin in Acute Ischemic Stroke

Globally, stroke (including ischemic stroke and hemorrhagic stroke) affects around 13.7 million individuals per year and is the second leading cause of death (Lindsay et al., 2019). Ischemic stroke caused by arterial occlusion is responsible for the majority of stroke cases (Campbell et al., 2019). After a stroke, a period of plasticity involving the neuronal genesis and synaptic modulation is essential to spontaneous recovery, which contains compensatory adaptation and real neurologic recovery (Felling and Song, 2015). A prospective study exploring Ng in paired CSF/plasma samples of AIS patients used both ELISA and single-molecule array (Simoa) technology for Ng measurement (De Vos et al., 2017). It showed that plasma Ng levels are only associated with the volume of cerebral infarction. Likewise, the levels of CSF Ng are significantly higher in patients with an infarction volume >5 ml than those in patients with smaller infarction volume. However, neither the symptoms severity nor long-term outcomes are correlated with Ng in plasma or CSF (De Vos et al., 2017).

In addition to AD, AIS is the only disease that has been studied to observe levels of CSF and blood Ng and their correlation. The discussed findings suggest the potential of blood Ng in reflecting brain tissue damage. However, whether the blood and CSF Ng have certain consistency is still an unavoidable issue for blood-based Ng. Recent studies have found that some kind of enzymes have the capacity of cleaving Ng and yielding specific fragments (Becker et al., 2018), which could influence the accuracy of the current detection methods. Therefore, the development of novel detection approaches is an urgent part of Ng clinical research.

DISCUSSION

Considerable evidence proves that a synaptic dysfunction is an early event in the pathogenesis of many neurodegenerative diseases, particularly in the AD (DeKosky and Scheff, 1990; Masliah, 2001) and PD (Jellinger, 2012). As a postsynaptic protein, Ng has been recommended as a promising biomarker for synapse loss or dysfunction (Brinkmalm et al., 2019). A series of clinical studies have confirmed the rationality, validity, sensitivity, and specificity of CSF Ng in the diagnosis for both AD dementia and prodromal AD. Thus, CSF Ng has been included in the A/T/(N) research framework of the biological definition of AD as an essential indicator of neurodegeneration (Jack et al., 2018).

However, there are still many problems in the development of the application of Ng from bench to bedside. So far, no clear evidence proves that Ng is a disease-specific biomarker, as the change of function and structure of synapse is common in the pathogenesis of different kinds of CNS diseases, indicating that the current research reports are insufficient to uncover the profile and potential application values of Ng in clinical practice. Besides, the consistency of the present results about Ng and AD is acceptable, but the other findings need more interpretation, such as the association of Ng with PD (Selnes et al., 2017; Hall et al., 2020), neuro-HIV (Guha et al., 2018; Yilmaz et al., 2019), FTD (Byrne et al., 2018; Clarke et al., 2019), etc., which in part attributes to the current measuring methods lacking high accuracy in detecting the proteins of extremely low levels both in CSF and blood.

Given that the blood samples are more accessible than CSF samples, striving has been made for replacing CSF-based biomarkers with blood-based biomarkers, especially for the diagnosis of NDs such as AD. For instance, the blood and CSF levels of neurofilament light (a promising biomarker for

AD diagnosis) has been proved consistent (Fortea et al., 2018; Jack et al., 2018), indicating that it is potential to develop a blood-based, rapid, simple, portable, and easily accessible testing method for the AD screening in community populations. Frustratingly, only scattered studies have investigated blood Ng levels but failed to show a significant difference between AD patients and HCs. Also, no significant correlation between CSF and blood Ng was reported previously (De Vos et al., 2015). Besides, blood Ng has also been poorly studied in other neurodegenerative diseases. Given that the concentration of CNS biomarkers outside of the CNS is often extremely low, it is difficult to be conducted using conventional clinical assays.

Other important factors complicating the analysis include peripheral expression of Ng, the endogenous antibodies interfering with the measured results, and the proteases influence the catabolism of Ng (Zetterberg and Burnham, 2019). Since the discovery of Ng in 1990 (Watson et al., 1990), there are, to our knowledge, very few studies investigating its metabolic profile. Mass spectrometry analyses suggested that Ng is catabolized into several short C-terminal peptides, which can be identified in CSF, and only minute amounts of full-length Ng is present in CSF. Furthermore, it showed that Ng in human plasma exists as several endogenous peptides via analyzing paired plasma and CSF samples from patients with AD and HCs. Among the endogenous Ng peptides detected, CSF Ng 48–76 shows the most pronounced increase in patients with AD compared with the controls. Importantly, Ng 48–76 is also proved to be dominant in the brain tissues of AD patients. However, Ng 48–76 is not detected in plasma. These findings indicate that this particular peptide is probably brain-specific (Kvartsberg et al., 2015b). Conversely, four of the Ng peptides found only in plasma are not generated after incubation of full-length Ng in Ng-depleted plasma, indicating that some certain enzymes existing in plasma have the capacity of cleaving Ng at different sites (Kvartsberg et al., 2015b).

On the basis of current studies, Ng is expressed in the lung, spleen, bone marrow, and platelets, which may contribute to its high concentrations in blood. Due to the high plasma concentrations of Ng in normal individuals (Kvartsberg et al., 2015b), the subtle alteration is probably not detected in blood in case of chronic progressive neurodegeneration like AD. Besides, Ng is catabolized into several short C-terminal peptides; the levels of which vary in CSF and plasma, implying that the development of monoclonal anti-Ng antibodies-based testing methods is relatively difficult.

In summary, blood-based biomarkers are an important development direction in the diagnosis of neurological and mental diseases due to their many advantages compared with CSF based biomarkers. Currently, the transformation process of Ng from bench to bed has been developing rapidly in the NDs, especially in AD. In other kinds of diseases, such as PD and schizophrenia, it also has a very great potential value of transformation and application. As an essential synaptic component, Ng is a potential and promising biomarker to improve the diagnosis, prognosis and severity evaluation of the neurological and mental diseases in the future with the development of detection approaches and sample processing.

AUTHOR CONTRIBUTIONS

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

FUNDING

This study was supported in part by the National Natural Science Foundation of China Fund (Grant No. 81601112), Sichuan Department of Science and Technology Fund (Grant No. 2018SZ0141), Top Project of Youth Incubation Program of Military Medical Science and Technology (Grant No. 19QNP065), and China's Post-doctoral Science Fund (Grant No. 2017M623357).

ACKNOWLEDGMENTS

We are deeply appreciative of the participants in this study, and thank all staff for their support and assistance.

REFERENCES

Antonell, A., Tort-Merino, A., Rios, J., Balasa, M., Borrego-Ecija, S., Auge, J. M., et al. (2020). Synaptic, axonal damage and inflammatory cerebrospinal fluid biomarkers in neurodegenerative dementias. *Alzheimers Dement.* 16, 262–272. doi: 10.1016/j.jalz.2019.09.001

Barker, R. A., and Transeuro consortium. (2019). Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease. *Nat. Med.* 25, 1045–1053. doi: 10.1038/s41591-019-0507-2

Bates, G. P., Dorsey, R., Gusella, J. F., Hayden, M. R., Kay, C., Leavitt, B. R., et al. (2015). Huntington disease. *Nat. Rev. Dis. Primers.* 1:15005. doi: 10.1038/nrdp.2015.5

Becker, B., Nazir, F. H., Brinkmalm, G., Camporesi, E., Kvartsberg, H., Portelius, E., et al. (2018). Alzheimer-associated cerebrospinal fluid fragments of neurogranin are generated by Calpain-1 and prolyl endopeptidase. *Mol. Neurodegener.* 13:47. doi: 10.1186/s13024-018-0279-z

Bereczki, E., Bogstedt, A., Hoglund, K., Tsitsi, P., Brodin, L., Ballard, C., et al. (2017). Synaptic proteins in CSF relate to Parkinson's disease stage markers. *NPJ. Parkinsons. Dis.* 3:7. doi: 10.1038/s41531-017-0008-2

Bereczki, E., Francis, P. T., Howlett, D., Pereira, J. B., Hoglund, K., Bogstedt, A., et al. (2016). Synaptic proteins predict cognitive decline in Alzheimer's disease and Lewy body dementia. *Alzheimers Dement.* 12, 1149–1158. doi: 10.1016/j.jalz.2016.04.005

Blennow, K., de Leon, M. J., and Zetterberg, H. (2006). Alzheimer's disease. *Lancet* 368, 387–403. doi: 10.1016/s0140-6736(06)69113-7

Blennow, K., Diaz-Lucena, D., Zetterberg, H., Villar-Pique, A., Karch, A., Vidal, E., et al. (2019). CSF neurogranin as a neuronal damage marker in CJD: a comparative study with AD. *J. Neurol. Neurosurg. Psych.* 90, 846–853. doi: 10.1136/jnnp-2018-320155

Brinkmalm, A., Portelius, E., Brinkmalm, G., Pannee, J., Dahlen, R., Gobom, J., et al. (2019). Fluid-based proteomics targeted on pathophysiological processes and pathologies in neurodegenerative diseases. *J. Neurochem.* 151, 417–434. doi: 10.1111/jnc.14594

Broadbelt, K., Ramprasaud, A., and Jones, L. B. (2006). Evidence of altered neurogranin immunoreactivity in areas 9 and 32 of schizophrenic prefrontal cortex. *Schizophr. Res.* 87, 6–14. doi: 10.1016/j.schres.2006.04.028

Byrne, L. M., Rodrigues, F. B., Johnson, E. B., De Vita, E., Blennow, K., Scahill, R., et al. (2018). Cerebrospinal fluid neurogranin and TREM2 in Huntington's disease. *Sci. Rep.* 8:4260. doi: 10.1038/s41598-018-21788-x

Cain, M. D., Salimi, H., Diamond, M. S., and Klein, R. S. (2019). Mechanisms of Pathogen Invasion into the Central Nervous System. *Neuron* 103, 771–783. doi: 10.1016/j.neuron.2019.07.015

Campbell, B. C. V., De Silva, D. A., Macleod, M. R., Coutts, S. B., Schwamm, L. H., Davis, S. M., et al. (2019). Ischaemic stroke. *Nat. Rev. Dis. Primers.* 5:70. doi: 10.1038/s41572-019-0118-8

Cheriyan, V. T., Alfaidi, M., Jorgensen, A. N., Alam, M. A., Abdullah, C. S., Kolluru, G. K., et al. (2020). Neurogranin regulates eNOS function and endothelial activation. *Redox. Biol.* 34:101487. doi: 10.1016/j.redox.2020.101487

Clarke, M. T. M., Brinkmalm, A., Foiani, M. S., Woollacott, I. O. C., Heller, C., Heslegrave, A., et al. (2019). CSF synaptic protein concentrations are raised in those with atypical Alzheimer's disease but not frontotemporal dementia. *Alzheimers Res. Ther.* 11:105. doi: 10.1186/s13195-019-0564-2

Clinton, J., Forsyth, C., Royston, M. C., and Roberts, G. W. (1993). Synaptic degeneration is the primary neuropathological feature in prion disease: a preliminary study. *NeuroReport.* 4, 65–68. doi: 10.1097/00001756-199301000-00017

De Vos, A., Bjerke, M., Brouns, R., De Roeck, N., Jacobs, D., Van den Abbeele, L., et al. (2017). Neurogranin and tau in cerebrospinal fluid and plasma of patients with acute ischemic stroke. *BMC Neurol.* 17:170. doi: 10.1186/s12883-017-0945-8

De Vos, A., Jacobs, D., Struyfs, H., Fransen, E., Andersson, K., Portelius, E., et al. (2015). C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. *Alzheimers Dement.* 11, 1461–1469. doi: 10.1016/j.jalz.2015.05.012

DeKosky, S. T., and Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann. Neurol.* 27, 457–464. doi: 10.1002/ana.410270502

Diez-Guerra, F. J. (2010). Neurogranin, a link between calcium/calmodulin and protein kinase C signaling in synaptic plasticity. *IUBMB Life.* 62, 597–606. doi: 10.1002/iub.357

Dugger, B. N., and Dickson, D. W. (2017). Pathology of Neurodegenerative Diseases. *Cold Spring Harb. Perspect. Biol.* 9:a028035. doi: 10.1101/cshperspect.a028035

Everall, I. P., Heaton, R. K., Marcotte, T. D., Ellis, R. J., McCutchan, J. A., Atkinson, J. H., et al. (1999). Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. *Brain Pathol.* 9, 209–217. doi: 10.1111/j.1750-3639.1999.tb00219.x

Felling, R. J., and Song, H. (2015). Epigenetic mechanisms of neuroplasticity and the implications for stroke recovery. *Exper. Neurol.* 268, 37–45. doi: 10.1016/j.expneurol.2014.09.017

Fortea, J., Carmona-Iragui, M., Benejam, B., Fernández, S., Videla, L., Barroeta, I., et al. (2018). Plasma and CSF biomarkers for the diagnosis of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *Lancet Neurol.* 17, 860–869. doi: 10.1016/s1474-4422(18)30285-0

Fyfe, I. (2015). Neurogranin in the CSF signals early Alzheimer disease and predicts disease progression. *Nat. Rev. Neurol.* 11, 609–609. doi: 10.1038/nrneurol.2015.178

Gao, X., Deng, P., Xu, Z. C., and Chen, J. (2011). Moderate traumatic brain injury causes acute dendritic and synaptic degeneration in the hippocampal dentate gyrus. *PLoS One.* 6:e24566. doi: 10.1371/journal.pone.0024566

Garrido-Garcia, A., De Andres, R., Jimenez-Pompa, A., Soriano, P., Sanz-Fuentes, D., Martinez-Blanco, E., et al. (2019). Neurogranin Expression Is Regulated by Synaptic Activity and Promotes Synaptogenesis in Cultured Hippocampal Neurons. *Mol. Neurobiol.* 56, 7321–7337. doi: 10.1007/s12035-019-1593-3

Glynne, R., Ghandour, G., Rayner, J., Mack, D. H., and Goodnow, C. C. (2000). B-lymphocyte quiescence, tolerance and activation as viewed by global gene expression profiling on microarrays. *Immunol. Rev.* 176, 216–246. doi: 10.1034/j.1600-065x.2000.00614.x

Gnatenko, D. V., Dunn, J. J., McCorkle, S. R., Weissmann, D., Perrotta, P. L., and Bahou, W. F. (2003). Transcript profiling of human platelets using microarray and serial analysis of gene expression. *Blood* 101, 2285–2293. doi: 10.1182/blood-2002-09-2797

Green, M. V., Raybuck, J. D., Zhang, X., Wu, M. M., and Thayer, S. A. (2019). Scaling Synapses in the Presence of HIV. *Neurochem. Res.* 44, 234–246. doi: 10.1007/s11064-018-2502-2

Guha, D., Wagner, M. C. E., and Ayyavoo, V. (2018). Human immunodeficiency virus type 1 (HIV-1)-mediated neuroinflammation dysregulates neurogranin and induces synaptodendritic injury. *J. Neuroinflamm.* 15:126. doi: 10.1186/s12974-018-1160-2

Hall, S., Janelidze, S., Zetterberg, H., Brix, B., Mattsson, N., Surova, Y., et al. (2020). Cerebrospinal fluid levels of neurogranin in Parkinsonian disorders. *Mov. Disord.* 35, 513–518. doi: 10.1002/mds.27950

Hellwig, K., Kvartsberg, H., Portelius, E., Andreasson, U., Oberstein, T. J., Lewczuk, P., et al. (2015). Neurogranin and YKL-40: independent markers of synaptic degeneration and neuroinflammation in Alzheimer's disease. *Alzheimers Res. Ther.* 7:74. doi: 10.1186/s13195-015-0161-y

Hodges, A., Strand, A. D., Aragaki, A. K., Kuhn, A., Sengstag, T., Hughes, G., et al. (2006). Regional and cellular gene expression changes in human Huntington's disease brain. *Hum. Mol. Genet.* 15, 965–977. doi: 10.1093/hmg/ddl013

Jack, C. R. Jr., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., et al. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 14, 535–562. doi: 10.1016/j.jalz.2018.02.018

Jellinger, K. A. (2012). Neuropathology of sporadic Parkinson's disease: evaluation and changes of concepts. *Mov. Disord.* 27, 8–30. doi: 10.1002/mds.23795

Jeon, S. G., Kang, M., Kim, Y. S., Kim, D. H., Nam, D. W., Song, E. J., et al. (2018). Intrahippocampal injection of a lentiviral vector expressing neurogranin enhances cognitive function in 5XFAD mice. *Exp. Mol. Med.* 50:e461. doi: 10.1038/emm.2017.302

Jia, L., Zhu, M., Kong, C., Pang, Y., Zhang, H., Qiu, Q., et al. (2020). Blood neuro-exosomal synaptic proteins predict Alzheimer's disease at the asymptomatic stage. *Alzheimer's & Dement.* 6:78. doi: 10.1002/alz.12166

Jin, L., An, Z., Xu, B., Mu, D., Fu, S., Hu, H., et al. (2019). The association between rs12807809 polymorphism in neurogranin gene and risk of schizophrenia: A meta-analysis. *Medicine* 98:e18518. doi: 10.1097/MD.0000000000018518

Kester, M. I., Teunissen, C. E., Crimmins, D. L., Herries, E. M., Ladenson, J. H., Scheltens, P., et al. (2015). Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. *JAMA Neurol.* 72, 1275–1280. doi: 10.1001/jamaneurol.2015.1867

Klein, R. S., Garber, C., and Howard, N. (2017). Infectious immunity in the central nervous system and brain function. *Nat. Immunol.* 18, 132–141. doi: 10.1038/ni.3656

Koob, A. O., Shaked, G. M., Bender, A., Bisquertt, A., Rockenstein, E., and Masliah, E. (2014). Neurogranin binds alpha-synuclein in the human superior temporal cortex and interaction is decreased in Parkinson's disease. *Brain Res.* 1591, 102–110. doi: 10.1016/j.brainres.2014.10.013

Kovacs, G. G. (2016). Molecular Pathological Classification of Neurodegenerative Diseases: Turning towards Precision Medicine. *Int. J. Mol. Sci.* 17:189. doi: 10.3390/ijms17020189

Kovacs, G. G. (2017). Concepts and classification of neurodegenerative diseases. *Handb. Clin. Neurol.* 145, 301–307. doi: 10.1016/B978-0-12-802395-2.00021-3

Kranaster, L., Blennow, K., Zetterberg, H., and Sartorius, A. (2017). Electroconvulsive therapy does not alter the synaptic protein neurogranin in the cerebrospinal fluid of patients with major depression. *J. Neural. Transm.* 124, 1641–1645. doi: 10.1007/s00702-017-1802-z

Kvartsberg, H., Duits, F. H., Ingelsson, M., Andreasen, N., Ohrfelt, A., Andersson, K., et al. (2015a). Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. *Alzheimers Dement.* 11, 1180–1190. doi: 10.1016/j.jalz.2014.10.009

Kvartsberg, H., Portelius, E., Andreasson, U., Brinkmalm, G., Hellwig, K., Lelental, N., et al. (2015b). Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls. *Alzheimer's Res. Ther.* 7:40. doi: 10.1186/s13195-015-0124-3

Li, T., Li, Z., Chen, P., Zhao, Q., Wang, T., Huang, K., et al. (2010). Common Variants in Major Histocompatibility Complex Region and TCF4 Gene Are Significantly Associated with Schizophrenia in Han Chinese. *Biol. Psych.* 68, 671–673. doi: 10.1016/j.biopsych.2010.06.014

Lindsay, M. P., Norrving, B., Sacco, R. L., Brainin, M., Hacke, W., Martins, S., et al. (2019). World Stroke Organization (WSO): Global Stroke Fact Sheet 2019. *Int. J. Str.* 14, 806–817. doi: 10.1177/1747493019881353

Liu, W., Lin, H., He, X., Chen, L., Dai, Y., Jia, W., et al. (2020). Neurogranin as a cognitive biomarker in cerebrospinal fluid and blood exosomes for Alzheimer's disease and mild cognitive impairment. *Transl. Psych.* 10:125. doi: 10.1038/s41398-020-0801-2

Lora, A., Kohn, R., Levav, I., McBain, R., Morris, J., and Saxena, S. (2012). Service availability and utilization and treatment gap for schizophrenic disorders: a survey in 50 low- and middle-income countries. *Bull. World Health Organ.* 90, 47–54. doi: 10.2471/BLT.11.089284

Martinez, de Arrieta, C., Perez Jurado, L., Bernal, J., and Coloma, A. (1997). Structure, organization, and chromosomal mapping of the human neurogranin gene (NRGN). *Genomics* 41, 243–249. doi: 10.1006/geno.1997.4622

Masliah, E. (2001). Recent advances in the understanding of the role of synaptic proteins in Alzheimer's Disease and other neurodegenerative disorders. *J. Alzheimer's Dis.* 3, 121–129. doi: 10.3233/jad-2001-3117

Masliah, E., Mallory, M., Alford, M., DeTeresa, R., Hansen, L. A., and McKeel, D. W. (2001). Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. *Neurology* 56, 127–129. doi: 10.1212/wnl.56.1.127

Mavroudis, I. A., Petridis, F., Chatzikonstantinou, S., and Kazis, D. (2019). A meta-analysis on CSF neurogranin levels for the diagnosis of Alzheimer's disease and mild cognitive impairment. *Aging Clin. Exp. Res.* 28:31463927 doi: 10.1007/s40520-019-01326-z

Mons, N., Enderlin, V., Jaffard, R., and Higueret, P. (2001). Selective age-related changes in the PKC-sensitive, calmodulin-binding protein, neurogranin, in the mouse brain. *J. Neurochem.* 79, 859–867. doi: 10.1046/j.1471-4159.2001.00646.x doi: 10.1046/j.1471-4159.2001.00646.x

Pak, J. H., Huang, F. L., Li, J., Balschun, D., Reymann, K. G., Chiang, C., et al. (2000). Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: a study with knockout mice. *Proc. Natl. Acad. Sci. U S A.* 97, 11232–11237. doi: 10.1073/pnas.210184697

Park, K., and Biederer, T. (2013). Neuronal adhesion and synapse organization in recovery after brain injury. *Fut. Neurol.* 8, 555–567. doi: 10.2217/fnl.13.35

Peacock, W. F. T., Van Meter, T. E., Mirshahi, N., Ferber, K., Gerwien, R., Rao, V., et al. (2017). Derivation of a Three Biomarker Panel to Improve Diagnosis in Patients with Mild Traumatic Brain Injury. *Front. Neurol.* 8:641. doi: 10.3389/fneur.2017.00641

Portelius, E., Olsson, B., Hoglund, K., Cullen, N. C., Kvartsberg, H., Andreasson, U., et al. (2018). Cerebrospinal fluid neurogranin concentration in neurodegeneration: relation to clinical phenotypes and neuropathology. *Acta Neuropathol.* 136, 363–376. doi: 10.1007/s00401-018-1851-x

Portelius, E., Zetterberg, H., Skillbäck, T., Törnqvist, U., Andreasson, U., Trojanowski, J. Q., et al. (2015). Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. *Brain* 138, 3373–3385. doi: 10.1093/brain/awv267

Quintero, M., Stanisic, D., Cruz, G., Pontes, J. G. M., Costa, T., and Tasic, L. (2019). Metabolomic Biomarkers in Mental Disorders: Bipolar Disorder and Schizophrenia. *Adv. Exp. Med. Biol.* 1118, 271–293. doi: 10.1007/978-3-030-05542-4_14

Rafii, M. S. (2016). Targeting tau protein in Alzheimer's disease. *Lancet* 388, 2842–2844. doi: 10.1016/S0140-6736(16)32107-9

Represa, A., Deloulme, J. C., Sensenbrenner, M., Ben-Ari, Y., and Baudier, J. (1990). Neurogranin: immunocytochemical localization of a brain-specific protein kinase C substrate. *J. Neurosci.* 10, 3782–3792. doi: 10.1523/jneurosci.10-12-03782.1990

Ropper, A. H. (2019). Neurosyphilis. *N. Engl. J. Med.* 381, 1358–1363. doi: 10.1056/NEJMra1906228

Rotheneichner, P., Lange, S., O'Sullivan, A., Marschallinger, J., Zaunmair, P., Geretsegger, C., et al. (2014). Hippocampal neurogenesis and antidepressive therapy: shocking relations. *Neural. Plast.* 2014:723915. doi: 10.1155/2014/723915

Runne, H., Kuhn, A., Wild, E. J., Pratyaksha, W., Kristiansen, M., Isaacs, J. D., et al. (2007). Analysis of potential transcriptomic biomarkers for Huntington's disease in peripheral blood. *Proc. Natl. Acad. Sci. U S A.* 104, 14424–14429. doi: 10.1073/pnas.0703652104

Santillo, A. F., Lundgren, S., Xu, C., Orhan, F., Fatouros-Bergman, H., Blennow, K., et al. (2019). Neurogranin as a potential synaptic marker in the cerebrospinal fluid of patients with a first episode psychosis. *Schizophr. Res.* 208, 490–492. doi: 10.1016/j.schres.2019.01.010

Scheff, S. W., Price, D. A., Schmitt, F. A., DeKosky, S. T., and Mufson, E. J. (2007). Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* 68, 1501–1508. doi: 10.1212/01.wnl.0000260698.46517.8f

Selnes, P., Stav, A. L., Johansen, K. K., Bjornerud, A., Coello, C., Auning, E., et al. (2017). Impaired synaptic function is linked to cognition in Parkinson's disease. *Ann. Clin. Transl. Neurol.* 4, 700–713. doi: 10.1002/acn3.446

Sepers, M. D., and Raymond, L. A. (2014). Mechanisms of synaptic dysfunction and excitotoxicity in Huntington's disease. *Drug. Discov. Today.* 19, 990–996. doi: 10.1016/j.drudis.2014.02.006

Sinharay, S., and Hammoud, D. A. (2019). Brain PET Imaging: Value for Understanding the Pathophysiology of HIV-associated Neurocognitive Disorder (HAND). *Curr. HIV/AIDS Rep.* 16, 66–75. doi: 10.1007/s11904-019-00419-8

Smith, R., Brundin, P., and Li, J. Y. (2005). Synaptic dysfunction in Huntington's disease: a new perspective. *Cell Mol. Life Sci.* 62, 1901–1912. doi: 10.1007/s00018-005-5084-5

Stefansson, H., Ophoff, R. A., Steinberg, S., Andreassen, O. A., Cichon, S., Rujescu, D., et al. (2009). Common variants conferring risk of schizophrenia. *Nature* 460, 744–747. doi: 10.1038/nature08186

Sudesh, R., Priyadarshini, T., Preeti, R., John, S., Thara, R., Mowry, B., et al. (2017). Minor allele C of rs12807809 polymorphism in NRGN contributes to the severity of psychosis in patients with Schizophrenia in South Indian population. *Neurosci. Lett.* 649, 107–111. doi: 10.1016/j.neulet.2017.04.008

Svirsky, S., Henchir, J., Li, Y., Ma, X., Carlson, S., and Dixon, C. E. (2020). Neurogranin Protein Expression Is Reduced after Controlled Cortical Impact in Rats. *J. Neurotrauma.* 37, 939–949. doi: 10.1089/neu.2019.6759

Tarawneh, R., D'Angelo, G., Crimmins, D., Herries, E., Griest, T., Fagan, A. M., et al. (2016). Diagnostic and Prognostic Utility of the Synaptic Marker Neurogranin in Alzheimer Disease. *JAMA Neurol.* 73, 561–71. doi: 10.1001/jamaneurol.2016.0086

Uttley, L., Carroll, C., Wong, R., Hilton, D. A., and Stevenson, M. (2020). Creutzfeldt-Jakob disease: a systematic review of global incidence, prevalence, infectivity, and incubation. *Lancet Infect. Dis.* 20, e2–e10. doi: 10.1016/s1473-3099(19)30615-2

Valcour, V., Chalermchai, T., Sailasuta, N., Marovich, M., Lerdlum, S., Suttichom, D., et al. (2012). Central nervous system viral invasion and inflammation during acute HIV infection. *J. Infect. Dis.* 206, 275–282. doi: 10.1093/infdis/jis326

Vora, N. M., Holman, R. C., Mehal, J. M., Steiner, C. A., Blanton, J., and Sejvar, J. (2014). Burden of encephalitis-associated hospitalizations in the United States, 1998-2010. *Neurology* 82, 443–451. doi: 10.1212/WNL.0000000000000086

Watson, J. B., Battenberg, E. F., Wong, K. K., Bloom, F. E., and Sutcliffe, J. G. (1990). Subtractive cDNA cloning of RC3, a rodent cortex-enriched mRNA encoding a novel 78 residue protein. *J. Neurosci. Res.* 26, 397–408. doi: 10.1002/jnr.490260402

Wellington, H., Paterson, R. W., Portelius, E., Tornqvist, U., Magdalinou, N., Fox, N. C., et al. (2016). Increased CSF neurogranin concentration is specific to Alzheimer disease. *Neurology* 86, 829–835. doi: 10.1212/Wnl.0000000000002423

Winston, C. N., Goetzl, E. J., Akers, J. C., Carter, B. S., Rockenstein, E. M., Galasko, D., et al. (2016). Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimers Dement* 3, 63–72. doi: 10.1016/j.dadm.2016.04.001

Yang, J., Korley, F. K., Dai, M., and Everett, A. D. (2015). Serum neurogranin measurement as a biomarker of acute traumatic brain injury. *Clin. Biochem.* 48, 843–848. doi: 10.1016/j.clinbiochem.2015.05.015

Yarnall, A. J., Rochester, L., and Burn, D. J. (2013). Mild cognitive impairment in Parkinson's disease. *Age Ageing.* 42, 567–576. doi: 10.1093/ageing/aft085

Yilmaz, A., Fuchs, D., Price, R. W., Spudich, S., Blennow, K., Zetterberg, H., et al. (2019). Cerebrospinal Fluid Concentrations of the Synaptic Marker Neurogranin in Neuro-HIV and Other Neurological Disorders. *Curr. HIV/AIDS Rep.* 16, 76–81. doi: 10.1007/s11904-019-00420-1

Zetterberg, H., and Blennow, K. (2015). Neurogranin Levels in Cerebrospinal Fluid: A New Addition to the Alzheimer Disease Diagnostic Toolbox. *JAMA Neurol.* 72, 1237–1238. doi: 10.1001/jamaneurol.2015.2075

Zetterberg, H., and Burnham, S. C. (2019). Blood-based molecular biomarkers for Alzheimer's disease. *Mol. Brain.* 12:26. doi: 10.1186/s13041-019-0448-1

Zhang, M., Zhong, X., Shi, H., Vanmechelen, E., De Vos, A., Liu, S., et al. (2020). BACE1 and Other Alzheimer's-Related Biomarkers in Cerebrospinal Fluid and Plasma Distinguish Alzheimer's Disease Patients from Cognitively-Impaired Neurosyphilis Patients. *J. Alzheimers Dis.* 77(1), 313–322. doi: 10.3233/jad-200362

Zhong, L., Brown, J., Kramer, A., Kaleka, K., Petersen, A., Krueger, J. N., et al. (2015). Increased prefrontal cortex neurogranin enhances plasticity and extinction learning. *J. Neurosci.* 35, 7503–7508. doi: 10.1523/JNEUROSCI.0274-15.2015

Zhong, L., Cherry, T., Bies, C. E., Florence, M. A., and Gerges, N. Z. (2009). Neurogranin enhances synaptic strength through its interaction with calmodulin. *EMBO J.* 28, 3027–3039. doi: 10.1038/emboj.2009.236

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.

Copyright © 2020 Xiang, Xin, Le and Yang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

SYSTEMATIC REVIEW
published: 27 November 2020
doi: 10.3389/fnagi.2020.574023

Acupuncture for the Treatment of Alzheimer's Disease: An Overview of Systematic Reviews

*Jinke Huang[1], Min Shen[1], Xiaohui Qin[2], Manli Wu[1], Simin Liang[1] and Yong Huang[3]**

[1] The Second Clinical Medical College of Guangzhou University of Chinese Medicine, Guangzhou, China, [2] Department of Neurology, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, China, [3] School of Traditional Chinese Medicine, Southern Medical University, Guangzhou, China

Background: Acupuncture may be an effective complementary treatment for Alzheimer's disease (AD). The aim of this study was to summarize the evidence provided by systematic reviews (SRs)/meta-analyses (MAs) on the effect of acupuncture on AD.

Methods: Eight electronic databases were searched from their inception until October 19, 2020. The methodological quality, reporting quality, and risk of bias of the included SRs were assessed by the Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR-2), the Risk of Bias in Systematic Reviews (ROBIS) tool, and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Moreover, the evidence quality of the outcome measures was assessed by the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE).

Results: Eleven SRs/MAs met all inclusion criteria. According to the results of the AMSTAR-2, all included reviews were rated critically as being of low quality. With PRISMA, the reporting checklist was relatively complete, but some reporting weaknesses remained in the topics of the protocol and registration, search strategy, risk of bias, additional analyses, and funding. Based on the ROBIS tool, only two SRs/MAs had a low risk of bias. With the GRADE system, no high-quality evidence was found, and only seven outcomes provided moderate-quality evidence. Among the downgraded factors, the risk of bias within the original trials was ranked first, followed by inconsistency, imprecision, and publication bias.

Conclusions: Acupuncture is a promising complementary treatment for AD. However, due to the low quality of the SRs/MAs supporting these results, high-quality studies with rigorous study designs and larger samples are needed before widespread recommendations can be made.

Keywords: acupuncture, Alzheimer's disease, overview, systematic reviews, treatment

OPEN ACCESS

Edited by:
Yu-Hui Liu,
Third Military Medical University, China

Reviewed by:
Gabriel Natan Pires,
Santa Casa of Sao Paulo, Brazil
Ghulam Md. Ashraf,
King Abdulaziz University, Saudi Arabia

****Correspondence:***
Yong Huang
nanfanglihuang@163.com

Received: *18 June 2020*
Accepted: *29 October 2020*
Published: *27 November 2020*

Citation:
Huang J, Shen M, Qin X, Wu M, Liang S and Huang Y (2020) Acupuncture for the Treatment of Alzheimer's Disease: An Overview of Systematic Reviews. Front. Aging Neurosci. 12:574023. doi: 10.3389/fnagi.2020.574023

INTRODUCTION

Alzheimer's disease (AD) is a common progressive degenerative encephalopathy characterized by cognitive impairment, declining memory, emotional changes, and a language barrier (McKhann et al., 1984). AD seriously affects the physical health and quality of life of patients and places a heavy burden on families and society (Xu et al., 2017). With the extension of the average life

expectancy of the population, the incidence of AD is increasing annually; the number of patients worldwide is currently as high as 24 million, which is expected to increase 4-fold by 2050 (Reitz and Mayeux, 2014). Currently, no medication can prevent, halt, or reverse the progression of AD. The clinical drugs approved by the Food and Drug Administration (FDA) for AD only have modest symptomatic effects (Atri et al., 2008) and have been related to many adverse reactions (Tampi and Dyck, 2007). Hence, some patients choose complementary and alternative medicine to treat AD in an effort to improve their quality of life. Worldwide, acupuncture has been accepted as a popular and safe complementary therapy (Bodeker et al., 2005), and it has been widely used to treat AD by physicians aiming to reduce the side effects of medication and to increase its therapeutic effectiveness.

Systematic reviews (SRs)/meta-analyses (MAs) are important tools to guide evidence-based clinical practice, and they have been widely used in various medical fields in recent years. However, with the increasing number of SRs/MAs, their quality is uneven, and their conclusions on the same topic of SR/MAs are often contradictory; therefore, the clinical evidence they provide has been criticized. A systematic overview of SRs/MAs is a relatively new approach for synthesizing the outcomes from multiple SRs/MAs, evaluating their quality and attempting to address any inconsistent outcomes. The objective of our study was to critically assess the scientific quality of relevant SRs/MAs regarding the application of acupuncture in the treatment of PD using a systematic overview.

MATERIALS AND METHODS

Eligibility Criteria

Type of Studies

This study included SRs/MAs of randomized controlled trials (RCTs) of patients who were diagnosed with AD using definitive diagnostic criteria. Repeated publications, graduate dissertations, and SRs/MAs that were not rigorous were excluded.

Interventions

Studies of acupuncture (e.g., manual acupuncture, auricular acupuncture, or needling) or acupuncture plus conventional therapy (CT) as an intervention for AD were included. The following treatments were used in the control group: medication, placebo, and no treatment. The control group treatments were CT alone or placebo.

Outcome Indicators

SRs/MAs should have at least one clear outcome such as the effective rate, Ability of Daily Living (ADL), Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale-Cognition (ADAS-cog), Hasegawa's Dementia Scale (HDS), mood, or behavior.

Data Sources and Search Strategy

Eight electronic databases [Web of Science, The Cochrane Library, PubMed, EMBASE, Sino-Med, China National Knowledge Infrastructure (CNKI), Wanfang Database, and Chongqing VIP] from their inception until February 21, 2020, were searched for potential SRs/MAs, and we conducted an updated search on October 19, 2020, to provide more up-to-date and comprehensive evidence. The search strategies for each database are presented in **Appendix 1**.

Data Management and Extraction

All articles were read by two independent investigators, and data from the articles were validated and extracted according to the predefined criteria. Disagreements between the two investigators were resolved through discussion.

Quality Assessment

Two independent investigators assessed the methodological quality, reporting quality, risk of bias, and evidence quality by the Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR-2) (Shea et al., 2017), Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for the acupuncture checklist (Wang et al., 2019a), Risk of Bias in Systematic Reviews (ROBIS) (Whiting et al., 2016), and Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) (Atkins et al., 2004), respectively. Disagreements between the two investigators were resolved through discussion.

The AMSTAR-2 is a valid instrument composed of 16 items, and each item could be evaluated as "yes," "partial yes," or "no." After interpreting the weaknesses detected in all items, the overall rating of the quality can be rated as "high," "moderate," "low," or "critically low" (Shea et al., 2017). The PRISMA statement is a valid instrument composed of 27 items. Each item was evaluated as "yes," "partial yes," and "no," representing full reports, partial reports, and no reports (Moher et al., 2010). The completion of each item is presented as a ratio. The ROBIS is a valid tool composed of three phases for evaluating the level of bias present within an SR. The risk of bias can be rated as "low," "high," or "unclear" (Whiting et al., 2016). The GRADE system assesses evidence quality with four levels: high, moderate, low, or very low. The initial grading would be decreased if there were study limitations, inconsistencies, imprecision, indirectness, or publication bias (Atkins et al., 2004).

RESULTS

Results on Literature Search and Selection

In total, 226 publications were retrieved from the eight databases. After removing duplicates and title/abstract screening, 16 publications were retrieved for full-text assessment. Examining these full-text publications resulted in the exclusion of four publications (**Appendix 2**). Finally, 11 publications (Guo et al., 2008; Lee et al., 2009; Cao et al., 2014; Xu and Xie, 2015; Zhou

Abbreviations: AD, Alzheimer's disease; SR, systematic review; MA, meta-analysis; AMSTAR-2, Assessing the Methodological Quality of Systematic Reviews 2; ROBIS, Risk of Bias in Systematic Reviews; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; GRADE, Grading of Recommendations, Assessment, Development, and Evaluation; FDA, Food and Drug Administration; RCTs, randomized clinical trials; CT, conventional therapy; CNKI, China National Knowledge Infrastructure; MMSE, Mini-Mental State Examination Score; ADL, Activities of Daily Living Scale; ADAS-cog, Alzheimer's Disease Assessment Scale-Cognition; HDS, Hasegawa's Dementia Scale; PICO, Population, Intervention, Control Group, and Outcome.

et al., 2015, 2017; Zou et al., 2016; Lin et al., 2017; Huang et al., 2019; Wang et al., 2019b, 2020) were selected for inclusion in this overview. The screening and selection procedure is presented in **Figure 1**.

Description of Characteristics

The summarized data extracted from the 11 SRs/MAs are presented in **Table 1**. These included SRs/MAs that were published in the period from 2008 to 2020. Six of them were written in Chinese (Guo et al., 2008; Cao et al., 2014; Xu and Xie, 2015; Zou et al., 2016; Lin et al., 2017; Wang et al., 2019b), and the remaining five (Lee et al., 2009; Zhou et al., 2015, 2017; Huang et al., 2019; Wang et al., 2020) were written in English. These SRs/MAs were all published by authors from East Asia (10 from China and one from Korea). The number of trials ranged between three and 31, and the sample size ranged from 166 to 2,045. Interventions in the therapy group were mainly acupuncture or acupuncture combined with CT, while CT or sham acupuncture was used in the control group. In terms of the quality assessment scales, two (Guo et al., 2008; Xu and Xie, 2015) used Jadad, and the others used the Cochrane risk of bias criteria. Three (one was published in English and two were published in Chinese) of the 11 included SRs/MAs reached negative conclusions, and the remaining eight reached positive conclusions (four were published in English and four were published in Chinese).

Results of the Methodological Quality

Considering the methodological quality, all SRs/MAs were regarded as critically low quality because there was more than one critical item that was unmet in the included SRs/MAs. The methodological limitations arose from the following items: item 2 (only one SR/MA registered the protocol), item 4 (only three SRs/MAs provided a complete search strategy), item 7 (none of the SRs/MAs provided

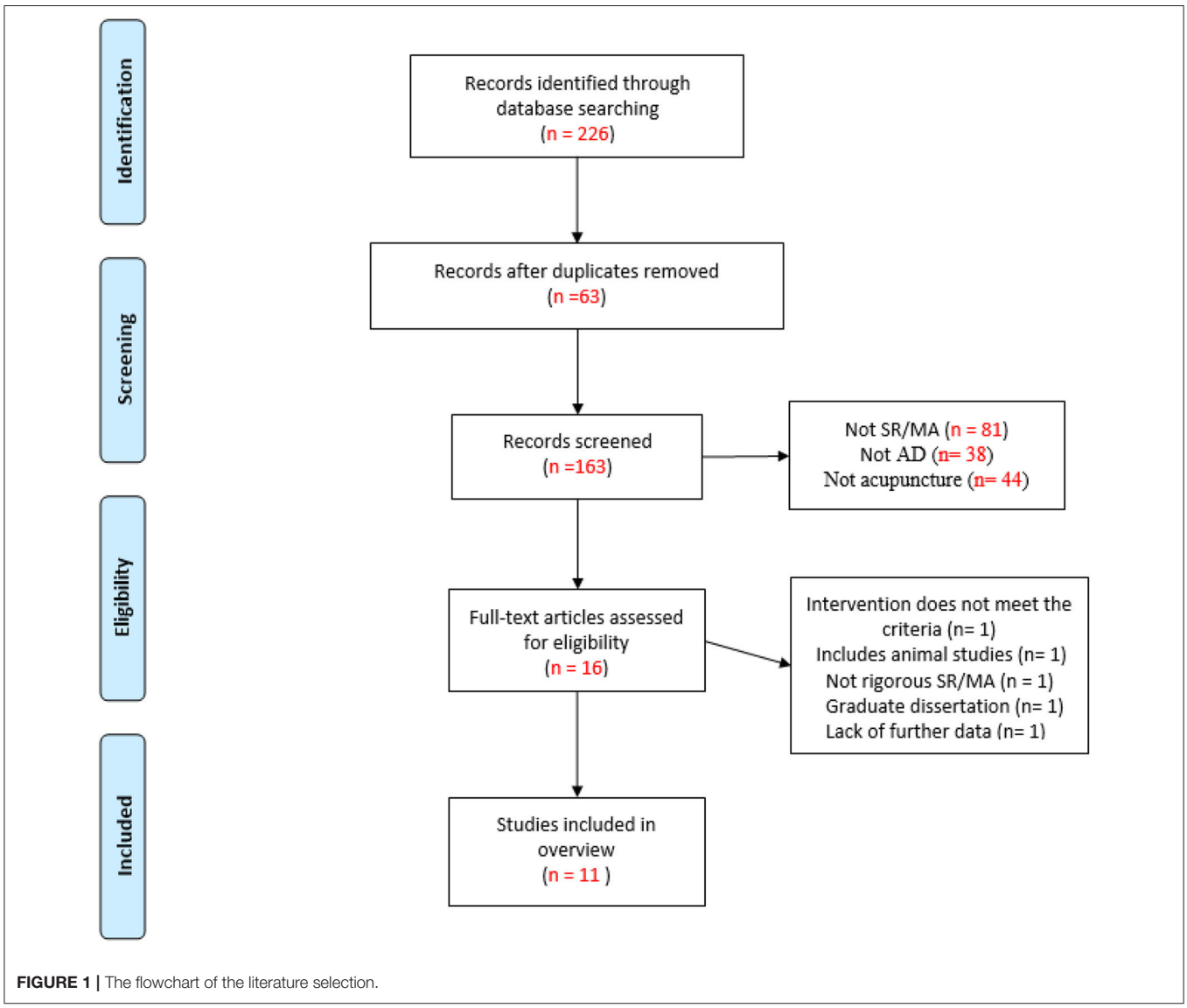

FIGURE 1 | The flowchart of the literature selection.

TABLE 1 | Main characteristics of the included reviews.

Author, year (country)	Language	Trials (subjects)	Treatment intervention	Control intervention	Quality assessment	Meta-analyses	Results	Results summary
Wang et al. (2019b) (China)	Chinese	8 (472)	CA + CT	CT	Cochrane criteria	Yes	Acupuncture combined with medicine for cognitive functions and life quality of AD patients is effective	Positive
Lin et al. (2017) (China)	Chinese	13 (730)	CA, EA, AT + CT	CT	Cochrane criteria	Yes	Acupuncture treatment can improve the learning and memory ability of patients with AD	Positive
Zou et al. (2016) (China)	Chinese	8 (349)	CA, EA	CT	Cochrane criteria	Yes	The advantages of acupuncture in treating AD compared with medication are unsure	Negative
Xu and Xie (2015) (China)	Chinese	10 (652)	CA, EA, SA, CA + CT	CT	Jadad	Yes	Acupuncture combined with medication in AD treatment is definitely effective	Positive
Cao et al. (2014) (China)	Chinese	5 (233)	CA	CT	Cochrane criteria	Yes	Compared with medication, the acupuncture cannot improve the MMSE and ADL score in patients with AD	Negative
Guo et al. (2008) (China)	Chinese	22 (1,368)	EA, SA, CA	CT	Jadad	Yes	Acupuncture is effective on AD according to the domestic clinical literatures	Positive
Wang et al. (2020) (China)	English	31 (2,045)	EA, CA + CT	CT, sham acupuncture, no treatment	Cochrane criteria	Yes	Acupuncture plus drug therapy may have a more beneficial effect for AD patients than drug therapy alone on general cognitive function in the short term and medium term and on ADL skills in the medium term	Positive
Huang et al. (2019) (China)	English	13 (777)	CA, EA	CT	Cochranecriteria	Yes	Acupuncture alone is superior to CT for AD in most of the studies assessed in the current MAs	Positive
Zhou et al. (2017) (China)	English	15 (1,217)	CA + CT	CT	Cochrane criteria	Yes	From the current results, acupuncture plus medicine may have advantages over CT for treating AD	Positive
Zhou et al. (2015) (China)	English	10 (585)	SA, EA, CA + CT	CT; no treatment	Cochrane criteria	Yes	Acupuncture may enhance the effect of CT for treating AD in terms of improving cognitive function. Acupuncture may also be more effective than CT at improving AD patients' ability to carry out their daily lives	Positive
Lee et al. (2009) (Korea)	English	3 (166)	EA + CT	CT	Cochrane criteria	Yes	The existing evidence does not demonstrate the effectiveness of acupuncture for AD	Negative

CA, conventional acupuncture; EA, electroacupuncture; SA, scalp acupuncture; EA, eye acupuncture; CT, conventional therapy.

a list of excluded studies), item 13 [three SRs/MAs did not account for risk of bias (RoB) in the primary studies when interpreting the results of the review], and item 15 (four SRs/MAs did not conduct a publication bias study or discuss its impact on the review). The details are given in **Table 2**.

TABLE 2 | Results of the AMSTAR-2 assessments.

References	AMSTAR-2																Quality
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	
Wang et al. (2019b)	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	CL
Lin et al. (2017)	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y	CL
Zou et al. (2016)	Y	PY	Y	PY	Y	Y	N	Y	Y	N	Y	N	N	Y	N	N	CL
Xu and Xie (2015)	Y	PY	Y	PY	N	N	N	Y	Y	Y	Y	N	Y	Y	Y	Y	CL
Cao et al. (2014)	Y	PY	Y	PY	Y	Y	N	Y	Y	N	Y	Y	Y	Y	N	N	CL
Guo et al. (2008)	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	CL
Wang et al. (2020)	Y	PY	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	CL
Huang et al. (2019)	Y	PY	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	CL
Zhou et al. (2017)	Y	PY	Y	PY	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	CL
Zhou et al. (2015)	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	CL
Lee et al. (2009)	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y	CL

Y, yes; PY, partial yes; N, no; CL, critically low; L, low; H, high.

Results of the Reporting Quality

Table 3 presents the overview of the PRISMA for the acupuncture checklist. In general, no SR/MA reported all items of the PRISMA, but both of them were adequately reported, over 60%. The results showed that more than half of the items were reported in 100% of all SRs/MAs, but there were still some reporting flaws in other items. In the section of the methods, the topic of the protocol and registration, diagnostic criteria in traditional medicine, search, risk of bias across studies, and additional analyses were reported inadequately ($\leq$50%); in the section of the results, no SR/MA reported details of the "de-qi," and the risk of bias and additional analyses were reported in only 54.5%; in the section of the discussion, limitations were reported in only 81.8%; in section of the funding, funding was reported in only 72.7%. More details are presented in **Table 3**.

Results of ROBIS Evaluation

For ROBIS, phase 1 assesses the relevance of the research topic, and all SRs/MAs were rated as having a low risk of bias. Domain 1 assessed the study eligibility criteria, and all SRs/MAs were rated at a low risk of bias. Domain 2 assessed the identification and selection studies, and 10 SRs/MAs had a low risk of bias. Domain 3 assessed the collection and study appraisal, and 10 SRs/MAs were at a low risk of bias. Domain 4 assessed the synthesis and findings, and 4 out of 11 SRs/MAs were rated as having a low risk of bias. Phase 3 considered the overall risk of bias in the reviews, and three SRs/MAs were at a low risk of bias. More details are presented in **Table 4**.

Evidence Quality

Thirty-three outcomes were evaluated by the GRADE system. According to the evaluation results, no high-quality evidence was found, and only seven outcomes provided moderate-quality evidence. The evidence was downgraded due to limitations within the RCTs, inconsistency, imprecision, and publication bias. The details are given in **Table 5**.

Efficacy Evaluation

Acupuncture vs. CT

Eight studies (Guo et al., 2008; Lee et al., 2009; Cao et al., 2014; Zhou et al., 2015; Zou et al., 2016; Lin et al., 2017; Huang et al., 2019; Wang et al., 2020) compared the effects of acupuncture with CT. The effective rate of acupuncture in the treatment of AD was reported in four of seven SRs/MAs (Guo et al., 2008; Zou et al., 2016; Lin et al., 2017; Huang et al., 2019), and the results showed that acupuncture was superior to CT. Four SRs/MAs (Zhou et al., 2015; Zou et al., 2016; Lin et al., 2017; Huang et al., 2019) found significantly greater reductions in MMSE scores in the acupuncture group than in the CT group; however, there was no significant difference in the other three reviews (Lee et al., 2009; Cao et al., 2014; Wang et al., 2020). One SR/MA (Lee et al., 2009) revealed a significantly greater reduction in ADL scores in the acupuncture group than in the CT group, while there was no significant difference in the other five reviews (Cao et al., 2014; Zhou et al., 2015; Zou et al., 2016; Huang et al., 2019; Wang et al., 2020). Three SRs/MAs (Zhou et al., 2015; Huang et al., 2019; Wang et al., 2020) revealed that the HDL score and ADAS-cog score were significantly lower in the acupuncture group than in the CT group. For the HDS score, three SRs/MAs (Zou et al., 2016; Huang et al., 2019; Wang et al., 2020) revealed a significant decrease in the acupuncture group compared with the CT group.

Acupuncture Plus CT vs. CT

Four SRs/MAs (Xu and Xie, 2015; Zhou et al., 2015, 2017; Wang et al., 2019b) compared the effects of acupuncture plus medication with medication. Two out of the four SRs/MAs (Xu and Xie, 2015; Zhou et al., 2017) reported the effective rate of acupuncture plus CT for AD, and the results indicated that the combined treatment was superior to CT alone. The MMSE score was used to evaluate the efficacy of acupuncture for AD in four SRs/MAs (Xu and Xie, 2015; Zhou et al., 2015, 2017; Wang et al., 2019b), and the results showed that the MMSE score was significantly reduced in the combined treatment group. Three SRs/MAs (Zhou et al., 2015, 2017; Wang et al., 2019b) used ADL scores to evaluate the efficacy of acupuncture plus CT vs. CT

TABLE 3 | Results of the PRISMA for the acupuncture checklist.

Section/ topic	Items	Wang et al. (2019b)	Lin et al. (2017)	Zou et al. (2016)	Xu and Xie (2015)	Cao et al. (2014)	Guo et al. (2008)	Wang et al. (2019b)	Huang et al. (2019)	Zhou et al. (2017)	Zhou et al. (2015)	Lee et al. (2009)	Compliance (%)
Title	Q1. Title	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Abstract	Q2. Structured summary	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Introduction	Q3. Rationale	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q4. Objectives	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Methods	Q5. Protocol and registration	N	N	N	N	N	N	N	N	N	Y	N	9.1
	Q6. Eligibility criteria	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q6a.1. Diagnostic criteria in Western medicine	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	100
	Q6a.2. Diagnostic criteria in traditional medicine	Y	N	Y	N	N	Y	N	N	Y	N	N	36.4
	Q6b. Types of acupuncture	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	63.6
	Q6c. Report measures for therapeutic effects	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q7. Information sources	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q8. Search	PY	PY	PY	PY	PY	PY	Y	Y	PY	Y	PY	27.3
	Q9. Study selection	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	90.9
	Q10. Data collection process	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	81.8
	Q11. Data items	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q12. Risk of bias in individual studies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q13. Summary measures	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q14. Synthesis of results	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q15. Risk of bias across studies	Y	N	N	Y	N	Y	Y	Y	Y	N	N	54.5
	Q16. Additional analyses	Y	N	Y	N	N	N	Y	Y	Y	Y	N	54.5
Results	Q17. Study selection	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q18. Study characteristics	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q18a. Describe details of "de-qi"	N	N	N	N	N	N	N	N	N	N	N	0
	Q19. Risk of bias within studies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q20. Results of individual studies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q21. Synthesis of results	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q22. Risk of bias across studies	N	N	Y	Y	N	Y	Y	Y	Y	N	N	54.5
	Q23. Additional analysis	N	N	Y	Y	N	N	Y	Y	Y	Y	N	54.5
Discussion	Q24. Summary of evidence	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q25. Limitations	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	81.8
	Q26. Conclusions	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Funding	Q27. Funding	Y	Y	N	Y	N	Y	Y	Y	N	Y	Y	72.7

TABLE 4 | Results of the ROBIS assessments.

Reviews	Phase 1	Phase 2				Phase 3
	Assessing relevance	Domain 1: study eligibility criteria	Domain 2: identification and selection of studies	Domain 3: collection and study appraisal	Domain 4: synthesis and findings	Risk of bias in the review
Wang et al. (2019b)	☺	☺	☺	☺	☹	☹
Lin et al. (2017)	☺	☺	☺	☺	☹	☹
Zou et al. (2016)	☺	☺	☺	☺	☹	☹
Xu and Xie (2015)	☺	☺	☹	☹	☹	☹
Cao et al. (2014)	☺	☺	☺	☺	☹	☹
Guo et al. (2008)	☺	☺	☺	☺	☹	☹
Wang et al. (2020)	☺	☺	☺	☺	☺	☺
Huang et al. (2019)	☺	☺	☺	☺	☺	☺
Zhou et al. (2017)	☺	☺	☺	☺	☺	☺
Zhou et al. (2015)	☺	☺	☺	☺	☺	☹
Lee et al. (2009)	☺	☺	☺	☺	☹	☺

☺, *low risk;* ☹, *high risk.*

in the treatment of AD, and the results showed that there was a significant decrease in ADL scores in the combined treatment group. Furthermore, one review (Wang et al., 2019b) reported that acupuncture plus medication was superior to medication alone for the ADAS-cog score.

DISCUSSION

SRs/MAs are considered the gold standard for assessing the effects of healthcare interventions, but their methodology must strictly comply with a series of guidelines to minimize the possibility of bias in answering a specific research question. That is, a high quality of SRs/MAs is crucial to ensure the validity, clarity, and accurate comprehension of evidence (Jadad et al., 1998; Balshem et al., 2011). In recent years, the number of SRs/MAs targeting the same topic has been increasing, but their quality is uneven, and their results are not always fully consistent. From the perspective of evidence-based medicine, this phenomenon might impair policy-making and healthcare decisions. Based on the above issues, the research methods of an overview of SR/MA has been proposed by experts in evidence-based medicine (Hunt et al., 2018). An SR/MA overview is a comprehensive research method for re-evaluating a comprehensive collection of SRs/MAs related to the same disease or health problem, and it enables more comprehensively integrating evidence, thus providing higher-quality evidence for clinicians. A literature search revealed that numerous SRs/MAs have been performed to clarify the efficacy and safety of acupuncture in the treatment of AD. However, their quality varied, and the results of these SRs/MAs have limitations. We conducted this systematic overview to synthesize the outcomes from multiple SRs/MAs and to evaluate their quality and attempted to address any inconsistent outcomes. To our knowledge, this overview was the first study to comprehensively evaluate SRs/MAs of acupuncture for AD, and some pivotal findings were found.

Summary of Main Findings

First, from this overview, we found that the methodological quality, reporting quality, risk of bias, and evidence quality of the included SRs/MAs were unsatisfactory. In AMSTAR-2, all included SRs/MAs were regarded as critically low quality, especially in items 2 (protocol registration), 4 (literature search strategy), 7 (literature screening), 13 (account for RoB), and 15 (publication bias). For reporting quality, inadequate reporting items focused on protocol registration, risk of bias, search, additional analyses, and risk of publication bias. For ROBIS, almost all included SRs/MAs were rated as high risk in phase 3, which results in a consequent decrease in the transparency of SRs/MAs and a consequent increase in the risk of bias. For GRADE, no high-quality evidence was found, and the risk of bias was the most common among the downgrading factors in the included SRs/MAs, followed by imprecision, inconsistency, publication bias, and indirectness. The assessment results of the above tools for the included SRs/MAs from different perspectives revealed common areas for improvement. First, almost all SRs/MAs did not register a protocol, which may result in a larger adjustment of the study process than expected, increasing the risk of bias and affecting the rigor of the systematic review. Second, 8 of 11 SRs/MAs provided only search keywords but no specific search strategy, likely contributing to making the comprehensiveness of the literature search difficult to ensure. Third, all included SRs/MAs did not provide a list of excluded trials with reasons for exclusion, which may undermine the transparency of the SRs/MAs and affect the reliability of their results. Furthermore, the included SRs/MAs have different degrees of shortcomings in the reasonable explanation of bias risk, the data synthesis process, publication bias, and funding support information, which affect the quality of SRs/MAs and reduce the utility of the evidence.

Second, no definitive conclusions can be drawn, and caution is required when acupuncture is recommended as an alternative treatment for AD based on the published results. Among

TABLE 5 | Results of evidence quality.

Reviews	Outcomes	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Relative effect (95% CI)	*P*-value	Quality
Wang et al. (2019b)	MMSE score	−1	−1	0	0	0	MD 0.76 (0.42, 1.10)	<0.0001	L
	ADAS-cog score	−1	0	0	−1	−1	MD −0.32 (−0.61, −0.03)	0.03	CL
	ADL score	−1	−1	0	−1	−1	MD −0.66 (−1.06, −0.27)	0.001	CL
Lin et al. (2017)	Effective rate	−1	−1	0	0	0	RR 1.16 (1.03, 1.31)	0.01	L
	MMSE score	−1	−1	0	−1	0	MD −0.99 (−3.45, 1.46)	>0.01	CL
Zou et al. (2016)	Effective rate	−1	0	0	−1	0	OR 1.15 (0.69, 1.91)	0.60	L
	MMSE score	−1	−1	0	0	0	MD 0.40 (−2.18, 2.97)	0.78	L
	ADL score	−1	−1	0	−1	0	MD 0.60 (−0.54, 1.74)	0.30	CL
	HDL score	−1	−1	0	−1	0	MD −0.20 (−1.19, 0.80)	0.70	CL
Xu and Xie (2015)	Effective rate	−1	0	0	0	0	RR 1.25 (1.14, 1.38)	<0.01	M
	MMSE score	−1	−1	0	−1	−1	MD 2.87 (0.64, 5.10)	0.01	CL
Cao et al. (2014)	MMSE score	−1	−1	0	−1	−1	WMD −0.61 (−1.34, 0.13)	0.11	CL
	ADL score	−1	−1	0	−1	−1	WMD −0.48 (−1.72, 0.76)	0.45	CL
Guo et al. (2008)	Effective rate	−1	0	0	0	0	OR 3.72 (2.73, 5.07)	<0.0001	M
Wang et al. (2020)	MMSE score	0	−1	0	0	0	MD 0.83 (0.14, 1.52)	0.02	M
	ADAS-cog score	0	−1	0	−1	0	MD −3.21 (−5.53, −0.89)	<0.01	L
	HDS score	0	0	0	−1	0	MD 0.58 (0.18, 0.99)	<0.01	M
	ADL score	0	0	0	−1	0	MD 0.21 (−0.74, 1.16)	0.66	M
Huang et al. (2019)	Effective rate	−1	0	0	0	0	RR 1.17 (1.06, 1.29)	0.001	M
	MMAE score	−1	−1	0	0	0	MD 1.96 (0.66, 3.26)	0.003	L
	ADAS-cog score	−1	−1	−1	0	−1	MD 3.56 (1.10, 6.03)	0.005	CL
	HDS score	−1	−1	0	0	0	MD −0.17 (−0.26, 0.90)	0.728	L
	ADL score	−1	−1	0	0	0	MD 1.99 (0.65, 3.34)	0.004	L
Zhou et al. (2017)	Effective rate	−1	0	0	0	0	OR 2.72 (2.04, 3.62)	<0.0001	M
	MMSE score	−1	−1	0	0	0	MD 2.10 (0.69, 3.51)	0.004	L
	ADL score	−1	−1	0	−1	−1	MD −3.59 (−7.18, 0.01)	0.05	CL
Zhou et al. (2015)	MMSE score	−1	−1	0	0	0	MD 1.05 (0.16, 1.93)	0.02	L
	HDS score	−1	0	0	−1	−1	SMD 0.09 (−0.28, 0.46)	0.62	CL
	ADL score	−1	0	0	−1	0	MD −2.80 (−4.57, −1.02)	0.002	L
	MMSE score	−1	0	0	−1	−1	MD 2.37 (1.53, 3.21)	<0.0001	CL
	ADL score	−1	0	0	−1	−1	MD −2.64 (−4.95, 0.32)	0.03	CL
Lee et al. (2009)	MMSE score	−1	0	0	−1	−1	MD −0.55 (−1.31, 0.21)	0.15	CL
	ADL score	−1	0	0	−1	−1	MD −1.29 (−1.77, −0.80)	<0.0001	CL

−1, downgrade; 0, not downgrade; CL, critically low; L, low; M, moderate; H, high; MMSE, Mini-Mental State Examination Score; ADL, Activities of Daily Living Scale; ADAS-cog, Alzheimer's Disease Assessment Scale-Cognition; HDS, Hasegawa's Dementia Scale; MD, mean difference; RR, relative risk/risk ratio; OR, odds ratio; WMD, weighted mean difference; SMD, standardized mean difference.

the included SRs/MAs, 8 of 11 reached positive results on acupuncture for AD, and the remaining came to a negative conclusion. Though the research topics of the included SRs/MAs were consistent, all were about acupuncture for AD, and they drew upon the same pool of articles. However, the research conclusions of these SRs/MAs were not consistent. Possible reasons for the inconsistency in conclusions are as follows. First, there was the same article pool among different SRs/MAs on the same topic, and when the number of articles in the article pool accounts for the majority of articles included in all of the SRs/MAs, they are more likely to draw consistent conclusions. We conducted an extraction analysis of all original RCTs included in the SRs/MAs of acupuncture for AD. It was found that 11 SRs/MAs included a total of 137 articles, of which 97 (70.8%) articles appeared only once in all of the SRs/MAs, which means that these 97 articles were not included in the common article pool. The same article pool contained 40 (29.2%) articles. Of these 40 articles, the same article was included from one to five times in the SRs/MAs, including 16 articles that were repeatedly included once, 6 articles that were repeatedly included twice, and 3 articles that were repeatedly included four times. This finding suggests that although the included SRs/MAs are all about acupuncture treatment of AD, their number of articles drawn from the same article pool is too small, and the overall differences among the included studies were large, so this may be one of the reasons why they came to inconsistent conclusions. Second, for GRADE, a risk of bias was the most common (29/33, 87.9%) downgrading factors in the included SRs/MAs, which means that the original trials included in the SRs/MAs were of poor quality. Assessing the methodological quality of the original RCTs, most

of them refer only to randomization and do not provide a random sequence generation method; most of the RCTs do not explicitly state that treatment allocation was concealed; only a few RCTs mentioned blinding, and most of the subjects and doctors were not blinded. Well-designed and implemented RCTs are considered the gold standard for evaluating interventions to minimize or avoid bias (Moher et al., 2010). Therefore, when the quality of the included RCTs is unsatisfactory, the risk of bias increases and may ultimately affect the authenticity of the results of SRs/MAs. Furthermore, it is worth noting that although most of the included SRs/MAs indicated that acupuncture appears to be an effective treatment for AD, most authors did not wish to draw definitive conclusions due to the small sample size of the included trials or their low quality. Therefore, more high-quality RCTs with large sample sizes are essential to determine whether acupuncture is beneficial for AD.

Third, all of the SRs/MAs included in this overview were conducted in two Asian countries (10 from China and one from Korea), and no unpublished studies using patients of different races were found, which may lead to a risk of publication bias. The included SRs/MAs were published in both Chinese and English languages, and the articles published in both languages contained negative and positive results. No significant risk of publication bias was found in the Chinese and English language publication forms. Acupuncture is currently used to relieve AD symptoms in many clinics in the West as well as the East, but there has been little research on its effectiveness; thus, this may affect the application of the results for an international population. Further studies on this topic should be carried out in both the East and the West in the future.

Implications for Future Research

Assessment of various aspects of the included SRs/MAs using the AMSTAR-2, PRISMA, and ROBIS assessments identified areas for common improvement. For example, the reviewer should register or publish the study protocol in advance to avoid any risk of bias and to ensure the rigor of the SR/MA process. In terms of the literature search and selection, the gray literature should be taken into account, and a list of excluded literature with explanations should be provided to guarantee transparency and to avoid publication bias. When conducting data analyses, if the heterogeneity is significant, subgroup analysis or meta-regression should be performed. Funding sources should be mentioned in the reviews because the results of business-funded studies might be biased toward the funder. Researchers should follow the relevant norms of the AMSTAR-2, PRISMA, and ROBIS assessments as much as possible to minimize the possibility of bias in answering a specific research question and to further improve the study quality. For GRADE, future RCTs should address the methodological issues through rigorous trial designs, reasonable appraisals, and critical analyses, and researchers should follow the basic guidelines for reporting clinical trials, such as the CONSORT statement and the STRICTA recommendations. Moreover, studies on this topic should be carried out in both the East and the West in the future.

Strength and Limitations

As an overview of acupuncture for AD, this study can provide a comprehensive evidence reference for clinical practice. Based on the current results, it may be useful for decision-making for AD treatment in the clinic. In addition, the evaluation process through AMSTAR-2, PRISMA, ROBIS, and GRADE revealed obvious limitations in SRs/MAs and RCTs, which may help guide future high-quality studies. However, it is also limited since the evaluation of quality is a subjective process, and different authors may have their own judgment on each factor, so the results may be different from other reviews, although our overview has been evaluated and checked by two independent authors.

CONCLUSION

This overview suggests that acupuncture is a promising complementary treatment for AD. However, the low quality of the SRs/MAs supporting these results is of concern. Future studies can be improved by adequately reporting the methodological details and adhering to the guidelines for conducting such reviews. The clinical effectiveness of acupuncture for AD should be tested in future RCTs with larger sample sizes.

AUTHOR CONTRIBUTIONS

JH and MS planned and designed the study. MW, SL, and XQ screened potential studies and extracted data from the included studies. JH and MS assessed the quality and summarized the evidence. JH wrote the first draft. YH revised the draft. All authors approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2020.574023/full#supplementary-material

REFERENCES

Atkins, D., Best, D., Briss, P. A., Eccles, M., Falck-Ytter, Y., Flottorp, S., et al. (2004). Grading quality of evidence and strength of recommendations. *BMJ* 328:1490. doi: 10.1136/bmj.328.7454.1490

Atri, A., Shaughnessy, L., W., and Locascio, J. J. (2008). Long-term course and effectiveness of combination therapy in Alzheimer's disease. *Alzheimer Dis. Assoc. Disord.* 22, 209–221. doi: 10.1097/WAD.0b013e31816653bc

Balshem, H., Helfand, M., Schünemann, H. J., Oxman, A. D., Kunz, R., Brozek, J., et al. (2011). GRADE guidelines: 3. Rating the quality of evidence. *J. Clin. Epidemiol.* 64, 401–406. doi: 10.1016/j.jclinepi.2010.07.015

Bodeker, G., Burford, G., Grundy, C., Ong, C.-K., and Shein, K. (2005). *WHO Global Atlas of Traditional, Complementary and Alternative Medicine*. Geneva: WHO.

Cao, F., Pan, X. L., Li, X. C., Chen, B., Jin, Y., and Huang, P. (2014). Systematic review and meta-analysis on acupuncture for Alzheimer's disease in Chinese population. *J. Nanchang Univ.* 54, 59–65. doi: 10.13764/j.cnki.ncdm.2014.09.016

Guo, X. X., Jin, H.-S., Huo, L., Zheng, J., and Zhou, X. M. (2008). Meta-analysis on acupuncture for treatment of dementia. *Chin. Acup. Moxib.* 28, 140–144. doi: 10.13703/j.0255-2930.2008.02.002

Huang, Q., Luo, D., Chen, L., Liang, F. X., and Chen, R. (2019). Effectiveness of acupuncture for Alzheimer's disease: an updated systematic review and meta-analysis. *Curr. Med. Sci.* 39, 500–511. doi: 10.1007/s11596-019-2065-8

Hunt, H., Pollock, A., Campbell, P., Estcourt, L., and Brunton, G. (2018). An introduction to overviews of reviews: planning a relevant research question and objective for an overview. *Syst. Rev.* 7:39. doi: 10.1186/s13643-018-0695-8

Jadad, A. R., Cook, D. J., and Jones, A. (1998). Methodology and reports of systematic reviews and meta-analysis: a comparison of Cochrane paper-based journals. *JAMA* 280, 278–280. doi: 10.1001/jama.280.3.278

Lee, M. S., Shin, B.-C., and Ernst, E. (2009). Acupuncture for Alzheimer's disease: a systematic review. *Int. J. Clin. Pract.* 63, 874–879. doi: 10.1111/j.1742-1241.2009.02043.x

Lin, L., Li, H., Shi, H. K., Huang, Y. L., Ding, X., and Li, M. Q. (2017). Meta-analysis of acupuncture for Senile Dementia. *Chin. J. Geriatrics* 37, 5014–5016. doi: 10.3969/j.issn.1005-9202.2017.20.026

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 4, 939–944. doi: 10.1212/WNL.34.7.939

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., et al. (2010). CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomized trials. *BMJ* 10, 28–55. doi: 10.1016/j.ijsu.2011.10.001

Reitz, C., and Mayeux, R. (2014). Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem. Pharmacol.* 88, 640–651. doi: 10.1016/j.bcp.2013.12.024

Shea, B. J., Reeves, B. C., Wells, G., Thuku, M., Hamel, C., Tugwell, P., et al. (2017). AMSTAR 2: a critical appraisal tool for systematic reviews that include randomized or non-randomized studies of healthcare interventions, or both. *BMJ* 358:4008. doi: 10.1136/bmj.j4008

Tampi, R. R., and Dyck, C. H. (2007). Memantine: efficacy and safety in mild-to-severe Alzheimer's disease. *Neuropsychiatr. Dis. Treat.* 3, 245–258. doi: 10.2147/nedt.2007.3.2.245

Wang, X., Chen, Y., Liu, Y., Yao L., Estill, J., Bian, Z., et al. (2019a). Reporting items for systematic reviews and meta-analyses of acupuncture: the PRISMA for acupuncture checklist. *BMC Complement. Altern. Med.* 19:208. doi: 10.1186/s12906-019-2624-3

Wang, Y. S., Xc, J., Fu, Q. H., and Pei, J. (2019b). Acupuncture combined with medicine for cognitive functions and life quality of patients with Alzheimer's disease: a systematic review. *Shanghai J. Tradit. Chin. Med.* 53, 19–25. doi: 10.16305/j.1007-1334.2019.01.005

Wang, Y. Y., Yu, S. F., Xue, H. Y., Li, Y., Zhao, C., and Jin, Y. H. (2020). Effectiveness and safety of acupuncture for the treatment of Alzheimer's disease: a systematic review and meta-analysis. *Front. Aging Neurosci.* 12:98. doi: 10.3389/fnagi.2020.00098

Whiting, P., Savović, J., Higgins, J. P., Caldwell, D. M., Reeves, B. C., Shea, B., et al. (2016). ROBIS: a new tool to asses srisk of bias in systematic reviews was developed. *J. Clin. Epidemiol.* 69, 225–234. doi: 10.1016/j.jclinepi.2015.06.005

Xu, J., Wang, J., Wimo, A., Fratiglioni, L., and Qiu, C. (2017). The economic burden of dementia in China, 1990-2030: implications for health policy. *Bull. World Health Organ.* 95, 18–26. doi: 10.2471/BLT.15.167726

Xu, X. T., and Xie, W. (2015). Meta-analysis on acupuncture combined with western medicine for treatment of Alzheimer's disease. *Modern. Tradit. Chin. Med. Mater. Med. World Sci. Technol.* 17, 836–840. doi: 10.11842/wst.2015.04.014

Zhou, J., Peng, W., Xu, M., Li, W., and Liu, Z. (2015). The effectiveness and safety of acupuncture for patients with Alzheimer's disease: a systematic review and meta-analysis of randomized controlled trials. *Medicine* 94:e933. doi: 10.1097/MD.0000000000000933

Zhou, S., Dong, L., He, Y., and Xiao, H. (2017). Acupuncture plus herbal medicine for Alzheimer's disease: a systematic review and meta-analysis. *Am. J. Chin. Med.* 45, 1327–1344. doi: 10.1142/S0192415X17500732

Zou, J. F., Xie, K., Guo, P. Y., Dong, X. S., and Zhao, F. (2016). Meta analysis of randomized controlled clinical trial about acupuncture in treating Alzheimer's disease. *Western J. Tradit. Chin. Med.* 29, 80–84.

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.

Copyright © 2020 Huang, Shen, Qin, Wu, Liang and Huang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

REVIEW
published: 07 December 2020
doi: 10.3389/fnagi.2020.583884

The Role of Chronic Inflammatory Bone and Joint Disorders in the Pathogenesis and Progression of Alzheimer's Disease

*Robert A. Culibrk and Mariah S. Hahn**

Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, United States

Late-onset Alzheimer's Disease (LOAD) is a devastating neurodegenerative disorder that causes significant cognitive debilitation in tens of millions of patients worldwide. Throughout disease progression, abnormal secretase activity results in the aberrant cleavage and subsequent aggregation of neurotoxic Aβ plaques in the cerebral extracellular space and hyperphosphorylation and destabilization of structural tau proteins surrounding neuronal microtubules. Both pathologies ultimately incite the propagation of a disease-associated subset of microglia—the principle immune cells of the brain—characterized by preferentially pro-inflammatory cytokine secretion and inhibited AD substrate uptake capacity, which further contribute to neuronal degeneration. For decades, chronic neuroinflammation has been identified as one of the cardinal pathophysiological driving features of AD; however, despite a number of works postulating the underlying mechanisms of inflammation-mediated neurodegeneration, its pathogenesis and relation to the inception of cognitive impairment remain obscure. Moreover, the limited clinical success of treatments targeting specific pathological features in the central nervous system (CNS) illustrates the need to investigate alternative, more holistic approaches for ameliorating AD outcomes. Accumulating evidence suggests significant interplay between peripheral immune activity and blood-brain barrier permeability, microglial activation and proliferation, and AD-related cognitive decline. In this work, we review a narrow but significant subset of chronic peripheral inflammatory conditions, describe how these pathologies are associated with the preponderance of neuroinflammation, and posit that we may exploit peripheral immune processes to design interventional, preventative therapies for LOAD. We then provide a comprehensive overview of notable treatment paradigms that have demonstrated considerable merit toward treating these disorders.

Keywords: Alzheimer's disease, osteoarthritis, osteoporosis, rheumatoid arthritis, inflammation, mesenchymal stem cells

OPEN ACCESS

Edited by:
Yu-Hui Liu,
Third Military Medical University, China

Reviewed by:
Sudhanshu P. Raikwar,
University of Missouri, United States
Olga Kokiko-Cochran,
The Ohio State University, United States

****Correspondence:***
Mariah S. Hahn
hahnm@rpi.edu

Received: *15 July 2020*
Accepted: *06 November 2020*
Published: *07 December 2020*

Citation:
Culibrk RA and Hahn MS (2020) The Role of Chronic Inflammatory Bone and Joint Disorders in the Pathogenesis and Progression of Alzheimer's Disease. Front. Aging Neurosci. 12:583884. doi: 10.3389/fnagi.2020.583884

INTRODUCTION

Canonical CNS Targets for AD Therapy

Pathological β-amyloid accumulations were among the earliest recorded physiological manifestations of AD (Tomlinson et al., 1970) and, along with neurofibrillary tangles, are considered a hallmark of AD-related neurodegeneration. It is therefore understandable that herculean efforts have been made in past decades to uncover their etiological contributions and

evaluate whether interventions targeting plaque accumulations ameliorate patient outcomes. While numerous animal studies have demonstrated significant dose-dependent attenuation of Aβ accumulation when such therapies were administered to a variety of transgenic disease models (Wiessner et al., 2011; Eketjäll et al., 2016), these results were rarely—if ever—recapitulated in clinical trials (Holmes et al., 2008; Salloway et al., 2011; Egan et al., 2018).

Interest in tau-targeting therapies has drastically increased in recent years, in part due to the overall failure of Aβ-directed treatment paradigms. Under neurotypical conditions, tau proteins surround neuronal microtubules in an organized lattice, affording structural integrity and facilitating inter-neuronal nutrient transport (Vershinin et al., 2007). In AD, however, through a series of complex immunological and neurophysiological events that transpire well before the onset of cognitive impairment (Braak et al., 2006), tau proteins undergo a series of post-translational modifications—including and chiefly hyperphosphorylation. These not only disrupt their standard microstructure but also promote aggregation into fragments which both directly and indirectly incite neuronal necrosis (Gong and Iqbal, 2008). While tauopathies present as a heterogenous mixture of paired helical filaments, straight filaments, twisted ribbons and oligomeric aggregates in the AD brain, oligomeric tau has recently emerged as the current research focus, owing to its strong cytotoxic effect in preclinical models and its prominence in early stages of AD and mild cognitive impairment (MCI) (Mufson et al., 2014; Guerrero-Muñoz et al., 2015). Unfortunately, as with Aβ-targeting therapeutics, a variety of promising drugs and immune therapies designed to target tau protein modification, prevent tau aggregation, or promote phagocytosis of cytosolic tau have either produced modest or negligible clinical benefits, resulted in adverse effects, or demonstrated suboptimal long-term pharmacokinetics. The results of these recent clinical studies, representing a broad gamut of tau-targeting therapies, have been comprehensively reviewed elsewhere (Congdon and Sigurdsson, 2018).

Of unclear significance to the neurodegenerative cascade in the AD brain is the generation, activation, and proliferation of disease-associated microglia (DAMs). Despite the intrinsic phenotypic heterogeneity of microglia, DAMs are functionally and pathologically distinct from their neurotypical counterparts: they express significantly lower levels of genes related to microglial homeostasis (including a host of purinergic receptors) and express far greater levels of genes associated with AD risk, including Apolipoprotein E *(APOE),* Lipoprotein lipase *(LPL),* and Triggering Receptor on Myeloid Cells *2 (TREM2)* (Keren-Shaul et al., 2017; Ofengeim et al., 2017). Likely due to sustained neuroinflammation, microglia proximal to sites of neuronal necrosis or pathological protein aggregation transition to a semi-activated state, demonstrating abrogated expression of homeostatic regulatory genes and robustly upregulated chemotactic cytokine activity. A TREM2-mediated secondary activation event then occurs, wherein microglia are rendered incapable of phagocytizing AD substrates, develop a "frustrated" phenotype, and subsequently contribute to the secretion of neuroinflammatory factors (Michaud and Rivest, 2015; Kabba et al., 2018). It is yet uncertain whether the net effects of DAMs in the early- and late-stage AD brain are beneficial yet insufficient, or altogether detrimental. While microglia-mediated neuroinflammation has garnered tremendous interest in recent years, no specific microglia-targeting therapy has reached clinical trials at the time of this review.

The dysregulation of microglial behavior in late stage AD has recently been credited to a series of missense mutations in TREM2-encoding genes at various loci. TREM2 is a transmembrane glycoprotein commonly expressed on granulocytes and monocytes. Its primary function is the modulation of leukocytic function; specifically, immunocyte activation following antigen recognition (Martin and Delarasse, 2018). A mutation at exon 2 of *TREM2*—which encodes a substitution of histidine for arginine at index 47 (R47H)—has been shown to result in abrogated TREM2 signaling potential. This loss of function prevents effective microglial phagocytosis of AD substrates and is believed to be one of the main sources of pathogenic effects in the AD brain (Doens and Fernández, 2014). Microglia-mediated neuronal degradation could be attributed to the aggressive encircling of synaptic clefts when microglia carrying the mutated variant interact with AD substrates—an effect further intensified by the microglial spread of insoluble tau. Indeed, TREM2 missense has been implicated in the spread of tau aggregates via cyclical failed phagocytosis and subsequent exocytosis, irrespective of synaptic transmission (Colonna and Holtzmann, 2017). Clinical deficits associated with the R47H mutation are apparent: patients carrying the missense variant exhibited lower-than-average performance on a series of cognitive assessments, particularly those involving a series of temporal memory tasks (Jonsson et al., 2013). The observed decline implicates TREM2 missense in the early cognitive deterioration that results in AD and is in line with the prevailing hypothesis that the disease manifests in accelerated mental aging. Nonetheless, despite the abundance of correlative genome-wide and preclinical studies involving this key microglial receptor, the specific mechanisms by which TREM2 missense propagates in a neuro-degenerative pathology are poorly understood and methodologies for targeted therapeutic intervention have yet to be realized. Moreover, while *TREM2* research has undoubtedly offered new insight into the underlying pathological processes of AD, its mutation, along with mutations in Presenilin 1 (*PS1*), Presenilin 2 (*PS2*), and Amyloid precursor protein (*APP*) account for <5% of all AD cases (National Institute of Aging, 2011).

Chronic Peripheral Inflammation—A Novel Paradigm for Therapeutic Intervention

The limited clinical success of the above treatment paradigms illustrates the need to investigate alternative features implicated in AD pathogenesis and exacerbation. Considerable evidence has recently emerged that chronic systemic inflammation originating in the periphery is associated with the neurodegenerative cascade in AD. Multiple systematic meta-analyses indicate elevated peripheral whole blood concentrations of inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), IL-1β, transforming growth factor beta (TGF-β), IL-12, and IL-18 in AD patients relative to age-matched healthy

controls (Swardfager et al., 2010; King et al., 2018; Walker et al., 2019a). Increases in C-X-C motif chemokine 10 (CXCL10), a chemokine that binds to C-X-C receptor 3 (CXCR3) and subsequently primes T-cell proliferation and natural killer cell maintenance, and vascular cell adhesion protein 1 (VCAM-1), a molecule involved in microvasculature permeability, have also been observed (Lai et al., 2017). Importantly, several studies have indicated that AD patients begin presenting aberrant pro-inflammatory cytokine profiles at *early* disease stages—levels which drop precipitously with disease progression (Engelhart et al., 2004; Kuo et al., 2005). Recent investigations have reiterated these findings in whole peripheral blood and plasma samples: in a heterogenous population of patients with Lewy-body dementia (LBD), advanced AD, and MCI, significantly higher levels of IL-1β, IL-4, and IL-2 were observed in MCI patients relative to healthy controls. The severity of cognitive decline—evaluated through performance assessments including the Mini Mental State Examination (MMSE) and Addenbrooke's Cognitive Examination Revised (ACE-R)—was found to be inversely proportional to serum levels of inflammatory markers (King et al., 2018). Longitudinal clinical studies further implicate early chronic peripheral inflammation in the preponderance of neurodegenerative disease: individuals with higher levels of pro-inflammatory cytokines in midlife are at a significantly higher risk for cognitive decline as they age (Walker et al., 2019b)—those who maintained aberrant levels for multiple decades were found to be especially prone to debilitating neurodegeneration via reduced brain volume and abnormal white matter microstructural integrity (Walker et al., 2017, 2018). Altogether, these data posit a putatively temporal relationship between chronic, systemic immune activation and cognitive deterioration and suggest that inflammation—which may occur decades before the onset of AD symptoms—exacerbates or directly mediates neurodegeneration.

Integral to our current understanding of the elaborate interplay between CNS immune activity and that of the periphery is the discovery that inflammatory cytokines are capable of traversing the blood-brain barrier (BBB) (Gutierrez et al., 1993; Banks et al., 1995). Where it was once believed that the tight junctions formed by capillary endothelial lining, astrocyte sheaths, and pericytes embedded in the capillary basement membrane conferred nearly complete immune privilege (Pollack and Lund, 1990), numerous studies indicate that circulating cytokines can induce signaling in the CNS through multiple mechanisms: (1) traversal of circumventricular organs (Buller, 2001; Roth et al., 2004), (2) vagus nerve stimulation (Borovikova et al., 2000; Das, 2007), and (3) direct cytokine-endothelial interactions, which result in tight junction opening and subsequent cytokine diffusion (Walker et al., 2019a). Indeed, systemic inflammation—whether caused by infection, chronic illness, or sepsis—has been identified as the primary catalyst of BBB permeability (Le Page et al., 2018), is shown to simultaneously upregulate proinflammatory (chiefly TNF-α, IL-1β, and IL-6) and downregulate immunosuppressive (IL-1ra, IL-4, IL-10, TGF-β) markers in whole blood, serum, and plasma samples (Su et al., 2019), and activates resident microglia, which in turn locally release proinflammatory cytokines that interfere with hippocampal neurogenesis (Chesnokova et al., 2016).

The World Health Organization collectively classifies chronic inflammatory diseases as the greatest threat to human health. As of 2017, 92.1 million Americans either have doctor-diagnosed arthritis or frequently report symptoms consistent with an arthritis diagnosis—a metric predicted to increase 49% by 2040. Moreover, previous estimates, which largely rely on doctor diagnoses, drastically undervalue the prevalence of inflammatory arthritis in younger population segments: indeed, a recent study found that 1 in 3 people aged 18–64 suffer from arthritis (Jafarzadeh and Felson, 2018). The animal studies and clinical investigations reviewed herein demonstrate that conditions like rheumatoid arthritis, osteoarthritis, and osteoporosis significantly increase the risk of and putatively accelerate cognitive decline in AD-related neurodegeneration. Given their ubiquity, it is imperative to consider whether effective treatment of these peripheral disorders before AD onset can forestall or mitigate AD-related neurodegeneration and subsequent cognitive decline. Therefore, while the pathogenic mechanisms of immune dysfunction for these conditions remain elusive, a thorough review covering the pathophysiology of these disorders, identifying current treatment paradigms, and discussing evidence of comorbid associations may provide new insight into how systemic inflammation may contribute to and mediate cognitive decline.

INFLAMMATORY AD COMORBIDITIES

Rheumatoid Arthritis (RA)

Pathophysiology

RA is a heterogenous chronic inflammatory disease that develops as a consequence of complex interactions between the innate and adaptive immune systems and is characterized by synovial hyperplasia leading to painful joint swelling and functional impairment (Catrina et al., 2017; Ghoryani et al., 2019). Increasing evidence attributes excessive neutrophil extracellular trap (NET) formation to the stimulation and maintenance of autoimmunity and inflammation in RA (Angelotti et al., 2017). Upon initiation of the typical inflammatory cascade, neutrophils aggregate, sequester, and stimulate degradation of invading pathogens including bacteria, viruses, and some microorganisms. During a process called "NETosis," which is thought to be elicited at least partly via local IL-8 secretion (Yipp and Kubes, 2013), neutrophils undergo "beneficial controlled suicide," releasing a milieu of intracellular components—nucleotides, proteins, histones, and elastases—that facilitate the formation of web-like structures that bind pathogens, rendering them inert (Takei et al., 1996). In both early and late-stage RA, neutrophils demonstrate a marked proclivity toward spontaneous NET formation (Corsiero et al., 2016; Berthelot et al., 2017). Additionally, neutrophils isolated from RA patients have been shown to favor NET formation *in vitro* following antigenic and inflammatory cytokine stimulation compared to those isolated from healthy controls (Angelotti et al., 2017). Recent investigations have established that, in RA, NETs promote pathogenic interferon gamma (IFN-γ)-producing T helper subtype 1 (Th1) cell immune

responses by increasing secretion of dendritic cell costimulatory molecules cluster of differentiation 80 (CD80) and CD86, as well as pro-inflammatory cytokine IL-6 (Papadaki et al., 2016). Another pathological mechanism that has recently gained traction is cyclical NETosis-mediated autoantibody production. RA neutrophils strongly express protein-arginine deiminase 4 (PAD4), an enzyme that catalyzes the conversion of specific arginine residues to citrulline, and as a result manufacture citrullinated forms of fibrinogen and histones H2A and H2B (Berthelot et al., 2017). These proteins, in turn, are recognized by antibodies to citrullinated protein antigens (ACPAs) which incite an autoimmune response that prompts inflammatory cytokine secretion, further neutrophil infiltration, and NET formation (Yipp and Kubes, 2013). Thus, NET-produced citrullinated proteins fuel the ACPA autoimmune response within the RA synovium, producing a positive-feedback loop that stimulates exponential immune activity.

Substantial research has implicated aberrant pro-inflammatory macrophage activity in the pathogenesis and maintenance of synovitis in RA. TNF-α is heavily upregulated in the synovial fluid (SF) of RA patients (Chu et al., 1991), is known to directly impair endothelial function by inciting production of nuclear factor κB (NF-κB) and reactive oxygen species (ROS) (Di Minno et al., 2015), and plays a pivotal role in disease pathogenesis (Ursini et al., 2017). Immunohistological assessments of excised synovial tissues reveal that macrophages are the principal TNF-producing cells in the inflamed RA joint (Udalova et al., 2016). Localized abundance of TNF-α and macrophage secretion of IL-8 and monocyte chemoattractant protein 1 (MCP-1) results in the recruitment of peripheral monocytes and neutrophils and activation of synovial fibroblasts, which perpetuate the inflammatory response via addition of IL-1β (Hamilton et al., 1993; Shigeyama et al., 2000). Activated synovial fibroblasts, in turn, produce receptor activator of nuclear factor κB ligand (RANKL) and macrophage colony stimulating factor (M-CSF) which elicit osteoclast proliferation and enforce pro-inflammatory macrophage polarization, respectively (Braun and Zwerina, 2011). The pathological macrophage secretome is likewise implicated in the dysregulation of adaptive immune processes: IL-23 stimulates the activation and proliferation of Th17 cells, putative regulators of autoimmunity in RA (Miossec and Kolls, 2012). Sustained macrophage IL-12 expression has been shown to upregulate Th1 activity (Aarvak et al., 1999). Indeed, the maintenance of the pro-inflammatory environment in RA appears to be due, at least in part, to the disruption of Th(1,17)/Treg balance (Wang W. et al., 2012), but further investigation is required to delineate the source(s) of this phenotypic shift. **Figure 1** summarizes these mechanisms.

Advancements in Current Treatments

The central role of TNF-α in synovial hyperplasia is emphasized by the clinical benefits conferred by anti-TNF drugs. While the therapeutic efficacy of conventional synthetic and biologic disease-modifying antirheumatic drugs (DMARDs) are thoroughly discussed elsewhere (Aletaha and Smolen, 2018), of particular note are antibody-based TNF antagonists Etanercept, Golimumab, and Certolizumab pegol. In a randomized, controlled clinical trial involving 234 patients with active RA, twice-weekly subcutaneous injections of Etanercept significantly reduced disease activity in a dose-dependent fashion (Moreland et al., 1999). In patients that had discontinued use of conventional TNF-α inhibitors due to lack of effectiveness, Golimumab significantly improved multiple patient outcomes, including swollen joint count, tender joint count, and patient assessments of pain, throughout the entire 24-week trial period (Smolen et al., 2009). Its increased effectiveness is attributed to its entirely human architecture and ability to bind both soluble and transmembrane TNF (Radner and Aletaha, 2015). Comparable therapeutic efficacy was observed in DMARD inadequate responders with Certolizumab pegol which, significantly, required half the administration frequency relative to standard DMARDs (Fleischmann et al., 2009).

Recent preclinical studies have explored more targeted approaches for correcting deviant mechanisms within the innate and adaptive immune systems in RA. As mentioned above, M-CSF is heavily upregulated in the SF of RA patients. Binding of the cytokine to its cognate receptor CSF1R is required for osteoclastogenesis and TNF-α induced osteolysis. Prophylactic administration of muAB5, a CSF1R antagonist, significantly reduced production of IL-6, CXCXL8, C-C motif chemokine ligand 2 (CCL2), CCL7, and matrix metalloproteinase 9 (MMP-9) in a collagen-induced arthritis (CIA) mouse model of RA (Garcia et al., 2016). Yin yang 1 (YY1) is a transcription factor that regulates multiple complex biological functions, has recently gained attention as a mediator of autoimmune disease, and is over-expressed in both RA patients and CIA mice. Lentiviral YY1 deactivation attenuated IL-6 production, reduced Th17 activity, and slowed disease progression in CIA mice (Lin et al., 2017). Jiang et al. targeted synovial angiogenesis and discovered that subcutaneous IL-35 administration attenuated arthritis in CIA mice via Th17 suppression, Treg stimulation, and inhibition of VEGF-mediated angiogenesis (Jiang et al., 2016).

Relation to AD

Numerous preclinical, systematic, and meta-analysis studies have implicated RA in the pathogenesis of LOAD. Raised whole blood and serum levels of several pro-inflammatory cytokines and adaptive immune players (chiefly TNF-α, IL-1β, IL-6, IFN-γ, Th1, and Th17) have been extensively studied for their involvement in the pathogenesis of both RA and AD (Ravaglia et al., 2007; Aletaha et al., 2008; Trollor et al., 2012; Pope and Shahrara, 2013; Schoels et al., 2013; Chi et al., 2017). A recent study found that inducing arthritis in APP/PS1 mice—the canonical murine model of AD—led to glial activation and exacerbation of amyloid pathology (Kyrkanides et al., 2011). Perhaps more compellingly, a nationwide cohort study found that the incidence of AD and other dementia-related illnesses is higher in RA patients than that of the general population (Lin et al., 2018). An independent nested case-control study of more than 8.5 million adults validated this disparity and found that it was maintained in both the young (average age 42.1 years) and the elderly (aged 65+) (Chou et al., 2016). In fact, the presence of any inflammatory joint disorder (OR:

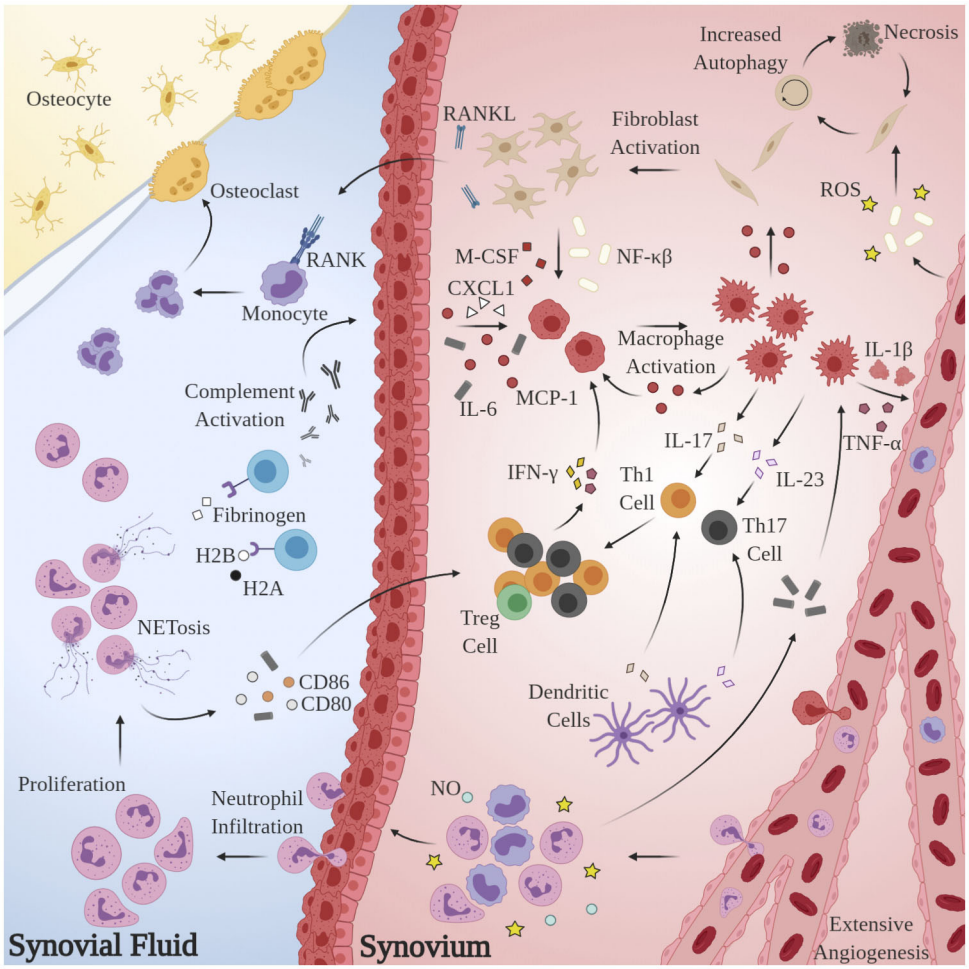

FIGURE 1 | Model for the generation and maintenance of chronic inflammation in RA. Pathological neutrophils manufacture citrullinated forms of fibrinogen and histones H2A and H2B which are recognized by proximal APCAs, prompting activation of the complement cascade and secretion of chemoattractant and pro-inflammatory cytokines CXCL1, IL-6, and MCP-1. Local macrophages are "activated" by this milieu, inciting extensive angiogenesis, activation, and proliferation of T-lymphocytes and synovial fibroblasts and further recruitment of circulating macrophages, monocytes, and neutrophils. Pro-inflammatory Th subsets dominate, owing largely to the byproducts of NETosis. Elevated RANKL production and osteoclast proliferation have also been observed.

1.96), but especially RA (OR: 2.77) is significantly associated with AD-related cognitive decline later in life—a correlation that remains significant when considering AD only and not general dementia (2.49) (Wallin et al., 2012). Interestingly, Vitturi et al. reported RA patients demonstrate evidence of cognitive impairment independent of canonical AD mechanisms earlier in life: RA patients scored significantly lower in MMSE and MoCA cognitive performance assessments relative to healthy controls ($p < 0.001$). General neuropsychiatric impairment was also found to be more prevalent in RA patients (59.5%) than in age-matched controls (17.1%; $p < 0.001$) (Vitturi et al., 2019). A recent systematic review recapitulated these findings and found that RA patients—predominantly women—exhibit significantly lower scores in attention, concentration, memory, and verbal function than age-matched controls. Finally, treatments including DMARD methotrexate (Judge et al., 2017) and prescription NSAIDs (Zandi and Breitner, 2001; Weaver and Carter, 2008), which target inflammation remission in RA patients, have been found to decrease risk of AD-related dementia particularly when administered early in disease. These data posit a putative, temporal relationship between chronic inflammation in RA and the onset and exacerbation of cognitive impairment in AD. Moreover, they highlight the importance of implementing treatments before AD symptom onset that target aging-associated systemic inflammation.

Osteoarthritis (OA)

Pathophysiology

OA is a progressive chronic inflammatory disease identified via gradual deterioration and loss of articular cartilage with concomitant structural and functional changes throughout the joint, including the synovium, meniscus, periarticular ligaments, and subchondral bone (Buckwalter and Mankin, 1998; Mobasheri and Batt, 2016). While chronic immune activation in

OA is considered low-grade relative to RA (Robinson et al., 2016), synovial explants and synovial fluid extracted from OA patients consistently demonstrate elevated levels of pro-inflammatory mediators TNF-α, IL-1β, IL-6, IL-8, IL-15, IL-17, IL-18, IL-21, PGE2, NO, and various complement components implicated in perpetuating immune activation (Blom et al., 2007; Robinson et al., 2016; Krishnasamy et al., 2018; Mora et al., 2018; Griffin and Scanzello, 2019). Unlike RA, the clinical manifestations of OA (pain, joint range of motion, radiographic pathology) are heterogenous; nevertheless, canonical features of inflammatory arthritis, including perivascular fibrosis and lymphoid follicles, are essentially conserved (Griffin and Scanzello, 2019). Moreover, like RA, synovial macrophages are considered key mediators of synovitis, synovial hyperplasia, osteophytosis, and inflammatory factor secretion (Blom et al., 2007; Bondeson et al., 2010). The number and concentration of macrophages is significantly upregulated in synovial tissue of OA patients and is known to be proportional to the severity of articular cartilage degradation (Kraus et al., 2016). Recent investigations posit a central role for macrophages in established destructive pathways: upon activation by damage-associated molecular patterns (DAMPs; the byproducts of cartilage degeneration) (Roh and Sohn, 2018), complement membrane attack complexes (MACs) (Ricklin et al., 2016), and pathological chondrocytes and synovial fibroblasts via toll-like receptors (TLRs) and CCR2, respectively, macrophages secrete IL-1, which induces activation and proliferation of matrix-metalloproteases MMP1, MMP3, and MMP13, as well as PGE2 (Bondeson et al., 2010; Griffin and Scanzello, 2019), cardinal mediators of cartilage catabolism. Various *in vivo* studies incriminate macrophages further: Blom et al. observed that macrophage depletion prior to OA induction via intra-articular administration of clodronate significantly decreased MMP-mediated cartilage damage in a CIA murine model (Blom et al., 2007). More recently, it was found that selective inhibition of macrophage pyroptosis—a novel apoptotic pathway implicated in OA (Vande Walle and Lamkanfi, 2016)—rescued synovial fibrosis and reduced inflammatory factor expression (Zhang L. et al., 2019).

The adaptive immune constituents in the OA synovium share many of the pathological features exhibited in inflammatory arthritis. $CD3^+$ T cells dominate synovial infiltrates, and $CD4^+/CD8^+$ cells propagate at levels comparable to those seen in RA synovial explants (Haseeb and Haqqi, 2013). Pro-inflammatory Th1 T cell subsets dominate their largely immunosuppressive (Th2) counterparts (Li et al., 2017) and directly contribute to the upregulation of inflammatory cytokines IL-2 and IFN-γ found in most OA patients. While no conclusive data exist identifying putative antigens responsible for autoantibody production, $CD20^+$ B-lymphocytes are found in significantly higher concentrations in sclerotic regions of subchondral bone (Weber et al., 2019a) and recent clonal analyses indicate OA B-cells undergo antigen driven activation suggestive of clonal selection (Da et al., 2007; Zhu et al., 2020).

Remarkably, osteoblasts have garnered increased attention as another key player in OA pathogenesis. Alterations in the physicochemical environment of subchondral bone may be linked to the progression of OA, as osteoblast phenotype is known to be modulated by a variety of stimuli including intraosseous pressure, fluid shear, mechanical loading, and local oxygen saturation (Hillsley and Frangos, 1994; Dodd et al., 1999; Warren et al., 2001). Indeed, preclinical studies in guinea pig models of OA have demonstrated that venous outlet syndrome and decreased perfusion directly precede and radiographically coincide with bone resorption and cartilage degeneration (Imhof et al., 1997; Watt, 2009). Additionally, Tanaka et al. observed that osteoblasts respond to changes in strain-induced fluid flow by synthesizing cytokines involved in the extracellular matrix (ECM) changes observed in OA including transcription factors c-Fos and Egr1, intracellular inflammatory mediators COX2, PGE2, and NO, and catabolic enzymes MMP1, MMP3, and MMP13 (Tanaka et al., 2005). Likely as a result of interactions with DAMPs and sustained exposure to the pro-inflammatory microenvironment observed in OA, osteoblasts undergo a discernable, pathological phenotypic shift that accelerates disease progression by interacting with key regulators of cartilage homeostasis: synovial chondrocytes. Conditioned media taken from OA-derived osteoblasts has been shown to enhance GAG release from normal cartilage (Westacott et al., 1997). Further, co-cultures of OA-derived osteoblasts and chondrocytes result in reduced expression of COL2A1, aggrecan, PTHrP/PTH-R, and SOX9 (Sanchez et al., 2005b), increased expression of OSF-1, MMP3, and MMP13 (Sanchez et al., 2005a), and induction of chondrocyte hypertrophy and matrix calcification via p38 and ERK-1/2 suppression (Aaron et al., 2017). See **Figure 2** for an overview of the pertinent pathological mechanisms.

Advancements in Current Treatments

Current clinical therapeutic goals for OA include inflammation and concomitant pain remission, ameliorating existing damage to or stimulating regeneration in articular cartilage, maximizing range of motion, and in general enhancing patient quality of life. Of the currently available treatments, topically and orally administered NSAIDs still represent the most prescribed medications for managing OA-related pain (Mora et al., 2018; Nakata et al., 2018), though other treatments paradigms have demonstrated some clinical success. Duloxetine, a serotonin and norepinephrine reuptake inhibitor originally prescribed for severe depression disorders, performed better than placebo at reducing pain and improving function when administered for at least 10 weeks (Citrome and Weiss-Citrome, 2012; Wang Z. Y. et al., 2015). Correction of dysfunctional pain pathways is considered the primary mechanism of action. Corticoids, another common therapy modality, exert anti-inflammatory effects by acting directly on nuclear receptors, decreasing production of IL-1, leukotrienes, prostaglandins (PGs), and MMPs (Levy et al., 2018). Significantly, >50 mg doses of prednisone (an NF-κB inhibitor) have been shown in multiple clinical trials to confer more lasting pain relief compared to other corticoid-based therapies (Bellamy et al., 2006; Law et al., 2015; Buyuk et al., 2017); however, a recent meta-analysis suggests that longitudinal corticoid administration may contribute to cartilage loss and degenerative OA pathology, suggesting that systemic anti-inflammatories possess limited efficacy in chronic conditions (Zeng et al., 2019).

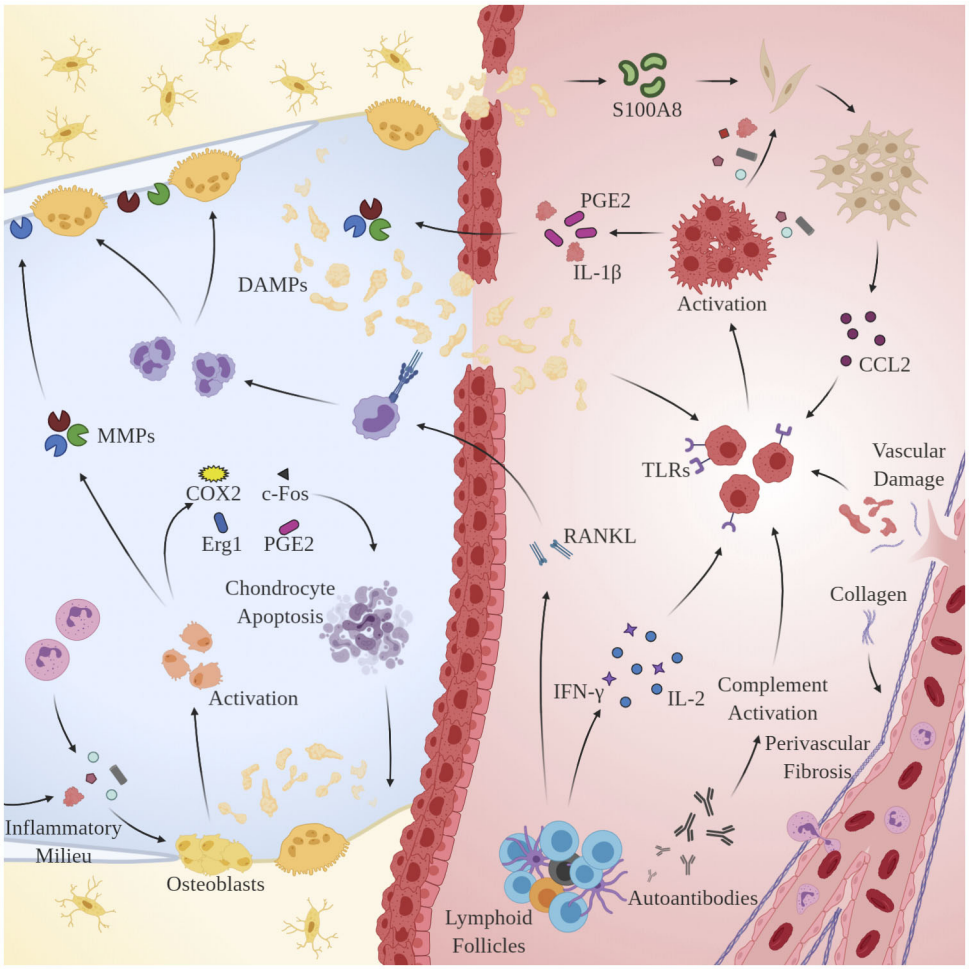

FIGURE 2 | Mechanisms of chronic immune hyperactivity in OA. Clinical features of inflammatory arthritis, including perivascular fibrosis and lymphoid follicles, are conserved. Upon activation by DAMPs, MACs, chondrocytes and synovial fibroblasts via TLRs and CCR2, respectively, macrophages secrete IL-1β, inducing proliferation of MMPs 1, 3, and 13, and PGE2 production. Th1 cells dominate and directly contribute to upregulation of inflammatory cytokines IL-2 and IFN-γ. B-cells undergo clonal selection and are implicated in RANKL production.

Hyaluronic acid (HA)—a glycosaminoglycan that provides viscous lubrication and shock-absorbing properties in healthy synovial tissue—is a common intraarticular supplement for the management of mild to severe OA (Altman et al., 2016). While some studies purport exogenous HA enhances endogenous HA and proteoglycan synthesis, promotes articular cartilage regeneration, and inhibits synovial production of pro-inflammatory cytokines (Migliore and Procopio, 2015), evidence for long-term clinical efficacy is conflicting (Richards et al., 2016; Altman et al., 2018; Pelletier et al., 2018) and the American Academy of Orthopedic Surgeons no longer recommends IA HA injection for clinical use (Jevsevar, 2013). Platelet-rich plasma—whole blood fractions prepared via centrifugation of autologous blood—may provide a viable alternative. Multiple favorable patient outcomes were observed in a series of randomized controlled trials (Cerza et al., 2012; Patel et al., 2013; Vaquerizo et al., 2013) due largely to its regenerative effect and anti-inflammatory potential (Shen et al., 2017).

Relation to AD

Systemic chronic inflammation has been implicated in the initiation and cyclical aggravation of a variety of age-related disorders including OA and AD (Weber et al., 2019b). Systematic reviews have identified multiple potential mechanisms through which the chronic low-grade inflammation in OA may contribute to AD-related neurodegeneration: (1) disruption of the BBB and subsequent influx of peripheral pro-inflammatory cytokines, (2) active transport of pro-inflammatory cytokines across the BBB, (3) a chain of activation events including brain endothelial cells, perivascular cells, and brain parenchymal cells, and (4) aberrant peripheral nervous system (PNS) activity (e.g., communications between peritoneal cavity and neuronal populations in the brain stem) (Banks et al., 2002; Konsman et al., 2002). Various studies have demonstrated significant overlap between the pathological cytokine profiles observed in OA and AD: one such work found that high-mobility group box 1 (HMGB1) and its cognate receptor for advanced glycation end products (RAGE) are found

at greatly elevated levels in both OA (Sun et al., 2016) and AD (Festoff et al., 2016). Multiple animal studies have linked OA to AD exacerbation and pathogenesis: induction of OA in APP/PS1 mice resulted in accelerated development of Aβ plaques and greater plaque deposition at later timepoints compared to OA^- controls (Kyrkanides et al., 2011). Moreover, transgenic mice expressing the human APOE ϵ4 allele—an allele associated with greater risk of LOAD onset—exhibited significantly greater synovial thickening and 32% more cartilage damage relative to APOE ϵ3 mice following 42 days of OA induction (de Munter et al., 2017). This suggests that patients carrying the pathological ϵ4 allele may be more susceptible to OA and other peripheral inflammatory diseases in addition to AD.

The results of epidemiological and longitudinal meta-analyses paint a similar picture. Age- and gender-adjusted cohorts of OA patients were found to be at a significantly greater risk for developing dementia later in life (OR: 1.36, $p < 0.0001$) (Weber et al., 2019b). A recent nationwide cohort study in Taiwan established a similar correlation, finding that OA patients were 25% more likely to have dementia (Adjusted HR: 1.25, $p < 0.001$) (Huang et al., 2015). In a study including 21,982 Appalachian adults aged 40 and older, participants with OA were found to be 80% more likely to report frequent memory loss independent of sleep or mood disorders (OR: 1.8, $p < 0.001$) (Innes and Sambamoorthi, 2018). Interestingly, in an investigation representing nearly 42.7 million Americans aged 65 or older, patient-reported pain, and the extent to which pain interfered with activities of daily living, was found to be significantly and positively correlated with the incidence of AD and related dementias, both in the presence (OR: 1.37) and absence (OR: 1.44) of OA ($p < 0.005$) (Ikram et al., 2019). Further investigation is required to decouple the contributions of pain to disease pathology.

Osteoporosis (OP)

Pathophysiology

OP is an age-related bone disorder characterized by reduction in bone mass and impairment of microarchitecture resulting in fragility fractures and a preponderance in activity of osteoclasts over osteoblasts (Pietschmann et al., 2016). Pathological bone resorption in OP is caused in part by changes in relative concentrations of RANKL and osteoprotegerin (OPG): RANKL is a type II transmembrane protein expressed by osteoblasts, proximal T-lymphocytes, and bone marrow stromal cells. Binding of RANKL to its cognate receptor RANK induces terminal differentiation of preosteoclasts and subsequent bone resorption. OPG, produced by osteoblasts (Hofbauer et al., 1999) and select B-cells, acts as a competitive inhibitor for RANKL (Awasthi et al., 2018). Under normal circumstances, completion of bone resorption initiates bone formation via recruitment of preosteoblast cells, during which factors including TGF-β, IGF-1, IGF-2, BMP-2, PDGF, and FGF inform differentiation of mesenchymal stem cells into osteoblasts (Clarke, 2008). Alternatively in OP, chronic pathological levels of pro-inflammatory cytokines and mediators promote bone resorption via osteoclast differentiation and activation, enhancement of RANKL expression, and the inhibition osteoblast survival (Clowes et al., 2005). Indeed, systemic inflammation is implicated in the dysregulation of multiple processes related to bone homeostasis: OP pathology propagates through a complex interplay of endocrine (estrogen; Almeida et al., 2017; Levin et al., 2018; Wu et al., 2018; Farr et al., 2019, parathyroid hormone; Camirand et al., 2016; Noordin and Glowacki, 2016; Williams et al., 2018; Lou et al., 2019, androgen; Shin et al., 2018; Joseph et al., 2019) immune (T-lymphocytes, cytokines), small molecule (Vitamin D; Ebeling and Eisman, 2018; Shill et al., 2019), and canonical signal pathway (Wnt/β-catenin; Johnson and Recker, 2017; Amjadi-Moheb and Akhavan-Niaki, 2019) regulators (see **Figure 3**).

Inflammatory cytokine-mediated bone resorption appears to occur through a variety of mechanisms. Both T- and B-lymphocytes have been shown to constitutively overexpress RANKL in pro-inflammatory conditions (Pietschmann et al., 2016; Srivastava et al., 2018). The inflammatory milieu produced by macrophages, dendritic cells, and local fibroblasts (TGF-β, IL-6, IL-1β, IL-23) incites proliferation of Th17 cells, which in turn promote bone resorption via RANKL expression (Dar et al., 2018c), upregulation of RANKL expression by osteoblasts and fibroblasts via IL-17 (Raphael et al., 2015), and exacerbation of the pro-inflammatory polarization of macrophages through secretion of IL-6, IL-17, TNF-α, and IFN-γ (Komatsu and Takayanagi, 2012). Activated B-lymphocytes, in addition to secreting TNF-α (Weitzmann, 2014), generates autoantibodies implicated in accelerating osteoclastogenesis (Pietschmann et al., 2016). TNF-α secreted by dendritic cells, macrophages, and $CD4^+/CD8^+$ cells is purported to act both indirectly by activating stromal cell secretion of RANKL, M-CSF, and IL-1, and directly by promoting osteoclast differentiation through activation of TGF-β (Al-Daghri et al., 2017). While data concerning inflammatory cytokine expression profiles in OP patients is limited, one study of over 100 post-menopausal OP patients found elevated pro-inflammatory cytokine levels (TNF-α, IL-1β, IL-6) were inversely correlated with expression of markers involved in inflammation remission (IL-4) and osteogenesis (osteocalcin) (Al-Daghri et al., 2017). STAT3 and the CXC (L1/R1) axis also merit further investigation: in an RA-induced murine population, STAT3 activation driven by pro-inflammatory cytokine expression led to increased RANKL-mediated bone loss, and STAT3 inhibition via cycloheximide significantly reduced expression of IL-6 family cytokines and RANKL (Mori et al., 2011). In a study comparing pre- and post-menopausal healthy controls to post-menopausal OP patients, CXCL1 concentrations were inversely correlated to bone mineral density and were directly proportional to bone turnover (TRACP-5b, NTx) and inflammatory (IL-1β, IL-6) markers (Chen et al., 2016), suggesting CXCL1 may be correlated to degree of OP development. Viral-mediated suppression of CXCR1 transcription resulted in a distinct reduction in RANKL-induced osteoclastogenesis (Wojdasiewicz et al., 2019).

Advancement in Current Treatments

Antiresorptive drugs are the most common therapy for osteoporotic patients and include selective estrogen response modulators (SERMs), bisphosphonates, and antibody-based RANKL inhibitors (Chapurlat and Genant, 2015). Estrogen is known to mediate bone turnover by directing calcium

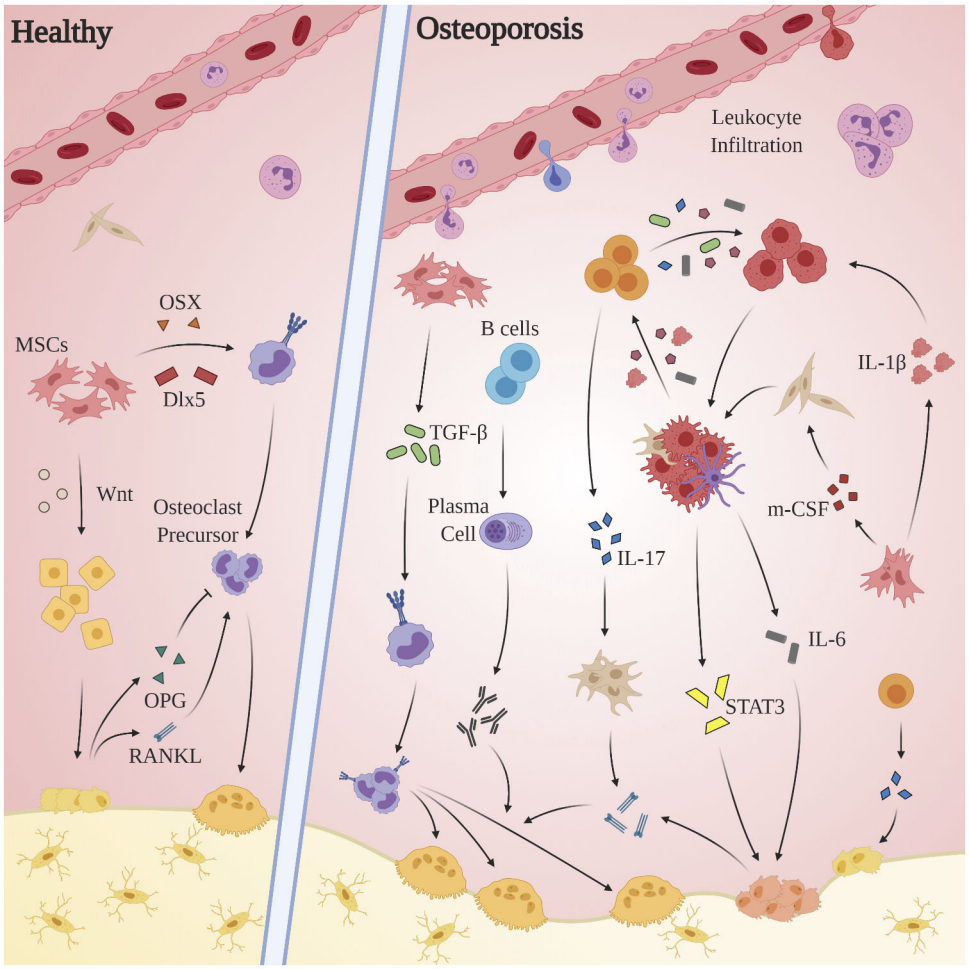

FIGURE 3 | Aberrant immune mechanisms in OP. Chronic pathological levels of pro-inflammatory cytokines and mediators promote bone resorption via osteoclast differentiation and activation, enhancement of RANKL expression, and the inhibition of osteoblast survival. The inflammatory milieu produced by macrophages, dendritic cells, and local fibroblasts incites proliferation of Th17 cells, which in turn express RANKL, promote upregulation of RANKL expression by osteoblasts and fibroblasts, and exacerbate the M1 polarization of macrophages. Multiple players contribute to osteoclastogenesis—both indirectly, as through activated stromal cell secretion of RANKL, and directly as by promoting osteoclast differentiation through activation of TGF-β. The STAT3 and CXCL1/R1 axes, while clearly of clinical significance, remain obfuscated and merit further investigation.

and Vitamin D homeostasis and conditionally promoting upregulation of cytokines that either incite or inhibit bone resorption (Lizneva et al., 2018). That OP is overwhelmingly presented by post-menopausal women (four times more common in women over 50 than similarly aged men) further solidifies the preponderance of estrogen in disease pathology. Thus, until recently, exogenous estrogen administration was a popular antiresorptive therapy: several controlled trials have demonstrated its ability to prevent bone mineral density (BMD) loss and reduce the risk of hip fractures by ∼30% (Chapurlat and Genant, 2015). Unfortunately, bone loss resumes at post-menopausal levels following cessation of therapy (Greendale et al., 2002) and prolonged treatment is linked to aberrant blood coagulant activity and significant breast cancer risk (Rossouw et al., 2009; Gennari et al., 2016).

The therapeutic mechanisms and longitudinal efficacies of SERMs, bisphosphonates, and RANKL inhibitors have been extensively reviewed elsewhere (Gennari et al., 2016). Moreover, the limited clinical potency and risk factors associated with existing treatments underscores the need to target alternative pathways contributing to disease pathology. Recent investigations have identified multiple treatments that remediate bone loss through modulating immune activity. B cell depletion via rituximab reduced synovial RANKL, expression of $RANK^+$ osteoblasts, and sera levels of bone turnover markers in patients with inflammatory OP (Wheater et al., 2011; Boumans et al., 2012). In ovariectomized and post-menopausal murine models of OP, administration of D-mannose (Liu et al., 2020), *Bacillus calusii* (Dar et al., 2018a), and *Lactobacillus acidophilus* (Dar et al., 2018b) attenuated bone loss, reduced expression of pro-inflammatory cytokines IL-6, IL-17, TNF-α, and IFN-γ, and increased expression of anti-osteoclastogenic factor IL-10 by stimulating the proliferation of Treg cells, restoring Treg/Th17 balance. Antibody-based TNF-α inhibition in a rat

model of OP elevated bone density, simultaneously increased and decreased OPG and RANKL expression, respectively, and enhanced osteogenic differentiation of endogenous stromal cells (Yu et al., 2019). Collectively, these and other studies purport inflammation as a viable target for therapeutic intervention in OP.

Relation to AD

While AD and OP initially appear pathologically and immunologically distinct, the results of numerous studies suggest a bidirectional and mutually antagonistic interplay between the two age-related disorders. Patients with AD exhibit, on average, significantly reduced hip BMD and retain a nearly 2-fold risk of hip fracture relative to healthy age-matched controls (Chen and Lo, 2017). Elevated TNF-α levels observed in AD patients, even before the onset of cognitive impairment, are known to induce osteoclastogenesis, inhibit bone formation by suppressing Wnt signaling, and accelerate cartilage destruction via production of MMPs and disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) (Osta et al., 2014). Bone turnover proteins osteocalcin (OCN), osteopontin (OPN), and sclerostin have been shown to exert potent neurological effects *in vivo* (Yuan et al., 2019a): OCN can traverse the BBB, enhance synthesis of serotonin, dopamine, and noradrenaline, inhibit GABA secretion, and bind to neurons in the brainstem, midbrain, and hippocampus (Oury et al., 2013). In a recent study, intravenous (IV) injection of plasma derived from $OCN^{+/+}$ 3 month-old mice rescued cognitive function of 16 month-old WT mice, but this therapeutic effect could not be replicated with $OCN^{-/-}$ mice, suggesting OCN may play a role in mitigating age-related cognitive deficits (Khrimian et al., 2017). OPN is found in higher levels in the plasma of AD patients (Comi et al., 2010; Carecchio and Comi, 2011), is known to enhance bone resorption (Luukkonen et al., 2019), and reduces Aβ burden in murine models of AD (Rentsendorj et al., 2018). Moreover, pathological variants of Aβ propagate in osteoporotic bone (Xia et al., 2013) and BMD has been shown to be inversely correlated with Aβ and APP expression in vertebral trabecular bone specimens ($r = -0.617$ and -0.531 for $A\beta_{42}$ and APP, respectively) (Li et al., 2014). *In vitro*, $A\beta_{42}$ enhances RANKL-induced bone resorption by silencing inhibitor of nuclear factor kappa B (IκB), subsequently enhancing NF-κB activity (Li et al., 2016). Finally, in AD, microglia-like cells originating from bone marrow traverse the BBB and migrate into the brain in a chemokine-dependent manner (El Khoury and Luster, 2008), giving further credence to the hypothesis that peripheral inflammation can directly contribute to pathological microgliosis and subsequent neuronal degradation.

ADDITIONAL MECHANISMS UNDERLYING SYSTEMIC PATHOLOGY

While no single theory exists concerning the underlying mechanisms of these systemic disorders, cellular senescence, dysregulation in peripheral nervous system activity, and a disruption in autophagic homeostasis are considered hallmark artifacts of arguably the most significant risk factor for all the conditions discussed above: aging. Cellular and immuno-senescence are highly conserved features in age-related inflammatory bone and joint disorders, as well as in CNS diseases including LOAD (Rodier and Campisi, 2011; Muñoz-Espín and Serrano, 2014). Cell cycle suppression genes are heavily upregulated with age and are implicated in the generation and maintenance of chronic systemic inflammation and exacerbation of disease pathology (Lebrasseur et al., 2015). Aberrant peripheral nervous system activity provides a physiological basis for the intimate, bidirectional relationship between bone and joint disorders and dysregulated neurological homeostasis. Not only are autonomic structures among the first impacted in neurodegenerative illness, but select neuropeptides highly expressed in bone are known to drive a host of osteo-homeostatic processes including bone reformation, hematopoietic progenitor cell (HPSC) niche maintenance, and innate and adaptive immune activity (Asada et al., 2013). Finally, global reduction in autophagy has been reported to exacerbate age-associated inflammation and accelerate the progression of degenerative diseases (Cuervo and Dice, 2000). Conversely, the maintenance of proper autophagic activity has been credited with heightened longevity and resistance to a host of age-related conditions (Arensman and Eng, 2018). Therefore, while complex disorders like AD result from a wide range of multifactorial mechanisms, a comprehensive understanding of these age-related phenomena is crucial to the development of effective interventional therapies.

Age-Related Cellular/Immuno-Senescence

Tissues affected in a variety of age-related diseases exhibit a preponderance of senescent cells characterized by cell cycle arrest, apoptosis resistance, and chronic secretion of preferentially pro-inflammatory molecules (Rodier and Campisi, 2011; Muñoz-Espín and Serrano, 2014). In conventional aging, cells assume a senescence-associated secretory phenotype (SASP) which is believed to contribute to age-related tissue inflammation (Lebrasseur et al., 2015). While SASPs surface from a variety of factors, including cell type, mechanism of senescence induction, and hormonal milieu (Wiley et al., 2016), inflammatory cytokines, including IL-6 and IL-8, are highly conserved in SASPs and are believed to play a crucial role in maintaining SASP signaling and senescence (Acosta et al., 2008; Coppé et al., 2008; Lasry and Ben-Neriah, 2015).

In AD, aggregating $A\beta_{1-42}$ peptides have been shown to directly trigger expression of senescence-associated marker β-galactosidase in oligodendrocyte precursor cells (OPCs) (Osso and Chan, 2019), indicating that senescent OPCs commonly observed in AD brains may be generated through direct stress from Aβ aggregates rather than, or in addition to, canonical replicative senescence mechanisms. Indeed, in 5XFAD murine models of AD, the expression of cell cycle suppression genes *p53*, *p16*, and *p21* were all upregulated in hippocampal homogenates. *P16* demonstrated the most significant discrepancy, increasing over 5-fold following the onset of pathology—a result consistent for both genomic and proteomic characterizations. Importantly, *p16* expression was inversely correlated with cognitive performance and

immunofluorescent staining revealed predominantly neuronal localization of the gene. These results were recapitulated *in vitro*: the administration of 10 μM oligomeric Aβ significantly upregulated *p16* (but not *p53*) in neuron monoculture (Wei et al., 2016). A similar senescent phenotype was discovered in aged APP/PS1ΔE9 mice and both short- and long-term administration of quercitin, a senolytic drug providing targeted ablation of senescent cells, conferred multiple benefits. Short-term treatment eliminated senescent OPCs, ramified pathological microglia proximal to plaques, and reduced IL-6 levels. Long-term therapeutic intervention before the onset of plaque pathology reduced overall hippocampal plaque burden later in disease and attenuated overall inflammatory cytokine secretion (Zhang P. et al., 2019).

P16 and *p21* are also known to be heavily upregulated in OP bone (Farr et al., 2019). In highly enriched cell populations derived from murine bone and bone marrow with no *in vitro* culture, expression of *p16* was greater in B- and T-lymphocytes, myeloid cells, osteoblast progenitors, osteoblasts, and osteocytes in 24 month-old mice relative to 6 month-old mice. Conventional aging was attributed to an over 500% increase in senescent osteocytes (Khosla et al., 2018). Indeed, as they age, bone marrow derived MSCs are known to not only lose their functional and regenerative capabilities, but also develop an increased propensity toward replicative senescence, contributing to chronic systemic inflammation and exacerbation of disease pathology (Qadir et al., 2020). Targeting senescent cells using genetic (Baker et al., 2011), senolytic (Yi et al., 2016), or inflammatory SASP-inhibiting compounds (Xu et al., 2015) for 2–4 months markedly improved bone mass and microarchitecture in trabecular and cortical bone.

Aberrant Peripheral Nervous System (PNS) Activity

Autonomic dysfunction—postulated to surface due to deficits in cholinergic function—is common in patients with dementia (Allan et al., 2007; Femminella et al., 2014). In an observational study, MCI patients were found to be ∼5.6 times more likely to demonstrate parasympathetic dysfunction than age-matched healthy controls (Collins et al., 2012). Central autonomic structures, including the hypothalamus, amygdala insula, and locus coeruleus are among the first neural structures afflicted in neurodegenerative illnesses like AD (Ahmed et al., 2015). These alterations culminate in markedly changed neuropeptide levels in the brains and cerebrospinal fluid (CSF) of AD patients—among them, reduced cortical calcitonin gene-related peptide (GCRP) (Choi et al., 2014) and vasoactive intestinal peptide (VIP) (Zhou et al., 1995; Sterniczuk et al., 2010), diminished CSF Substance P (SP) (Friedberg et al., 1991; Quigley and Kowall, 1991; Waters and Davis, 1997), and elevated norepinephrine (NE) (Gannon and Wang, 2019), tyrosine hydroxylase (TH) (Szot et al., 2006, 2007), dopamine β hydroxylase (DBH) (Giubilei et al., 2004), and neuropeptide Y (NPY) (Allen et al., 1984).

Importantly, these and other neuronal products are expressed in bone and have been shown to exert multiple immunomodulatory and osteo-homeostatic pathological deviations in the periphery (Asmus et al., 2000). CGRP increases proliferation and reduces apoptosis of osteoblast progenitors, enhances osteogenic gene expression, and stimulates osteoblast activity via cAMP and Wnt/β-catenin signaling (Mrak et al., 2010). A decrease in VIP levels induces a concomitant increase (>50%) in osteoclast-covered surface in rat mandible and calvariae (Elefteriou, 2005). SP inhibition exacerbates bone loss via decreased MSC recruitment, as evidenced by increased osteoblast activity and decreased OPG/RANKL ratio in ovariectomized murine models of OP (Elefteriou, 2005). Adrenergic (NE) signaling directly stimulates osteoclast differentiation through upregulation of RANKL by binding β2AR, a β-adrenergic receptor highly expressed in osteoblasts (Brazill et al., 2019). Inhibiting DBH signaling lowers sympathetic tone, induces osteoblast proliferation, and increases mean BMD in murine bones (Elefteriou, 2005). Deletion of NPY and its major receptor, Y2, in selective knockout mice stimulates osteoblast activity and increases both cortical and trabecular bone formation (Baldock et al., 2002).

Given the above, that the hypothalamus enjoys a central role in regulating bone homeostasis comes as no surprise. Neural-osteo interplay appears to occur through two distinct channels: (1) well-defined hormonal signals generated in the hypothalamus and subsequently processed in the pituitary; (2) efferent neuronal discharges originating from the hypothalamus and processed through the brainstem (Driessler and Baldock, 2010). Chronic stimulation of sympathetic outflow is known to have detrimental effects on bone: indeed, sustained β2AR signaling on osteoblasts and osteocytes disrupts their capacity to maintain the endosteal HPSC niche. Various immune players have been implicated in regulating local neuropeptide secretion (Serre et al., 1999) and, under certain conditions, uptake (Pirzgalska et al., 2017), but further investigation is required to determine whether these mechanisms can be exploited for designing therapeutic interventions.

Dysregulated Autophagic Homeostasis

Autophagic lysosome deficits occur early in AD onset and are hypothesized to be significant contributors to disease pathology (Zare-shahabadi et al., 2015). As early as 1967, abnormal aggregations of subcellular vesicles—subsequently identified as immature autophagic vacuoles—were reported to accumulate in dystrophic neurites in the AD brain (Suzuki and Terry, 1967). Aberrant lysosomal activity in AD resembles that induced by knocking out specific cathepsins or by administering lysosomal protease inhibitors. Prevailing theory suggests that failed protein and organelle catabolism by dystrophic autophagosomes induces a compensatory mechanism whereby autophagy is upregulated via ROS-dependent activation of type III PI3 kinase. Unfortunately, because downstream degradative pathways (chiefly lysosomal acidification) are already dysregulated, this only accelerates disease pathology. Promoting cathepsin activity via deletion of cystatin B (a cathepsin inhibitor) rescues autophagic-lysosomal pathology, reduces pathological Aβ accumulations, ubiquitinates proteins within autophagosomes, and reduces intraneural Aβ peptide (Yang et al., 2011). The pathological associations between

dysregulated autophagic processes and neurodegeneration in AD are emphasized by the similar clinical features observed in certain lysosomal storage disorders: neurofibrillary tangles are seen in human Niemann Pick Type C disease and mucopolysaccharidosis type IIB (Ryazantsev et al., 2007). Further, evidence suggests that APOE-ϵ4, considered a risk factor toward the onset of sporadic AD, may work in concert with Aβ peptides to incite lysosomal membrane disruption, release of lysosomal enzymes, and subsequent neuronal degradation. While counterintuitive, global *inhibition* of autophagy, when deviant as in neurodegenerative disease, may be beneficial (Tung et al., 2012).

While the role of autophagy in the pathogenesis of age-related chronic inflammatory diseases like OP and OA requires elucidation, autophagic processes are intimately ingrained in the maintenance of bone and cartilage homeostasis. Increased autophagy is assumed critical in osteogenesis due to the requirement for rapid organelle recycling, preservation of nutrients, and the increased environmental susceptibility to hypoxia inherent to the osteoblast-to-osteocyte transition (Manolagas and Parfitt, 2010). In articular cartilage, primarily characterized by low cell turnover and limited vascularization, autophagy is essential for maintaining cellular integrity, function, and survival. Indeed, expression of ULK1, Beclin1, and LC3, an inducer, regulator, and executor of autophagy, respectively, was found to decrease with GAG loss in both age-related and surgically induced OA (Caramés et al., 2010). Importantly, autophagosome formation is heavily upregulated in the superficial and medial zones of OA cartilage in early disease stages and apoptotic factors dominate with disease progression, suggesting a shift toward an apoptotic phenotype that may be due, at least in part, to failed autophagy similar to that observed in AD (Almonte-Becerril et al., 2010).

Recent studies have purported autophagy inhibition as a novel treatment paradigm for inflammation-mediated osteoclastogenesis. Overall resorptive activity decreased in osteoclast monoculture following bafilomycin (potent autophagy inhibitor) administration (Neutzsky-Wulff et al., 2010). These findings were later recapitulated in a murine model of bone loss induced by both ovariectomy and glucocorticoid treatment, where pharmacological (chloroquine) and genetic (*Atg7* deletion) suppression of autophagy in monocytes reduced osteoclastogenesis and subsequent bone resorption (Lin et al., 2016). Others, however, have reported the opposite: promoting autophagy in osteoblasts rescued viability following glucocorticoid treatment and reduced bone loss (Yao et al., 2016). Deletion of *FIP200* (involved in autophagosome formation) in osteoblasts induces osteopenia in rats (Yao et al., 2016). *Atg7* osteocyte knockout was shown to promote BMD loss in both male and female mice, not unlike that seen during natural aging (Onal et al., 2013). It appears that, overall, upregulation of autophagy in osteocytes and osteoblasts relieves oxidative stress, promotes cellular viability, and decreases bone resorption, while increased autophagy in osteoclasts exacerbates and accelerates bone and articular cartilage degradation in OP and OA. This precludes the use of systemic autophagy inhibitors for the treatment of these pathologies and underscores the need to develop vehicles for targeted stimulation or inhibition of autophagy in defined cell types. Moreover, the net effect of aging on autophagy on the microscopic scale requires further investigation: while age-related senescence contributes to a global reduction in autophagy (Caramés et al., 2010), the resulting accumulation of oxidative stress may induce autophagy predominantly in inflammatory mediators involved in disease pathology. A study delineating the propensity of different cell types toward increased autophagy following ROS stimulation at varied disease stages may provide some insight.

Pathological MicroRNA Profiles

Micro ribonucleic acids (miRNAs) are sentinels of post-transcriptional regulation of gene expression: by binding the 3'-untranslated regions (UTRs) of their target genes, miRNAs prevent translation—either through direct translation suppression or mRNA cleavage (Llave et al., 2002). Due to the ubiquity of 3'-UTR motifs and the wide gamut of complementary microRNAs discovered in recent years, these short nucleotide strands are estimated to target and modulate expression of over 80% of all genes in humans (Herrera-Espejo et al., 2019). Dysregulation of miRNA profiles has thus garnered considerable interest as a prominent driving force of several systemic pathologies, including those discussed herein. Indeed, a host of miRNAs regulate genes involved in production of amyloid plaques (Jahangard et al., 2020) and hyperphosphorylated tau (Femminella et al., 2015; Moncini et al., 2017), as well as those encoding cytokines canonically associated with chronic neuroinflammation (Ravari et al., 2017; Liu et al., 2019)—most of which are downregulated in the AD brain (Reddy et al., 2017b). Multiple target genes implicated in the inception and maintenance of chronic peripheral inflammation (Zhu et al., 2012; Bogunia-Kubik et al., 2016) and concurrent cartilage degradation (Park S. J. et al., 2013) and osteopenia (Kelch et al., 2017) likewise continue to be evaluated. While dysregulated miRNA profiles in AD (Herrera-Espejo et al., 2019), RA (Reyes-Long et al., 2020), OA (Sondag and Haqqi, 2016), and OP (Ko et al., 2020) have been thoroughly reviewed elsewhere, **Table 1** lists the miRNAs prominently referenced in recent literature, identifies whether they are up- or down-regulated in each condition, and provides a succinct overview of their respective targets and putative contributions to disease pathology. Inconsistencies in the expression of these miRNAs taken from different patient cohorts and procurement sites exemplify the complexity of miRNA biology: greater standardization and experimentation is required to uncover any direct correlation between those miRNAs differentially expressed in peripheral inflammatory bone and joint disorders and the onset and exacerbation of neurodegenerative disease.

MESENCHYMAL STEM CELL (MSC) THERAPY

Cumulatively, the results of the above studies suggest that effective treatment of RA, OA, and OP may delay and ameliorate AD-related neurodegeneration and that they may do

TABLE 1 | Dysregulated miRNA profiles implicated in the pathologies discussed herein.

Disease	miRNA	Expression vs. Control	Relevant Target(s) ‖ Putative contribution to pathology	Source(s)
AD	9	Down	*FGFR1, SIRT1, REST* ‖ Downregulation correlates directly with reduced cortical thickness and cognitive performance in AD patients	Kumar and Reddy, 2016; Maldonado-Lasuncion et al., 2019
	16-5p	Down	*APP* ‖ Inhibition leads to accumulation of APP, subsequent dysregulation of insulin pathway and heighted expression of Raf and/or NF-$\kappa\beta$	Liu et al., 2012; Kirouac et al., 2017
	29	Down	*BACE1*, *BIM* ‖ Expression inversely correlated with BACE1; Treatment with exogenous miR-29b has been shown to reduce expression of Aβ, and its pathological effects, *in vitro*	Jahangard et al., 2020
	34a-5p	Up	*p53* ‖ Heightened miR-34 expression associated with tau hyperphosphorylation; Downregulation has been found to rescue some cognitive abilities in murine models	Zovoilis et al., 2011; Femminella et al., 2015
	106	Down	*APP*, *ABCA1* ‖ Overexpression may inhibit amyloid-associated tau aggregation	Kim et al., 2012; Liu et al., 2016b
	107	Down	*CDK5* ‖ Heavily downregulated in the hippocampus and temporal cortex of AD patients; CDK5 involved in tau hyperphosphorylation	Shukla et al., 2012; Moncini et al., 2017
	125-5p	Up	*DUSP6, PPP1CA, Bcl-W* ‖ Upregulation associated with heightened neuroinflammation	Banzhaf-Strathmann et al., 2014; Reddy et al., 2017a
	132-3p	Down	*SIRT1, FOXO1, p250GAP* ‖ Reduction in miR-132 appears preclude neuron loss; *in vitro* miR-132 protects neurons against both Aβ and glutamate; In early AD, expression is increased and correlated to higher MMSE scores; In late AD, expression is abrogated in both AD brain and neural exosomes	Wong et al., 2013; Hadar et al., 2018; Cha et al., 2019
	146a	Up	*TLR2* ‖ Key regulator of AD-related immune response and implicated in multiple inflammatory pathologies including AD; Murine models demonstrate positive correlation between miR expression, senile plaque density, and cognitive impairment	Li et al., 2011; Ravari et al., 2017; Ansari et al., 2019
	155	Up	*c-Maf, IFNGR1, SHIP1* ‖ Regulates microglial inflammatory response; Heavily upregulated in 3xTg murine models of AD; elevated levels coincide with c-Jun expression, microglial and astrocyte activation, and upregulated secretion of inflammatory mediators; Has also been implicated in activation of a wide gamut of T lymphocytes	Song and Lee, 2015; Liu et al., 2019; Zhao et al., 2019
	181a/c/d	Down	*SPTLC1, c-Fos, SIRT1* ‖ Regulates cell proliferation, apoptosis, autophagy, mitochondrial function, and immune response; Loss increases serine palmitoyltransferase	Ouyang et al., 2012; Rodriguez-Ortiz et al., 2014; Indrieri et al., 2020
	212-3p	Down	*SIRT1* ‖ Correlations similar to those observed in 132-3p	Hadar et al., 2018
RA	16-5p	Up	*A2AaR* ‖ Results in upregulation and activation of NF-$\kappa\beta$ pathways; upregulation in Th17 cells; treatment with anti-TNF agents or DMARDs led to significantly increased expression	Reyes-Long et al., 2020
	23b-3p	Up/Down	*NOX4, TAB2, TAB3, IKK-*α ‖ Immunosuppressive via regulation of NOX4, which in turn inhibits expression of proinflammatory cytokines COX2, TNF-a, and IL-1β; Shown to be protective of GABAergic and motor neurons; Regulates NF-$\kappa\beta$ via TAB2, TAB3, and IKK-a (genes through which TNF-α, IL-17, IL-1β activate the NF-$\kappa\beta$ pathway); IL-17 creates dysregulated feedback loop between 23b-3p and NF-$\kappa\beta$, leading to increased expression of both	Zhu et al., 2012
	124-3p	Up/Down	*I*$\kappa\beta$*, MCP-1, SIRT1* ‖ Pathological downregulation leads to repression of inhibitors of $\kappa\beta$ (i$\kappa\beta$), ultimately increasing NF-kB expression	Chiu et al., 2020
	146-5p	Down	*TRAF6, JNK/CCL2, NF-*$\kappa\beta$ ‖ Serum expression significantly reduced in RA patients compared to controls, but found significantly increased in synovial tissue and synovial fluid-derived monocytes; expression induced by TNF-α and IL-1β	Bogunia-Kubik et al., 2016; Reyes-Long et al., 2020
	155-5p	Up	*SOCS1* ‖ Overexpressed in synovial joints of RA patients, leading to suppression of SOCS1 and triggering expression of TNF-α and IL-6	Bogunia-Kubik et al., 2016
	223-3p	Up	*E2F1* ‖ Predominantly expressed in Th cells; Overexpression in RA decreases E2F1 levels, leading to dysregulation of T-lymphocyte phenotype and subsequent autoimmunity	Pawlik et al., 2003; Fulci et al., 2010
OA	9	Down	NF-$\kappa\beta$ ‖ Overexpression has been implicated in reduction of NF-$\kappa\beta$ pathway signaling factors including NF-$\kappa\beta$, TNF-α, IL-1β	Bazzoni et al., 2009; Liu et al., 2016a
	34a	Up	*SIRT1* ‖ Upregulation results in concomitant decrease in SIRT1 expression; injection of a lentiviral vector encoding anti-miR-34a effectively abrogated OA progression in rat models	Yan et al., 2016
	130	Down	*TNF* ‖ Downregulated in OA patients, with concomitant upregulation in TNF	Li et al., 2015; Panagopoulos and Lambrou, 2018
	146a	Up	*CAMK2D, PPP3R2* ‖ Upregulation exacerbates proinflammatory cytokine secretion; miR-146a overexpression murine model demonstrated significantly higher TNF-α, IL-1β expression; TNF-α, IL-1β, and IL-17 administration appear to elevate miR levels in a positive feedback loop manner	Zhang X. et al., 2017

(Continued)

TABLE 1 | Continued

Disease	miRNA	Expression vs. Control	Relevant Target(s) ‖ Putative contribution to pathology	Source(s)
	149	Down	*MyD88, STAT3* ‖ Effective modulator of a wide variety of pro-inflammatory factors; inhibits hepatic inflammatory response via STAT3 pathway; Overexpression in macrophages linked to inhibition of NF-Kb, TNF-a, and IL-6	Xu et al., 2014; Zhang Q. et al., 2017; Tahamtan et al., 2018
	199	Down	*SMAD1, MAPK* ‖ Involved in promotion of chondrogenesis; Promotes osteoblastic differentiation of hMSCs via down- and up-regulation of SOX9 and aggrecan, respectively; Downregulation in OA results in increased expression of COX-2	Laine et al., 2012; Zhang et al., 2012
	558	Down	*COX2* ‖ Directly suppresses COX-2 mRNA activity and IL-1β induced catabolic events in chondrocytes to promote homeostasis	Park S. J. et al., 2013
OP	9-5p	Up	WNT3A ‖ Found to be highly expressed in OP patients relative to negative controls; Promotes adipogenesis and inhibits osteogenesis	Zhang H. G. et al., 2019
	21	Up/Down	*PDCD4* ‖ Upregulated in senile osteoporosis, which leads to c-Fos expression and subsequent osteoclastogenesis; Role in osteoblast differentiation is more controversial	Sugatani et al., 2011; Cheng et al., 2019
	23-3p	Up	*RUNX2-SATB2* ‖ Inhibition of SATB2 expression may incite osteoclastogenesis	Yavropoulou et al., 2020
	29b	Down	*HDAC4, RUNX2* ‖ Decrease in miR-29b causes concomitant increase in HDAC4 and subsequent reduction in osteoblastic differentiation	Li Z. et al., 2009; Bellavia et al., 2019
	100	Up	*BMPR2, SMAD1* ‖ Inhibits osteogenic differentiation of MSCs; *Ex vivo* study of osteoclasts taken from OP patients revealed an inverse correlation between miR-100 expression and BMD at the femoral neck	Fu et al., 2016; Bellavia et al., 2019
	124	Up	*Dlx2, Dlx3, Dlx5* ‖ MiR-124 overexpression has been shown to drive MSC adipogenesis; Significantly elevated in patients with low bone mass	Qadir et al., 2015; Yavropoulou et al., 2017
	125	Up	*CBFβ* ‖ Inhibition of osteogenesis via RUNX2 suppression; MiR-125 level found to be inversely correlated with patient femoral head BMD; Circulating miR-125 found to be significantly upregulated in osteoporotic patients	Huang et al., 2014; Liu et al., 2015; Panach et al., 2015
	133	Up	*RUNX2, CXCL11, CXCR3, SLC39A1, TCF-7* ‖ Suppression of osteoblastic differentiation via RUNX2 inhibition	Li et al., 2008; Wang Y. et al., 2012; Liao et al., 2013
	187	Down	*IL6, TNF* ‖ Elevated expression may lead to increased pro-inflammatory cytokine expression and inhibited osteogenesis	Garmilla-Ezquerra et al., 2015
	2861	Down	*HDAC5* ‖ Significantly downregulated in osteoporosis patients, leading to HDAC5-mediated inhibition of RUNX2 and BMD loss	Li H. et al., 2009

so through targeting mechanisms associated with aging. The limited longitudinal clinical benefits conferred by the currently administered pharmacological and antibody-based therapies highlights the need to investigate novel paradigms for the treatment of these disorders. Moreover, while conservative, systemic anti-inflammatory treatments may suffice for short-term improvement in patient-reported pain and range of motion, their role in ameliorating the underlying structural abnormalities in bone and cartilage remain limited (Jevotovsky et al., 2018). The introduction and usage of stem cells represents an important advance in regenerative cellular therapy: a number of works report preclinical benefits through differentiation of induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) into targeted cell types and these findings have been thoroughly reviewed elsewhere (Burke et al., 2016; Duncan and Valenzuela, 2017); however, their tendency to incite teratoma growth (Zakrzewski et al., 2019) as well as their immunogenicity (Deuse et al., 2019) has precluded their mainstream usage. MSCs possess excellent therapeutic potential for a broad range of chronic inflammatory and neurodegenerative conditions, owing to their accessibility relative to embryonic and induced pluripotent stem cells, their relatively predictable behavior, and their inherent ability to differentiate into osteoblasts, chondrocytes, and adipocytes. Typically isolated from bone marrow, adipose tissue, and more recently, the umbilical cord (Xu et al., 2018), MSCs mediate wound-healing by exerting pro-angiogenic, anti-fibrotic, and anti-inflammatory activity through direct cell-cell interactions and via the secretion of potent trophic factors (Shi et al., 2018). In addition to retaining oxidative stress resistance in inflammatory environments (Cui et al., 2017), MSCs modulate the activation, proliferation, and function of key mediators of both the innate and adaptive immune systems (see **Figure 4**). As such, they are uniquely suited to serve as the foundation for multiple therapies designed to ameliorate AD-related neurodegeneration, chronic systemic inflammation, and arthritis-associated bone and cartilage degradation. Over past decades, a plurality of studies have found that direct intracerebral (AD) and intraarticular (RA, OP, OA) injection of MSCs confers multiple benefits evidenced by three key features: (1) inflammation remission, (2) stimulation of neotissue formation, and (3) measurable improvements in behavioral outcomes. While these investigations are thoroughly reviewed elsewhere (Duncan and Valenzuela, 2017; Kim and Shon, 2020), we here discuss current developments toward maximizing the clinical usability and therapeutic potential of MSCs as they apply to AD, inflammatory arthritis, and OP.

Systemic MSC Injection

Systemic or intravenous (IV), MSC administration confers a key clinical benefit: minimization of the proximal tissue damage

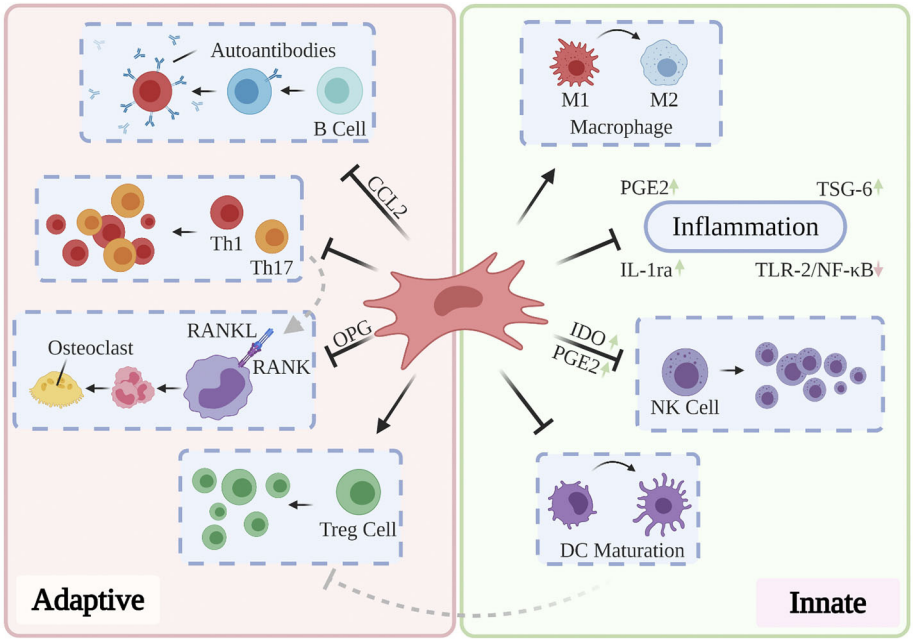

FIGURE 4 | Partly hypothetical model for the key immunosuppressive mechanisms of MSCs, which modulate the activation, proliferation, and function of prominent mediators of both the innate and adaptive immune systems. In addition to quelling production of local inflammatory cytokines via secretion of PGE2, IL-1ra, and TSG-6, primed MSCs have been shown to reverse the pro-inflammatory polarization of macrophages, inhibit proliferation of NK cells via IDO and PGE2, and prevent the maturation of DCs. Local and systemic administration of MSCs has also been shown to restore normal Th1/Th17:Treg ratios, prevent the production of pathogenic autoantibodies via CCL2, and inhibit osteoclastogenesis through OPG production. These and other findings contribute to the hypothesis that MSCs are uniquely suited to treat a variety of chronic inflammatory diseases.

inherent to local MSC injection boluses. This coupled with accumulating evidence that intravenously injected MSCs home to sites of interest (Sui et al., 2016) and retain therapeutic efficacy comparable to direct injection routes (Cui et al., 2017; Harach et al., 2017) renders systemic administration an appealing and viable treatment paradigm. In AD, intravenously injected MSCs have been shown to traverse the BBB and, importantly, display no evidence of eliciting a tumorigenic or immune response (Duncan and Valenzuela, 2017). While the mechanisms by which MSCs exert their therapeutic potential in the AD brain have yet to be clarified, multiple studies have shown that MSCs can differentiate into a plurality of neural cell types and enhance neurogenesis through the secretion of neurotrophic factors (Park D. et al., 2013; Garcia et al., 2014; Kim et al., 2015). Recent animal studies reflect this paradigm: intravenous transplantation of 2×10^6 human umbilical cord (hUC)-MSCs into 12 month-old Tg2576 mice improved cognitive performance as assessed by the Morris water maze 4 weeks after transplantation, attenuated oxidative stress, promoted neuronal proliferation, supported neurogenesis in the hippocampus, and increased expression of neurotrophic factors Sirt1, brain-derived neurotrophic factor (BDNF), and α-synuclein (SYN) (Cui et al., 2017). APP/PS1 mice given an equivalent treatment of bone marrow (BM)-MSCs demonstrated a significantly reduced escape latency in the Morris water maze, decreased concentrations of pathological $A\beta_{1-42}$ and beta-secretase 1 (BACE1), and attenuated expression of inflammatory cytokines IL-1, IL-2, TNF-α, and IFN-γ in whole blood samples (Wei et al., 2018). Collectively, IV MSC treatment appears to ameliorate cognitive dysfunction by promoting neurogenesis and synaptic plasticity, increasing secretion of neurotrophic factors, decreasing hippocampal oxidative stress, and modulating expression of Aβ-related genes.

Similarly encouraging results have emerged for its application in inflammatory arthritis. In a Phase Ia clinical trial including predominantly post-menopausal women, IV injection of 1×10^8 hUC-MSCs reduced whole blood levels of IL-1β, IL-6, IL-8, and TNF-α 24 h post-injection (Park et al., 2018). In another controlled trial including 53 patients, nearly 50% of those receiving a single injection of autologous adipose-derived (A)-MSCs achieved ACR20—a clinical benchmark for treatment efficacy—within the first month; however, benefits were found to diminish after 3 months, suggesting longitudinal efficacy would require repeated treatments (Álvaro-Gracia et al., 2017). In a macaque model of OA receiving 2 weekly injections of 1×10^7 allogenic MSCs, immunohistochemical staining revealed that the peripherally administered cells localized in and around the injured synovium (Fernandez-Pernas et al., 2017). Surprisingly, proliferation and activation of endogenous MSCs was heavily upregulated 2 weeks post-infusion, suggesting injected MSCs exert their therapeutic affects partly by recruiting and activating endogenous senescent MSCs. Finally, systemic infusion of 1×10^6 allogenic BM-MSCs via the caudal vein maintained

trabecular bone mass in glucocorticoid-challenged murine models of OP and promoted osteoblast and osteoprogenitor survival (Sui et al., 2016). As with previous studies, donor MSCs were found to specifically home and engraft to recipient bone marrow 4 weeks post-infusion.

Despite these encouraging findings, systemic MSC injection is not free of limitations. While IV administration is less invasive than established local injection paradigms and allows for the therapeutic cells to disseminate throughout the body, significant pulmonary MSC entrapment has been observed in a number of animal models (Fischer et al., 2009; Ankrum and Karp, 2010; Zheng et al., 2016). Before they attain systemic circulation, MSCs pass through and agglutinate in the lungs, largely due to interactions between the abundance of pulmonary fibronectin and vitronectin, and select adhesion integrins on the MSC surface (Wang S. et al., 2015). While promising advancements have been made in mitigating this phenomenon—be it via antibody-mediated integrin blockade (Wang S. et al., 2015) or strategic culturing practices during the *in vitro* expansion of MSCs from select sources (Nystedt et al., 2013)—pulmonary MSC entrapment represents a significant clinical obstacle which merits further investigation.

MSC Conditioned Medium

While MSCs exert potent immunosuppressive functions following exposure to an inflammatory microenvironment (Noronha Nc et al., 2019), recent studies suggest that longitudinal interactions with pro-inflammatory cytokines and their mediators may gradually reduce their clinical efficacy (Shi et al., 2018). Researchers have identified several attributes inherent to MSCs that limit their therapeutic efficiency in injections, including low survival rates in pathological microenvironments and the concomitant requirement for substantial overexpansion prior to injection, and considerable variability in donor properties, *in vitro* culture conditions, and clinical performance assessment procedures (Noronha Nc et al., 2019). These and other deficiencies have prompted investigation into the purely paracrine modality of MSC-mediated immunosuppression through utilization of MSC-conditioned medium (MSC-CM). Conditioned medium extracted from primed MSCs presents several hypothetical advantages: (1) CM can be manufactured in tightly controlled *in vitro* culture conditions optimized for mass production; (2) It can be freeze-dried, packaged, and subsequently transported far easier than live MSC populations; (3) A single CM batch preparation can be used for multiple therapeutic injections; and (4) CM vastly decreases the probability of host rejection and the aberrant immune response inherent to allogenic stem cell transplantation (Chen et al., 2018).

The results of numerous animal studies support the clinical efficacy of MSC-CM. Sustained microglial activation has been implicated in the pathogenesis and exacerbation of AD (Colonna and Holtzmann, 2017; Perea et al., 2018). Both murine carcinoma (BV2) and primary human microglia showed a ∼50% reduction in the secretion of pro-inflammatory cytokines TNF-α and IL-6 and increased IL-10 production following LPS activation when cultured in MSC-CM for 24 and 6 h, respectively (Ooi et al., 2015). A separate study found that MSC-CM protected BV2 microglia from Aβ_{35-45} challenge by reducing BV2 proliferation and apoptosis, promoting Aβ phagocytosis, correcting aberrant autophagic profiles, and upregulating expression of Aβ-degrading enzymes (Xu et al., 2018). Intraarticular injection of concentrated MSC-CM into antigen-induced arthritis (AIA) murine models reduced TNF-α sera concentration, attenuated aggrecan breakdown, increased production of IL-4 and FOXP3, and restored Treg:Th17 balance (Kay et al., 2017). Incubation of LPS-activated chondrocytes in concentrated MSC-CM decreased transcription of proinflammatory genes at both 24 and 72 h post-treatment, increased expression of ECM markers AGG and COL1, and increased global chondrocyte viability relative to untreated controls (Chen et al., 2018). Intriguingly, multiple reports have proposed that MSC-CM can induce a similar or stronger osteogenic effect than transplanted cells (Osugi et al., 2012; Chen et al., 2018). *In vivo* imaging and immunohistopathological staining of transgenic OP rats revealed that MSC-CM treatment groups displayed a larger area of newly regenerated bone and greater recruitment of native MSCs to the defect area compared to MSC-injected groups (Osugi et al., 2012). This finding adds further credence to the hypothesis that MSCs exert their regenerative effects partly through the mobilization of endogenous stem cells.

In the short term, MSC-CM exerts powerful neuroprotective, chondroprotective, and anti-inflammatory effects; however, the relatively short experimental timepoints of the above works (∼3–7 days) highlight the need for elucidating the longitudinal effects of MSC-CM treatment, dose requirements, and treatment frequency to produce optimal therapeutic outcomes. Moreover, tightly regulated manufacturing standards (e.g., basal media formulations, MSC incubation period, MSC seeding density, MSC age, donor, etc.) must be enforced to rigorously test clinical efficacy. Isolation and utilization of MSC-derived exosomes further diminishes potential immunogenicity concerns associated with MSCs and their derivatives. Indeed, MSC-secreted exosomes have recently been found to orchestrate—to a significant degree—MSCs' therapeutic mechanisms of action. This exciting field of MSC therapy has been thoroughly reviewed elsewhere (Mendt et al., 2019; Yin et al., 2019; Forsberg et al., 2020).

Biomaterial-Based Approaches

The field of tissue engineering is dominated by two primary strategies for creating regenerative tissue constructs: scaffolds and spheroids. While spheroid architectures intrinsically promote cell-cell interactions and cellular fusion into cohesive constructs that endogenously produce ECM, they often demonstrate inadequate mechanical properties, especially when used to regenerate load-bearing tissues. Alternatively, scaffolds are suitable for applications requiring compressive and torsional strength, native cellular infiltration, and neotissue deposition. In addition, scaffolds are remarkably versatile, enabling a broad range of mechanical and degradative properties, and can be tailored to release therapeutic molecules either via controlled release or surface immobilization (McMasters et al., 2017; Ovsianikov et al., 2018). Finally, combinatorial approaches utilizing multiple substrates afford advanced characteristics

like shape-memory and endogenous induction of targeted cell phenotypes. Engineered scaffolds thus represent an appealing paradigm for maximizing the therapeutic efficiency of MSCs, either through directed differentiation or stimulation of immunosuppressive phenotypes.

OP

Electrospun gelatin scaffolds, which demonstrate structural properties similar to native collagen, have been shown to promote MSC proliferation, survival, and osteogenic differentiation in the absence of exogenous growth factors (Chang et al., 2012). Following 21 days of osteogenic induction, BM-MSCs seeded in pure gelatin scaffolds demonstrated significantly increased mineralization relative to 2D controls (Moll et al., 2017). Poly(ϵ-caprolactone) (PCL) is used commonly in general scaffold design owing to its biocompatibility, biodegradability, low immune reactivity, optimal biomechanical properties and the ability to form complex 3D shapes; however, its usage in osteogenic induction is limited as it lacks the surface reactivity necessary for cell attachment. Application of composites like hydroxyapatite, and more recently, powdered oyster shells (OS), overcome these detriments by conferring hydrophilicity and topographical variance. Seeding MSCs on OS-coated PCL scaffolds enhanced MSC proliferation, significantly promoted osteogenic differentiation, increased long-term MSC viability, and demonstrated higher levels of alkaline phosphatase (ALP) activity and calcium deposition than bare PCL scaffolds (Didekhani et al., 2020).

Numerous studies have reported that applying HA coating to polymer meshes supports osteoblast function and osteogenic differentiation (Sato et al., 2006; Nguyen et al., 2013; Venugopal et al., 2013). The application of HA nanoparticles to electrospun PCL scaffolds dramatically increased hydrophilicity in the absence of plasma treatment, increased ALP activity by 20% compared to PCL/collagen controls, markedly increased mineralization, and incited noticeable changes in cellular morphology associated with osteogenesis (Venugopal et al., 2013). Indeed, HA and other major bone constituents including α- and β-tricalcium phosphate (TCP) are the most widely investigated ceramic scaffold supplements for osteogenic stimulation of MSCs; however, both lack vital elements related to bone metabolism and TCP generates alkaline degradation products that lead to proximal cytotoxicity and reduction in scaffold mechanical properties. Zinc-containing hardystonite (HS) has been shown in PCL scaffolds to surpass HA coating in mechanical strength, MSC proliferation, scaffold infiltration, ALP activity, and mineralization (Jaiswal et al., 2013). These effects were later recapitulated in electrospun PLLA scaffolds, which demonstrated increased osteonectin and OCN expression (Tavangar et al., 2018). Other studies have indicated that HA and TCP supplementation with various phytocompounds enhances their osteogenic effects: incorporation of 20 μM diosmin augmented ALP activity and calcium deposition, and increased expression of RUNX2, ALP, COL1, OCN, and osterix following 14 days of culture (Chandran et al., 2019). After 11 days, 10^{-8}–10^{-6} M icariin increased ALP expression and bone mineralization (Fan et al., 2011). 5–10 μM chrysin enhanced ALP activity, produced a marked increase in mineralization and calcium deposition, and sustained upregulation of RUNX2 expression (Menon et al., 2018).

OA

The mechanical stimuli produced in tissue microenvironments is known to direct differentiation of stem cells to terminal, specific fates. Hydrogel systems have demonstrated an excellent capacity to enhance chondrogenesis of MSCs by approximating the fibrous nanostructure of articular cartilage, but they are limited in their ability to simultaneously recapitulate the most crucial physiological properties of cartilage: compressive and viscoelastic moduli, porosity, and complex *in vivo* geometry. To illustrate, while the compressive modulus of human articular cartilage ranges from 240 to 1,000 kPa, that of typical hydrogel systems is lower by at least an order of magnitude (Beck et al., 2016). Bioengineers and material scientists are thus often presented with a "tightrope walk," where they must balance the high viscoelastic moduli needed to intrinsically promote chondrogenesis with requisite scaffold porosities enabling effective seeding, native cell perfusion, and nutrient diffusion. To that end, Aliabouzar et al. tested multiple pore geometries and found that small (700 $\times$ 690 μm) square pores stimulated significantly higher MSC proliferation than hexagonal pores (Aliabouzar et al., 2018). This finding coincided with those of other investigations which indicated that MSCs preferentially adhere to and proliferate more rapidly on larger curvatures (Knychala et al., 2013; Zhou et al., 2016).

In a recent study, various popular polymer chemistries were evaluated for their inherent potential to direct chondrogenic differentiation in seeded MSCs. Of the six formulations tested, poly-L-lysin-coated polydioxanone (PDO) and poly-L-ornithine (PLO) scaffolds best supported chondrogenic fate commitment, resulting in increased sulfated GAG concentration and chondrogenic matrix deposition. Notably, these effects persisted in the absence of supplemented chondrogenic growth factors (San-Marina et al., 2017). Scaffolds are frequently implanted to sequester therapeutic cells to injury sites. Poly-lactic-co-glycolic acid (PLGA) nano fibers (NFs) promote MSC proliferation and differentiation into osteoblasts under osteogenic culture conditions. Immunohistology revealed that MSCs seeded in columnar-shaped PLGA NFs enjoyed greater chondrogenic potential compared to 2D controls in equivalent media, as shown by heavily upregulated SOX9 and COL10A1 mRNA expression (Sonomoto et al., 2016).

AD

Intracranially transplanted cells typically generate sparse amounts of non-neuronal cells or unexpectedly die (Menon et al., 2018). Additionally, limitations in real-time imaging and human error often produce spatial disparities between injury and therapeutic injection sites. Although there currently exists no published literature pertaining to scaffold-based tissue regeneration in AD, various pre-clinical studies in models of related neurodegenerative disorders have produced encouraging results. Collagen scaffold implantation of MSCs in a rat model of TBI improved cell survival and neurite outgrowth *in vivo*, limited

distribution of MSCs to the transplanted region, improved brain metabolism, and resulted in improved neurite functional recovery compared to direct MSC injection (Menon et al., 2018). Following surgical resection-simulated chronic TBI in rats, fibrous collagen scaffolds obtained from bovine aponeuroses and seeded with hUC-MSCs improved locomotion, promoted neural regeneration and remyelination, induced proximal neurogenesis, and blocked astrocyte proliferation outside the lesion area (Wang et al., 2018). Self-assembling nanofiber scaffolds (SANs), characterized by a repetitive peptide sequence which mimics native ECM properties, have garnered recent attention in AD therapeutics owing to their inherent capacity to augment cellular adhesion, enhance axon growth impacts, and stimulate synapse development (Liedmann et al., 2012). Perhaps most importantly, SANs have been shown to innately interfere with APP processing by inhibiting BACE1 signaling, reducing the expression of $A\beta_{1-40}$ and $A\beta_{1-42}$ in APP/PS1 transgenic mice.

ALTERNATIVE TREATMENT PARADIGMS

As of this review, MSCs are a predominant non-pharmacological therapy applied toward peripheral chronic inflammatory conditions including RA, OA, and OP. The plurality of investigations reviewed herein support their usage, owing to their tissue regenerative properties and their intrinsic propensity to drive inflammation remission; unfortunately, current MSC procurement and administration methods limit their therapeutic efficacy and pose multiple clinical challenges. Firstly, the accepted clinical procedure for the harvesting and intra-articular injection of autologous MSCs is invasive—often entailing a prolonged recovery period (Harrell et al., 2019). Moreover, MSC injections are only approved for those with severe disease. When the procedure is ultimately approved, IV-injected MSCs have been shown to home to therapeutic areas of interest (Sui et al., 2016); however, undesired cell scattering is inevitable and degrades therapeutic efficiency (Zheng et al., 2016). Finally, few of the clinical studies enumerated above involve sufficiently long investigatory timepoints and thus fail to assess the longitudinal (>6 months) effects of MSC administration.

While drawing attention to the prevailing need to investigate novel methods for maximizing the clinical utility of MSCs is a focus of this review, a plurality of other therapeutic paradigms are under continual development and demonstrate tremendous potential for treating and/or modeling the complex, multifactorial conditions described herein. Following their discovery in the seminal works of Takahashi et al. (2007), iPSCs have contributed to astounding advancements in personalized medicine, disease modeling, and cellular therapy; indeed, iPSCs have been used as model system and treatment paradigm for AD (Devineni et al., 2016; Tcw, 2019; Penney et al., 2020), RA (Cassotta et al., 2020), OA (Dubey et al., 2018; Nakayama et al., 2020); and OP (Paspaliaris and Kolios, 2019; Rana et al., 2019). iPSCs retain several theoretical advantages over MSCs when applied to cellular therapies: in addition to being capable of unrestrained growth, they demonstrate relatively minimal immunogenicity and can be differentiated into a wide gamut of specific cell niches. When coupled with recent advances in genome editing, iPSCs enable interrogation of the consequences of various genetic and environmental perturbations in tightly controlled settings. While their embryo-derived counterparts, ESCs, represent the ideal source for cellular differentiation studies, they are generally considered unsuitable for clinical treatments due to their low differentiation efficiency, observed overgrowth within tissue grafts, tumorigenicity, and the host of ethical and safety concerns associated with their procurement and usage (Rana et al., 2019). Interestingly, iPSC-derived MSCs have gained abundant interest in recent years, as they integrate the benefits of both iPSCs and MSCs: autologous somatic cells can be harvested via relatively non-invasive procedures, and differentiated MSCs ostensibly confer immunosuppression and tissue regeneration with minimal risk of eliciting a host immune response (Khan et al., 2019).

Systems for targeted genomic modification have likewise gained considerable traction—particularly after the discovery of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 paradigm (Jinek et al., 2012). The Cas9 endonuclease, when paired with a single guide (sg)RNA, can efficiently cleave specific sites of double-stranded (ds)DNA, ultimately rendering specific gene segments inert (Lino et al., 2018). These gene knock-out models have been employed extensively toward refining searches for genetic disease susceptibility loci. In preclinical models of AD, most CRISPR-Cas9 mediated therapies are directed toward inhibiting the neurotoxic form of $A\beta$ proteins and deactivation of γ-secretase activating protein (Karimian et al., 2020), and have demonstrated encouraging results toward their therapeutic application in both FAD and LOAD. The CRISPR-Cas9 system has also been utilized to generate numerous knockout models of inflammatory arthritis and OP. Following the identification of putatively pathological gene segments via genome-wide association studies, these genes of interest are either deactivated, modified, or spliced into model systems (often incorporating iPSCs) to faithfully recapitulate the diseased phenotype in controlled *in vitro* and *in vivo* settings—these findings have been thoroughly reviewed elsewhere (Adkar et al., 2017; Ding and Orozco, 2019; Wu et al., 2019; Yuan et al., 2019b). The simplicity, low cost, and high cleavage efficiency of CRISPR-Cas9 compared to earlier Transcription Activator-Like Effector Nuclease and Zinc Finger Nuclease based systems is appealing; however, its limitations merit serious consideration and currently preclude its use in mainstream clinical settings. Firstly, Cas9 is known to generate off-target modifications: a few mismatches distal to the protospacer adjacent motif do not prevent activation of the CRISPR-Cas9 system. Screening alternative Cas orthologs with enhanced specificity and target range has been attempted to mitigate this artifact (Wu et al., 2019). Founder mosaicism is another boundary: in knockout and transgenic models of disease, CRISPR-Cas9 components are injected into the fertilized zygote and continuously target and cleave genes during embryonic development, often causing mosaicism in the introduced mutations. Certain strategies are being investigated to combat mosaicism, including quickening the editing process (introducing Cas9 at very early zygote stages), shortening the

longevity of Cas9, and CRISPR-mediated germline modification (Mehravar et al., 2019); these investigations are ongoing.

The conditions discussed in this review are complex and multifactorial, resulting from a wide array of genetic, epigenetic, and environmental factors; however, given that specific gene segments have been shown to drastically increase the risk and severity of these conditions, gene therapy has recently emerged as another exciting avenue of research. Gene therapy entails the delivery of therapeutic genes via specialized carriers, or vectors, that are typically viral, polymeric, or lipid-based in design (Deviatkin et al., 2020). Viral vectors have historically enabled remarkably stable and longitudinal transgene expression, but also demonstrate a host of safety concerns, including the risk of insertional mutagenesis inherent to retroviruses and activation of innate and adaptive immune mechanisms. Polymeric and lipid-based vector systems effectively eliminate these safety concerns, but are generally overlooked due to their comparatively low induction of transgene expression (Young et al., 2020). As with CRISPR-mediated treatments, the vast majority of gene therapy strategies for AD involve inhibition of the pathological variants of Aβ peptides, either through functional gene knockouts, Aβ immunization, or viral-mediated overexpression of genes encoding for enzymes that efficiently degrade Aβ (Choong et al., 2016). Adeno-associated viruses (AAVs) are the most popular vehicles for genetic therapies, owing to their proven efficacy and safety in a large number of animal models (Naso et al., 2017). Indeed, AAV-mediated enforcement of osteogenic and chondrogenic gene overexpression in transplanted somatic cells and infusion of exogenous recombinant growth and survival factors appears to be the focus of current clinical investigations in arthritis (Deviatkin et al., 2020; Young et al., 2020) as well as OP (Ball et al., 2018). Unfortunately, the results of multiple preclinical and clinical trials have made it yet unclear whether viral-mediated delivery of growth/survival factors is beneficial in these conditions (Ball et al., 2018; Honig, 2018; Deviatkin et al., 2020). Nonetheless, these drug and gene delivery vehicles—in conjunction with CRISPR-Cas9 and iPSCs—enable the development of combinatorial therapeutic strategies that bring us ever closer to truly personalized medicine.

CONCLUSION

Accumulating evidence from preclinical, clinical, systematic, and meta-analysis studies reports that peripheral chronic inflammatory conditions including rheumatoid arthritis, osteoarthritis, and osteoporosis may contribute to AD pathogenesis and exacerbate inflammatory neurodegeneration with disease progression. While the mechanisms underlying these disease pathologies remain elusive, chronic inflammation is clearly implicated as a predominant driving force of bone, cartilage, and neuron degeneration, and numerous immunosuppressive therapeutic agents have produced positive clinical outcomes in AD—especially when administered prior to the onset of cognitive impairment. The chronic systemic inflammatory conditions discussed herein are prevalent—crucially, even among the young—and therefore merit serious consideration as significant factors contributing to AD pathogenesis. Moreover, the limited clinical success of treatments geared toward canonical AD targets in the CNS illustrates the need to consider more holistic approaches toward generating interventional therapies. Given the above data, we submit that effective treatment of these prominent peripheral immune disorders in early- to mid-life may significantly decrease the risk of and ameliorate inflammation-mediated cognitive decline in AD. MSCs are uniquely suited to serve as the foundation for multiple therapies designed to address these aberrant aging-related inflammatory conditions due to their robust immunosuppressive properties, their unique ability to activate and recruit senescent cells, and their excellent accessibility relative to their embryonic and pluripotent stem cell counterparts. Nonetheless, current administration methods limit their clinical and longitudinal efficacy, and increase the risk of donor site morbidity and host rejection of transplanted allogenic cells. Future studies, therefore, should investigate methods of maximizing the therapeutic efficiency of MSCs and their conditioned medium via isolation and concentration of select paracrine factors and combinatorial biomaterials to improve MSC localization to diseased regions of interest. Toward this end, decoupling the direct cell-cell and paracrine mechanisms by which MSCs exert immunomodulation is crucial. Emerging technologies and treatment paradigms such as CRISPR-Cas9 and vector-mediated gene therapy should be utilized in conjunction with MSCs to generate combinatorial, personalized therapies. Indeed, a holistic consideration of the contributions of peripheral immune processes to changes in the CNS may incite a paradigm shift in our understanding of AD pathogenesis and treatment.

AUTHOR CONTRIBUTIONS

RC performed all data acquisition, interpretation, and manuscript drafting. MH assisted with data interpretation and performed manuscript revision. All authors contributed to the article and approved the submitted version.

FUNDING

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE 1744655.

ACKNOWLEDGMENTS

The author would like to thank MH and Dr. Ryan Gilbert for providing continued guidance and critical feedback. Dr. Timothy Kamaldinov and Mr. Ahmad Arabiyat offered valuable organizational and conceptual assistance throughout the drafting of this work. Their contributions substantially improved the clarity and impact of this manuscript.

REFERENCES

Aaron, R. K., Racine, J., and Dyke, J. P. (2017). Contribution of circulatory disturbances in subchondral bone to the pathophysiology of osteoarthritis. *Curr. Rheumatol. Rep.* 19:49. doi: 10.1007/s11926-017-0660-x

Aarvak, T. T., Chabaud, M., Källberg, E., Miossecdagger, P., and Natvig, J. B. (1999). Change in the Th1/Th2 phenotype of memory T-cell clones from rheumatoid arthritis synovium. *Scand. J. Immunol.* 50, 1–9. doi: 10.1046/j.1365-3083.1999.00581.x

Acosta, J. C., O'Loghlen, A., Banito, A., Guijarro, M. V., Augert, A., Raguz, S., et al. (2008). Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* 133, 1006–1018. doi: 10.1016/j.cell.2008.03.038

Adkar, S. S., Brunger, J. M., Willard, V. P., Wu, C.-L., Gersbach, C. A., and Guilak, F. (2017). Genome engineering for personalized arthritis therapeutics. *Trends Mol. Med.* 23, 917–931. doi: 10.1016/j.molmed.2017.08.002

Ahmed, R. M., Iodice, V., Daveson, N., Kiernan, M. C., Piguet, O., and Hodges, J. R. (2015). Autonomic dysregulation in frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* 86, 1048–1049. doi: 10.1136/jnnp-2014-309424

Al-Daghri, N. M., Aziz, I., Yakout, S., Aljohani, N. J., Al-Saleh, Y., Amer, O. E., et al. (2017). Inflammation as a contributing factor among postmenopausal Saudi women with osteoporosis. *Medicine* 96:e5780. doi: 10.1097/MD.0000000000005780

Aletaha, D., Landewe, R., Karonitsch, T., Bathon, J., Boers, M., Bombardier, C., et al. (2008). Reporting disease activity in clinical trials of patients with rheumatoid arthritis: EULAR/ACR collaborative recommendations. *Ann. Rheum. Dis.* 67, 1360–1364. doi: 10.1136/ard.2008.091454

Aletaha, D., and Smolen, J. S. (2018). Diagnosis and management of rheumatoid arthritis: a review. *JAMA* 320, 1360–1372. doi: 10.1001/jama.2018.13103

Aliabouzar, M., Lee, S. J., Zhou, X., Zhang, G. L., and Sarkar, K. (2018). Effects of scaffold microstructure and low intensity pulsed ultrasound on chondrogenic differentiation of human mesenchymal stem cells. *Biotechnol. Bioeng.* 115, 495–506. doi: 10.1002/bit.26480

Allan, L. M., Ballard, C. G., Allen, J., Murray, A., Davidson, A. W., McKeith, I. G., et al. (2007). Autonomic dysfunction in dementia. *J. Neurol. Neurosurg. Psychiatry* 78, 671–677. doi: 10.1136/jnnp.2006.102343

Allen, J. M., Ferrier, I. N., Roberts, G. W., Cross, A. J., Adrian, T. E., Crow, T. J., et al. (1984). Elevation of neuropeptide Y (NPY) in substantia innominata in Alzheimer's type dementia. *J. Neurol. Sci.* 64, 325–331. doi: 10.1016/0022-510X(84)90180-1

Almeida, M., Laurent, M. R., Dubois, V., Claessens, F., O'Brien, C. A., Bouillon, R., et al. (2017). Estrogens and androgens in skeletal physiology and pathophysiology. *Physiol. Rev.* 97, 135–187. doi: 10.1152/physrev.00033.2015

Almonte-Becerril, M., Navarro-Garcia, F., Gonzalez-Robles, A., Vega-Lopez, M. A., Lavalle, C., and Kouri, J. B. (2010). Cell death of chondrocytes is a combination between apoptosis and autophagy during the pathogenesis of osteoarthritis within an experimental model. *Apoptosis* 15, 631–638. doi: 10.1007/s10495-010-0458-z

Altman, R., Hackel, J., Niazi, F., Shaw, P., and Nicholls, M. (2018). Efficacy and safety of repeated courses of hyaluronic acid injections for knee osteoarthritis: a systematic review. *Semin. Arthritis Rheum.* 48, 168–175. doi: 10.1016/j.semarthrit.2018.01.009

Altman, R. D., Devji, T., Bhandari, M., Fierlinger, A., Niazi, F., and Christensen, R. (2016). Clinical benefit of intra-articular saline as a comparator in clinical trials of knee osteoarthritis treatments: a systematic review and meta-analysis of randomized trials. *Semin. Arthritis Rheum.* 46, 151–159. doi: 10.1016/j.semarthrit.2016.04.003

Álvaro-Gracia, J. M., Jover, J. A., García-Vicuña, R., Carreño, L., Alonso, A., Marsal, S., et al. (2017). Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): results of a multicentre, dose escalation, randomised, singleblind, placebo-controlled phase Ib/IIa clinical trial. *Ann. Rheum. Dis.* 76, 196–202. doi: 10.1136/annrheumdis-2015-208918

Amjadi-Moheb, F., and Akhavan-Niaki, H. (2019). Wnt signaling pathway in osteoporosis: epigenetic regulation, interaction with other signaling pathways, and therapeutic promises. *J. Cell. Physiol.* 234, 14641–14650. doi: 10.1002/jcp.28207

Angelotti, F., Parma, A., Cafaro, G., Capecchi, R., Alunno, A., and Puxeddu, I. (2017). One year in review 2017: pathogenesis of rheumatoid arthritis. *Clin. Exp. Rheumatol.* 35, 368–378.

Ankrum, J., and Karp, J. M. (2010). Mesenchymal stem cell therapy: two steps forward, one step back. *Trends Mol. Med.* 16, 203–209. doi: 10.1016/j.molmed.2010.02.005

Ansari, A., Maffioletti, E., Milanesi, E., Marizzoni, M., Frisoni, G. B., Blin, O., et al. (2019). miR-146a and miR-181a are involved in the progression of mild cognitive impairment to Alzheimer's disease. *Neurobiol. Aging* 82, 102–109. doi: 10.1016/j.neurobiolaging.2019.06.005

Arensman, M. D., and Eng, C. H. (2018). Self-digestion for lifespan extension: enhanced autophagy delays aging. *Mol. Cell* 71, 485–486. doi: 10.1016/j.molcel.2018.08.002

Asada, N., Katayama, Y., Sato, M., Minagawa, K., Wakahashi, K., Kawano, H., et al. (2013). Matrix-embedded osteocytes regulate mobilization of hematopoietic stem/progenitor cells. *Cell Stem Cell* 12, 737–747. doi: 10.1016/j.stem.2013.05.001

Asmus, S. E., Parsons, S., and Landis, S. C. (2000). Developmental changes in the transmitter properties of sympathetic neurons that innervate the periosteum. *J. Neurosci.* 20, 1495–1504. doi: 10.1523/JNEUROSCI.20-04-01495.2000

Awasthi, H., Mani, D., Singh, D., and Gupta, A. (2018). The underlying pathophysiology and therapeutic approaches for osteoporosis. *Med. Res. Rev.* 38, 2024–2057. doi: 10.1002/med.21504

Baker, D. J., Wijshake, T., Tchkonia, T., Lebrasseur, N. K., Childs, B. G., Van De Sluis, B., et al. (2011). Clearance of p16 Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 479, 232–236. doi: 10.1038/nature10600

Baldock, P. A., Sainsbury, A., Couzens, M., Enriquez, R. F., Thomas, G. P., Gardiner, E. M., et al. (2002). Hypothalamic Y2 receptors regulate bone formation. *J. Clin. Invest.* 109, 915–921. doi: 10.1172/JCI0214588

Ball, A. N., Donahue, S. W., Wojda, S. J., McIlwraith, C. W., Kawcak, C. E., Ehrhart, N., et al. (2018). The challenges of promoting osteogenesis in segmental bone defects and osteoporosis. *J. Orthop. Res.* 36, 1559–1572. doi: 10.1002/jor.23845

Banks, W. A., Farr, S. A., and Morley, J. E. (2002). Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation* 10, 319–327. doi: 10.1159/000071472

Banks, W. A., Kastin, A. J., and Broadwell, R. D. (1995). Passage of cytokines across the blood-brain barrier. *Neuroimmunomodulation* 2, 241–248. doi: 10.1159/000097202

Banzhaf-Strathmann, J., Benito, E., May, S., Arzberger, T., Tahirovic, S., Kretzschmar, H., et al. (2014). Micro RNA-125b induces tau hyperphosphorylation and cognitive deficits in Alzheimer's disease. *EMBO J.* 33, 1667–1680. doi: 10.15252/embj.201387576

Bazzoni, F., Rossato, M., Fabbri, M., Gaudiosi, D., Mirolo, M., Mori, L., et al. (2009). Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. *Proc. Natl. Aacd. Sci. U.S.A.* 106, 5282–5287. doi: 10.1073/pnas.0810909106

Beck, E. C., Barragan, M., Tadros, M. H., Gehrke, S. H., and Detamore, M. S. (2016). Approaching the compressive modulus of articular cartilage with a decellularized cartilage-based hydrogel. *Acta Biomater.* 38, 94–105. doi: 10.1016/j.actbio.2016.04.019

Bellamy, N., Campbell, J., Welch, V., Gee, T. L., Bourne, R., and Wells, G. A. (2006). Intraarticular corticosteroid for treatment of osteoarthritis of the knee. *Cochrane Database Syst. Rev.* CD005328. doi: 10.1002/14651858.CD005328.pub2

Bellavia, D., De Luca, A., Carina, V., Costa, V., Raimondi, L., Salamanna, F., et al. (2019). Deregulated miRNAs in bone health: epigenetic roles in osteoporosis. *Bone* 122, 52–75. doi: 10.1016/j.bone.2019.02.013

Berthelot, J. M., Le Goff, B., Neel, A., Maugars, Y., and Hamidou, M. (2017). NETosis: at the crossroads of rheumatoid arthritis, lupus, and vasculitis. *Jt. Bone Spine* 84, 255–262. doi: 10.1016/j.jbspin.2016.05.013

Blom, A. B., Van Lent, P. L., Libregts, S., Holthuysen, A. E., Van Der Kraan, P. M., Van Rooijen, N., et al. (2007). Crucial role of macrophages in matrix metalloproteinase-mediated cartilage destruction during experimental osteoarthritis: involvement of matrix metalloproteinase 3. *Arthritis Rheum.* 56, 147–157. doi: 10.1002/art.22337

Bogunia-Kubik, K., Wysoczańska, B., Piatek, D., Iwaszko, M., Ciechomska, M., and Swierkot, J. (2016). Significance of polymorphism and expression of miR-146a and NFkB1 genetic variants in patients with rheumatoid arthritis. *Arch. Immunol. Ther. Exp.* 64, 131–136. doi: 10.1007/s00005-016-0443-5

Bondeson, J., Blom, A. B., Wainwright, S., Hughes, C., Caterson, B., and Van Den Berg, W. B. (2010). The role of synovial macrophages and macrophage-produced mediators in driving inflammatory and destructive responses in osteoarthritis. *Arthritis Rheum.* 62, 647–657. doi: 10.1002/art.27290

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., et al. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405, 458–462. doi: 10.1038/35013070

Boumans, M. J. H., Thurlings, R. M., Yeo, L., Scheel-Toellner, D., Vos, K., Gerlag, D. M., et al. (2012). Rituximab abrogates joint destruction in rheumatoid arthritis by inhibiting osteoclastogenesis. *Ann. Rheum. Dis.* 71, 108–113. doi: 10.1136/annrheumdis-2011-200198

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., and Tredici, K. (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 112, 389–404. doi: 10.1007/s00401-006-0127-z

Braun, T., and Zwerina, J. (2011). Positive regulators of osteoclastogenesis and bone resorption in rheumatoid arthritis. *Arthritis Res. Ther.* 13:235. doi: 10.1186/ar3380

Brazill, J. M., Beeve, A. T., Craft, C. S., Ivanusic, J. J., and Scheller, E. L. (2019). Nerves in bone: evolving concepts in pain and anabolism. *J. Bone Miner. Res.* 34, 1393–1406. doi: 10.1002/jbmr.3822

Buckwalter, J. A., and Mankin, H. J. (1998). Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. *Instr. Course Lect.* 47, 487–504.

Buller, K. M. (2001). Role of circumventricular organs in pro-inflammatory cytokine-induced activation of the hypothalamic-pituitary-adrenal axis. *Clin. Exp. Pharmacol. Physiol.* 28, 581–589. doi: 10.1046/j.1440-1681.2001.03490.x

Burke, J., Hunter, M., Kolhe, R., Isales, C., Hamrick, M., and Fulzele, S. (2016). Therapeutic potential of mesenchymal stem cell based therapy for osteoarthritis. *Clin. Transl. Med.* 5:27. doi: 10.1186/s40169-016-0112-7

Buyuk, A. F., Kilinc, E., Camurcu, I. Y., Camur, S., Ucpunar, H., and Kara, A. (2017). Compared efficacy of intra-articular injection of methylprednisolone and triamcinolone. *Acta Ortop. Bras.* 25, 206–208. doi: 10.1590/1413-785220172505172581

Camirand, A., Goltzman, D., Gupta, A., Kaouass, M., Panda, D., and Karaplis, A. (2016). The role of parathyroid hormone-related protein (PTHrP) in osteoblast response to microgravity: mechanistic implications for osteoporosis development. *PLoS ONE* 11:e0160034. doi: 10.1371/journal.pone.0160034

Caramés, B., Taniguchi, N., Otsuki, S., Blanco, F. J., and Lotz, M. (2010). Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis. *Arthritis Rheum.* 62, 791–801. doi: 10.1002/art.27305

Carecchio, M., and Comi, C. (2011). The role of osteopontin in neurodegenerative diseases. *J. Alzheimer's Dis.* 25, 179–185. doi: 10.3233/JAD-2011-102151

Cassotta, M., Pistollato, F., and Battino, M. (2020). Rheumatoid arthritis research in the 21st century: limitations of traditional models, new technologies, and opportunities for a human biology-based approach. *ALTEX* 37, 223–242. doi: 10.14573/altex.1910011

Catrina, A. I., Svensson, C. I., Malmström, V., Schett, G., and Klareskog, L. (2017). Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 13, 79–86. doi: 10.1038/nrrheum.2016.200

Cerza, F., Carnì, S., Carcangiu, A., Di Vavo, I., Schiavilla, V., Pecora, A., et al. (2012). Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. *Am. J. Sports Med.* 40, 2822–2827. doi: 10.1177/0363546512461902

Cha, D. J., Mengel, D., Mustapic, M., Liu, W., Selkoe, D. J., Kapogiannis, D., et al. (2019). miR-212 and miR-132 are downregulated in neurally derived plasma exosomes of Alzheimer's patients. *Front. Neurosci.* 13:1208. doi: 10.3389/fnins.2019.01208

Chandran, S. V., Vairamani, M., and Selvamurugan, N. (2019). Osteostimulatory effect of biocomposite scaffold containing phytomolecule diosmin by integrin/FAK/ERK signaling pathway in mouse mesenchymal stem cells. *Sci. Rep.* 9:11900. doi: 10.1038/s41598-019-48429-1

Chang, J., Lei, H., Liu, Q., Qin, S., Ma, K., Luo, S., et al. (2012). Optimization of culture of mesenchymal stem cells: a comparison of conventional plate and microcarrier cultures. *Cell Prolif.* 45, 430–437. doi: 10.1111/j.1365-2184.2012.00836.x

Chapurlat, R. D., and Genant, H. K. (2015). "Osteoporosis," in *Endocrinology: Adult and Pediatric*, eds J. Larry Jameson and L. J. DeGroot (Philadelphia, PA: Elsevier Inc.), 1184–1213.e6.

Chen, Y. C., Chang, Y. W., Tan, K. P., Shen, Y. S., Wang, Y. H., and Chang, C. H. (2018). Can mesenchymal stem cells and their conditioned medium assist inflammatory chondrocytes recovery? *PLoS ONE* 13:e0205563. doi: 10.1371/journal.pone.0205563

Chen, Y. D., Huang, C. Y., Liu, H. Y., Yao, W. F., Wu, W. G., Lu, Y. L., et al. (2016). Serum CX3CL1/fractalkine concentrations are positively associated with disease severity in postmenopausal osteoporotic patients. *Br. J. Biomed. Sci.* 73, 121–128. doi: 10.1080/09674845.2016.1209897

Chen, Y. H., and Lo, R. Y. (2017). Alzheimer's disease and osteoporosis. *Tzu Chi Med. J.* 29, 138–142. doi: 10.4103/tcmj.tcmj_54_17

Cheng, V. K.-F., Au, P. C.-M., Tan, K. C., and Cheung, C.-L. (2019). MicroRNA and human bone health. *JBMR Plus* 3, 2–13. doi: 10.1002/jbm4.10115

Chesnokova, V., Pechnick, R. N., and Wawrowsky, K. (2016). Chronic peripheral inflammation, hippocampal neurogenesis, and behavior. *Brain. Behav. Immun.* 58, 1–8. doi: 10.1016/j.bbi.2016.01.017

Chi, G. C., Fitzpatrick, A. L., Sharma, M., Jenny, N. S., Lopez, O. L., and DeKosky, S. T. (2017). Inflammatory biomarkers predict domain-specific cognitive decline in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 72, 796–803. doi: 10.1093/gerona/glw155

Chiu, Y. S., Wu, J. L., Yeh, C. T., Yadav, V. K., Huang, H. S., and Wang, L. S. (2020). γ-Mangostin isolated from *Garcinia mangostana* L. suppresses inflammation and alleviates symptoms of osteoarthritis via modulating miR-124-3p/IL-6/NF-κB signaling. *Aging* 12, 6630–6643. doi: 10.18632/aging.103003

Choi, Y., Lee, K., Ryu, J., Kim, H. G., Jeong, A. Y., Woo, R. S., et al. (2014). Neuritin attenuates cognitive function impairments in Tg2576 mouse model of Alzheimer's disease. *PLoS ONE* 9:e104121. doi: 10.1371/journal.pone.0104121

Choong, C. J., Baba, K., and Mochizuki, H. (2016). Gene therapy for neurological disorders. *Expert Opin. Biol. Ther.* 16, 143–159. doi: 10.1517/14712598.2016.1114096

Chou, R. C., Kane, M., Ghimire, S., Gautam, S., and Gui, J. (2016). Treatment for rheumatoid arthritis and risk of Alzheimer's disease: a nested case-control analysis. *CNS Drugs* 30, 1111–1120. doi: 10.1007/s40263-016-0374-z

Chu, C. Q., Field, M., Feldmann, M., and Maini, R. N. (1991). Localization of tumor necrosis factor α in synovial tissues and at the cartliage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheum.* 34, 1125–1132. doi: 10.1002/art.1780340908

Citrome, L., and Weiss-Citrome, A. (2012). A systematic review of duloxetine for osteoarthritic pain: what is the number needed to treat, number needed to harm, and likelihood to be helped or harmed? *Postgrad. Med.* 124, 83–93. doi: 10.3810/pgm.2012.01.2521

Clarke, B. (2008). Normal bone anatomy and physiology. *Clin. J. Am. Soc. Nephrol.* 3, S131–S139. doi: 10.2215/CJN.04151206

Clowes, J. A., Riggs, B. L., and Khosla, S. (2005). The role of the immune system in the pathophysiology of osteoporosis. *Immunol. Rev.* 208, 207–227. doi: 10.1111/j.0105-2896.2005.00334.x

Collins, O., Dillon, S., Finucane, C., Lawlor, B., and Kenny, R. A. (2012). Parasympathetic autonomic dysfunction is common in mild cognitive impairment. *Neurobiol. Aging* 33, 2324–2333. doi: 10.1016/j.neurobiolaging.2011.11.017

Colonna, M., and Holtzmann, D. (2017). Microglia in Alzheimer's disease. *J. Clin. Invest.* 3, 3240–3249. doi: 10.1172/JCI90606

Comi, C., Carecchio, M., Chiocchetti, A., Nicola, S., Galimberti, D., Fenoglio, C., et al. (2010). Osteopontin is increased in the cerebrospinal fluid of patients with Alzheimer's disease and its levels correlate with cognitive decline. *J. Alzheimer's Dis.* 19, 1143–1148. doi: 10.3233/JAD-2010-1309

Congdon, E. E., and Sigurdsson, E. M. (2018). Tau-targeting therapies for Alzheimer disease. *Nat. Rev. Neurol.* 14, 399–415. doi: 10.1038/s41582-018-0013-z

Coppé, J. P., Patil, C. K., Rodier, F., Sun, Y., Muñoz, D. P., Goldstein, J., et al. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* 6, 2853–2868. doi: 10.1371/journal.pbio.0060301

Corsiero, E., Pratesi, F., Prediletto, E., Bombardieri, M., and Migliorini, P. (2016). NETosis as source of autoantigens in rheumatoid arthritis. *Front. Immunol.* 7:485. doi: 10.3389/fimmu.2016.00485

Cuervo, A. M., and Dice, J. F. (2000). Age-related decline in chaperone-mediated autophagy. *J. Biol. Chem.* 275, 31505–31513. doi: 10.1074/jbc.M002102200

Cui, Y. B., Ma, S. S., Zhang, C. Y., Cao, W., Liu, M., Li, D. P., et al. (2017). Human umbilical cord mesenchymal stem cells transplantation improves cognitive function in Alzheimer's disease mice by decreasing oxidative stress and promoting hippocampal neurogenesis. *Behav. Brain Res.* 320, 291–301. doi: 10.1016/j.bbr.2016.12.021

Da, R.-R., Qin, Y., Baeten, D., and Zhang, Y. (2007). B cell clonal expansion and somatic hypermutation of Ig variable heavy chain genes in the synovial membrane of patients with osteoarthritis. *J. Immunol.* 178, 557–565. doi: 10.4049/jimmunol.178.1.557

Dar, H. Y., Pal, S., Shukla, P., Mishra, P. K., Tomar, G. B., Chattopadhyay, N., et al. (2018a). *Bacillus clausii* inhibits bone loss by skewing Treg-Th17 cell equilibrium in postmenopausal osteoporotic mice model. *Nutrition* 54, 118–128. doi: 10.1016/j.nut.2018.02.013

Dar, H. Y., Shukla, P., Mishra, P. K., Anupam, R., Mondal, R. K., Tomar, G. B., et al. (2018b). *Lactobacillus acidophilus* inhibits bone loss and increases bone heterogeneity in osteoporotic mice via modulating Treg-Th17 cell balance. *Bone Rep.* 8, 46–56. doi: 10.1016/j.bonr.2018.02.001

Dar, H. Y., Singh, A., Shukla, P., Anupam, R., Mondal, R. K., Mishra, P. K., et al. (2018c). High dietary salt intake correlates with modulated Th17-Treg cell balance resulting in enhanced bone loss and impaired bone-microarchitecture in male mice. *Sci. Rep.* 8:2503. doi: 10.1038/s41598-018-20896-y

Das, U. N. (2007). Vagus nerve stimulation, depression, and inflammation. *Neuropsychopharmacology* 32, 2053–2054. doi: 10.1038/sj.npp.1301286

de Munter, W., Ascone, G., Blom, A., Sloetjes, A., van den Berg, W., van der Kraan, P., et al. (2017). Human APOε4 results in more severe experimental osteoarthritis in comparison to APOε3: apoe-isoforms as possible risk factor for inflammatory osteoarthritis development? *Osteoarthr. Cartil.* 25:S270. doi: 10.1016/j.joca.2017.02.455

Deuse, T., Hu, X., Agbor-Enoh, S., Koch, M., Spitzer, M. H., Gravina, A., et al. (2019). *De novo* mutations in mitochondrial DNA of iPSCs produce immunogenic neoepitopes in mice and humans. *Nat. Biotechnol.* 37, 1137–1144. doi: 10.1038/s41587-019-0227-7

Deviatkin, A. A., Vakulenko, Y. A., Akhmadishina, L. V., Tarasov, V. V., Beloukhova, M. I., Zamyatnin, A. A., et al. (2020). Emerging concepts and challenges in rheumatoid arthritis gene therapy. *Biomedicines* 8:9. doi: 10.3390/biomedicines8010009

Devineni, A., Tohme, S., Kody, M. T., Adams Cowley, R., and Harris, B. T. (2016). Stepping back to move forward: a current review of iPSCS in the fight against Alzheimer's disease. *Am. J. Stem Cells* 5, 99–106.

Di Minno, M. N. D., Ambrosino, P., Lupoli, R., Di Minno, A., Tasso, M., Peluso, R., et al. (2015). Clinical assessment of endothelial function in patients with rheumatoid arthritis: a meta-analysis of literature studies. *Eur. J. Intern. Med.* 26, 835–842. doi: 10.1016/j.ejim.2015.10.016

Didekhani, R., Sohrabi, M. R., Soleimani, M., Seyedjafari, E., and Hanaee-Ahvaz, H. (2020). Incorporating PCL nanofibers with oyster shell to improve osteogenic differentiation of mesenchymal stem cells. *Polym. Bull.* 77, 701–715. doi: 10.1007/s00289-019-02750-x

Ding, J., and Orozco, G. (2019). Identification of rheumatoid arthritis causal genes using functional genomics. *Scand. J. Immunol.* 89:e12753. doi: 10.1111/sji.12753

Dodd, J. S., Raleigh, J. A., and Gross, T. S. (1999). Osteocyte hypoxia: a novel mechanotransduction pathway. *Am. J. Physiol.* 277, C598–C602. doi: 10.1152/ajpcell.1999.277.3.C598

Doens, D., and Fernández, P. L. (2014). Microglia receptors and their implications in the response to amyloid β for Alzheimer's disease pathogenesis. *J. Neuroinflammation* 11:48. doi: 10.1186/1742-2094-11-48

Driessler, F., and Baldock, P. A. (2010). Hypothalamic regulation of bone. *J. Mol. Endocrinol.* 45, 175–181. doi: 10.1677/JME-10-0015

Dubey, N. K., Mishra, V. K., Dubey, R., Syed-Abdul, S., Wang, J. R., Wang, P. D., et al. (2018). Combating osteoarthritis through stem cell therapies by rejuvenating cartilage: a review. *Stem Cells Int.* 2018:5421019. doi: 10.1155/2018/5421019

Duncan, T., and Valenzuela, M. (2017). Alzheimer's disease, dementia, and stem cell therapy. *Stem Cell Res. Ther.* 8:111. doi: 10.1186/s13287-017-0567-5

Ebeling, P. R., and Eisman, J. A. (2018). "Vitamin D and osteoporosis," in *Vitamin D,* ed Feldman D (London: Elsevier), 203–220.

Egan, M. F., Kost, J., Tariot, P. N., Aisen, P. S., Cummings, J. L., Vellas, B., et al. (2018). Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* 378, 1691–1703. doi: 10.1056/NEJMoa1706441

Eketjäll, S., Janson, J., Kaspersson, K., Bogstedt, A., Jeppsson, F., Fälting, J., et al. (2016). AZD3293: a novel, orally active BACE1 inhibitor with high potency and permeability and markedly slow off-rate kinetics. *J. Alzheimer's Dis.* 50, 1109–1123. doi: 10.3233/JAD-150834

El Khoury, J., and Luster, A. D. (2008). Mechanisms of microglia accumulation in Alzheimer's disease: therapeutic implications. *Trends Pharmacol. Sci.* 29, 626–632. doi: 10.1016/j.tips.2008.08.004

Elefteriou, F. (2005). Neuronal signaling and the regulation of bone remodeling. *Cell. Mol. Life Sci.* 62, 2339–2349. doi: 10.1007/s00018-005-5175-3

Engelhart, M. J., Geerlings, M. I., Meijer, J., Kiliaan, A., Ruitenberg, A., Van Swieten, J. C., et al. (2004). Inflammatory proteins in plasma and the risk of dementia: the rotterdam study. *Arch. Neurol.* 61, 668–672. doi: 10.1001/archneur.61.5.668

Fan, J. J., Cao, L. G., Wu, T., Wang, D. X., Jin, D., Jiang, S., et al. (2011). The dose-effect of icariin on the proliferation and osteogenic differentiation of human bone mesenchymal stem cells. *Molecules* 16, 10123–10133. doi: 10.3390/molecules161210123

Farr, J. N., Rowsey, J. L., Eckhardt, B. A., Thicke, B. S., Fraser, D. G., Tchkonia, T., et al. (2019). Independent roles of estrogen deficiency and cellular senescence in the pathogenesis of osteoporosis: evidence in young adult mice and older humans. *J. Bone Miner. Res.* 34, 1407–1418. doi: 10.1002/jbmr.3729

Femminella, G. D., Ferrara, N., and Rengo, G. (2015). The emerging role of microRNAs in Alzheimer's disease. *Front. Physiol.* 6:40. doi: 10.3389/fphys.2015.00040

Femminella, G. D., Rengo, G., Komici, K., Iacotucci, P., Petraglia, L., Pagano, G., et al. (2014). Autonomic dysfunction in Alzheimer's disease: tools for assessment and review of the literature. *J. Alzheimer's Dis.* 42, 369–377. doi: 10.3233/JAD-140513

Fernandez-Pernas, P., Rodríguez-Lesende, I., De La Fuente, A., Mateos, J., Fuentes, I., De Toro, J., et al. (2017). CD105+-mesenchymal stem cells migrate into osteoarthritis joint: an animal model. *PLoS ONE* 12:e0188072. doi: 10.1371/journal.pone.0188072

Festoff, B. W., Sajja, R. K., van Dreden, P., and Cucullo, L. (2016). HMGB1 and thrombin mediate the blood-brain barrier dysfunction acting as biomarkers of neuroinflammation and progression to neurodegeneration in Alzheimer's disease. *J. Neuroinflamm.* 13, 1–12. doi: 10.1186/s12974-016-0670-z

Fischer, U. M., Harting, M. T., Jimenez, F., Monzon-Posadas, W. O., Xue, H., Savitz, S. I., et al. (2009). Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells Dev.* 18, 683–691. doi: 10.1089/scd.2008.0253

Fleischmann, R., Vencovsky, J., Van Vollenhoven, R. F., Borenstein, D., Box, J., Coteur, G., et al. (2009). Efficacy and safety of certolizumab pegol monotherapy every 4 weeks in patients with rheumatoid arthritis failing previous disease-modifying antirheumatic therapy: the FAST4WARD study. *Ann. Rheum. Dis.* 68, 805–811. doi: 10.1136/ard.2008.099291

Forsberg, M. H., Kink, J. A., Hematti, P., and Capitini, C. M. (2020). Mesenchymal stromal cells and exosomes: progress and challenges. *Front. Cell Dev. Biol.* 8:665. doi: 10.3389/fcell.2020.00665

Friedberg, R. C., Spyker, D. A., and Herold, D. A. (1991). Massive overdoses with sustained-release lithium carbonate preparations: pharmacokinetic model based on two case studies. *Clin. Chem.* 37, 1205–1209. doi: 10.1093/clinchem/37.7.1205

Fu, H.-L., Pan, H.-X., Zhao, B., Dong, B.-C., Shao, L., Fu, G.-S., et al. (2016). MicroRNA-100 inhibits bone morphogenetic protein-induced osteoblast differentiation by targeting Smad1. *Eur. Rev. Med. Pharmacol. Sci.* 20, 3911–3919.

Fulci, V., Scappucci, G., Sebastiani, G. D., Giannitti, C., Franceschini, D., Meloni, F., et al. (2010). miR-223 is overexpressed in T-lymphocytes of patients affected by rheumatoid arthritis. *Hum. Immunol.* 71, 206–211. doi: 10.1016/j.humimm.2009.11.008

Gannon, M., and Wang, Q. (2019). Complex noradrenergic dysfunction in Alzheimer's disease: low norepinephrine input is not always to blame. *Brain Res.* 1702, 12–16. doi: 10.1016/j.brainres.2018.01.001

Garcia, K. O., Ornellas, F. L. M., Matsumoto Martin, P. K., Patti, C. L., Mello, L. E., Frussa-Filho, R., et al. (2014). Therapeutic effects of the transplantation

of VEGF overexpressing bone marrow mesenchymal stem cells in the hippocampus of murine model of alzheimer's disease. *Front. Aging Neurosci.* 6:30. doi: 10.3389/fnagi.2014.00030

Garcia, S., Hartkamp, L. M., Malvar-Fernandez, B., van Es, I. E., Lin, H., Wong, J., et al. (2016). Colony-stimulating factor (CSF) 1 receptor blockade reduces inflammation in human and murine models of rheumatoid arthritis. *Arthritis Res. Ther.* 18:75. doi: 10.1186/s13075-016-0973-6

Garmilla-Ezquerra, P., Sañudo, C., Delgado-Calle, J., Pérez-Nuñez, M. I., Sumillera, M., and Riancho, J. A. (2015). Analysis of the bone MicroRNome in osteoporotic fractures. *Calcif. Tissue Int.* 96, 30–37. doi: 10.1007/s00223-014-9935-7

Gennari, L., Rotatori, S., Bianciardi, S., Nuti, R., and Merlotti, D. (2016). Treatment needs and current options for postmenopausal osteoporosis. *Expert Opin. Pharmacother.* 17, 1141–1152. doi: 10.1080/14656566.2016.1176147

Ghoryani, M., Shariati-Sarabi, Z., Tavakkol-Afshari, J., Ghasemi, A., Poursamimi, J., and Mohammadi, M. (2019). Amelioration of clinical symptoms of patients with refractory rheumatoid arthritis following treatment with autologous bone marrow-derived mesenchymal stem cells: a successful clinical trial in Iran. *Biomed. Pharmacother.* 109, 1834–1840. doi: 10.1016/j.biopha.2018.11.056

Giubilei, F., Calderaro, C., Antonini, G., Sepe-Monti, M., Tisei, P., Brunetti, E., et al. (2004). Increased lymphocyte dopamine β-hydroxylase immunoreactivity in Alzheimer's disease: compensatory response to cholinergic deficit? *Dement. Geriatr. Cogn. Disord.* 18, 338–341. doi: 10.1159/000080128

Gong, C.-X., and Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated tau: a promising therapeutic target for Alzheimer disease. *Curr. Med. Chem.* 15, 2321–2328. doi: 10.2174/092986708785909111

Greendale, G. A., Espeland, M., Slone, S., Marcus, R., and Barrett-Connor, E. (2002). Bone mass response to discontinuation of long-term hormone replacement therapy. *Arch. Intern. Med.* 162, 665–672. doi: 10.1001/archinte.162.6.665

Griffin, T. M., and Scanzello, C. R. (2019). Innate inflammation and synovial macrophages in osteoarthritis pathophysiology. *Clin. Exp. Rheumatol.* 37, 57–63.

Guerrero-Muñoz, M. J., Gerson, J., and Castillo-Carranza, D. L. (2015). Tau oligomers: the toxic player at synapses in Alzheimer's disease. *Front. Cell. Neurosci.* 9:464. doi: 10.3389/fncel.2015.00464

Gutierrez, E. G., Banks, W. A., and Kastin, A. J. (1993). Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. *J. Neuroimmunol.* 47, 169–176. doi: 10.1016/0165-5728(93)90027-V

Hadar, A., Milanesi, E., Walczak, M., Puzianowska-Kuznicka, M., Kuznicki, J., Squassina, A., et al. (2018). SIRT1, miR-132 and miR-212 link human longevity to Alzheimer's disease. *Sci. Rep.* 8:8465. doi: 10.1038/s41598-018-26547-6

Hamilton, O. A., Filonzi, E. L., and Ianches, G. (1993). Regulation of macrophage colony-stimulating factor (m-CSF) production in cultured human synovial fibroblasts. *Growth Factors* 9, 157–165. doi: 10.3109/08977199309010831

Harach, T., Jammes, F., Muller, C., Duthilleul, N., Cheatham, V., Zufferey, V., et al. (2017). Administrations of human adult ischemia-tolerant mesenchymal stem cells and factors reduce amyloid beta pathology in a mouse model of Alzheimer's disease. *Neurobiol. Aging* 51, 83–96. doi: 10.1016/j.neurobiolaging.2016.11.009

Harrell, C. R., Markovic, B. S., Fellabaum, C., Arsenijevic, A., and Volarevic, V. (2019). Mesenchymal stem cell-based therapy of osteoarthritis: current knowledge and future perspectives. *Biomed. Pharmacother.* 109, 2318–2326. doi: 10.1016/j.biopha.2018.11.099

Haseeb, A., and Haqqi, T. M. (2013). Immunopathogenesis of osteoarthritis. *Clin. Immunol.* 146, 185–196. doi: 10.1016/j.clim.2012.12.011

Herrera-Espejo, S., Santos-Zorrozua, B., Álvarez-González, P., Lopez-Lopez, E., and Garcia-Orad, Á. (2019). A systematic review of microRNA expression as biomarker of late-onset Alzheimer's disease. *Mol. Neurobiol.* 56, 8376–8391. doi: 10.1007/s12035-019-01676-9

Hillsley, M. V., and Frangos, J. A. (1994). Bone tissue engineering: the role of interstitial fluid flow. *Biotechnol. Bioeng.* 43, 573–581. doi: 10.1002/bit.260430706

Hofbauer, L. C., Khosla, S., Dunstan, C. R., Lacey, D. L., Spelsberg, T. C., and Riggs, L. B. (1999). Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. *Endocrinology* 140, 4367–4370. doi: 10.1210/endo.140.9.7131

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., et al. (2008). Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 372, 216–223. doi: 10.1016/S0140-6736(08)61075-2

Honig, L. S. (2018). Gene therapy in Alzheimer disease - it may be feasible, but will it be beneficial? *JAMA Neurol.* 75, 791–793. doi: 10.1001/jamaneurol.2017.4029

Huang, K., Fu, J., Zhou, W., Li, W., Dong, S., Yu, S., et al. (2014). MicroRNA-125b regulates osteogenic differentiation of mesenchymal stem cells by targeting Cbfβ *in vitro*. *Biochimie* 102, 47–55. doi: 10.1016/j.biochi.2014.02.005

Huang, S. W., Wang, W. T., Chou, L. C., Liao, C. D., Liou, T. H., and Lin, H. W. (2015). Osteoarthritis increases the risk of dementia: a nationwide cohort study in Taiwan. *Sci. Rep.* 5:10145. doi: 10.1038/srep10145

Ikram, M., Innes, K., and Sambamoorthi, U. (2019). Association of osteoarthritis and pain with Alzheimer's diseases and related dementias among older adults in the United States. *Osteoarthr. Cartil.* 27, 1470–1480. doi: 10.1016/j.joca.2019.05.021

Imhof, H., Breitenseher, M., Kainberger, F., and Trattnig, S. (1997). Degenerative joint disease: cartilage or vascular disease? *Skeletal Radiol.* 26, 398–403. doi: 10.1007/s002560050254

Indrieri, A., Carrella, S., Carotenuto, P., Banfi, S., and Franco, B. (2020). The pervasive role of the MiR-181 family in development, neurodegeneration, and cancer. *Int. J. Mol. Sci.* 21:2092. doi: 10.3390/ijms21062092

Innes, K. E., and Sambamoorthi, U. (2018). The association of perceived memory loss with osteoarthritis and related joint pain in a large appalachian population. *Pain Med.* 19, 1340–1356. doi: 10.1093/pm/pnx107

Jafarzadeh, S. R., and Felson, D. T. (2018). Updated estimates suggest a much higher prevalence of arthritis in the United States adults than previous ones. *Arthritis Rheumatol.* 70, 185–192. doi: 10.1002/art.40355

Jahangard, Y., Monfared, H., Moradi, A., Zare, M., Mirnajafi-Zadeh, J., and Mowla, S. J. (2020). Therapeutic effects of transplanted exosomes containing miR-29b to a rat model of Alzheimer's disease. *Front. Neurosci.* 14:564. doi: 10.3389/fnins.2020.00564

Jaiswal, A. K., Chhabra, H., Kadam, S. S., Londhe, K., Soni, V. P., and Bellare, J. R. (2013). Hardystonite improves biocompatibility and strength of electrospun polycaprolactone nanofibers over hydroxyapatite: a comparative study. *Mater. Sci. Eng. C* 33, 2926–2936. doi: 10.1016/j.msec.2013.03.020

Jevotovsky, D. S., Alfonso, A. R., Einhorn, T. A., and Chiu, E. S. (2018). Osteoarthritis and stem cell therapy in humans: a systematic review. *Osteoarthr. Cartil.* 26, 711–729. doi: 10.1016/j.joca.2018.02.906

Jevsevar, D. S. (2013). Treatment of osteoarthritis of the knee: evidence-based guideline, 2nd edition. *J. Am. Acad. Orthop. Surg.* 21, 571–576. doi: 10.5435/JAAOS-21-09-571

Jiang, S., Li, Y., Lin, T., Yuan, L., Li, Y., Wu, S., et al. (2016). IL-35 inhibits angiogenesis through VEGF/Ang2/Tie2 pathway in rheumatoid arthritis. *Cell. Physiol. Biochem.* 40, 1105–1116. doi: 10.1159/000453165

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337, 816–821. doi: 10.1126/science.1225829

Johnson, M. L., and Recker, R. R. (2017). Exploiting the wnt signaling pathway for clinical purposes. *Curr. Osteoporos. Rep.* 15, 153–161. doi: 10.1007/s11914-017-0357-0

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P. V., Snaedal, J., et al. (2013). Variant of TREM2 associated with the risk of Alzheimer's disease. *N. Engl. J. Med.* 368, 107–116. doi: 10.1056/NEJMoa1211103

Joseph, J. S., Lam, V., and Patel, M. I. (2019). Preventing osteoporosis in men taking androgen deprivation therapy for prostate cancer: a systematic review and meta-analysis. *Eur. Urol. Oncol.* 2, 551–561. doi: 10.1016/j.euo.2018.11.001

Judge, A., Garriga, C., Arden, N. K., Lovestone, S., Prieto-Alhambra, D., Cooper, C., et al. (2017). Protective effect of antirheumatic drugs on dementia in rheumatoid arthritis patients. *Alzheimer's Dement. Transl. Res. Clin. Interv.* 3, 612–621. doi: 10.1016/j.trci.2017.10.002

Kabba, J. A., Xu, Y., Christian, H., Ruan, W., Chenai, K., Xiang, Y., et al. (2018). Microglia: housekeeper of the central nervous system. *Cell. Mol. Neurobiol.* 38, 53–71. doi: 10.1007/s10571-017-0504-2

Karimian, A., Gorjizadeh, N., Alemi, F., Asemi, Z., Azizian, K., Soleimanpour, J., et al. (2020). CRISPR/Cas9 novel therapeutic road for the treatment of neurodegenerative diseases. *Life Sci.* 259:118165. doi: 10.1016/j.lfs.2020.118165

Kay, A. G., Long, G., Tyler, G., Stefan, A., Broadfoot, S. J., Piccinini, A. M., et al. (2017). Mesenchymal stem cell-conditioned medium reduces disease severity and immune responses in inflammatory arthritis. *Sci. Rep.* 7:18019. doi: 10.1038/s41598-017-18144-w

Kelch, S., Balmayor, E. R., Seeliger, C., Vester, H., Kirschke, J. S., and Van Griensven, M. (2017). MiRNAs in bone tissue correlate to bone mineral density and circulating miRNAs are gender independent in osteoporotic patients. *Sci. Rep.* 7:15861. doi: 10.1038/s41598-017-16113-x

Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T. K., et al. (2017). A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* 169, 1276–1290. doi: 10.1016/j.cell.2017.05.018

Khan, M. A., Alanazi, F., Ahmed, H. A., Shamma, T., Kelly, K., Hammad, M. A., et al. (2019). IPSC-derived MSC therapy induces immune tolerance and supports long-term graft survival in mouse orthotopic tracheal transplants. *Stem Cell Res. Ther.* 10:290. doi: 10.1186/s13287-019-1397-4

Khosla, S., Farr, J. N., and Kirkland, J. L. (2018). Inhibiting cellular senescence: a new therapeutic paradigm for age-related osteoporosis. *J. Clin. Endocrinol. Metab.* 103, 1282–1290. doi: 10.1210/jc.2017-02694

Khrimian, L., Obri, A., Ramos-Brossier, M., Rousseaud, A., Moriceau, S., Nicot, A. S., et al. (2017). Gpr158 mediates osteocalcin's regulation of cognition. *J. Exp. Med.* 214, 2859–2873. doi: 10.1084/jem.20171320

Kim, D. H., Lee, D., Chang, E. H., Kim, J. H., Hwang, J. W., Kim, J. Y., et al. (2015). GDF-15 secreted from human umbilical cord blood mesenchymal stem cells delivered through the cerebrospinal fluid promotes hippocampal neurogenesis and synaptic activity in an Alzheimer's disease model. *Stem Cells Dev.* 24, 2378–2390. doi: 10.1089/scd.2014.0487

Kim, G. B., and Shon, O.-J. (2020). Current perspectives in stem cell therapies for osteoarthritis of the knee. *Yeungnam Univ. J. Med.* 37, 149–158. doi: 10.12701/yujm.2020.00157

Kim, J., Yoon, H., Ramírez, C. M., Lee, S. M., Hoe, H. S., Fernández-Hernando, C., et al. (2012). MiR-106b impairs cholesterol efflux and increases Aβ levels by repressing ABCA1 expression. *Exp. Neurol.* 235, 476–483. doi: 10.1016/j.expneurol.2011.11.010

King, E., O'Brien, J. T., Donaghy, P., Morris, C., Barnett, N., Olsen, K., et al. (2018). Peripheral inflammation in prodromal Alzheimer's and lewy body dementias. *J. Neurol. Neurosurg. Psychiatry* 89, 339–345. doi: 10.1136/jnnp-2017-317134

Kirouac, L., Rajic, A. J., Cribbs, D. H., and Padmanabhan, J. (2017). Activation of ras-ERK signaling and GSK-3 by amyloid precursor protein and amyloid beta facilitates neurodegeneration in Alzheimer's disease. *eNeuro* 4:e0149-16.2017. doi: 10.1523/ENEURO.0149-16.2017

Knychala, J., Bouropoulos, N., Catt, C. J., Katsamenis, O. L., Please, C. P., and Sengers, B. G. (2013). Pore geometry regulates early stage human bone marrow cell tissue formation and organisation. *Ann. Biomed. Eng.* 41, 917–930. doi: 10.1007/s10439-013-0748-z

Ko, N. Y., Chen, L. R., and Chen, K. H. (2020). The role of micro rna and long-non-coding rna in osteoporosis. *Int. J. Mol. Sci.* 21:4886. doi: 10.3390/ijms21144886

Komatsu, N., and Takayanagi, H. (2012). Autoimmune arthritis. the interface between the immune system and joints. *Adv Immunol.* 115, 45–71. doi: 10.1016/B978-0-12-394299-9.00002-3

Konsman, J. P., Parnet, P., and Dantzer, R. (2002). Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci.* 25, 154–159. doi: 10.1016/S0166-2236(00)02088-9

Kraus, V. B., McDaniel, G., Huebner, J. L., Stabler, T. V., Pieper, C. F., Shipes, S. W., et al. (2016). Direct *in vivo* evidence of activated macrophages in human osteoarthritis. *Osteoarthr. Cartil.* 24, 1613–1621. doi: 10.1016/j.joca.2016.04.010

Krishnasamy, P., Hall, M., and Robbins, S. R. (2018). The role of skeletal muscle in the pathophysiology and management of knee osteoarthritis. *Rheumatol* 57, iv22–iv33. doi: 10.1093/rheumatology/kex515

Kumar, S., and Reddy, P. H. (2016). Are circulating microRNAs peripheral biomarkers for Alzheimer's disease? *Biochim. Biophys. Acta Mol. Basis Dis.* 1862, 1617–1627. doi: 10.1016/j.bbadis.2016.06.001

Kuo, H. K., Yen, C. J., Chang, C. H., Kuo, C. K., Chen, J. H., and Sorond, F. (2005). Relation of C-reactive protein to stroke, cognitive disorders, and depression in the general population: systematic review and meta-analysis. *Lancet Neurol.* 4, 371–380. doi: 10.1016/S1474-4422(05)70099-5

Kyrkanides, S., Tallents, R. H., Miller, J. N. H., Olschowka, M. E., Johnson, R., Yang, M., et al. (2011). Osteoarthritis accelerates and exacerbates Alzheimer's disease pathology in mice. *J. Neuroinflamm.* 8:112. doi: 10.1186/1742-2094-8-112

Lai, K. S. P., Liu, C. S., Rau, A., Lanctôt, K. L., Köhler, C. A., Pakosh, M., et al. (2017). Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. *J. Neurol. Neurosurg. Psychiatry* 88, 876–882. doi: 10.1136/jnnp-2017-316201

Laine, S. K., Alm, J. J., Virtanen, S. P., Aro, H. T., and Laitala-Leinonen, T. K. (2012). MicroRNAs miR-96, miR-124, and miR-199a regulate gene expression in human bone marrow-derived mesenchymal stem cells. *J. Cell. Biochem.* 113, 2687–2695. doi: 10.1002/jcb.24144

Lasry, A., and Ben-Neriah, Y. (2015). Senescence-associated inflammatory responses: aging and cancer perspectives. *Trends Immunol.* 36, 217–228. doi: 10.1016/j.it.2015.02.009

Law, T. Y., Nguyen, C., Frank, R. M., Rosas, S., and McCormick, F. (2015). Current concepts on the use of corticosteroid injections for knee osteoarthritis. *Phys. Sportsmed.* 43, 269–273. doi: 10.1080/00913847.2015.1017440

Le Page, A., Dupuis, G., Frost, E. H., Larbi, A., Pawelec, G., Witkowski, J. M., et al. (2018). Role of the peripheral innate immune system in the development of Alzheimer's disease. *Exp. Gerontol.* 107, 59–66. doi: 10.1016/j.exger.2017.12.019

Lebrasseur, N. K., Tchkonia, T., and Kirkland, J. L. (2015). Cellular senescence and the biology of aging, disease, and frailty. *Nestle Nutr. Inst. Workshop Ser.* 83, 11–18. doi: 10.1159/000382054

Levin, V. A., Jiang, X., and Kagan, R. (2018). Estrogen therapy for osteoporosis in the modern era. *Osteoporos. Int.* 29, 1049–1055. doi: 10.1007/s00198-018-4414-z

Levy, D. M., Petersen, K. A., Scalley Vaught, M., Christian, D. R., and Cole, B. J. (2018). Injections for knee osteoarthritis: corticosteroids, viscosupplementation, platelet-rich plasma, and autologous stem cells. *Arthrosc. J. Arthrosc. Relat. Surg.* 34, 1730–1743. doi: 10.1016/j.arthro.2018.02.022

Li, H., Xie, H., Liu, W., Hu, R., Huang, B., Tan, Y. F., et al. (2009). A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J. Clin. Invest.* 119, 3666–3677. doi: 10.1172/JCI39832

Li, S., Liu, B., Zhang, L., and Rong, L. (2014). Amyloid beta peptide is elevated in osteoporotic bone tissues and enhances osteoclast function. *Bone* 61, 164–175. doi: 10.1016/j.bone.2014.01.010

Li, S., Yang, B., Teguh, D., Zhou, L., Xu, J., and Rong, L. (2016). Amyloid β peptide enhances RANKL-induced osteoclast activation through NF-κB, ERK, and calcium oscillation signaling. *Int. J. Mol. Sci.* 17:1683. doi: 10.3390/ijms17101683

Li, Y. S., Luo, W., Zhu, S. A., and Lei, G. H. (2017). T cells in osteoarthritis: alterations and beyond. *Front. Immunol.* 8:356. doi: 10.3389/fimmu.2017.00356

Li, Y. Y., Cui, J. G., Hill, J. M., Bhattacharjee, S., Zhao, Y., and Lukiw, W. J. (2011). Increased expression of miRNA-146a in Alzheimer's disease transgenic mouse models. *Neurosci. Lett.* 487, 94–98. doi: 10.1016/j.neulet.2010.09.079

Li, Z., Hassan, M. Q., Jafferji, M., Aqeilan, R. I., Garzon, R., Croce, C. M., et al. (2009). Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J. Biol. Chem.* 284, 15676–15684. doi: 10.1074/jbc.M809787200

Li, Z., Hassan, M. Q., Volinia, S., Van Wijnen, A. J., Stein, J. L., Croce, C. M., et al. (2008). A microRNA signature for a BMP2-induced osteoblast lineage commitment program. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13906–13911. doi: 10.1073/pnas.0804438105

Li, Z. C., Han, N., Li, X., Li, G., Liu, Y. Z., Sun, G. X., et al. (2015). Decreased expression of microRNA-130a correlates with TNF-α in the development of osteoarthritis. *Int. J. Clin. Exp. Pathol.* 8, 2555–2564.

Liao, X. B., Zhang, Z. Y., Yuan, K., Liu, Y., Feng, X., Cui, R. R., et al. (2013). MiR-133a modulates osteogenic differentiation of vascular smooth muscle cells. *Endocrinology* 154, 3344–3352. doi: 10.1210/en.2012-2236

Liedmann, A., Frech, S., Morgan, P. J., Rolfs, A., and Frech, M. J. (2012). Differentiation of human neural progenitor cells in functionalized hydrogel matrices. *Biores. Open Access* 1, 16–24. doi: 10.1089/biores.2012.0209

Lin, J., He, Y., Chen, J., Zeng, Z., Yang, B., and Ou, Q. (2017). A critical role of transcription factor YY1 in rheumatoid arthritis by regulation of interleukin-6. *J. Autoimmun.* 77, 67–75. doi: 10.1016/j.jaut.2016.10.008

Lin, N. Y., Chen, C. W., Kagwiria, R., Liang, R., Beyer, C., Distler, A., et al. (2016). Inactivation of autophagy ameliorates glucocorticoid-induced and ovariectomy-induced bone loss. *Ann. Rheum. Dis.* 75, 1203–1210. doi: 10.1136/annrheumdis-2015-207240

Lin, T. M., Chen, W. S., Sheu, J. J., Chen, Y. H., Chen, J. H., and Chang, C. C. (2018). Autoimmune rheumatic diseases increase dementia risk in middle-aged patients: a nationwide cohort study. *PLoS ONE* 13:e018675. doi: 10.1371/journal.pone.0186475

Lino, C. A., Harper, J. C., Carney, J. P., and Timlin, J. A. (2018). Delivering CRISPR: a review of the challenges and approaches. *Drug Deliv.* 25, 1234–1257. doi: 10.1080/10717544.2018.1474964

Liu, D., Zhao, D., Zhao, Y., Wang, Y., Zhao, Y., and Wen, C. (2019). Inhibition of microRNA-155 alleviates cognitive impairment in Alzheimer's disease and involvement of neuroinflammation. *Curr. Alzheimer Res.* 16, 473–482. doi: 10.2174/1567205016666190503145207

Liu, H., Gu, R., Zhu, Y., Lian, X., Wang, S., Liu, X., et al. (2020). D-mannose attenuates bone loss in mice via Treg cell proliferation and gut microbiota-dependent anti-inflammatory effects. *Ther. Adv. Chronic Dis.* 11, 1–17. doi: 10.1177/2040622320912661

Liu, W., Liu, C., Zhu, J., Shu, P., Yin, B., Gong, Y., et al. (2012). MicroRNA-16 targets amyloid precursor protein to potentially modulate Alzheimer's-associated pathogenesis in SAMP8 mice. *Neurobiol. Aging* 33, 522–534. doi: 10.1016/j.neurobiolaging.2010.04.034

Liu, W., Wang, X., Zheng, Y., Shang, G., Huang, J., Tao, J., et al. (2016a). Electroacupuncture inhibits inflammatory injury by targeting the miR-9-mediated NF-κB signaling pathway following ischemic stroke. *Mol. Med. Rep.* 13, 1618–1626. doi: 10.3892/mmr.2015.4745

Liu, W., Zhao, J., and Lu, G. (2016b). miR-106b inhibits tau phosphorylation at Tyr18 by targeting Fyn in a model of Alzheimer's disease. *Biochem. Biophys. Res. Commun.* 478, 852–857. doi: 10.1016/j.bbrc.2016.08.037

Liu, Y., Wang, Y., Yang, N., Wu, S., Lv, Y., and Xu, L. (2015). *In silico* analysis of the molecular mechanism of postmenopausal osteoporosis. *Mol. Med. Rep.* 12, 6584–6590. doi: 10.3892/mmr.2015.4283

Lizneva, D., Yuen, T., Sun, L., Kim, S. M., Atabiekov, I., Munshi, L. B., et al. (2018). Emerging concepts in the epidemiology, pathophysiology, and clinical care of osteoporosis across the menopausal transition. *Matrix Biol.* 71–72, 70–81. doi: 10.1016/j.matbio.2018.05.001

Llave, C., Xie, Z., Kasschau, K. D., and Carrington, J. C. (2002). Cleavage of scarecrow-like mRNA targets directed by a class of arabidopsis miRNA. *Science* 297, 2053–2056. doi: 10.1126/science.1076311

Lou, S., Lv, H., Yin, P., Li, Z., Tang, P., and Wang, Y. (2019). Combination therapy with parathyroid hormone analogs and antiresorptive agents for osteoporosis: a systematic review and meta-analysis of randomized controlled trials. *Osteoporos. Int.* 30, 59–70. doi: 10.1007/s00198-018-4790-4

Luukkonen, J., Hilli, M., Nakamura, M., Ritamo, I., Valmu, L., Kauppinen, K., et al. (2019). Osteoclasts secrete osteopontin into resorption lacunae during bone resorption. *Histochem. Cell Biol.* 151, 475–487. doi: 10.1007/s00418-019-01770-y

Maldonado-Lasuncion, I., Atienza, M., Sanchez-Espinosa, M. P., and Cantero, J. L. (2019). Aging-related changes in cognition and cortical integrity are associated with serum expression of candidate MicroRNAs for Alzheimer disease. *Cereb. Cortex* 29, 4426–4437. doi: 10.1093/cercor/bhy323

Manolagas, S. C., and Parfitt, A. M. (2010). What old means to bone. *Trends Endocrinol. Metab.* 21, 369–374. doi: 10.1016/j.tem.2010.01.010

Martin, E., and Delarasse, C. (2018). Complex role of chemokine mediators in animal models of Alzheimer's disease. *Biomed. J.* 41, 34–40. doi: 10.1016/j.bj.2018.01.002

McMasters, J., Poh, S., Lin, J. B., and Panitch, A. (2017). Delivery of anti-inflammatory peptides from hollow PEGylated poly(NIPAM) nanoparticles reduces inflammation in an *ex vivo* osteoarthritis model. *J. Control. Release* 258, 161–170. doi: 10.1016/j.jconrel.2017.05.008

Mehravar, M., Shirazi, A., Nazari, M., and Banan, M. (2019). Mosaicism in CRISPR/Cas9-mediated genome editing. *Dev. Biol.* 445, 156–162. doi: 10.1016/j.ydbio.2018.10.008

Mendt, M., Rezvani, K., and Shpall, E. (2019). Mesenchymal stem cell-derived exosomes for clinical use. *Bone Marrow Transplant.* 54, 789–792. doi: 10.1038/s41409-019-0616-z

Menon, A. H., Soundarya, S. P., Sanjay, V., Chandran, S. V., Balagangadharan, K., and Selvamurugan, N. (2018). Sustained release of chrysin from chitosan-based scaffolds promotes mesenchymal stem cell proliferation and osteoblast differentiation. *Carbohydr. Polym.* 195, 356–367. doi: 10.1016/j.carbpol.2018.04.115

Michaud, J. P., and Rivest, S. (2015). Anti-inflammatory signaling in microglia exacerbates Alzheimer's disease-related pathology. *Neuron* 85, 450–452. doi: 10.1016/j.neuron.2015.01.021

Migliore, A., and Procopio, S. (2015). Effectiveness and utility of hyaluronic acid in osteoarthritis. *Clin. Cases Miner. Bone Metab.* 12, 31–33. doi: 10.11138/ccmbm/2015.12.1.031

Miossec, P., and Kolls, J. K. (2012). Targeting IL-17 and Th17 cells in chronic inflammation. *Nat. Rev. Drug Discov.* 11, 763–776. doi: 10.1038/nrd3794

Mobasheri, A., and Batt, M. (2016). An update on the pathophysiology of osteoarthritis. *Ann. Phys. Rehabil. Med.* 59, 333–339. doi: 10.1016/j.rehab.2016.07.004

Moll, C. W. I., Schmiedinger, T., Moll, M. A., Seppi, T., Pfaller, K., Hess, M. W., et al. (2017). Extracellular matrix mimicking scaffold promotes osteogenic stem cell differentiation: a new approach in osteoporosis research. *Biomed. Mater. Eng.* 28, 87–103. doi: 10.3233/BME-171659

Moncini, S., Lunghi, M., Valmadre, A., Grasso, M., Del Vescovo, V., Riva, P., et al. (2017). The miR-15/107 family of microRNA genes regulates CDK5R1/p35 with implications for Alzheimer's disease pathogenesis. *Mol. Neurobiol.* 54, 4329–4342. doi: 10.1007/s12035-016-0002-4

Mora, J. C., Przkora, R., and Cruz-Almeida, Y. (2018). Knee osteoarthritis: pathophysiology and current treatment modalities. *J. Pain Res.* 11, 2189–2196. doi: 10.2147/JPR.S154002

Moreland, L. W., Schiff, M. H., Baumgartner, S. W., Tindall, E. A., Fleischmann, R. M., Bulpitt, K. J., et al. (1999). Etanercept therapy in rheumatoid arthritis. *Ann. Intern. Med.* 130, 478–486. doi: 10.7326/0003-4819-130-6-199903160-00004

Mori, T., Miyamoto, T., Yoshida, H., Asakawa, M., Kawasumi, M., Kobayashi, T., et al. (2011). IL-1β and TNFα-initiated IL-6-STAT3 pathway is critical in mediating inflammatory cytokines and RANKL expression in inflammatory arthritis. *Int. Immunol.* 23, 701–712. doi: 10.1093/intimm/dxr077

Mrak, E., Guidobono, F., Moro, G., Fraschini, G., Rubinacci, A., and Villa, I. (2010). Calcitonin gene-related peptide (CGRP) inhibits apoptosis in human osteoblasts by ß-catenin stabilization. *J. Cell. Physiol.* 225, 701–708. doi: 10.1002/jcp.22266

Mufson, E. J., Ward, S., and Binder, L. (2014). Prefibrillar tau oligomers in mild cognitive impairment and Alzheimer's disease. *Neurodegener. Dis.* 13, 151–153. doi: 10.1159/000353687

Muñoz-Espín, D., and Serrano, M. (2014). Cellular senescence: from physiology to pathology. *Nat. Rev. Mol. Cell Biol.* 15, 482–496. doi: 10.1038/nrm3823

Nakata, K., Hanai, T., Take, Y., Osada, T., Tsuchiya, T., Shima, D., et al. (2018). Disease-modifying effects of COX-2 selective inhibitors and non-selective NSAIDs in osteoarthritis: a systematic review. *Osteoarthr. Cartil.* 26, 1263–1273. doi: 10.1016/j.joca.2018.05.021

Nakayama, N., Pothiawala, A., Lee, J. Y., Matthias, N., Umeda, K., Ang, B. K., et al. (2020). Human pluripotent stem cell-derived chondroprogenitors for cartilage tissue engineering. *Cell. Mol. Life Sci.* 77, 2543–2563. doi: 10.1007/s00018-019-03445-2

Naso, M. F., Tomkowicz, B., Perry, W. L., and Strohl, W. R. (2017). Adeno-associated virus (AAV) as a vector for gene therapy. *BioDrugs* 31, 317–334. doi: 10.1007/s40259-017-0234-5

National Institute of Aging (2011). *Alzheimer's Disease Genetics [Fact Sheet]*. Bethesda, MD: National Institute of Aging.

Neutzsky-Wulff, A. V., Sørensen, M. G., Kocijancic, D., Leeming, D. J., Dziegiel, M. H., Karsdal, M. A., et al. (2010). Alterations in osteoclast function and phenotype induced by different inhibitors of bone resorption-implications for osteoclast quality. *BMC Musculoskelet. Disord.* 11:109. doi: 10.1186/1471-2474-11-109

Nguyen, N. K., Leoni, M., Maniglio, D., and Migliaresi, C. (2013). Hydroxyapatite nanorods: soft-template synthesis, characterization and preliminary *in vitro* tests. *J. Biomater. Appl.* 28, 49–61. doi: 10.1177/0885328212437065

Noordin, S., and Glowacki, J. (2016). Parathyroid hormone and its receptor gene polymorphisms: implications in osteoporosis and in fracture healing. *Rheumatol. Int.* 36, 1–6. doi: 10.1007/s00296-015-3319-9

Noronha Nc, N. D. C., Mizukami, A., Caliári-Oliveira, C., Cominal, J. G., Rocha, J. L. M., Covas, D. T., et al. (2019). Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. *Stem Cell Res. Ther.* 10:131. doi: 10.1186/s13287-019-1224-y

Nystedt, J., Anderson, H., Tikkanen, J., Pietil,ä, M., Hirvonen, T., Takalo, R., et al. (2013). Cell surface structures influence lung clearance rate of systemically infused mesenchymal stromal cells. *Stem Cells* 31, 317–326. doi: 10.1002/stem.1271

Ofengeim, D., Mazzitelli, S., Ito, Y., DeWitt, J. P., Mifflin, L., Zou, C., et al. (2017). RIPK1 mediates a disease-associated microglial response in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 114, E8788–E8797. doi: 10.1073/pnas.1714175114

Onal, M., Piemontese, M., Xiong, J., Wang, Y., Han, L., Ye, S., et al. (2013). Suppression of autophagy in osteocytes mimics skeletal aging. *J. Biol. Chem.* 288, 17432–17440. doi: 10.1074/jbc.M112.444190

Ooi, Y. Y., Dheen, S. T., and Sam Wah Tay, S. (2015). Paracrine effects of mesenchymal stem cells-conditioned medium on microglial cytokines expression and nitric oxide production. *Neuroimmunomodulation* 22, 233–242. doi: 10.1159/000365483

Osso, L. A., and Chan, J. R. (2019). Senescent glia spell trouble in Alzheimer's disease. *Nat. Neurosci.* 22, 683–684. doi: 10.1038/s41593-019-0395-2

Osta, B., Benedetti, G., and Miossec, P. (2014). Classical and paradoxical effects of TNF-α on bone homeostasis. *Front. Immunol.* 5:48. doi: 10.3389/fimmu.2014.00048

Osugi, M., Katagiri, W., Yoshimi, R., Inukai, T., Hibi, H., and Ueda, M. (2012). Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects. *Tissue Eng. A* 18, 1479–1489. doi: 10.1089/ten.tea.2011.0325

Oury, F., Khrimian, L., Denny, C. A., Gardin, A., Goeden, N., Huang, Y., et al. (2013). Maternal and offspring pools of osteocalcin influence brain development and functions. *Cell* 155, 228–241. doi: 10.1016/j.cell.2013.08.042

Ouyang, Y. B., Lu, Y., Yue, S., and Giffard, R. G. (2012). MiR-181 targets multiple Bcl-2 family members and influences apoptosis and mitochondrial function in astrocytes. *Mitochondrion* 12, 213–219. doi: 10.1016/j.mito.2011.09.001

Ovsianikov, A., Khademhosseini, A., and Mironov, V. (2018). The synergy of scaffold-based and scaffold-free tissue engineering strategies. *Trends Biotechnol.* 36, 348–357. doi: 10.1016/j.tibtech.2018.01.005

Panach, L., Mifsut, D., Tarín, J. J., Cano, A., and García-Pérez, M. Á. (2015). Serum circulating MicroRNAs as biomarkers of osteoporotic fracture. *Calcif. Tissue Int.* 97, 495–505. doi: 10.1007/s00223-015-0036-z

Panagopoulos, P., and Lambrou, G. (2018). The involvement of MicroRNAs in osteoarthritis and recent developments: a narrative review. *Mediterr. J. Rheumatol.* 29, 67–79. doi: 10.31138/mjr.29.2.67

Papadaki, G., Kambas, K., Choulaki, C., Vlachou, K., Drakos, E., Bertsias, G., et al. (2016). Neutrophil extracellular traps exacerbate Th1-mediated autoimmune responses in rheumatoid arthritis by promoting DC maturation. *Eur. J. Immunol.* 46, 2542–2554. doi: 10.1002/eji.201646542

Park, D., Yang, G., Bae, D. K., Lee, S. H., Yang, Y. H., Kyung, J., et al. (2013). Human adipose tissue-derived mesenchymal stem cells improve cognitive function and physical activity in ageing mice. *J. Neurosci. Res.* 91, 660–670. doi: 10.1002/jnr.23182

Park, E. H., Lim, H., suk, Lee, S., Roh, K., Seo, K. W., Kang, K. S., et al. (2018). Intravenous infusion of umbilical cord blood-derived mesenchymal stem cells in rheumatoid arthritis: a phase ia clinical trial. *Stem Cells Transl. Med.* 7, 636–642. doi: 10.1002/sctm.18-0031

Park, S. J., Cheon, E. J., and Kim, H. A. (2013). MicroRNA-558 regulates the expression of cyclooxygenase-2 and IL-1β-induced catabolic effects in human articular chondrocytes. *Osteoarthr. Cartil.* 21, 981–989. doi: 10.1016/j.joca.2013.04.012

Paspaliaris, V., and Kolios, G. (2019). Stem cells in osteoporosis: from biology to new therapeutic approaches. *Stem Cells Int.* 2019:1730978. doi: 10.1155/2019/1730978

Patel, S., Dhillon, M. S., Aggarwal, S., Marwaha, N., and Jain, A. (2013). Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am. J. Sports Med.* 41, 356–364. doi: 10.1177/0363546512471299

Pawlik, A., Ostanek, L., Brzosko, I., Brzosko, M., Masiuk, M., Machalinski, B., et al. (2003). The expansion of CD4+CD28- T cells in patients with rheumatoid arthritis. *Arthritis Res. Ther.* 5, R210–R213. doi: 10.1186/ar766

Pelletier, J. P., Raynauld, J. P., Abram, F., Dorais, M., Delorme, P., and Martel-Pelletier, J. (2018). Exploring determinants predicting response to intra-articular hyaluronic acid treatment in symptomatic knee osteoarthritis: 9-year follow-up data from the osteoarthritis initiative. *Arthritis Res. Ther.* 20, 1–10. doi: 10.1186/s13075-018-1538-7

Penney, J., Ralvenius, W. T., and Tsai, L. H. (2020). Modeling Alzheimer's disease with iPSC-derived brain cells. *Mol. Psychiatry* 25, 148–167. doi: 10.1038/s41380-019-0468-3

Perea, J. R., Llorens-Martín, M., Ávila, J., and Bolós, M. (2018). The role of microglia in the spread of tau: relevance for tauopathies. *Front. Cell. Neurosci.* 12:172. doi: 10.3389/fncel.2018.00172

Pietschmann, P., Mechtcheriakova, D., Meshcheryakova, A., Föger-Samwald, U., and Ellinger, I. (2016). Immunology of osteoporosis: a mini-review. *Gerontology* 62, 128–137. doi: 10.1159/000431091

Pirzgalska, R. M., Seixas, E., Seidman, J. S., Link, V. M., Sánchez, N. M., Mahú, I., et al. (2017). Sympathetic neuron-associated macrophages contribute to obesity by importing and metabolizing norepinephrine. *Nat. Med.* 23, 1309–1318. doi: 10.1038/nm.4422

Pollack, I. F., and Lund, R. D. (1990). The blood-brain barrier protects foreign antigens in the brain from immune attack. *Exp. Neurol.* 108, 114–121. doi: 10.1016/0014-4886(90)90017-M

Pope, R. M., and Shahrara, S. (2013). Possible roles of IL-12-family cytokines in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 9, 252–256. doi: 10.1038/nrrheum.2012.170

Qadir, A., Liang, S., Wu, Z., Chen, Z., Hu, L., and Qian, A. (2020). Senile osteoporosis: the involvement of differentiation and senescence of bone marrow stromal cells. *Int. J. Mol. Sci.* 21:349. doi: 10.3390/ijms21010349

Qadir, A. S., Um, S., Lee, H., Baek, K., Seo, B. M., Lee, G., et al. (2015). MiR-124 negatively regulates osteogenic differentiation and *in vivo* bone formation of mesenchymal stem cells. *J. Cell. Biochem.* 116, 730–742. doi: 10.1002/jcb.25026

Quigley, B. J., and Kowall, N. W. (1991). Substance P-like immunoreactive neurons are depleted in Alzheimer's disease cerebral cortex. *Neuroscience* 41, 41–60. doi: 10.1016/0306-4522(91)90199-X

Radner, H., and Aletaha, D. (2015). Anti-TNF in rheumatoid arthritis: an overview. *Wien. Med. Wochenschr.* 165, 3–9. doi: 10.1007/s10354-015-0344-y

Rana, D., Kumar, S., Webster, T. J., and Ramalingam, M. (2019). Impact of induced pluripotent stem cells in bone repair and regeneration. *Curr. Osteoporos. Rep.* 17, 226–234. doi: 10.1007/s11914-019-00519-9

Raphael, I., Nalawade, S., Eagar, T. N., and Forsthuber, T. G. (2015). T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine* 74, 5–17. doi: 10.1016/j.cyto.2014.09.011

Ravaglia, G., Forti, P., Maioli, F., Chiappelli, M., Montesi, F., Tumini, E., et al. (2007). Blood inflammatory markers and risk of dementia: the conselice study of brain aging. *Neurobiol. Aging* 28, 1810–1820. doi: 10.1016/j.neurobiolaging.2006.08.012

Ravari, A., Mirzaei, T., Kennedy, D., and Kazemi Arababadi, M. (2017). Chronoinflammaging in Alzheimer; a systematic review on the roles of toll like receptor 2. *Life Sci.* 171, 16–20. doi: 10.1016/j.lfs.2017.01.003

Reddy, P. H., Tonk, S., Kumar, S., Vijayan, M., Kandimalla, R., Kuruva, C. S., et al. (2017a). A critical evaluation of neuroprotective and neurodegenerative MicroRNAs in Alzheimer's disease. *Biochem. Biophys. Res. Commun.* 483, 1156–1165. doi: 10.1016/j.bbrc.2016.08.067

Reddy, P. H., Williams, J., Smith, F., Bhatti, J. S., Kumar, S., Vijayan, M., et al. (2017b). "MicroRNAs, aging, cellular senescence, and Alzheimer's disease," in *Progress inMolecular Biology and Translational Science*, ed D. B. Teplow (Cambridge, MA: Elsevier Inc.), 127–171.

Rentsendorj, A., Sheyn, J., Fuchs, D., Daley, D., Brenda, C., Schubloom, H. E., et al. (2018). A novel role for osteopontin in macrophage-mediated amyloid-(beta) clearance in Alzheimer's models. *Brain. Behav. Immun.* 67, 163–180. doi: 10.1016/j.bbi.2017.08.019

Reyes-Long, S., Cortes-Altamirano, J. L., Clavijio-Cornejo, D., Gutiérrez, M., Bertolazzi, C., Bandala, C., et al. (2020). Nociceptive related microRNAs and their role in rheumatoid arthritis. *Mol. Biol. Rep.* 47, 7265–7272. doi: 10.1007/s11033-020-05700-3

Richards, M. M., Maxwell, J. S., Weng, L., Angelos, M. G., and Golzarian, J. (2016). Intra-articular treatment of knee osteoarthritis: from anti-inflammatories to products of regenerative medicine. *Phys. Sportsmed.* 44, 101–108. doi: 10.1080/00913847.2016.1168272

Ricklin, D., Reis, E. S., and Lambris, J. D. (2016). Complement in disease: a defence system turning offensive. *Nat. Rev. Nephrol.* 12, 383–401. doi: 10.1038/nrneph.2016.70

Robinson, W. H., Lepus, C. M., Wang, Q., Raghu, H., Mao, R., Lindstrom, T. M., et al. (2016). Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. *Nat. Rev. Rheumatol.* 12, 580–592. doi: 10.1038/nrrheum.2016.136

Rodier, F., and Campisi, J. (2011). Four faces of cellular senescence. *J. Cell Biol.* 192, 547–556. doi: 10.1083/jcb.201009094

Rodriguez-Ortiz, C. J., Baglietto-Vargas, D., Martinez-Coria, H., LaFerla, F. M., and Kitazawa, M. (2014). Upregulation of miR-181 decreases c-Fos and SIRT-1 in the hippocampus of 3xTg-AD mice. *J. Alzheimer's Dis.* 42, 1229–1238. doi: 10.3233/JAD-140204

Roh, J. S., and Sohn, D. H. (2018). Damage-associated molecular patterns in inflammatory diseases. *Immune Netw.* 18, 1–14. doi: 10.4110/in.2018.18.e27

Rossouw, J. E., Anderson, G. L., Prentice, R. L., LaCroix, A. Z., Kooperberg, C., Stefanick, M. L., et al. (2009). Risks and benefits of estrogen plus progestin in healthy postmenopausal women. *JAMA* 288, 321–333. doi: 10.1001/jama.288.3.321

Roth, J., Harre, E.-M., Rummel, C., Gerstberger, R., and Hubschle, T. (2004). Signaling the brain in systemic inflammation: role of sensory circumventricular organs. *Front. Biosci.* 9, 290–300. doi: 10.2741/1241

Ryazantsev, S., Yu, W. H., Zhao, H. Z., Neufeld, E. F., and Ohmi, K. (2007). Lysosomal accumulation of SCMAS (subunit c of mitochondrial ATP synthase) in neurons of the mouse model of mucopolysaccharidosis III B. *Mol. Genet. Metab.* 90, 393–401. doi: 10.1016/j.ymgme.2006.11.006

Salloway, S., Sperling, R., Keren, R., Porsteinsson, A. P., Van Dyck, C. H., Tariot, P. N., et al. (2011). A phase 2 randomized trial of ELND005, scyllo-inositol, in mild to moderate Alzheimer disease. *Neurology* 77, 1253–1262. doi: 10.1212/WNL.0b013e3182309fa5

Sanchez, C., Deberg, M. A., Piccardi, N., Msika, P., Reginster, J. Y. L., and Henrotin, Y. E. (2005a). Osteoblasts from the sclerotic subchondral bone downregulate aggrecan but upregulate metalloproteinases expression by chondrocytes. This effect is mimicked by interleukin-6,−1β and oncostatin M pre-treated non-sclerotic osteoblasts. *Osteoarthr. Cartil.* 13, 979–987. doi: 10.1016/j.joca.2005.03.008

Sanchez, C., Deberg, M. A., Piccardi, N., Msika, P., Reginster, J. Y. L., and Henrotin, Y. E. (2005b). Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic chondrocytes. *Osteoarthr. Cartil.* 13, 988–997. doi: 10.1016/j.joca.2005.07.012

San-Marina, S., Sharma, A., Voss, S. G., Janus, J. R., and Hamilton, G. S. (2017). Assessment of scaffolding properties for chondrogenic differentiation of adipose-derived mesenchymal stem cells in nasal reconstruction. *JAMA Facial Plast. Surg.* 19, 108–114. doi: 10.1001/jamafacial.2016.1200

Sato, M., Sambito, M. A., Aslani, A., Kalkhoran, N. M., Slamovich, E. B., and Webster, T. J. (2006). Increased osteoblast functions on undoped and yttrium-doped nanocrystalline hydroxyapatite coatings on titanium. *Biomaterials* 27, 2358–2369. doi: 10.1016/j.biomaterials.2005.10.041

Schoels, M. M., Van Der Heijde, D., Breedveld, F. C., Burmester, G. R., Dougados, M., Emery, P., et al. (2013). Blocking the effects of interleukin-6 in rheumatoid arthritis and other inflammatory rheumatic diseases: systematic literature review and meta-analysis informing a consensus statement. *Ann. Rheum. Dis.* 72, 583–589. doi: 10.1136/annrheumdis-2012-202470

Serre, C. M., Farlay, D., Delmas, P. D., and Chenu, C. (1999). Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. *Bone* 25, 623–629. doi: 10.1016/S8756-3282(99)00215-X

Shen, L., Yuan, T., Chen, S., Xie, X., and Zhang, C. (2017). The temporal effect of platelet-rich plasma on pain and physical function in the treatment of knee osteoarthritis: systematic review and meta-analysis of randomized controlled trials. *J. Orthop. Surg. Res.* 12:16. doi: 10.1186/s13018-017-0521-3

Shi, Y., Wang, Y., Li, Q., Liu, K., Hou, J., Shao, C., et al. (2018). Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat. Rev. Nephrol.* 14, 493–507. doi: 10.1038/s41581-018-0023-5

Shigeyama, Y., Pap, T., Kunzler, P., Simmen, B. R., Gay, R. E., and Gay, S. (2000). Expression of osteoclast differentiation factor in rheumatoid arthritis. *Arthritis Rheum.* 43, 2523–2530. doi: 10.1002/1529-0131(200011)43:11<2523::AID-ANR20>3.0.CO;2-Z

Shill, L. C., Purba, N. H., Sultana, M., Hasan, T., Rahman, M., and Sultana, N. (2019). An update on osteoporosis research: effect of calcium plus vitamin D supplementation. *Int. J. Commun. Med. Public Health.* 6:4120. doi: 10.18203/2394-6040.ijcmph20194028

Shin, D. E., Ahn, T. K., Kim, J. W., Oh, C. H., and Choi, S. J. (2018). Testosterone and male osteoporosis. *Clin. Rev. Bone Miner. Metab.* 16, 49–56. doi: 10.1007/s12018-018-9245-0

Shukla, V., Skuntz, S., and Pant, H. C. (2012). Deregulated Cdk5 activity is involved in inducing Alzheimer's disease. *Arch. Med. Res.* 43, 655–662. doi: 10.1016/j.arcmed.2012.10.015

Smolen, J. S., Kay, J., Doyle, M. K., Landew,é, R., Matteson, E. L., Wollenhaupt, J., et al. (2009). Golimumab in patients with active rheumatoid arthritis after treatment with tumour necrosis factor α inhibitors (GO-AFTER study): a multicentre, randomised, double-blind, placebo-controlled, phase III trial. *Lancet* 374, 210–221. doi: 10.1016/S0140-6736(09)60506-7

Sondag, G. R., and Haqqi, T. M. (2016). The role of MicroRNAs and their targets in osteoarthritis. *Curr. Rheumatol. Rep.* 18:56. doi: 10.1007/s11926-016-0604-x

Song, J., and Lee, J. E. (2015). MiR-155 is involved in Alzheimer's disease by regulating T lymphocyte function. *Front. Aging Neurosci.* 7:61. doi: 10.3389/fnagi.2015.00061

Sonomoto, K., Yamaoka, K., Kaneko, H., Yamagata, K., Sakata, K., Zhang, X., et al. (2016). Spontaneous differentiation of human mesenchymal stem cells on poly-lactic-co-glycolic acid nano-fiber scaffold. *PLoS ONE* 11:e0153231. doi: 10.1371/journal.pone.0153231

Srivastava, R. K., Dar, H. Y., and Mishra, P. K. (2018). Immunoporosis: immunology of osteoporosis-role of T cells. *Front. Immunol.* 9:657. doi: 10.3389/fimmu.2018.00657

Sterniczuk, R., Dyck, R. H., Laferla, F. M., and Antle, M. C. (2010). Characterization of the 3xTg-AD mouse model of Alzheimer's disease: part 1. Circadian changes. *Brain Res.* 1348, 139–148. doi: 10.1016/j.brainres.2010.05.013

Su, C., Zhao, K., Xia, H., and Xu, Y. (2019). Peripheral inflammatory biomarkers in Alzheimer's disease and mild cognitive impairment: a systematic review and meta-analysis. *Psychogeriatrics* 19, 300–309. doi: 10.1111/psyg.12403

Sugatani, T., Vacher, J., and Hruska, K. A. (2011). A microRNA expression signature of osteoclastogenesis. *Blood* 117, 3648–3657. doi: 10.1182/blood-2010-10-311415

Sui, B., Hu, C., Zhang, X., Zhao, P., He, T., Zhou, C., et al. (2016). Allogenic mesenchymal stem cell therapy promotes osteoblastogenesis and prevents glucocorticoid-induced osteoporosis. *Stem Cells Transl. Med.* 5, 1238–1246. doi: 10.5966/sctm.2015-0347

Sun, X. H., Liu, Y., Han, Y., and Wang, J. (2016). Expression and significance of high-mobility group protein B1 (HMGB1) and the receptor for advanced glycation end-product (RAGE) in knee osteoarthritis. *Med. Sci. Monit.* 22, 2105–2112. doi: 10.12659/MSM.895689

Suzuki, K., and Terry, R. D. (1967). Fine structural localization of acid phosphatase in senile plaques in Alzheimer's presenile dementia. *Acta Neuropathol.* 8, 276–284. doi: 10.1007/BF00688828

Swardfager, W., Lanctt, K., Rothenburg, L., Wong, A., Cappell, J., and Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biol. Psychiatry* 68, 930–941. doi: 10.1016/j.biopsych.2010.06.012

Szot, P., White, S. S., Lynne Greenup, J., Leverenz, J. B., Peskind, E. R., and Raskind, M. A. (2006). Compensatory changes in the noradrenergic nervous system in the locus ceruleus and hippocampus of postmortem subjects with Alzheimer's disease and dementia with lewy bodies. *J. Neurosci.* 26, 467–478. doi: 10.1523/JNEUROSCI.4265-05.2006

Szot, P., White, S. S., Lynne Greenup, J., Leverenz, J. B., Peskind, E. R., and Raskind, M. A. (2007). Changes in adrenoreceptors in the prefrontal cortex of subjects with dementia: evidence of compensatory changes. *Neuroscience* 146, 471–480. doi: 10.1016/j.neuroscience.2007.01.031

Tahamtan, A., Teymoori-Rad, M., Nakstad, B., and Salimi, V. (2018). Anti-inflammatory MicroRNAs and their potential for inflammatory diseases treatment. *Front. Immunol.* 9:1377. doi: 10.3389/fimmu.2018.01377

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872. doi: 10.1016/j.cell.2007.11.019

Takei, H., Araki, A., Watanabe, H., Ichinose, A., and Sendo, F. (1996). Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate

(PMA) accompanied by changes different from typical apoptosis or necrosis. *J. Leukoc. Biol.* 59, 229–240. doi: 10.1002/jlb.59.2.229

Tanaka, S. M., Sun, H. B., Roeder, R. K., Burr, D. B., Turner, C. H., and Yokota, H. (2005). Osteoblast responses one hour after load-induced fluid flow in a three-dimensional porous matrix. *Calcif. Tissue Int.* 76, 261–271. doi: 10.1007/s00223-004-0238-2

Tavangar, B., Arasteh, S., Edalatkhah, H., Salimi, A., Doostmohammadi, A., and Seyedjafari, E. (2018). Hardystonite-coated poly(L-lactide) nanofibrous scaffold and efficient osteogenic differentiation of adipose-derived mesenchymal stem cells. *Artif. Organs* 42, E335–E348. doi: 10.1111/aor.12891

Tcw, J. (2019). Human iPSC application in Alzheimer's disease and Tau-related neurodegenerative diseases. *Neurosci. Lett.* 699, 31–40. doi: 10.1016/j.neulet.2019.01.043

Tomlinson, B. E., Blessed, G., and Roth, M. (1970). Observations on the brains of demented old people. *J. Neurol. Sci.* 11, 205–242. doi: 10.1016/0022-510X(70)90063-8

Trollor, J. N., Smith, E., Agars, E., Kuan, S. A., Baune, B. T., Campbell, L., et al. (2012). The association between systemic inflammation and cognitive performance in the elderly: the sydney memory and ageing study. *Age* 34, 1295–1308. doi: 10.1007/s11357-011-9301-x

Tung, Y. T., Wang, B. J., Hu, M. K., Hsu, W. M., Lee, H., Huang, W. P., et al. (2012). Autophagy: a double-edged sword in Alzheimer's disease. *J. Biosci.* 37, 157–165. doi: 10.1007/s12038-011-9176-0

Udalova, I. A., Mantovani, A., and Feldmann, M. (2016). Macrophage heterogeneity in the context of rheumatoid arthritis. *Nat. Rev. Rheumatol.* 12, 472–485. doi: 10.1038/nrrheum.2016.91

Ursini, F., Leporini, C., Bene, F., D'Angelo, S., Mauro, D., Russo, E., et al. (2017). Anti-TNF-alpha agents and endothelial function in rheumatoid arthritis: a systematic review and meta-analysis. *Sci. Rep.* 7:5346. doi: 10.1038/s41598-017-05759-2

Vande Walle, L., and Lamkanfi, M. (2016). Pyroptosis. *Curr. Biol.* 26, R568–R572. doi: 10.1016/j.cub.2016.02.019

Vaquerizo, V., Plasencia, M. Á., Arribas, I., Seijas, R., Padilla, S., Orive, G., et al. (2013). Comparison of intra-articular injections of plasma rich in growth factors (PRGF-Endoret) versus durolane hyaluronic acid in the treatment of patients with symptomatic osteoarthritis: a randomized controlled trial. *Arthrosc. J. Arthrosc. Relat. Surg.* 29, 1635–1643. doi: 10.1016/j.arthro.2013.07.264

Venugopal, J., Rajeswari, R., Shayanti, M., Low, S., Bongso, A., Giri Dev, V. R., et al. (2013). Electrosprayed hydroxyapatite on polymer nanofibers to differentiate mesenchymal stem cells to osteogenesis. *J. Biomater. Sci. Polym. Ed.* 24, 170–184. doi: 10.1163/156856212X629845

Vershinin, M., Carter, B. C., Razafsky, D. S., King, S. J., and Gross, S. P. (2007). Multiple-motor based transport and its regulation by Tau. *Proc. Natl. Acad. Sci. U.S.A.* 104, 87–92. doi: 10.1073/pnas.0607919104

Vitturi, B. K., Nascimento, B. A. C., Alves, B. R., de Campos, F. S. C., and Torigoe, D. Y. (2019). Cognitive impairment in patients with rheumatoid arthritis. *J. Clin. Neurosci.* 69, 81–87. doi: 10.1016/j.jocn.2019.08.027

Walker, K. A., Ficek, B. N., and Westbrook, R. (2019a). Understanding the role of systemic inflammation in Alzheimer's disease. *ACS Chem. Neurosci.* 10, 3340–3342. doi: 10.1021/acschemneuro.9b00333

Walker, K. A., Gottesman, R. F., Wu, A., Knopman, D. S., Gross, A. L., Mosley, T. H., et al. (2019b). Systemic inflammation during midlife and cognitive change over 20 years: the ARIC study. *Neurology* 92, E1256–E1267. doi: 10.1212/WNL.0000000000007094

Walker, K. A., Hoogeveen, R. C., Folsom, A. R., Ballantyne, C. M., Knopman, D. S., Windham, B. G., et al. (2017). Midlife systemic inflammatory markers are associated with late-life brain volume: the ARIC study. *Neurology* 89, 2262–2270. doi: 10.1212/WNL.0000000000004688

Walker, K. A., Windham, B. G., Power, M. C., Hoogeveen, R. C., Folsom, A. R., Ballantyne, C. M., et al. (2018). The association of mid- to late-life systemic inflammation with white matter structure in older adults: the ARIC study. *Neurobiol. Aging* 68, 26–33. doi: 10.1016/j.neurobiolaging.2018.03.031

Wallin, K., Solomon, A., Kreholt, I., Tuomilehto, J., Soininen, H., and Walin, K. (2012). Midlife rheumatoid arthritis increases the risk of cognitive impairment two decades later: a population-based study. *J. Alzheimer's Dis.* 31, 669–676. doi: 10.3233/JAD-2012-111736

Wang, N., Xiao, Z., Zhao, Y., Wang, B., Li, X., Li, J., et al. (2018). Collagen scaffold combined with human umbilical cord-derived mesenchymal stem cells promote functional recovery after scar resection in rats with chronic spinal cord injury. *J. Tissue Eng. Regen. Med.* 12, e1154–e1163. doi: 10.1002/term.2450

Wang, S., Guo, L., Ge, J., Yu, L., Cai, T., Tian, R., et al. (2015). Excess integrins cause lung entrapment of mesenchymal stem cells. *Stem Cells* 33, 3315–3326. doi: 10.1002/stem.2087

Wang, W., Shao, S., Jiao, Z., Guo, M., Xu, H., and Wang, S. (2012). The Th17/Treg imbalance and cytokine environment in peripheral blood of patients with rheumatoid arthritis. *Rheumatol. Int.* 32, 887–893. doi: 10.1007/s00296-010-1710-0

Wang, Y., Li, L., Moore, B. T., Peng, X. H., Fang, X., Lappe, J. M., et al. (2012). Mir-133a in human circulating monocytes: a potential biomarker associated with postmenopausal osteoporosis. *PLoS ONE* 7:e34641. doi: 10.1371/journal.pone.0034641

Wang, Z. Y., Shi, S. Y., Li, S. J., Chen, F., Chen, H., Lin, H. Z., et al. (2015). Efficacy and safety of duloxetine on osteoarthritis knee pain: a meta-analysis of randomized controlled trials. *Pain Med.* 16, 1373–1385. doi: 10.1111/pme.12800

Warren, S. M., Steinbrech, D. S., Mehrara, B. J., Saadeh, P. B., Greenwald, J. A., Spector, J. A., et al. (2001). Hypoxia regulates osteoblast gene expression. *J. Surg. Res.* 99, 147–155. doi: 10.1006/jsre.2001.6128

Waters, S. M., and Davis, T. P. (1997). Alterations of peptide metabolism and neuropeptidase activity in senile dementia of the Alzheimer's type. *Ann. N. Y. Acad. Sci.* 814, 30–39. doi: 10.1111/j.1749-6632.1997.tb46142.x

Watt, I. (2009). Osteoarthritis revisited - again! *Skeletal Radiol.* 38, 419–423. doi: 10.1007/s00256-008-0637-y

Weaver, D. F., and Carter, M. D. (2008). Does NSAID use modify cognitive trajectories in the elderly? The cache county study. *Neurology* 70:895. doi: 10.1212/01.wnl.0000307673.96325.ab

Weber, A., Chan, P. M. B., and Wen, C. (2019a). Do immune cells lead the way in subchondral bone disturbance in osteoarthritis? *Prog. Biophys. Mol. Biol.* 148, 21–31. doi: 10.1016/j.pbiomolbio.2017.12.004

Weber, A., Mak, S. H., Berenbaum, F., Sellam, J., Zheng, Y. P., Han, Y., et al. (2019b). Association between osteoarthritis and increased risk of dementia: a systemic review and meta-analysis. *Medicine* 98:e14355. doi: 10.1097/MD.0000000000014355

Wei, Y., Xie, Z., Bi, J., and Zhu, Z. (2018). Anti-inflammatory effects of bone marrow mesenchymal stem cells on mice with alzheimer's disease. *Exp. Ther. Med.* 16, 5015–5020. doi: 10.3892/etm.2018.6857

Wei, Z., Chen, X. C., Song, Y., Pan, X. D., Dai, X. M., Zhang, J., et al. (2016). Amyloid β protein aggravates neuronal senescence and cognitive deficits in 5XFAD mouse model of Alzheimer's disease. *Chin. Med. J.* 129, 1835–1844. doi: 10.4103/0366-6999.186646

Weitzmann, N. M. (2014). T-cells and B-cells in osteoporosis. *Curr. Opin. Endocrinol. Diabetes Obes.* 21, 461–467. doi: 10.1097/MED.0000000000000103

Westacott, C. I., Webb, G. R., Warnock, M. G., Sims, J. V., and Elson, C. J. (1997). Alteration of cartilage metabolism by cells from osteoarthritic bone. *Arthritis Rheum.* 40, 1282–1291.

Wheater, G., Hogan, V. E., Teng, Y. K. O., Tekstra, J., Lafeber, F. P., Huizinga, T. W. J., et al. (2011). Suppression of bone turnover by B-cell depletion in patients with rheumatoid arthritis. *Osteoporos. Int.* 22, 3067–3072. doi: 10.1007/s00198-011-1607-0

Wiessner, C., Wiederhold, K. H., Tissot, A. C., Frey, P., Danner, S., Jacobson, L. H., et al. (2011). The second-generation active Aβ immunotherapy CAD106 reduces amyloid accumulation in APP transgenic mice while minimizing potential side effects. *J. Neurosci.* 31, 9323–9331. doi: 10.1523/JNEUROSCI.0293-11.2011

Wiley, C. D., Velarde, M. C., Lecot, P., Liu, S., Sarnoski, E. A., Freund, A., et al. (2016). Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab.* 23, 303–314. doi: 10.1016/j.cmet.2015.11.011

Williams, A. J., Jordan, F., King, G., Lewis, A. L., Illum, L., Masud, T., et al. (2018). *In vitro* and preclinical assessment of an intranasal spray formulation of parathyroid hormone PTH 1–34 for the treatment of osteoporosis. *Int. J. Pharm.* 535, 113–119. doi: 10.1016/j.ijpharm.2017.10.029

Wojdasiewicz, P., Turczyn, P., Dobies-Krzesniak, B., Frasunska, J., and Tarnacka, B. (2019). Role of CX3CL1/CX3CR1 signaling axis activity in osteoporosis. *Mediators Inflamm.* 2019:7570452. doi: 10.1155/2019/7570452

Wong, H. K. A., Veremeyko, T., Patel, N., Lemere, C. A., Walsh, D. M., Esau, C., et al. (2013). De-repression of FOXO3a death axis by microRNA-132 and−212 causes neuronal apoptosis in Alzheimer's disease. *Hum. Mol. Genet.* 22, 3077–3092. doi: 10.1093/hmg/ddt164

Wu, G., Xu, R., Zhang, P., Xiao, T., Fu, Y., Zhang, Y., et al. (2018). Estrogen regulates stemness and senescence of bone marrow stromal cells to prevent osteoporosis via ERβ-SATB2 pathway. *J. Cell. Physiol.* 233, 4194–4204. doi: 10.1002/jcp.26233

Wu, N., Liu, B., Du, H., Zhao, S., Li, Y., Cheng, X., et al. (2019). The progress of CRISPR/Cas9-mediated gene editing in generating mouse/zebrafish models of human skeletal diseases. *Comput. Struct. Biotechnol. J.* 17, 954–962. doi: 10.1016/j.csbj.2019.06.006

Xia, W.-F., Jung, J.-U., Shun, C., Xiong, S., Xiong, L., Shi, X.-M., et al. (2013). Swedish mutant APP suppresses osteoblast differentiation and causes osteoporotic deficit, which are ameliorated by N-Acetyl-L-cysteine. *J. Bone Miner. Res.* 28, 2122–2135. doi: 10.1002/jbmr.1954

Xu, G., Zhang, Z., Xing, Y., Wei, J., Ge, Z., Liu, X., et al. (2014). MicroRNA-149 negatively regulates TLR-triggered inflammatory response in macrophages by targeting MyD88. *J. Cell. Biochem.* 115, 919–927. doi: 10.1002/jcb.24734

Xu, M., Tchkonia, T., Ding, H., Ogrodnik, M., Lubbers, E. R., Pirtskhalava, T., et al. (2015). JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc. Natl. Acad. Sci. U.S.A.* 112, E6301–E6310. doi: 10.1073/pnas.1515386112

Xu, Z., Nan, W., Zhang, X., Sun, Y., Yang, J., Lu, K., et al. (2018). Umbilical cord mesenchymal stem cells conditioned medium promoted AB25-35 phagocytosis by modulating autophagy and AB-degrading enzymes in BV2 cells. *J. Mol. Neurosci.* 65, 222–233. doi: 10.1007/s12031-018-1075-5

Yan, S., Wang, M., Zhao, J., Zhang, H., Zhou, C., Jin, L., et al. (2016). MicroRNA-34a affects chondrocyte apoptosis and proliferation by targeting the SIRT1/p53 signaling pathway during the pathogenesis of osteoarthritis. *Int. J. Mol. Med.* 38, 201–209. doi: 10.3892/ijmm.2016.2618

Yang, D. S., Stavrides, P., Mohan, P. S., Kaushik, S., Kumar, A., Ohno, M., et al. (2011). Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. *Brain* 134, 258–277. doi: 10.1093/brain/awq341

Yao, W., Dai, W., Jiang, L., Lay, E. Y. A., Zhong, Z., Ritchie, R. O., et al. (2016). Sclerostin-antibody treatment of glucocorticoid-induced osteoporosis maintained bone mass and strength. *Osteoporos. Int.* 27, 283–294. doi: 10.1007/s00198-015-3308-6

Yavropoulou, M. P., Anastasilakis, A. D., Makras, P., Papatheodorou, A., Rauner, M., Hofbauer, L. C., et al. (2020). Serum profile of microRNAs linked to bone metabolism during sequential treatment for postmenopausal osteoporosis. *J. Clin. Endocrinol. Metab.* 105, e2885–e2894. doi: 10.1210/clinem/dgaa368

Yavropoulou, M. P., Anastasilakis, A. D., Makras, P., Tsalikakis, D. G., Grammatiki, M., and Yovos, J. G. (2017). Expression of microRNAs that regulate bone turnover in the serum of postmenopausal women with low bone mass and vertebral fractures. *Eur. J. Endocrinol.* 176, 169–176. doi: 10.1530/EJE-16-0583

Yi, J.-S., Huang, Y., Kwaczala, A. T., Kuo, I. Y., Ehrlich, B. E., Campbell, S. G., et al. (2016). Low-dose dasatinib rescues cardiac function in noonan syndrome. *JCI Insight* 1, 1–16. doi: 10.1172/jci.insight.90220

Yin, K., Wang, S., and Zhao, R. C. (2019). Exosomes from mesenchymal stem/stromal cells: a new therapeutic paradigm. *Biomark. Res.* 7:8. doi: 10.1186/s40364-019-0159-x

Yipp, B. G., and Kubes, P. (2013). NETosis: how vital is it? *Blood* 122, 2784–2794. doi: 10.1182/blood-2013-04-457671

Young, E., Gould, D., and Hart, S. (2020). Toward gene therapy in rheumatoid arthritis. *Expert Rev. Precis. Med. Drug Dev.* 5, 123–133. doi: 10.1080/23808993.2020.1736942

Yu, Y., Lu, L., and Guo, Z. (2019). Effect of TNF-α on osteoporosis by regulating the RANKL/OPG signaling pathway. *Int. J. Clin. Exp. Med.* 12, 6925–6931. doi: 10.3892/mmr.2018.8698

Yuan, J., Meloni, B. P., Shi, T., Bonser, A., Papadimitriou, J. M., Mastaglia, F. L., et al. (2019a). The potential influence of bone-derived modulators on the progression of Alzheimer's disease. *J. Alzheimer's Dis.* 69, 59–70. doi: 10.3233/JAD-181249

Yuan, J., Tickner, J., Mullin, B. H., Zhao, J., Zeng, Z., Morahan, G., et al. (2019b). Advanced genetic approaches in discovery and characterization of genes involved with osteoporosis in mouse and human. *Front. Genet.* 10:288. doi: 10.3389/fgene.2019.00288

Zakrzewski, W., Dobrzyński, M., Szymonowicz, M., and Rybak, Z. (2019). Stem cells: past, present, and future. *Stem Cell Res. Ther.* 10:68. doi: 10.1186/s13287-019-1165-5

Zandi, P. P., and Breitner, J. C. S. (2001). Do NSAIDs prevent Alzheimer's disease? And, if so, why? The epidemiological evidence. *Neurobiol. Aging* 22, 811–817. doi: 10.1016/S0197-4580(01)00297-4

Zare-shahabadi, A., Masliah, E., Johnson, G. V. W., and Rezaei, N. (2015). Autophagy in Alzheimer's disease. *Rev. Neurosci.* 26, 385–395. doi: 10.1515/revneuro-2014-0076

Zeng, C., Lane, N. E., Hunter, D. J., Wei, J., Choi, H. K., McAlindon, T. E., et al. (2019). Intra-articular corticosteroids and the risk of knee osteoarthritis progression: results from the osteoarthritis initiative. *Osteoarthr. Cartil.* 27, 855–862. doi: 10.1016/j.joca.2019.01.007

Zhang, H. G., Wang, X. B., Zhao, H., and Zhou, C. N. (2019). MicroRNA-9-5p promotes osteoporosis development through inhibiting osteogenesis and promoting adipogenesis via targeting Wnt3a. *Eur. Rev. Med. Pharmacol. Sci.* 23, 456–463. doi: 10.26355/eurrev_201901_16855

Zhang, L., Xing, R., Huang, Z., Zhang, N., Zhang, L., Li, X., et al. (2019). Inhibition of synovial macrophage pyroptosis alleviates synovitis and fibrosis in knee osteoarthritis. *Mediators Inflamm.* 2019:2165918. doi: 10.1155/2019/2165918

Zhang, P., Kishimoto, Y., Grammatikakis, I., Gottimukkala, K., Cutler, R. G., Zhang, S., et al. (2019). Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. *Nat. Neurosci.* 22, 719–728. doi: 10.1038/s41593-019-0372-9

Zhang, Q., Su, J., Wang, Z., Qi, H., Ge, Z., Li, Z., et al. (2017). MicroRNA-149* suppresses hepatic inflammatory response through antagonizing STAT3 signaling pathway. *Oncotarget* 8, 65397–65406. doi: 10.18632/oncotarget.18541

Zhang, X., Wang, C., Zhao, J., Xu, J., Geng, Y., Dai, L., et al. (2017). MiR-146a facilitates osteoarthritis by regulating cartilage homeostasis via targeting Camk2d and Ppp3r2. *Cell Death Dis.* 8, 1–11. doi: 10.1038/cddis.2017.146

Zhang, Z., Kang, Y., Zhang, Z., Zhang, H., Duan, X., Liu, J., et al. (2012). Expression of microRNAs during chondrogenesis of human adipose-derived stem cells. *Osteoarthr. Cartil.* 20, 1638–1646. doi: 10.1016/j.joca.2012.08.024

Zhao, Y., Zhang, Y., Zhang, L., Dong, Y., Ji, H., and Shen, L. (2019). The potential markers of circulating micrornas and long non-coding RNAs in Alzheimer's disease. *Aging Dis.* 10, 1293–1301. doi: 10.14336/AD.2018.1105

Zheng, B., Von See, M. P., Yu, E., Gunel, B., Lu, K., Vazin, T., et al. (2016). Quantitative magnetic particle imaging monitors the transplantation, biodistribution, and clearance of stem cells *in vivo*. *Theranostics* 6, 291–301. doi: 10.7150/thno.13728

Zhou, J. N., Hofman, M. A., and Swaab, D. F. (1995). VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. *Neurobiol. Aging* 16, 571–576. doi: 10.1016/0197-4580(95)00043-E

Zhou, X., Castro, N. J., Zhu, W., Cui, H., Aliabouzar, M., Sarkar, K., et al. (2016). Improved human bone marrow mesenchymal stem cell osteogenesis in 3D bioprinted tissue scaffolds with low intensity pulsed ultrasound stimulation. *Sci. Rep.* 6:32876. doi: 10.1038/srep32876

Zhu, S., Pan, W., Song, X., Liu, Y., Shao, X., Tang, Y., et al. (2012). The microRNA miR-23b suppresses IL-17-associated autoimmune inflammation by targeting TAB2, TAB3 and IKK-α. *Nat. Med.* 18, 1077–1086. doi: 10.1038/nm.2815

Zhu, W., Zhang, X., Jiang, Y., Liu, X., Huang, L., Wei, Q. J., et al. (2020). Alterations in peripheral T cell and B cell subsets in patients with osteoarthritis. *Clin. Rheumatol.* 39, 523–532. doi: 10.1007/s10067-019-04768-y

Zovoilis, A., Agbemenyah, H. Y., Agis-Balboa, R. C., Stilling, R. M., Edbauer, D., Rao, P., et al. (2011). MicroRNA-34c is a novel target to treat dementias. *EMBO J.* 30, 4299–4308. doi: 10.1038/emboj.2011.327

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.

Copyright © 2020 Culibrk and Hahn. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

GLOSSARY

ABCA1, ATP-binding cassette transporter sub-family A; ACE-R, Addenbrooke's Cognitive Examination Revised; ACPA, Antibodies to citrullinated protein antigens; AD, Alzheimer's Disease; ADAMTS, Disintegrin and metalloproteinase with thrombospondin motifs; AGG, Aggrecan; AIA, Antigen-induced arthritis; ALP, Alkaline phosphatase; A-MSC, Adipose-derived mesenchymal stem cell; APOE, Apolipoprotein E; APP, Apolipoprotein; BACE1, Beta-secretase; BBB, Blood-brain barrier; BDNF, Brain-derived neurotrophic factor; BIM, Bcl-2-like protein 11; BMD, Bone mineral density; BM-MSC, Bone marrow-derived mesenchymal stem cell; CCL, C-C motif chemokine ligand; CD##, Cluster of differentiation ##; CDK5, Cyclin dependent kinase 5; CIA, Collagen-induced arthritis; CNS, Central nervous system; Col1, Collagen-1; CSF, Cerebrospinal fluid; CXCL, C-X-C motif chemokine; CXCR; C-X-C motif receptor; DAM, Disease-associated microglia; DAMP, Damage-associated molecular pattern; DBH, Dopamine beta-hydroxylase; DMARD, Disease-modifying anti-rheumatic drugs; ECM, Extracellular matrix; FGFR1, Fibroblast growth factor receptor 1; GABA, Gamma aminobutyric acid; GCRP, Calcitonin gene-related peptide; HA, Hydroxyapatite; HMGB1, High-mobility group box 1; HPSC, Hematopoietic progenitor cell; HS, Hardystonite; hUC-MSC, Human umbilical cord-derived mesenchymal stem cell; IFN-γ, Interferon gamma; IL, Interleukin; IV, Intravenous; IκB, Inhibitor of nuclear factor κB; LBD, Lewy body dementia; LPL, Lipoprotein lipase; LOAD, Late-onset Alzheimer's Disease; LPS, Lipopolysaccharide; MAC, Membrane attack complex; MCI, Mild cognitive impairment; MCP-1, Macrophage chemoattractant protein 1; M-CSF, Macrophage colony stimulating factor; MMP, Matrix metalloproteinase; MMSE, Mini Mental State Examination; MSC, Mesenchymal stem cell; MSC-CM, Mesenchymal stem cell conditioned medium; NE, Norepinephrine; NET, Neutrophil extracellular trap; NF, Nanofiber; NF-κB, Nuclear factor κB; NPY, Neuropeptide Y; OA, Osteoarthritis; OCN, Osteocalcin; OP, Osteoporosis; OPC, Oligodendrocyte precursor cell; OPG, Osteoprotegerin; OPN, Osteopontin; OS, Oyster shell; PAD4, Protein-arginine deiminase 4; PCL, Poly(ϵ-caprolactone); PDO, Polydioxanone; PLGA, Poly-lactic-co-glycolic acid; PLO, Poly-L-ornithine; PNS, Peripheral nervous system; RA, Rheumatoid arthritis; RAF, RAF proto-oncogene serine/threonine-protein kinase; RAGE, Receptor for advanced glycated end products; RANKL, Receptor activation of nuclear factor κB; REST, RE1-silencing transcription factor; ROS, Reactive oxygen species; SAN, Self-assembling nanofiber scaffold; SASP, Senescence-associated secretory phenotype; SERM, Selective estrogen response modulator; SF, Synovial fluid; SIRT1, Sirtuin 1; SP, Substance P; SYN, Alpha-synuclein; TCP, beta-tricalcium phosphate; TGF, Transforming growth factor; TH, Tyrosine hydroxylase; Th1, T helper subtype 1; TLR, Toll-like receptor; TNF, Tumor necrosis factor; Treg, Regulatory T-cell; TREM2, Triggering receptor on myeloid cells 2; VCAM, Vascular cell adhesion protein; VIP, Vasoactive intestinal peptide; YY1, Yin yang 1.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 25 January 2021
doi: 10.3389/fnagi.2020.584863

Dynamics and Concordance Abnormalities Among Indices of Intrinsic Brain Activity in Individuals With Subjective Cognitive Decline: A Temporal Dynamics Resting-State Functional Magnetic Resonance Imaging Analysis

Yiwen Yang[1,2] Xinyi Zha[1], Xiaodong Zhang[3], Jun Ke[1,2], Su Hu[1,2], Ximing Wang[1,2], Yunyan Su[1,2] and Chunhong Hu[1,2]* for the Alzheimer's Disease Neuroimaging Initiative[†]*

[1] Department of Radiology, The First Affiliated Hospital of Soochow University, Suzhou, China, [2] Institute of Medical Imaging, Soochow University, Suzhou, China, [3] Department of Radiology, Tianjin First Central Hospital, Tianjin, China

OPEN ACCESS

Edited by:
Yang Xiang,
Western Theater General Hospital, China

Reviewed by:
Indira García Cordero,
Universidad de San Andrés, Argentina
Ying Han,
Capital Medical University, China
Bin Jing,
Capital Medical University, China

***Correspondence:**
Chunhong Hu
hch5305@163.com
Yunyan Su
suyunyanhappy@163.com

[†] Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. A complete list of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgment_List.pdf

Received: 18 July 2020
Accepted: 03 December 2020
Published: 25 January 2021

Citation:
Yang Y, Zha X, Zhang X, Ke J, Hu S, Wang X, Su Y and Hu C (2021) Dynamics and Concordance Abnormalities Among Indices of Intrinsic Brain Activity in Individuals With Subjective Cognitive Decline: A Temporal Dynamics Resting-State Functional Magnetic Resonance Imaging Analysis. Front. Aging Neurosci. 12:584863. doi: 10.3389/fnagi.2020.584863

Individuals with subjective cognitive decline (SCD) are more likely to develop into Alzheimer disease (AD) in the future. Resting-state functional magnetic resonance imaging (rs-fMRI) studies have shown alterations of intrinsic brain activity (IBA) in SCD individuals. However, rs-fMRI studies to date have mainly focused on static characteristics of IBA, with few studies reporting dynamics- and concordance-related changes in IBA indices in SCD individuals. To investigate these aberrant changes, a temporal dynamic analysis of rs-fMRI data was conducted on 94 SCD individuals (71.07 ± 6.18 years, 60 female), 75 (74.36 ± 8.42 years, 35 female) mild cognitive impairment (MCI) patients, and 82 age-, gender-, and education-matched controls (NCs; 73.88 ± 7.40 years, 49 female) from the Alzheimer's Disease Neuroimaging Initiative database. The dynamics and concordance of the rs-fMRI indices were calculated. The results showed that SCD individuals had a lower amplitude of low-frequency fluctuations dynamics in bilateral hippocampus (HP)/parahippocampal gyrus (PHG)/fusiform gyrus (FG) and bilateral cerebellum, a lower fractional amplitude of low-frequency fluctuation dynamics in bilateral precuneus (PreCu) and paracentral lobule, and a lower regional homogeneity dynamics in bilateral cerebellum, vermis, and left FG compared with the other two groups, whereas those in MCI patients were higher (Gaussian random field–corrected, voxel-level $P < 0.001$, cluster-level $P < 0.05$). Furthermore, SCD individuals had higher concordance in bilateral HP/PHG/FG, temporal lobe, and left midcingulate cortex than NCs, but those in MCI were lower than those in NCs. No correlation between concordance values and neuropsychological scale scores was found. SCD individuals

showed both dynamics and concordance-related alterations in IBA, which indicates a compensatory mechanism in SCD individuals. Temporal dynamics analysis offers a novel approach to capturing brain alterations in individuals with SCD.

Keywords: subjective cognitive decline, Alzheimer's disease, resting-state functional MRI, temporal dynamics analysis, intrinsic brain activity

INTRODUCTION

The neurodegenerative changes that eventually develop into dementia due to Alzheimer disease (AD) begin to accumulate approximately 20 years before clinical symptoms appear (Sperling et al., 2011). Subjective cognitive decline (SCD) refers to the subjective experience of cognitive decline without any objective impairments detectable by cognitive assessments, and they are more likely to develop into AD in the future (Jessen et al., 2014; Rabin et al., 2017). The early diagnosis of AD-related SCD individuals is particularly important for AD prevention and intervention in clinical settings (Lin et al., 2019). In recent years, neuroimaging biomarkers capable of detecting the early neurodegenerative changes and dynamic alterations in neurological disorders on the AD spectrum have been identified, including SCD duration (Yang et al., 2018). A previous structural magnetic resonance imaging (sMRI) study demonstrated that a thinner cortical layer, particularly in the temporal cortex, is associated with steeper memory decline in SCD individuals (Verfaillie et al., 2018). Other researchers have also reported that SCD individuals have a greater white matter (WM) hyperintensity volume (van Rooden et al., 2018) and more severe WM fiber tract injury than controls (Brueggen et al., 2019). Moreover, SCD individuals exhibit higher amplitude of low-frequency fluctuations (ALFF) values in some brain regions compared with controls (Sun et al., 2016). Additionally, SCD individuals show alterations in their whole-brain functional connectivity strength (FCS), with the relative FCS found to be higher in posterior cingulate cortex (PCC)/precuneus (PreCu) (Dong et al., 2018). Furthermore, Rodda et al. found that while executing a divided attention task, SCD individuals showed increased activation in some brain regions compared to controls (Rodda et al., 2011). Li et al. assessed the intrinsic connectivity network of SCD individuals and found that SCD individuals show higher degree centrality (DC) in bilateral hippocampus (HP) and left fusiform gyrus (FG), but lower DC in the inferior parietal region than controls (Li et al., 2018). All these studies indicate a possible compensatory mechanism affecting the intrinsic brain activity (IBA) of SCD individuals. In addition, research has revealed no significant differences in cortical thickness in SCD individuals compared with healthy people (Sun et al., 2016), suggesting that the change in IBA might be a more sensitive biomarker for SCD than structure alterations.

To date, resting state functional MRI (rs-fMRI) studies have mostly focused on static characteristics, which involve calculations based on all time points during the scan as a whole. However, human brain activity is context-sensitive and activity-dependent, meaning that IBA is dynamic and fluctuates over time; this underlies functional integration in the brain (Park et al., 2018). Recently, studies of the temporal dynamics of IBA using analyses such as the sliding window method have yielded some important results. For instance, Mao et al. found gender differences in dynamic functional connectivity in healthy individuals (Mao et al., 2017). Similarly, dynamic alterations have also been reported in patients with depression (Kaiser et al., 2016; Li et al., 2019b; Wu et al., 2019), epilepsy (Wang et al., 2019), and Parkinson disease (Fiorenzato et al., 2019; Zhang et al., 2019). In addition, Yan et al. found that different rs-fMRI indices of IBA tend to exhibit a relatively high degree of concordance within and between individuals; this difference in concordance appears to be stable and is negatively related to age when taken as an interindividual measurement (Yan et al., 2017). However, to our knowledge, few studies to date have analyzed changes in the dynamics and concordance of IBA indices in SCD individuals.

To investigate changes in the dynamics of IBA in SCD individuals, we conducted a temporal dynamics analysis based on rs-fMRI data obtained from SCD individuals, and compared these with data from mild cognitive impairment (MCI) patients and controls (NCs), respectively. We hypothesized that there would be some differences in the dynamics and concordance of IBA among the three groups and that the alterations in SCD individuals would be intermediate between those in MCI patients and NCs.

MATERIALS AND METHODS

Alzheimer's Disease Neuroimaging Initiative

Data used in this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). ADNI was launched in 2003 as a public–private partnership, led by principal investigator Michael W. Weiner, MD. The primary goal of ADNI is to test whether data from clinical and neuropsychological assessments, serial MRI, positron emission tomography, and other biological markers can be combined to measure the progression of AD.

Participants

The research plan was approved by the review committee at each institution participating in ADNI. All subjects fully understood the research aims and signed an informed consent form. Individuals with self-report significant memory concern are recruited into significant memory concern cohort in ADNI. In the current study, this part of the population was included as the SCD group. SCD, MCI, and NC subjects who underwent structural, rs-fMRI scans on 3.0 T MRI (Siemens, Germany), in addition to receiving neuropsychological assessments in the

same visit, were included (see **Supplementary Material 1** for the specific inclusion criteria for subjects). Exclusion criteria were as follows: incomplete image or neuropsychological assessment data, severe neuropsychiatric disease, cerebral organic disease, history of brain injury or other persistent neurological diseases, or known structural abnormalities of the brain. According to the criteria above, a total of 291 were collected in this study; 40 individuals were excluded because of excessive head motion. Finally, 94 SCD individuals, 75 MCI patients, and 82 well-matched NCs were included in this study.

Neuropsychological and MRI Data Acquisition

All subjects underwent neuropsychological tests, comprising the following assessments: to evaluate general cognition, the Mini-Mental State Examination and the Montreal Cognitive Assessment Scale were used; to evaluate memory function, the delayed recall test of Webster's Memory Scale–Logical Memory II and the immediate and delayed memory parts of the Auditory Verbal Learning Test (AVLT) were used; to evaluate executive function, parts A and B of the Trail-Making Test were used; to evaluate daily cognition, the Everyday Cognition test (patient and informant version) was used; and to evaluate visuospatial function, the Clock-Drawing Test was used.

Magnetization-prepared rapid gradient echo (MPRAGE), 3D T_1-weighted image (T_1WI), and echo-planar imaging (EPI) rs-fMRI sequences were included in this study. The parameters of each sequence were as follows: sagittal MPRAGE 3D-T_1WI: repetition time (TR)/echo time (TE) = 2,300 ms/2.95 ms; field of view = 240 × 256 mm; flip angle = 9°; thickness = 1.2 mm; 176 slices; and EPI rs-fMRI: TR/TE = 3,000 ms/30 ms; spatial resolution = 3.4 × 3.4 × 3.4 mm; 48 slices; 197 time points. All subjects were instructed to keep their eyes open as normal and to rest calmly during the scan.

MRI Data Preprocessing and Calculation of rs-fMRI Indices

Image preprocessing was performed using the Data Processing Assistant for Resting-State fMRI toolbox (DPARSF; http://rfmri.org/DPARSF). The first 10 volumes were removed to allow for adaptation of participants to the environment, and the remaining 187 volumes were corrected for timing differences. The corrected functional sequences from each subject were motion-corrected using a six-parameter (rigid body) linear transformation. Forty subjects were removed based on exclusion criteria of head movements leading to >2-mm translation or >2° rotation in any direction. Next, individual T_1WI images were coregistered to the mean functional images and then segmented into gray matter, WM, and cerebrospinal fluid (CSF). Based on these segmented images, the functional volumes of each individual were spatially normalized to the Montreal Neurological Institute space using the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) toolbox and then resampled to 3-mm isotropic voxels. To prepare the data for extracting rs-fMRI indices, functional volumes were selectively smoothed with a 6-mm full width at half maximum (FWHM) Gaussian kernel, and the linear trend of the time course was removed, or the volumes were filtered using a bandpass filter of 0.01–0.08 Hz. In order to eliminate the influence of FWHM on the results, we also adopted FWHM kernel of 4 and 8 mm. Finally,

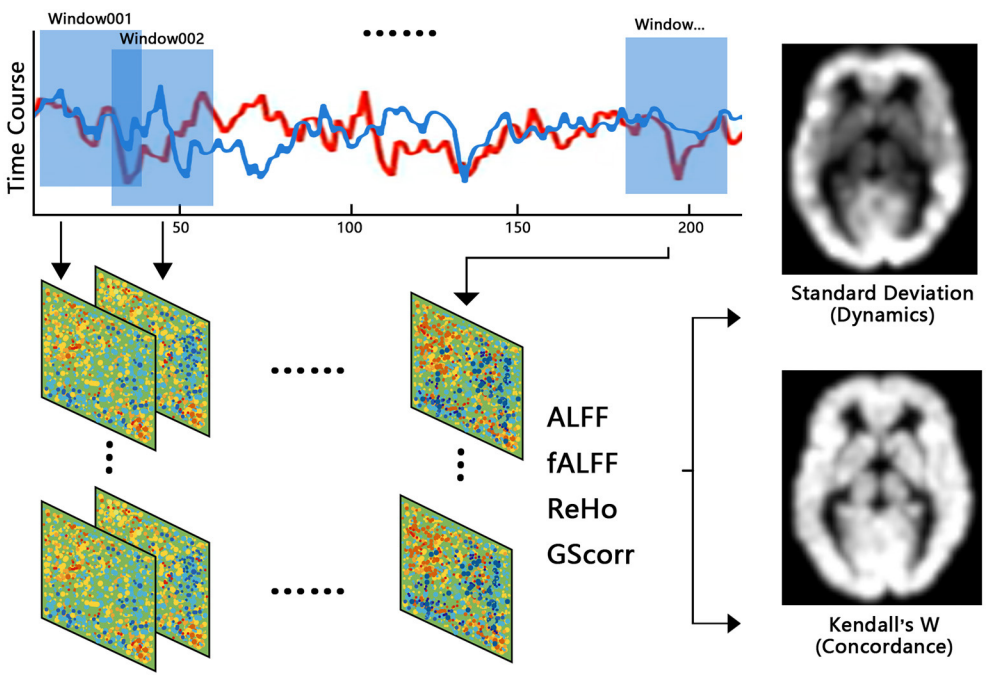

FIGURE 1 | Brief information about dynamic and concordance indices calculation.

nuisance signals including Friston 24-head motion parameters and CSF signals were extracted and regressed out from the data to reduce the effects of non-neuronal signals. For studies that have dispute over whether the mean global and WM signal regress (Murphy and Fox, 2017; Ding et al., 2018; Li et al., 2019a), we have studied the data with removal and non-removal of the mean global signal and WM signal, respectively. The resultant residual time series were used for further analyses (**Figure 1**).

Calculation of rs-fMRI Indices of Dynamics

Sliding window analysis was used to generate rs-fMRI indices of brain dynamics using the Temporal Dynamic Analysis toolkit in the Data Processing & Analysis of Brain Imaging (DPABI; http://rfmri.org/DPABI) toolbox. Moderate Hamming windows with a length of 30 TR and an overlap of 1 TR were applied to the preprocessed time series to obtain windowed time series. For each subject, 187 postprocessed volumes were segmented into 158 windows in total. Within each window, the following indices of IBA were calculated: (1) ALFF, (2) fractional ALFF (fALFF), (3) regional homogeneity (ReHo), and (4) global signal correlation (GSCorr). It is worth noting that data for ALFF and fALFF were smoothed but not filtered, and that data for the other indices were filtered but not smoothed. Standard deviation (SD) maps of each index across the windows were later calculated to characterize the dynamics of the rs-fMRI indices (yielding dALFF, dfALFF, dReHo, and dGSCorr). Finally, *Z* standardization and smoothing (except for dALFF and dfALFF, which were smoothed during preprocessing) were performed on these SD maps. Other window sizes of 60 and 90 TR were also conducted in this study.

Concordance Index of rs-fMRI Indices

The concordance indices were calculated based on Kendall's *W* among each rs-fMRI index. We calculated two kinds of concordance index: (1) the volume-wise concordance index, with Kendall's *W* of the rs-fMRI indices across all brain voxels for each subject across all time windows during the scan, was calculated as the global-level concordance index; (2) the voxel-wise concordance index, with Kendall's *W* for the rs-fMRI indices of each voxel for each subject across time windows was calculated as the voxel-level concordance index. Considering the high similarity between ALFF and fALFF, and fALFF is less susceptible to artifactual contributions of motion and pulsatile effects

TABLE 1 | Comparison of demographic and neuropsychological data among the SCD, MCI, and NC groups.

	NC ($n = 82$)	SCD ($n = 94$)	MCI ($n = 75$)	F/χ^2	*P*
Demographics					
Gender (female/male)	49/33	60/34	35/40	5.308	0.070
APOE ε4 (+/–)	19/61	33/47	26/42	6.140	0.046
Age (years)	73.88 ± 7.40	71.07 ± 6.18	74.36 ± 8.42	1.680	0.191
Education (years)	16.70 ± 2.18	16.91 ± 2.17	16.03 ± 2.84	0.792	0.454
Jenkinson mean FD	0.11 ± 0.07	0.12 ± 0.06	0.12 ±0.07	0.770	0.680
Global cognition					
MMSE	28.88 ± 1.53	29.03 ± 1.14	26.96 ± 3.71	19.574	<0.001
MoCA	26.40 ± 2.78	26.03 ± 2.65	22.37 ± 4.10	37.441	<0.001
Memory					
WMS-LM II delayed recall	14.13 ± 3.67	13.05 ± 3.89	7.20 ± 4.48	66.965	<0.001
AVLT immediate recall	9.23 ± 4.78	7.76 ± 4.40	4.35 ± 4.20	23.982	<0.001
AVLT delayed recall	13.48 ± 1.97	12.92 ± 2.40	10.72 ± 3.63	22.274	<0.001
Executive function					
TMT-A (s)	31.07 ± 9.68	30.71 ± 8.80	41.78 ± 27.18	11.091	<0.001
TMT-B (s)	74.98 ± 40.53	74.64 ± 28.12	109.19 ± 75.69	12.039	<0.001
Everyday cognition					
ECog memory (patient)	1.40 ± 0.48	1.82 ± 0.54	2.30 ± 0.80	41.363	<0.001
ECog total (patient)	1.23 ± 0.30	1.52 ± 0.41	1.84 ± 0.61	34.824	<0.001
ECog memory (informant)	1.28 ± 0.39	1.40 ± 0.51	2.28 ± 0.91	56.833	<0.001
ECog total (informer)	1.14 ± 0.22	1.22 ± 0.30	1.88 ± 0.76	55.901	<0.001
Visuospatial function					
CDT	4.66 ± 0.67	4.77 ± 0.51	4.33 ± 1.10	6.966	0.001

AVLT, Auditory Verbal Learning Test; CDT, Clock Drawing Test; ECog, Everyday Cognition scale; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment Scale; NC, normal control; SCD, subjective cognitive decline; TMT-A and -B, Trail-Making Test, parts A and B; WMS-LM II, Webster's Memory Scale–Logical Memory II.

compared to ALFF (Yan et al., 2017), only the fALFF, ReHo, and GSCorr indices were used to calculate the concordance index in this study, in order to avoid artificially improving the concordance.

Statistical Analysis

Data regarding the gender and genetics of subjects were compared among groups using the χ^2-test. The demographic data, neuropsychological scale scores, and volume-wise concordance indices of each group were analyzed using one-way analyses of variance (ANOVAs), with the least significant difference (LSD) test used for *post-hoc* comparisons. $P < 0.05$ was considered as statistically significant. One-way ANOVAs were performed to compare the standardized SD maps of each rs-fMRI index and the voxel-wise concordance maps among the three groups. In order to reduce the influence of factors such as age, gender, education, head motion, and scanning site on the results, these factors were used as covariates and removed by regression during statistical analysis. Gaussian random field correction was used to correct for multiple comparisons, using a voxel-level $P < 0.001$ and cluster-level $P < 0.05$. Finally, the concordance indices in regions with significant differences in each SCD individual

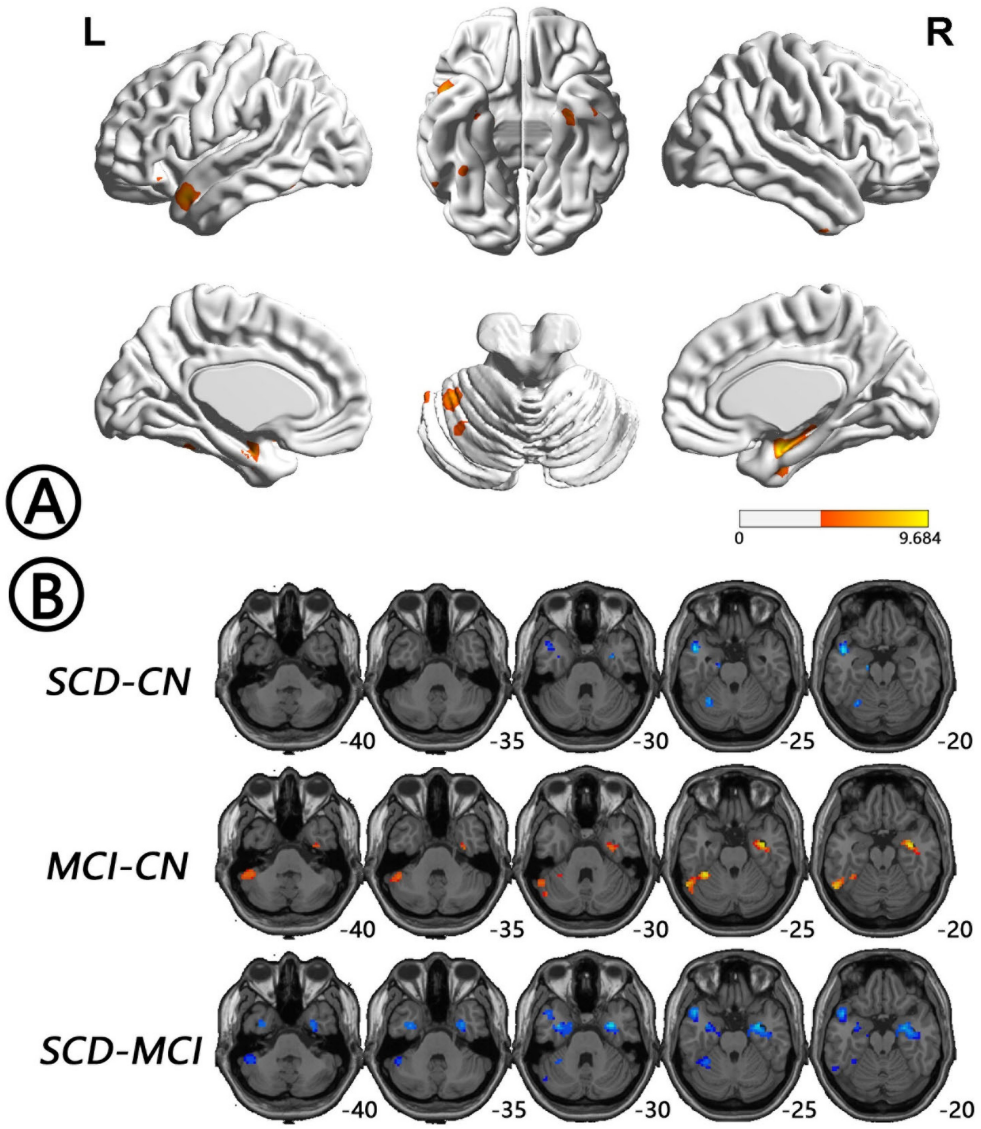

FIGURE 2 | Regions with differences in dALFF between the SCD, MCI, and NC groups and *post-hoc* analysis brain maps (GRF-corrected, voxel-level $P < 0.001$, cluster-level $P < 0.05$). **(A)** Differences in dALFF were shown in bilateral HP/PHG/FG, which extended to the left STG/MTG/TP and bilateral cerebellum among groups (red). **(B)** dALFF values in the MCI group were higher than those in the NC group (red), whereas those in the SCD group were lower than those in the other two groups (blue). dALFF, dynamics of amplitude of low-frequency fluctuations; FG, fusiform gyrus; GRF, Gaussian random field; HP, hippocampus; MCI, mild cognitive impairment; MTG, middle temporal gyrus; NC, normal control; PHG, parahippocampal gyrus; SCD, subjective cognitive decline; STG, superior temporal gyrus; TP, temporal pole.

were extracted, and correlations between the concordance indices of each brain area and scores on the neuropsychological scales were analyzed using a general linear model:

$$Y = \beta_0 + \beta_1 \times V_{\text{scores}} + \beta_2 \times V_{\text{age}} + \beta_3 \times V_{\text{sex}} + \beta_4 \times V_{\text{edu}} + \beta_5 \times V_{\text{meanFD}} + \beta_6 \times V_{\text{site}} + \text{error} \quad (1)$$

RESULTS

Demographic and Neuropsychological Data

There were no significant differences in age, gender, and education level among the SCD, MCI, and NC groups ($P > 0.05$) (**Table 1**), whereas difference in *APOE ε4* status of the three groups was significant ($P = 0.046$). In contrast, all neuropsychological test scores differed significantly among the three groups ($P < 0.05$, **Table 1**). LSD *post-hoc* analysis revealed that only the AVLT immediate memory scores ($P = 0.033$) were significantly lower in the SCD group than those in NCs; all of the other significant neuropsychological differences resulted from comparisons between the MCI group and the other two groups, respectively ($P < 0.001$) (**Supplementary Table 1**).

Dynamics of rs-fMRI indices

The intergroup differences in ALFF dynamics (dALFF) included bilateral HP/parahippocampal gyrus (PHG)/FG, which extended to the left superior temporal gyrus/middle temporal gyrus)/temporal pole (TP), and bilateral cerebellum. Differences in fALFF dynamics (dfALFF) among the three groups included bilateral PreCu and paracentral lobule. And differences in ReHo dynamics (dReHo) among the three groups included left cerebellum posterior lobe, which extended to the left FG, vermis, and right cerebellum anterior lobe. There were no significant differences in dGSCorr among the

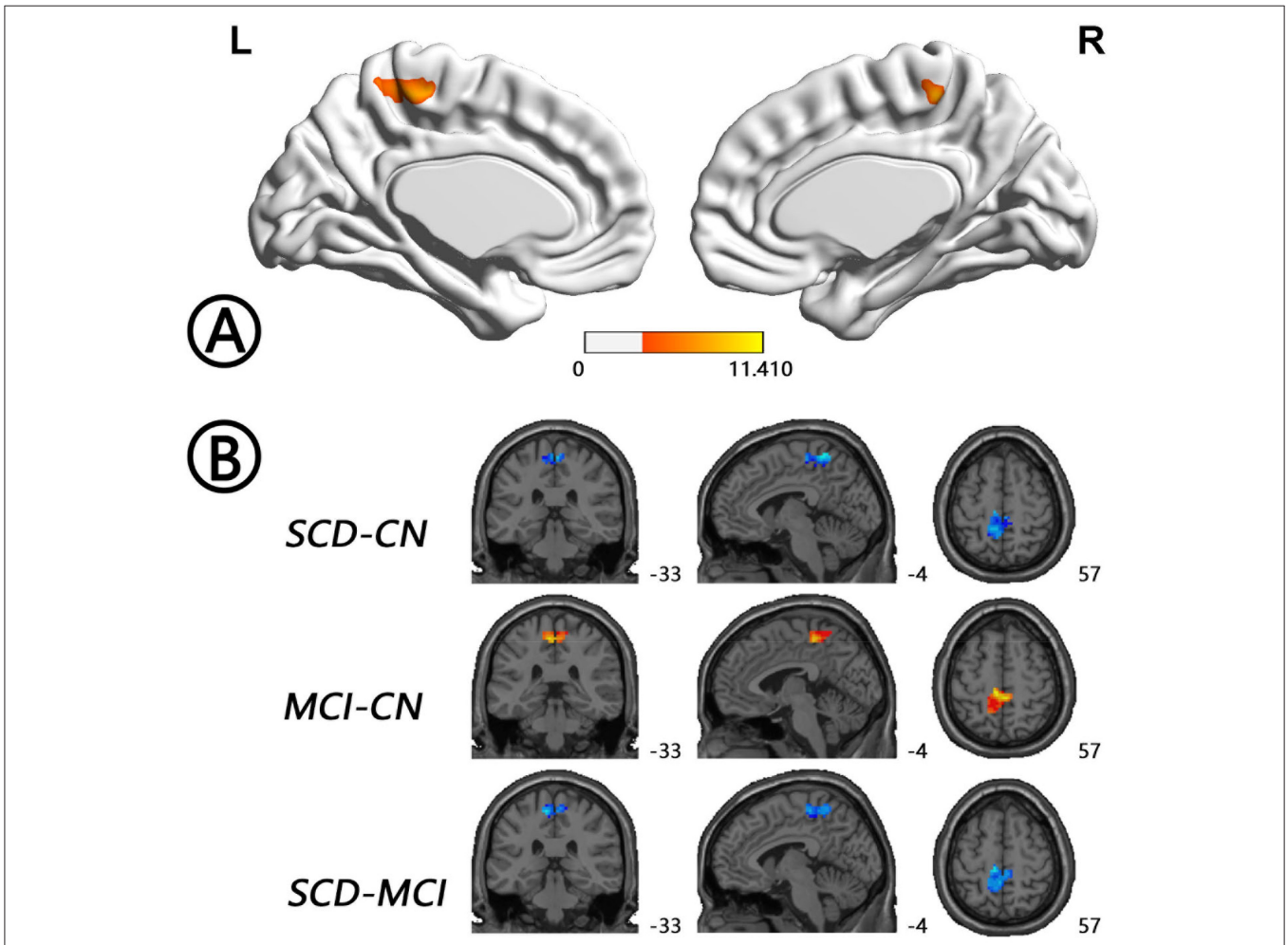

FIGURE 3 | Regions with differences in dfALFF between the SCD, MCI, and NC groups and *post-hoc* analysis brain maps (GRF-corrected, voxel-level $P < 0.001$, cluster-level $P < 0.05$). **(A)** Differences in dfALFF were shown in bilateral PreCu and paracentral lobule (red). **(B)** dfALFF values in the MCI group were higher than those in the NC group (red), whereas those in the SCD group were lower than those in the other two groups (blue). dfALFF, dynamics of fractional amplitude of low-frequency fluctuations; GRF, Gaussian random field; MCI, mild cognitive impairment; NC, normal control; PreCu, precuneus; SCD, subjective cognitive decline.

three groups. *Post-hoc* analysis using LSD testing showed that dALFF, dfALFF, and dReHo in the MCI group were higher than those in the NC group, whereas these measures were lower in the SCD group than those in the other two groups (**Figures 2–4**, **Table 2**). All of the results were preserved when we omitted different FWHM Gaussian kernel or different Hamming window size (**Supplementary Figures 1–4**). Results of whether the WM or mean global signal regresses were similar (**Supplementary Figures 5, 6**).

Concordance of rs-fMRI Indices

The mean values of the volume-wise concordance in the SCD, MCI, and NC groups show significant differences ($P = 0.005$). Subsequent analyses showed that the mean concordance of the MCI group was lower than that of the NC group ($P = 0.035$), whereas that of the SCD group was higher than those of the MCI ($P = 0.002$) and NC groups ($P = 0.022$). There was no statistical difference in the SD of the volume-wise concordance among the three groups ($P = 0.445$) (**Figure 5**, **Table 3**).

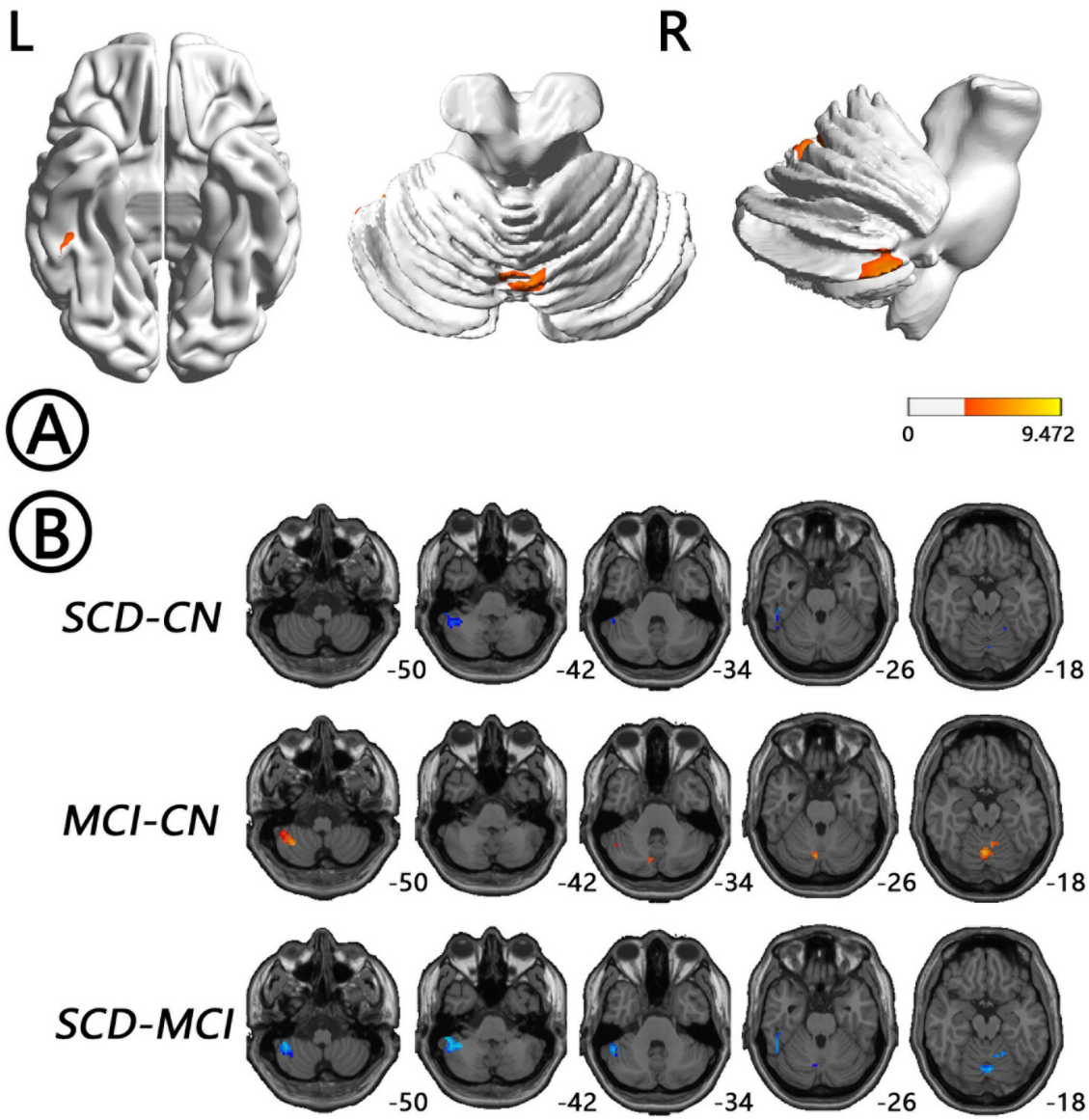

FIGURE 4 | Regions with differences in dReHo between the SCD, MCI, and NC groups and *post-hoc* analysis brain maps (GRF-corrected, voxel-level $P < 0.001$, cluster-level $P < 0.05$). **(A)** Differences in dReHo were shown in left cerebellum posterior lobe, which extended to the left FG, vermis, and right cerebellum anterior lobe (red). **(B)** dReHo values in the MCI group were higher than those in the NC group (red), whereas those in the SCD group were lower than those in the other two groups (blue). dReHo, dynamics of regional homogeneity; FG, fusiform gyrus; GRF, Gaussian random field; MCI, mild cognitive impairment; NC, normal control; SCD, subjective cognitive decline.

TABLE 2 | Regions with differences in rs-fMRI indices of dynamics among the SCD, MCI, and NC groups.

Brain regions	Brodmann area	Size (voxels)	MNI coordinates (mm)			Peak value
			X	*Y*	*Z*	
dALFF						
Right HP/PHG/FG	20/28/36	281	30	−6	−24	7.0327
Left HP/PHG/FG extend to STG/MTG/TP	20/21/36/38	270	−51	12	−21	9.6840
Left cerebellum anterior/ posterior lobe	—	226	−37	−48	−23	7.2869
dfALFF						
Bilateral PreCu/paracentral lobule	5	184	1	−37	54	11.2712
dReHo						
Left cerebellum posterior lobe extend to FG	—	190	−42	−48	−48	6.2432
Right cerebellum anterior lobe and vermis	—	104	−3	−63	−15	8.1379

dALFF, dynamics of amplitude of low-frequency fluctuations; dfALFF, dynamics of fractional amplitude of low-frequency fluctuations; dReHo, dynamics of regional homogeneity; FG, fusiform gyrus; HP, hippocampus; MNI, Montreal Neurological Institute; MTG, middle temporal gyrus; PHG, parahippocampal gyrus; PreCu, precuneus; STG, superior temporal gyrus; TP, temporal pole.

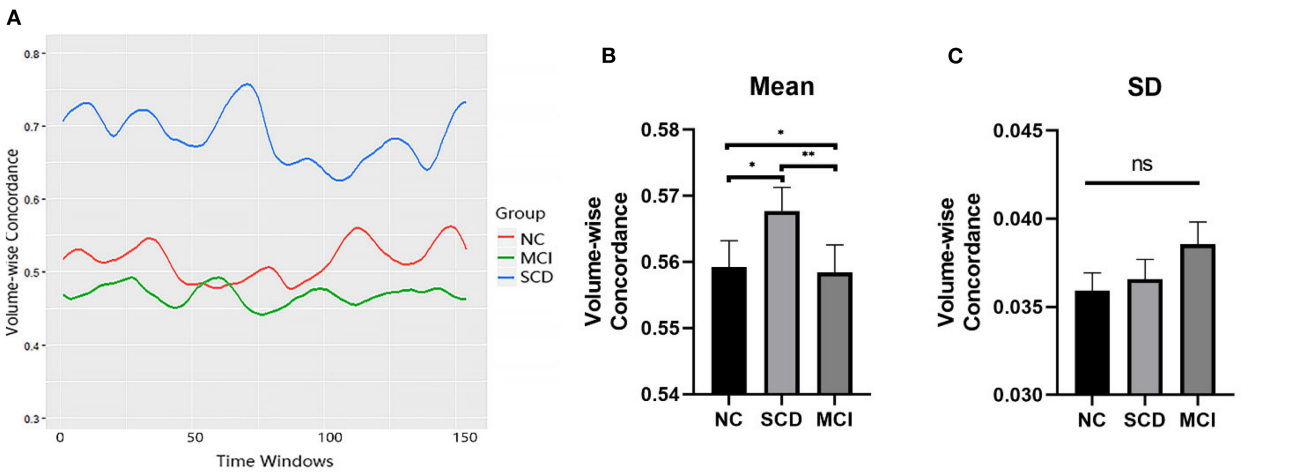

FIGURE 5 | Comparison of volume-wise concordance indices among the SCD, MCI, and NC groups. **(A)** Volume-wise concordance time series of typical SCD, MCI, and NC subjects. **(B)** Comparison of mean volume-wise concordance indices. **(C)** Comparison of the SD of volume-wise concordance indices. MCI, mild cognitive impairment; NC, normal control; SCD, subjective cognitive decline; SD, Standard deviation. *$P < 0.05$; **$P < 0.01$; ns, no significance.

TABLE 3 | Comparison of volume-wise concordance indices among the SCD, MCI, and NC groups.

	NC	SCD	MCI	*F*	*P*
Mean	0.56 ± 0.04	0.57 ± 0.03	0.55 ± 0.03	5.510	0.005
SD	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.811	0.445

MCI, mild cognitive impairment; NC, normal control; SCD, subjective cognitive decline; SD, standard deviation.

Comparing the voxel-wise concordance maps among the three groups, significant differences were found in left HP/PHG, left insula/TP, left midcingulate cortex (MCC), right TP/FG, and right Rolandic operculum/insula. Subsequent analysis showed that the concordance of these regions in the MCI patients was lower than that in the NCs, whereas the concordance in SCD patients was higher than those in the other two groups (**Figure 6**, **Table 4**). All of the results were preserved with different FWHM Gaussian kernel or different Hamming window size (**Supplementary Figures 7, 8**). Results of whether the WM or mean global signal regresses were similar as well (**Supplementary Figure 9**).

No correlation between concordance values and neuropsychological scale scores was found in the SCD.

DISCUSSION

In this study, we found that SCD individuals had lower dALFF and dReHo values and higher concordance in memory-related brain regions such as HP, PHG, and FG compared with the other

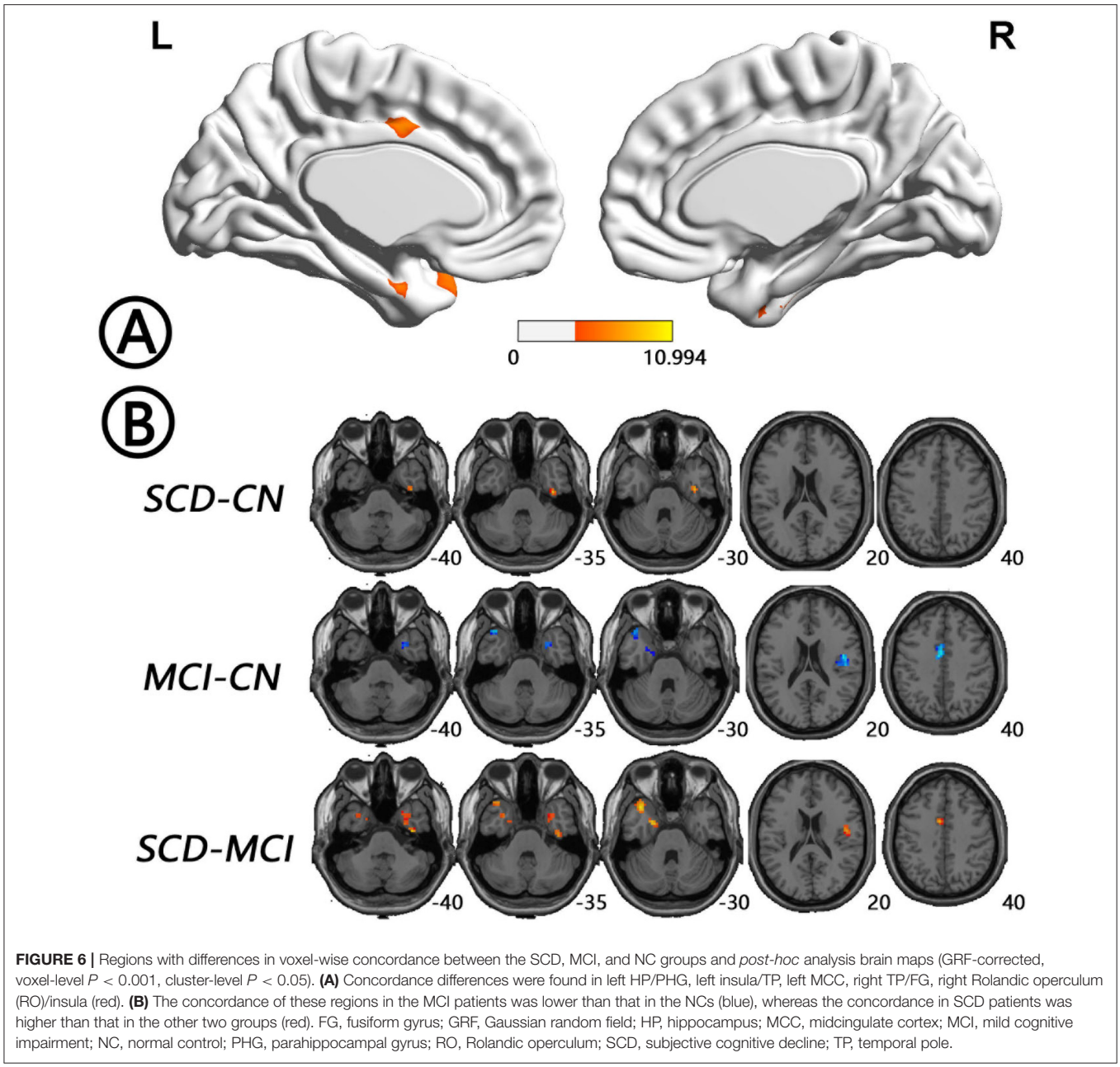

FIGURE 6 | Regions with differences in voxel-wise concordance between the SCD, MCI, and NC groups and *post-hoc* analysis brain maps (GRF-corrected, voxel-level $P < 0.001$, cluster-level $P < 0.05$). **(A)** Concordance differences were found in left HP/PHG, left insula/TP, left MCC, right TP/FG, right Rolandic operculum (RO)/insula (red). **(B)** The concordance of these regions in the MCI patients was lower than that in the NCs (blue), whereas the concordance in SCD patients was higher than that in the other two groups (red). FG, fusiform gyrus; GRF, Gaussian random field; HP, hippocampus; MCC, midcingulate cortex; MCI, mild cognitive impairment; NC, normal control; PHG, parahippocampal gyrus; RO, Rolandic operculum; SCD, subjective cognitive decline; TP, temporal pole.

TABLE 4 | Regions with differences in the concordance of rs-fMRI indices among the SCD, MCI, and NC groups.

Brain regions	Brodmann area	Size (voxels)	MNI coordinates (mm)			Peak value
			X	*Y*	*Z*	
Left PHG/HP	28/36	53	−18	−3	−27	7.4466
Left MCC	23/24	62	−6	−6	39	6.6607
Left insula/TP	38/48	133	−44	10	−12	7.3861
Right FG/TP	20/36	89	40	−15	−32	6.7529
Right RO/insula	48	58	45	−9	21	8.9552

FG, fusiform gyrus; HP, hippocampus; MCC, midcingulate cortex; MNI, Montreal Neurological Institute; PHG, parahippocampal gyrus; RO, Rolandic operculum; TP, temporal pole.

two groups. MCI patients showed high dynamics-related indices in these areas, suggesting insufficient stability of IBA in these areas. In contrast, SCD individuals showed IBA dynamics values that were even lower than those in the NC group, indicating stable but inflexible brain activity. This alteration might affect the efficient, rapid, and complex processes related to memory coding, resulting in memory loss in SCD patients. The concordance of rs-fMRI indices reflects the integrative function of the brain (Yan et al., 2017). Concordance in MCI patients was significantly lower than that in NCs, indicating that this integrative function might be impaired in MCI patients or in those who suffer from a cognitive disorder. However, the concordance was high in SCD individuals, suggesting that their integrative function was intact; indeed, it was even higher than that in NCs, which is not consistent with the memory loss observed in SCD individuals. We infer that this finding might reflect ineffective integration or be the result of a compensatory mechanism (Elman et al., 2014).

Brain regions showing differences in dALFF among the three groups were more extensive, including bilateral HP/PHG/FG and bilateral cerebellum. All these regions are consistent with brain areas reported for tau protein deposition in AD patients (Congdon and Sigurdsson, 2018). HP and PHG are essential structures of the medial temporal lobe and play a role in the coding of episodic memory (Eichenbaum et al., 2007). Visible HP and PHG atrophy can be found in MCI and AD patients (Yang et al., 2012), and furthermore, the functional connectivity between HP, PHG, and other brain regions has been shown to be reduced in MCI and AD patients (Liu et al., 2015). The FG is the center of face recognition (Weiner and Zilles, 2016), with recent studies showing that FG also plays a vital role in semantic memory (Forseth et al., 2018) and that abnormalities in left FG can result in semantic dementia (Merck et al., 2017). In this study, we found increased dynamics in bilateral HP/PHG/FG in SCD patients, which is consistent with results of Li et al. (2018). In addition to dALFF, dReHo also showed differences among groups in the bilateral cerebellum. The cerebellum is the center of motor regulation and forms a cognitive and motor loop with the telencephalon (Salmi et al., 2010), with both cerebellum (Jacobs et al., 2018; Schmahmann, 2019) and vermis (Wang et al., 2017) critical to cognitive function. Abnormal IBA in cerebellar regions has been reported in SCD, MCI, and AD patients (Yang et al., 2012; Sun et al., 2016). Besides, bilateral PreCu also showed dfALFF differences among groups. PreCu locates in the posteromedial portion of the parietal lobe. Functional imaging findings in healthy subjects suggest a central role for the PreCu in a wide spectrum of highly integrated tasks, including episodic memory retrieval (Cavanna and Trimble, 2006). The PreCu and PCC regions together constitute a key hub of the default mode network, which is also an area associated with the accumulation of amyloid-β plaque (Buckner et al., 2009). The PCC/PreCu exhibits reduced functional connectivity in mild AD patients (Zhang et al., 2009). After 12 weeks of moderate-intensity walking exercise training, functional connectivity of the PCC/PreCu was increased in individuals with MCI (Chirles et al., 2017).

Our results showed intergroup concordance differences in left HP/PHG, MCC, and bilateral temporal lobe regions. The temporal lobe is involved in memory and language function (Eichenbaum et al., 2007), whereas the cingulate cortex plays a key role in cognitive, motor, and emotional function, with activity in MCC correlating with cognitive and sensorimotor networks (Kragel et al., 2018). Studies have shown significant atrophy of the temporal lobe in patients with MCI (Sheelakumari et al., 2018; DeVivo et al., 2019). Moreover, the global functional connectivity of the cingulate cortex, including MCC, shows deterioration in MCI patients (Cera et al., 2019).

No correlation between concordance values and neuropsychological scale scores was found in the SCD group in our research. This may be that the cognitive changes of SCD individuals caused by abnormal functional integration ability were too subtle, and simple cognitive scales cannot reflect such subtle changes. Therefore, we need to find a scale that can identify SCD individuals more efficiently.

There are several limitations to this study. First, the sample size was insufficient; thus, further analyses with more participants are warranted. Second, ADNI is a multicenter study, and although the influence of site was regressed out as a covariate in our statistical analyses, its effect still cannot be removed completely. Third, only ALFF, fALFF, ReHo, and GSCorr indices were used in this study, and more parameters could be analyzed in future studies. Finally, the sliding window method in this study requires a fixed window width, but in the future, a more comprehensive analysis without a fixed window width could be conducted.

SCD individuals showed both alterations to the dynamics and concordance of their IBA. These results suggest that analysis of temporal dynamics based on rs-fMRI data is a novel approach for investigating brain alterations in individuals with SCD. Furthermore, these alterations in dynamics and concordance might help us to better understand the mechanisms underlying brain activity changes in SCD individuals.

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 in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Alzheimer's Disease Neuroimaging Initiative Coordinating Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YY contributed to the design the work, analyzed the data, and wrote the manuscript. XZhan provided technical support for the data analysis and revised the manuscript. XZha agreed to be accountable for all aspects of the work. JK provided technical support for the data analysis and revised the manuscript. SH

gave final approval of the version to be published. XW agreed to be accountable for all aspects of the work. YS revised the manuscript and gave final approval of the version to be published. CH agreed to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

FUNDING

The authors gratefully acknowledge grants from: the National Key Research and Development Program of China (no. 2017YFC0114300 to CH); the National Natural Science Foundation of China (no. 81701667 to YS, no. 81701669 to JK, and no. 81701679 to XZhan); the Natural Science Foundation of Jiangsu Province (no. BK20170367 to YS and no. BK20170368 to JK); the Natural Science Foundation of Tianjin (no. 19JCQNJC09800 to XZhan); the Jiangsu Provincial Key Research and Development Program (no. BE2019660 to CH); and the Medical Research Project of Jiangsu Commission of Health (no. H2017063 to SH). Data collection and sharing for this project was funded by ADNI (National Institutes of Health grant no. U01 AG024904) and DOD ADNI (Department of Defense award no. W81XWH-12-2-0012).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2020.584863/full#supplementary-material

REFERENCES

Brueggen, K., Dyrba, M., Cardenas-Blanco, A., Schneider, A., Fliessbach, K., and Buerger, K., et al. (2019). Structural integrity in subjective cognitive decline, mild cognitive impairment and Alzheimer's disease based on multicenter diffusion tensor imaging. *J. Neurol.* 266, 2465–2474. doi: 10.1007/s00415-019-09429-3

Buckner, R. L., Sepulcre, J., Talukdar, T., Krienen, F. M., and Liu, H., et al. (2009). Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J. Neurosci.* 29, 1860–1873. doi: 10.1523/JNEUROSCI.5062-08.2009

Cavanna, A. E., and Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 129, 564–583. doi: 10.1093/brain/awl004

Cera, N., Esposito, R., Cieri, F., and Tartaro, A. (2019). Altered cingulate cortex functional connectivity in normal aging and mild cognitive impairment. *Front. Neurosci.* 13:857. doi: 10.3389/fnins.2019.00857

Chirles, T. J., Reiter, K., Weiss, L. R., Alfini, A. J., and Nielson, K. A. (2017). Exercise training and functional connectivity changes in mild cognitive impairment and healthy elders. *J. Alzheimers Dis.* 57, 845–856. doi: 10.3233/JAD-161151

Congdon, E. E., and Sigurdsson, E. M. (2018). Tau-targeting therapies for Alzheimer disease. *Nat. Rev. Neurol.* 14, 399–415. doi: 10.1038/s41582-018-0013-z

DeVivo, R., Zajac, L., Mian, A., Cervantes-Arslanian, A., Steinberg, E., and Alosco, M. L., et al. (2019). Differentiating between healthy control participants and those with mild cognitive impairment using volumetric MRI data. *J. Int. Neuropsychol. Soc.* 25, 800–810. doi: 10.1017/S135561771900047X

Ding, Z., Huang, Y., Bailey, S. K., Gao, Y., and Cutting, L. E., et al. (2018). Detection of synchronous brain activity in white matter tracts at rest and under functional loading. *Proc. Natl. Acad. Sci. U.S.A.* 115, 595–600. doi: 10.1073/pnas.1711567115

Dong, C., Liu, T., Wen, W., Kochan, N. A., Jiang, J., and Li, Q., et al. (2018). Altered functional connectivity strength in informant-reported subjective cognitive decline: a resting-state functional magnetic resonance imaging study. *Alzheimers Dement.* 10, 688–697. doi: 10.1016/j.dadm.2018.08.011

Eichenbaum, H., Yonelinas, A. P., and Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu. Rev. Neurosci.* 30, 123–152. doi: 10.1146/annurev.neuro.30.051606.094328

Elman, J. A., Oh, H., Madison, C. M., Baker, S. L., Vogel, J. W., and Marks, S. M., et al. (2014). Neural compensation in older people with brain amyloid-β deposition. *Nat. Neurosci.* 17, 1316–1318. doi: 10.1038/nn.3806

Fiorenzato, E., Strafella, A. P., Kim, J., Schifano, R., Weis, L., and Antonini, A., et al. (2019). Dynamic functional connectivity changes associated with dementia in Parkinson's disease. *Brain* 142, 2860–2872. doi: 10.1093/brain/awz192

Forseth, K. J., Kadipasaoglu, C. M., Conner, C. R., Hickok, G., Knight, R. T., and Tandon, N. (2018). A lexical semantic hub for heteromodal naming in middle fusiform gyrus. *Brain* 141, 2112–2126. doi: 10.1093/brain/awy120

Jacobs, H. I. L., Hopkins, D. A., Mayrhofer, H. C., Bruner, E., van Leeuwen, F. W., and Raaijmakers, W., et al. (2018). The cerebellum in Alzheimer's disease: evaluating its role in cognitive decline. *Brain* 141, 37–47. doi: 10.1093/brain/awx194

Jessen, F., Amariglio, R. E., van Boxtel, M., Breteler, M., Ceccaldi, M., and Chételat, G., et al. (2014). A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimers Dement.* 10, 844–852. doi: 10.1016/j.jalz.2014.01.001

Kaiser, R. H., Whitfield-Gabrieli, S., Dillon, D. G., Goer, F., Beltzer, M., and Minkel, J., et al. (2016). Dynamic resting-state functional connectivity in major depression. *Neuropsychopharmacology* 41, 1822–1830. doi: 10.1038/npp.2015.352

Kragel, P. A., Kano, M., Van Oudenhove, L., Ly, H. G., Dupont, P., and Rubio, A., et al. (2018). Generalizable representations of pain, cognitive control, and negative emotion in medial frontal cortex. *Nat. Neurosci.* 21, 283–289. doi: 10.1038/s41593-017-0051-7

Li, J., Biswal, B. B., Wang, P., Duan, X., Cui, Q., and Chen, H., et al. (2019a). Exploring the functional connectome in white matter. *Hum. Brain Mapp.* 40, 4331–4344. doi: 10.1002/hbm.24705

Li, J., Duan, X., Cui, Q., Chen, H., and Liao, W. (2019b). More than just statics: temporal dynamics of intrinsic brain activity predicts the suicidal ideation in depressed patients. *Psychol. Med.* 49, 852–860. doi: 10.1017/S0033291718001502

Li, K., Luo, X., Zeng, Q., Jiaerken, Y., Xu, X., and Huang, P., et al. (2018). Aberrant functional connectivity network in subjective memory complaint individuals relates to pathological biomarkers. *Transl. Neurodegener.* 7:27. doi: 10.1186/s40035-018-0130-z

Lin, Y., Shan, P., Jiang, W., Sheng, C., and Ma, L. (2019). Subjective cognitive decline: preclinical manifestation of Alzheimer's disease. *Neurol. Sci.* 40, 41–49. doi: 10.1007/s10072-018-3620-y

Liu, J., Zhang, X., Yu, C., Duan, Y., Zhuo, J., and Cui, Y., et al. (2015). Impaired parahippocampus connectivity in mild cognitive impairment and Alzheimer's disease. *J. Alzheimers Dis.* 49, 1051–1064. doi: 10.3233/JAD-150727

Mao, N., Zheng, H., Zhiying, L., Li, Y., and Xia, W. (2017). Gender differences in dynamic functional connectivity based on resting-state fMRI. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2017, 2940–2943. doi: 10.1109/EMBC.2017.8037473

Merck, C., Corouge, I., Jonin, P., Desgranges, B., Gauvrit, J., and Belliard, S. (2017). What semantic dementia teaches us about the functional organization of the left posterior fusiform gyrus. *Neuropsychologia* 106, 159–168. doi: 10.1016/j.neuropsychologia.2017.09.023

Murphy, K., and Fox, M. D. (2017). Towards a consensus regarding global signal regression for resting state functional connectivity MRI. *Neuroimage* 154, 169–173. doi: 10.1016/j.neuroimage.2016.11.052

Park, H., Friston, K. J., Pae, C., Park, B., and Razi, A. (2018). Dynamic effective connectivity in resting state fMRI. *NeuroImage* 180, 594–608. doi: 10.1016/j.neuroimage.2017.11.033

Rabin, L. A., Smart, C. M., and Amariglio, R. E. (2017). Subjective cognitive decline in preclinical Alzheimer's disease. *Annu. Rev Clin. Psychol.* 13, 369–396. doi: 10.1146/annurev-clinpsy-032816-045136

Rodda, J., Dannhauser, T., Cutinha, D. J., Shergill, S. S., and Walker, Z. (2011). Subjective cognitive impairment: functional MRI during a divided attention task. *Eur. Psychiatry* 26, 457–462. doi: 10.1016/j.eurpsy.2010.07.003

Salmi, J., Pallesen, K. J., Neuvonen, T., Brattico, E., Korvenoja, A., and Salonen, O., et al. (2010). Cognitive and motor loops of the human cerebro-cerebellar system. *J. Cogn. Neurosci.* 22, 2663–2676. doi: 10.1162/jocn.2009.21382

Schmahmann, J. D. (2019). The cerebellum and cognition. *Neurosci. Lett.* 688, 62–75. doi: 10.1016/j.neulet.2018.07.005

Sheelakumari, R., Kesavadas, C., Lekha, V. S., Justus, S., Sarma, P. S., and Menon, R. (2018). Structural correlates of mild cognitive impairment: a clinicovolumetric study. *Neurol. India* 66, 370–376. doi: 10.4103/0028-3886.227298

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., and Fagan, A. M., et al. (2011). Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National institute on Aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 280–292. doi: 10.1016/j.jalz.2011.03.003

Sun, Y., Dai, Z., Li, Y., Sheng, C., Li, H., and Wang, X., et al. (2016). Subjective cognitive decline: mapping functional and structural brain changes–a combined resting-state functional and structural MR imaging study. *Radiology* 281, 185–192. doi: 10.1148/radiol.2016151771

van Rooden, S., van den Berg-Huysmans, A. A., Croll, P. H., Labadie, G., Hayes, J. M., and Viviano, R., et al. (2018). Subjective cognitive decline is associated with greater white matter hyperintensity volume. *J. Alzheimers Dis.* 66, 1283–1294. doi: 10.3233/JAD-180285

Verfaillie, S. C. J., Slot, R. E., Tijms, B. M., Bouwman, F., Benedictus, M. R., and Overbeek, J. M., et al. (2018). Thinner cortex in patients with subjective cognitive decline is associated with steeper decline of memory. *Neurobiol. Aging* 61, 238–244. doi: 10.1016/j.neurobiolaging.2017.09.009

Wang, J., Zhou, L., Cui, C., Liu, Z., and Lu, J. (2017). Gray matter morphological anomalies in the cerebellar vermis in first-episode schizophrenia patients with cognitive deficits. *BMC Psychiatry* 17:374. doi: 10.1186/s12888-017-1543-4

Wang, Y., Berglund, I. S., Uppman, M., and Li, T. (2019). Juvenile myoclonic epilepsy has hyper dynamic functional connectivity in the dorsolateral frontal cortex. *NeuroImage Clin.* 21:101604. doi: 10.1016/j.nicl.2018.11.014

Weiner, K. S., and Zilles, K. (2016). The anatomical and functional specialization of the fusiform gyrus. *Neuropsychologia* 83, 48–62. doi: 10.1016/j.neuropsychologia.2015.06.033

Wu, X., He, H., Shi, L., Xia, Y., Zuang, K., and Feng, Q., et al. (2019). Personality traits are related with dynamic functional connectivity in major depression disorder: a resting-state analysis. *J. Affect Disord.* 245, 1032–1042. doi: 10.1016/j.jad.2018.11.002

Yan, C., Yang, Z., Colcombe, S. J., Zuo, X., and Milham, M. P. (2017). Concordance among indices of intrinsic brain function: insights from inter-individual variation and temporal dynamics. *Sci. Bull.* 62, 1572–1584. doi: 10.1016/j.scib.2017.09.015

Yang, J., Pan, P., Song, W., Huang, R., Li, J., and Chen, K., et al. (2012). Voxelwise meta-analysis of gray matter anomalies in Alzheimer's disease and mild cognitive impairment using anatomic likelihood estimation. *J. Neurol. Sci.* 316, 21–29. doi: 10.1016/j.jns.2012.02.010

Yang, L., Yan, Y., Wang, Y., Hu, X., Lu, J., and Chan, P., et al. (2018). Gradual disturbances of the amplitude of low-frequency fluctuations (ALFF) and fractional ALFF in Alzheimer spectrum. *Front. Neurosci.* 12:975. doi: 10.3389/fnins.2018.00975

Zhang, C., Dou, B., Wang, J., Xu, K., Zhang, H., and Sami, M. U., et al. (2019). Dynamic alterations of spontaneous neural activity in Parkinson's disease: a resting-state fMRI study. *Front. Neurol.* 10:1052. doi: 10.3389/fneur.2019.01052

Zhang, H. Y., Wang, S. J., Xing, J., Liu, B., and Ma, Z. L. (2009). Detection of PCC functional connectivity characteristics in resting-state fMRI in mild Alzheimer's disease. *Behav. Brain Res.* 197, 103–108. doi: 10.1016/j.bbr.2008.08.012

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.

Copyright © 2021 Yang, Zha, Zhang, Ke, Hu, Wang, Su and Hu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 25 February 2021
doi: 10.3389/fnagi.2021.638922

TNFRSF1B Gene Variants and Related Soluble TNFR2 Levels Impact Resilience in Alzheimer's Disease

Jagan A. Pillai[1,2,3]*, *Gurkan Bebek*[4,5], *Maria Khrestian*[6,7], *James Bena*[8], *Cornelia C. Bergmann*[7], *William S. Bush*[9], *James B. Leverenz*[1,2,3] and *Lynn M. Bekris*[6,7] *for the Alzheimer's Disease Neuroimaging Initiative*†

[1] Department of Neurology, Cleveland Clinic, Cleveland, OH, United States, [2] Lou Ruvo Center for Brain Health, Cleveland Clinic, Cleveland, OH, United States, [3] Neurological Institute, Cleveland Clinic, Cleveland, OH, United States, [4] Center for Proteomics and Bioinformatics, Case Western Reserve University, Cleveland, OH, United States, [5] Department of Nutrition, Case Western Reserve University, Cleveland, OH, United States, [6] Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH, United States, [7] Department of Neuroscience, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States, [8] Department of Quantitative Health Science, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States, [9] Department of Population and Quantitative Health Sciences, Case Western Reserve University, Cleveland, OH, United States

Tumor necrosis factor receptor 2 (TNFR2) promotes neuronal survival downstream. This longitudinal study evaluated whether the *TNFRSF1B* gene encoding TNFR2 and levels of its soluble form (sTNFR2) affect Alzheimer disease (AD) biomarkers and clinical outcomes. Data analyzed included 188 patients in the Alzheimer's Disease Neuroimaging Initiative (ADNI) who had mild cognitive impairment (MCI) and AD dementia. Further, a replication study was performed in 48 patients with MCI with positive AD biomarkers who were treated at a memory clinic. Cerebrospinal fluid (CSF) sTNFR2 levels along with two related *TNFRSF1B* gene single nucleotide polymorphisms (SNPs) rs976881 and rs1061622 were assessed. General linear models were used to evaluate the effect of CSF sTNFR2 levels and each SNP in relationship to CSF t-tau and p-tau, cognitive domains, MRI brain measures, and longitudinal cognitive changes after adjustments were made for covariates such as *APOE ε4* status. In the ADNI cohort, a significant interaction between rs976881 and CSF sTNFR2 modulates CSF t-tau and p-tau levels; hippocampal and whole brain volumes; and Digit Span Forwards subtest scores. In the replication cohort, a significant interaction between rs976881 and CSF sTNFR2 modulates CSF p-tau. A significant interaction between rs976881 and CSF sTNFR2 also impacts Clinical Dementia Rating Sum of Boxes scores over 12 months in the ADNI cohort. The interaction between *TNFRSF1B* variant rs976881 and CSF sTNFR2 levels was noted to modulate multiple AD-associated severity markers and cognitive domains. This interaction impacts resilience-related clinical outcomes in AD and lends support to sTNFR2 as a promising candidate for therapeutic targeting to improve clinical outcomes of interest.

Keywords: Alzheimer disease, neuroinflammation, TNFRSF1B, resilience, executive function, MCI, digit span

OPEN ACCESS

Edited by:
Yang Xiang,
Western Theater General Hospital, China

Reviewed by:
Atsushi Iwata,
Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology (TMGH-IG), Japan
Guoyuan Qi,
University of Arizona, United States

***Correspondence:**
Jagan A. Pillai
pillaij@ccf.org

†*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf*

Received: *07 December 2020*
Accepted: *01 February 2021*
Published: *25 February 2021*

Citation:
Pillai JA, Bebek G, Khrestian M, Bena J, Bergmann CC, Bush WS, Leverenz JB and Bekris LM (2021) TNFRSF1B Gene Variants and Related Soluble TNFR2 Levels Impact Resilience in Alzheimer's Disease. Front. Aging Neurosci. 13:638922. doi: 10.3389/fnagi.2021.638922

INTRODUCTION

Alzheimer disease (AD) is paradigmatically characterized by the presence of amyloid β plaques and tau neurofibrillary tangles in the brain. However, the cognitive and behavioral phenotypes of AD and their related CSF and MRI biomarker signatures can vary among patients, and molecular factors beyond amyloid β and tau likely play a role in this heterogeneity (van der Vlies et al., 2009; Whitwell et al., 2012; Dubois et al., 2014; Risacher et al., 2017; Pillai et al., 2019a). Multiple studies have evaluated *APOE ε*4 and other genes that may be related to the various MRI and clinical endophenotypes of AD (Hohman et al., 2014; Saykin et al., 2015; Louwersheimer et al., 2016; Therriault et al., 2020). Elucidating the interplay between genetic factors and these clinical phenotypes is crucial to developing precision medicine interventions and understanding cognitive "resilience," a term used to describe better-than-expected cognitive performance in relation to the degree of AD pathology (Negash et al., 2013; Arenaza-Urquijo and Vemuri, 2018).

Accumulating evidence supports that inflammation-related changes may play a role in AD (Perry et al., 2010; Wyss-Coray and Rogers, 2012). We recently reported that key inflammatory analytes in the CSF related to rapid cognitive decline are pro-inflammatory among patients with the mild cognitive impairment (MCI) stage of AD (MCI-AD) (Pillai et al., 2020), whereas a cell-protective analyte profile is noted in the CSF correlating to neurodegeneration markers (Pillai et al., 2019b). Among the analytes that showed consistently high correlation with CSF t-tau, p-tau, and neuron-specific enolase was CSF soluble tumor necrosis factor receptor 2 (sTNFR2) (Pillai et al., 2019b). TNFR2 activation has been shown to promote downstream antiapoptotic responses and protect against oxidative stress-induced neuronal death and neurodegeneration (Fischer et al., 2011; Dong et al., 2016). sTNFR2 results from alternative splicing/shedding from membrane bound TNFR2 and, upregulation of the TNFR2 receptor is thought to be reflected in elevated sTNFR2 levels during inflammation (DeBerge et al., 2015). The *TNFRSF1B* gene (also known as *TNFR2*) and its variants have previously been linked with inflammatory responses, inflammatory disease outcomes, and rate of cancer progression (Fairfax et al., 2011; Steenholdt et al., 2012; Singhal et al., 2016), but the role of these gene variants in AD has yet to be elucidated.

We therefore sought to determine whether sTNFR2 and related *TNFRSF1B* gene variants affect the relationship between the biomarkers of tau pathology, MRI measures of disease severity, and cognitive outcomes. Our main hypothesis was that key *TNFRSF1B* gene variants and associated CSF sTNFR2 levels would modify AD biomarker levels of t-tau and p-tau, MRI brain volumes, and cognitive outcomes. After performing this analysis for patients enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI), we evaluated the reliability of the findings by assessing the same factors in a group of patients with MCI-AD who were treated at a memory clinic.

MATERIALS AND METHODS

For the ADNI study, all patient consent was obtained according to the Declaration of Helsinki, and the study was approved by local Institutional Review Boards. For the replication memory clinic cohort, all patients provided written informed consent, and CSF, plasma, and DNA samples were collected for the Lou Ruvo Center for Brain Health Aging and Neurodegeneration Biobank following approval by the Cleveland Clinic Institutional Review Board.

Study Design

A multistage study design was used to collect and replicate findings in two independent cohorts (the ADNI cohort and the replication memory clinic cohort) for which data regarding *TNFRSF1B* variants, CSF sTNFR2, and CSF AD biomarkers and cognitive variables were available. The CSF data used in this analysis were cleaned and quality controlled according to methodology described previously[1].

ADNI Cohort

ADNI is a longitudinal multicenter study designed to develop clinical, imaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. The eligibility criteria for ADNI-1 (the first phase of the project) are described in the ADNI-1 protocol (available at http://adni.loni.usc.edu/methods/documents/). Briefly, eligible participants were aged 55 to 90 years, spoke either English or Spanish, and had an informant who was able to provide an independent evaluation of functioning. Eligible participants had also completed at least 6 years of education (or had a work history sufficient to exclude intellectual disability).

Our final analysis cohort consisted of 188 patients from ADNI-1 for whom CSF sTNFR2 levels and genetic status related to the *TNFRSF1B* SNPs of interest were available. In addition CSF Aβ42 levels were available for 177 and t-tau and p-tau levels for 182 of these patients. Details regarding the Elecsys method used to measure ADNI AD biomarkers are provided elsewhere (Shaw et al., 2019).

ADNI Cohort: sTNFR2 Levels

Levels of sTNFR2 were measured in ADNI CSF samples using the RBM Discovery Multi-Analyte Profiling (MAP) v.1.0 panel (Myriad Genetics, Salt Lake City, Utah), which uses a Luminex platform (Myriad Genetics). Data obtained from "Biomarkers Consortium CSF Proteomics Project RBM Multiplex Data and Primer."

ADNI Cohort: Genotyping

ADNI-1 patients were genotyped using Illumina's10 Human610-Quad BeadChip (San Diego, California), and intensity data were processed with GenomeStudio v2009.1 (Illumina). For this study, we analyzed two *TNFRSF1B* SNPs: rs976881 and rs1061622, which were found to be in linkage equilibrium in all populations

[1] *Biomarkers Consortium ADNI CSF Targeted Proteomics Project - Data Primer Version 28 Dec 2011*. Available online at: http://adni.loni.usc.edu/methods/ (accessed December 1, 2018).

($R^2 = 0.022$ and $D' = 0.50$; chi square = 108.34; $p < 0.0001$) according to LDlink analysis (Machiela and Chanock, 2015). These two SNPs are located within or in close proximity to the *TNFRSF1B* gene in the chr1p36 intronic 5' region (rs976881) and a missense coding variant in exon 6 (rs1061622). These SNPs were chosen based on their association with *TNFRSF1B*-related clinical outcomes and peripheral sTNFR2 levels (Glossop et al., 2005; Vistoropsky et al., 2010; Medrano et al., 2014; Cohen-Woods et al., 2018). Reference, heterozygous, and alternate alleles were T/T, T/C, and C/C, respectively, for rs976881, and G/G, G/T, and T/T, respectively, for rs 1061622. The reference and alternate allele notations were determined per the ADNI dataset based on calls from the International HapMap Project phase 3 data (Biffi et al., 2010). *APOE ε4* genotyping data were obtained from the ADNIMERGE table.

ADNI Cohort: Structural MRI Acquisition and Processing

In ADNI-1 patients with MCI or dementia, results from 1.5T MRI scans were performed at baseline, and these results (taken from the ADNIMERGE primary table) were used for this analysis (Jack et al., 2008). The whole brain, ventricular volume, and hippocampal volume were the primary dependent variables, with intracranial volume used as a covariate in statistical models. These data are available as part of the ADNIMERGE package (downloaded on May 6, 2020).

ADNI Cohort: Neurocognitive Measures

Baseline neurocognitive scores for ADNI-1 patients with MCI or dementia were analyzed. All major cognitive domains included in ADNI were evaluated, including the Logical Memory delayed recall score to assess verbal episodic memory (Wechsler Memory Scale, Logical Memory subtest) (Wechsler, 1981). Attention was assessed using the Digit Span subtest (Digit Span Forward and Digit Span Backward), and executive functioning was quantified using the Trail Making Test Part B, both from the Wechsler Adult Intelligence Scale (Wechsler, 1981). Object naming was assessed using the Boston Naming Test (Kaplan et al., 1983), and verbal fluency was assessed using a category fluency test (animals) (Strauss et al., 2006). The Mini Mental State Examination (MMSE) (Folstein et al., 1975) and Clinical Dementia Rating Sum of Boxes (CDR-SB) score (Morris, 1993) were used to assess global cognition, and change in CDR-SB score over 12 months was used to assess longitudinal change in cognition.

Replication Memory Clinic Cohort

A cross-sectional replication cohort consisting of 48 patients in the MCI stage of AD (MCI-AD) were recruited from a specialized memory clinic at Cleveland Clinic (Lou Ruvo Center for Brain Health, Cleveland site). Recruitment details have been described previously (Pillai et al., 2019b).

The diagnosis of MCI-AD in these patients was confirmed by the presence of CSF Aβ42 and p-tau levels consistent with a diagnosis of AD as the primary etiology; the diagnosis was also based on consensus evaluation from two neurologists using published criteria (Albert et al., 2011). A commercially available test (ADmark Alzheimer's Evaluation, Athena Diagnostics, Marlborough, Massachusetts) was used to measure CSF levels of Aβ42, t-tau, and p-tau. All patients met the CSF cutoffs of $\leq$530 pg/mL for Aβ42 and $\geq$60 pg/mL for p-tau, which are consistent with amyloid-positive status on Amyvid (florbetapir F18 injection) at our center. *APOE* status was determined with blood samples (10 ng per patient) dispensed into 96-well plates for TaqMan (Applied Biosystems, Waltham, Massachusetts) allelic discrimination assays for the detection of SNPs associated with *APOE* alleles (*rs429358, rs7412*). PCR was performed using a 9700 Gene Amp PCR system (Applied Biosystems) and an end-point read in a 7500 Real-Time PCR system (Applied Biosystems) (Bekris et al., 2008).

Replication Memory Clinic Cohort: sTNFR2 Levels

CSF levels of sTNFR2 were assessed as described previously (Pillai et al., 2019b). In brief, CSF was collected and analyzed by an independent laboratory via the RBM HumanMAP v.2.0, which is a subset of the RBM DiscoveryMAP v.1.0 used in the ADNI cohort; these platforms have the same quality control and thresholding process and are comparable. The lowest detectable dose of sTNFR2 was 0.0017 ng/mL. Samples of CSF were frozen within 15 min after collection and were processed (at −70°C in dry ice) and maintained at −80°C (in a non-frost-free refrigerator). The samples were shipped frozen in a Styrofoam container with sufficient dry ice to maintain the temperature at <-70°C for at least 48 h. Samples therefore underwent a single freeze-thaw cycle before analysis.

Replication Memory Clinic Cohort: Genotyping

Genomic DNA was extracted from peripheral whole blood using standard protocols. Two *TNFRSF1B* SNPs (rs976881 and rs1061622) were characterized. *TNFRSF1B* variant allelic discrimination was performed using the 7500 Real Time PCR System and TaqMan SNP Genotyping Assays. The genotypes were determined based on sample clustering using the autocaller function of the Genotyping Application within Thermo Fisher Connect software.

Replication Memory Clinic Cohort: Cognitive and Functional Measures

MMSE and Montreal Cognitive Assessment (MoCA) scores (Nasreddine et al., 2005) obtained <6 months before AD biomarker testing were available for all patients. In addition baseline CDR-SB scores (Morris, 1993) were available to characterize patients' cognitive and functional deficits.

Statistical Analysis

Participants in ADNI-1 who had either MCI or AD dementia at baseline were merged into one group to ensure adequate power of analysis. The normality of biomarkers was evaluated using graphical methods and the Shapiro-Wilk test. A log (base 2) transformation allowed Pearson correlations to be fit; the sTNFR2 and AD biomarker levels described are therefore dimensionless. Along with estimates of correlation, 95% CIs and p-values were calculated. ANOVA models were used to

estimate the mean differences between CSF sTNFR2 and t-tau and p-tau levels among reference, alternate, and heterozygous alleles of the *TNFRSF1B* SNPs. Multivariate general linear models were used to test the effect of CSF sTNFR2 levels and each of the two *TNFRSF1B* SNPs on CSF t-tau and p-tau, MRI brain measures, and cognitive domains after adjustments were made for covariates such as age, sex, years of education, *APOE ε4* status, and CSF Aβ42/p-tau ratio. Baseline MMSE and t-tau were also included as covariates in evaluations of cognitive domains as dependent variables, and baseline intracranial volumes and t-tau were included as covariates in evaluations of MRI measures as dependent variables.

First, we tested the effect of sTNFR2 levels on CSF t-tau and p-tau, MRI volumetric measures, and cognitive variables. Second, we tested the effect of each *TNFRSF1B* variant of interest on CSF t-tau and p-tau, MRI volumetric measures, and cognitive variables. Third, we tested whether the interaction effects between sTNFR2 and each *TNFRSF1B* variant and between sTNFR2 and t-tau were present for CSF t-tau and p-tau, MRI volumetric measures, and cognitive variables. Levene's test of equality of error variances, lack-of-fit F-test, and residual plots were used to assess model linearity and fit.

Model 1, evaluated the dependent variables CSF t-tau and p-tau. The main effects were CSF sTNFR2, *TNFRSF1B* SNP, and interaction effect between sTNFR2 and *TNFRSF1B* SNP. Covariates for this model included age, sex, CSF Aβ42, and *APOEε4* status.

Model 2, evaluated the dependent variables hippocampal volume, ventricular volume, and whole brain volume. The main effects were CSF sTNFR2, *TNFRSF1B* SNP, CSF t-tau, and interaction effects between sTNFR2 and *TNFRSF1B* SNP and between sTNFR2 and t-tau. Covariates for this model included age, sex, years of education, CSF Aβ42/p-tau ratio, *APOE ε4* status, and baseline intracranial volume.

Model 3, evaluated the dependent variables Logical Memory delayed recall score, Digit Span subtest score, Trail Making Test Part B score, category fluency test (animals), and Boston Naming Test score. The main effects were CSF sTNFR2, *TNFRSF1B* SNP, CSF t-tau, and interaction effects between sTNFR2 and *TNFRSF1B* SNP and between sTNFR2 and t-tau. The covariates for this model included age, sex, years of education, CSF Aβ42/p-tau ratio, *APOE ε4* status, and baseline MMSE score.

Model 4, CDR-SB scores were log transformed because Levene's test demonstrated that the variances were significantly different for CDR-SB scores for the *TNFRSF1B* SNPs. A univariate general linear model was used to evaluate the interaction effect for each *TNFRSF1B* SNP and CSF sTNFR2 on change in CDR-SB score over 1 year in the ADNI cohort. CDR-SB score at 1 year was the dependent variable; the main effects were disease state (MCI or AD dementia), CSF t-tau, and interaction effect between sTNFR2 and *TNFRSF1B* SNP. The covariates included age, sex, years of education, *APOE ε4* status, baseline CDR-SB score, and CSF Aβ42/p-tau.

Replication Cohort Model

Paralleling Model 1, here the dependent variables were again CSF t-tau and p-tau. The main effects were CSF sTNFR2, *TNFRSF1B* SNP, and interaction effect between sTNFR2 and *TNFRSF1B* SNP. Covariates for this model included age, sex, CSF Aβ42, and *APOE ε4* status.

Sensitivity analysis with and without covariates were conducted. Proportion of variance associated with one or more main effects was calculated using eta squared. All tests were two-tailed, and statistical significance was set at $p < 0.05$. For model 3 (with more than three dependent variables in the outcome), the Benjamini-Hochberg false discovery rate was used to assess significance. IBM SPSS Statistics for Windows, Version 22.0 (Armonk, New York) and RStudio Team (2020) RStudio (Version 1.2.5042) were used for all analyses.

Data Availability

ADNI data are available on request at loni.ADNI.org.

RESULTS

Our analysis included a total of 188 ADNI-1 participants (59 with AD dementia; 129 with MCI) (**Table 1**).There were no differences in sTNFR2 or in CSF Aβ42, t-tau, or p-tau biomarker levels within the allelic subgroups of rs976881 or rs1061622.

A total of 48 participants with MCI-AD were included in the replication memory clinic cohort (**Table 2**). There were again no differences in sTNFR2 or in CSF t-tau, or p-tau biomarker levels within the allelic subgroups of rs976881 and rs1061622. The differences between ADNI and replication cohort is noted in **Supplementary Table 1**. Both cohorts had similar baseline CDR-SB scores, years of education and were predominantly White and a slightly higher frequency of men. The replication cohort had a lower age, higher frequency of *APOE* ε4 carriers and a slightly lower baseline MMSE.

ADNI Cohort: Model 1

A significant interaction effect between rs976881 and CSF sTNFR2 was associated with CSF t-tau levels, but this association with CSF t-tau levels was not seen for the interaction effect between rs1061622 and CSF sTNFR2 (**Table 3** and **Figure 1A**). The effect sizes (by partial eta squared values) were 0.046 for CSF t-tau and 0.044 for CSF p-tau (i.e., 4.6% of all variance in CSF t-tau and 4.4% of all variance in CSF p-tau were attributable to the interaction effect between rs976881 and CSF sTNFR2). The T/T group was noted to have a shallower slope and poorer fit ($R^2 = 0.1$) than C/C ($R^2 = 0.38$), suggesting that sTNFR2 levels are not as closely related to t-tau levels in T/T as in C/C. The direct main effects of sTNFR2 on CSF t-tau and p-tau are shown in **Supplementary Table 2**.

ADNI Cohort: Model 2

A significant interaction effect between rs976881 and CSF sTNFR2 was associated with hippocampal volume and whole brain volume but not with ventricular volume (**Table 3**). 8.8% of all variance in hippocampal volume and 5.9% of all variance in whole brain volume were attributable to the interaction effect between rs976881 and CSF sTNFR2.

TABLE 1 | Demographics of ADNI cohort with patients divided into allelic subgroups, Mean (Std dev), percent of total and counts are presented.

ADNI cohort (MCI + AD dementia)	rs976881 Homozygous reference T/T *N* = 16	rs976881 Heterozygous T/C *N* = 78	rs976881 Homozygous alternate C/C *N* =94	rs976881 Total 188	*P*-value	rs1061622 Homozygous reference G/G *N* = 8	rs1061622 Heterozygous G/T *N* = 45	rs1061622 Homozygous alternate T/T *N* = 130	rs1061622 Total 183	*P*-value
MCI, N/total	11/16	51/78	67/94	129/188	0.70	4/8	31/45	92/130	127/183	0.46
Age, yrs	71.01 (8.8)	73.53 (7.9)	76.50 (6.1)	188	0.014*	75.95 (9.7)	76.27 (5.9)	74.29 (7.5)	183	0.23
Female, %F	18.7%	35.9%	37.2%	66/188	0.35	37.5%	44.4%	31.5%	64/183	0.29
Education, yrs	15.31 (2.9)	15.64 (3.0)	15.86 (2.8)	188	0.75	14.88 (3.4)	16.16 (2.6)	15.62 (2.9)	183	0.66
APOE ε4 +*ve*	7	50	58	115/188	0.58	5	29	77	111/183	0.80
Log_2 CSF Aβ42	9.42 (0.5)	9.22 (0.5)	9.32 (0.5)	171	0.48	9.50 (0.7)	9.28 (0.4)	9.28 (0.5)	166	0.87
Log_2 CSF t-tau	8.17 (0.4)	8.34 (0.4)	8.25 (0.6)	182	0.36	8.56 (0.4)	8.36 (0.5)	8.24 (0.5)	177	0.24
Log_2 CSF p-tau	4.76 (0.5)	4.99 (0.5)	4.89 (0.7)	188	0.39	5.18 (0.5)	5.01 (0.6)	4.88 (0.6)	177	0.36
Log_2 CSF sTNFR2	−0.13 (0.1)	−0.11 (0.1)	−0.147 (0.1)	188	0.59	−0.136 (0.1)	−0.11 (0.1)	−0.13 (0.1)	183	0.86
MMSE	26.56 (2.7)	25.85 (2.2)	25.74 (2.5)	188	0.37	25.38 (3.6)	26 (2.4)	25.93 (2.3)	183	0.94
CDR-SB	2.18 (1.3)	2.59 (1.8)	2.36 (1.7)	188	0.53	3.31 (2.1)	2.53 (2.0)	2.32 (1.5)	183	0.22
CDR-SB change at 12 months	0.25 (0.9)	1.20 (1.8)	0.81 (1.2)	188	0.19	0.93 (1.8)	1.18 (1.8)	0.83 (1.3)	183	0.44
Logical memory delayed	3.13 (2.9)	2.58 (2.4)	2.79 (2.7)	188	0.70	3 (3.1)	2.56 (2.6)	2.75 (2.6)	183	0.62
Digit span forward length	6.31 (1.3)	6.55 (0.9)	6.33 (1.1)	188	0.35	6.38 (0.9)	6.49 (1.1)	6.38 (1.0)	183	0.82
Digit span backward length	4.13 (1.4)	4.46 (1.0)	4.39 (1.2)	188	0.46	4.62 (0.7)	4.87 (1.1)	4.24 (1.2)	183	0.007*
Trails B score	143.87 (87.7)	161 (90.4)	160.52 (84.9)	188	0.98	181.25 (106.6)	146.59 (78.6)	158.36 (86.8)	183	0.51
Category fluency Animals	15.69 (6.0)	14.13 (4.6)	14.65 (4.8)	188	0.62	15.13 (6.8)	15.49 (5.3)	14.27 (4.5)	183	0.39
Boston naming test Total	26.06 (4.1)	24.81 (4.5)	24.12 (5.6)	188	0.24	24.38 (3.2)	24.93 (6.0)	24.62 (4.6)	183	0.39

*Independent samples Kurskal Wallis test p-values, *p < 0.05. All cognitive scores at baseline unless otherwise stated.*

The MRI measures were not affected by rs1061622, nor by the interaction effect between sTNFR2 and CSF t-tau. CSF t-tau did have a significant direct main effect on ventricular volume and whole brain volume but not on hippocampal volume (**Supplementary Table 2**).

ADNI Cohort: Model 3

In Model 3, a significant interaction effect between rs976881 and CSF sTNFR2 was associated with the Digit Span subtest score, the Trail Making Test Part B score, and the category fluency test (animals) score (**Table 3**). 9.9% of all variance for the Digit Span Forward score and 4.7% of all variance for the category fluency (animals) score were attributable to the interaction effect between rs976881 and CSF sTNFR2. There was no significant interaction effect between sTNFR2 and CSF t-tau, nor was there a significant main effect of CSF t-tau on these cognitive measures (**Figure 2A**). As shown in **Figure 2B**, the T/T group had a steeper slope and a larger regression coefficient for the Digit Span Forward score ($R^2 = 0.33$), suggesting that lower sTNFR2 levels (but not CSF t-tau levels) relate to poorer Digit Span Forward scores for this group, unlike for the C/C group ($R^2 = 0.004$). The interaction effect between rs1061622 and CSF sTNFR2 had no effect on these cognitive measures. The Digit Span Forward score was significant even after false discovery rate correction (false discovery rate–corrected *p*-values are provided in the **Supplementary Material**).

ADNI Cohort: Model 4

A significant interaction effect between rs976881 and sTNFR2 was associated with change in CDR-SB scores over 1 year (df 3, 175; mean square = 0.21; $F = 2.83$; adjusted $R^2 = 0.36$). A

TABLE 2 | Demographics of replication memory clinic cohort with patients divided into allelic subgroups.

Replication Cohort (MCI only)	rs976881 Homozygous reference T/T *N* = 8	rs976881 Heterozygous T/C *N* = 19	rs976881 Homozygous alternate C/C *N* = 21	rs976881 Total	*P*-value	rs1061622 Homozygous reference G/G *N* = 2	rs1061622 Heterozygous G/T *N* = 38	rs1061622 Homozygous alternate T/T *N* = 8	rs1061622 Total	*P*-value
Age, yrs	67.9 (6.7)	69.6 (7.9)	64.3 (7.4)	48	0.088	65.0 (7.6)	66.8 (7.9)	67.3 (7.0)	48	0.65
Female, %F	22.2%	47.4%	45%	20/48	0.50	50%	39.5%	50%	20/48	0.90
Education, yrs	16.1 (2.4)	15.0 (3.1)	15.4 (2.9)	48	0.65	13.0 (1.4)	15.8 (2.7)	14.1 (3.3)	48	0.20
APOE ε4 +*ve*	5	16	15	36/48	0.46	2	28	6	36/48	0.72
Log_2 CSF Aβ42	8.5 (0.26)	8.04 (0.47)	8.9 (0.63)	48	0.018*	8.0 (0.56)	8.2 (0.52)	7.7 (0.58)	48	0.12
Log_2 CSF t-tau	8.7 (0.50)	9.3 (1.02)	8.7 (0.88)	48	0.066	9.07 (1.09)	8.9 (0.91)	9.7 (0.74)	48	0.41
Log_2 CSF p-tau	6.2 (0.39)	6.6 (0.72)	6.1 (0.63)	48	0.075	6.8 (0.58)	6.3 (0.67)	6.4 (0.67)	48	0.41
Log_2 CSF sTNFR2	1.06 (0.33)	1.21 (0.59)	1.25 (0.4)	48	0.54	0.96 (0.28)	1.19 (0.49)	1.2 (0.00)	48	0.32
MMSE Baseline	24.5 (3.7)	25.1 (3.1)	23.4 (6.1)	48	0.39	25.0 (1.4)	24.2 (5.1)	24.3 (3.3)	48	0.59
MOCA Baseline	19.3 (4.2)	18.2 (4.8)	18.2 (2.4)	48	0.79	13.0 (1.4)	18.5 (4.5)	18.0 (4.1)	48	0.14
CDR-SB Baseline	2.07 (0.87)	2.05 (1.30)	2.22 (1.22)	47	0.43	2.3 (1.8)	2.1 (1.2)	2.3 (1.1)	47	0.90

*Mean (Std dev), percent of total and counts are presented. Independent samples Kurskal Wallis test p-values, *p < 0.05.*

total of 4.6% of all variance in CDR-SB change over 1 year was attributable to the interaction effect between rs976881 and CSF sTNFR2 (**Figure 3A**). As shown in **Figure 3B**, the T/T group had a smaller change (shallower slope) in CDR-SB change over 1 year than the C/C group ($R^2 = 0.52$ vs 0.70). There was no significant effect of rs1061622 on CDR-SB change over 1 year (df 3, 170; $F = 2.32$; adjusted $R^2 = 0.35$).

Replication Memory Clinic Cohort

A significant interaction effect between rs976881 and CSF sTNFR2 was associated with CSF p-tau levels (**Table 3**). Eleven percentage of all variance in CSF t-tau and 13% of all variance in CSF p-tau were attributable to the interaction effect between rs976881 and CSF sTNFR2. The homozygous reference (T/T) group had a shallower slope and a smaller regression coefficient ($R^2 = 0.37$) than the homozygous alternate allele (C/C) ($R^2 = 0.42$) (**Figure 1B**), suggesting that sTNFR2 levels are not as closely related to t-tau levels in the T/T replication memory clinic group as they are in the ADNI cohort. Pairwise comparisons for the rs976881 alleles demonstrated significant differences between the T/T and T/C groups in CSF t-tau levels (mean difference = −0.58; 95% CI: 0.074 to 1.09) and CSF p-tau levels (mean difference = −0.39; 95% CI: 0.050–0.747). **Supplementary Table 2** provides details regarding the direct main effects of significance.

A significant interaction effect between rs1061622 and CSF sTNFR2 was associated with CSF t-tau and p-tau levels. Fourteen percentage of all variance in CSF t-tau and 14% of all variance in CSF p-tau were attributable to the interaction effect between rs1061622 and CSF sTNFR2 (**Table 3**). Pairwise comparisons for the rs1061622 alleles demonstrated significant differences between the T/T and T/G groups in CSF t-tau levels (mean difference = −0.69; 95% CI: −0.079 to −1.29) and CSF p-tau levels (mean difference = −0.49; 95% CI: −0.067 to −0.91).

DISCUSSION

Immune-related genes and pathways have been found to play a role in AD pathophysiology (Fischer et al., 2011; Kunkle et al., 2019). Previous studies have assessed how these genetic immune and factors could affect AD biomarkers (Hohman et al., 2014; Louwersheimer et al., 2016; Deming et al., 2019) and clinical outcomes (Pillai et al., 2020), but little research has addressed their potential contribution to resilience. The inflammatory analyte TNF has been found to play a role in synaptic plasticity and modulating responses to neural injury and neurodegeneration (Beattie et al., 2002; Ellwardt et al., 2018), suggesting that it may also be involved in cognitive resilience (Pape et al., 2019). The current study demonstrated that the interaction between TNF pathway–related sTNFR2 CSF levels and *TNFRSF1B* gene variants contributed to significant differences in AD-associated severity markers such as CSF t-tau and p-tau, MRI measures of interest, and cognitive scores in the ADNI cohort. In a replication memory clinic cohort, the interaction between CSF sTNFR2 and rs976881 was also associated with differences in CSF p-tau biomarkers, with a significantly larger effect size than was seen in the ADNI cohort. In the ADNI cohort, rs976881 T/T allele (homozygous reference)

TABLE 3 | Key results of the general linear models: Results from the ADNI and replication memory clinic cohorts, *$p \leq 0.05$.

Rs976881 * sTNFR2	Df	Mean Square	F	*P*-value	Adjusted R^2	Partial Eta squared
Model 1: Dependent variables: CSF t-tau and p-tau, Main effects: CSF sTNFR2, TNFRSF1B SNP, Interaction effect: sTNFR2 X TNFRSF1B SNP, Covariates: age, sex, CSF Aβ42, APOEε4 status						
ADNI cohort						
Log CSF t-tau	2,165	0.21	3.94	0.021*	0.27	0.046
Log CSF p-tau	2,165	0.33	3.83	0.024*	0.24	0.044
Replication cohort						
Log CSF t-tau	2,44	1.30	2.82	0.07	0.47	0.11
Log CSF p-tau	2,44	0.70	3.20	0.05*	0.51	0.13
Rs1061622*sTNFR2						
Log CSF t-tau	2,44	1.60	3.58	0.036*	0.48	0.14
Log CSF p-tau	2,44	0.77	3.56	0.037*	0.52	0.14
Model 2: Dependent variables: hippocampal volume, ventricular volume and whole brain volume, Main effects: CSF sTNFR2, *TNFRSF1B* SNP, CSF t-tau, Interaction effects: sTNFR2 X *TNFRSF1B* SNP and sTNFR2 X t-tau, Covariates: age, sex, education, CSF Aβ42/p-tau ratio, *APOEε4* status and baseline intra cranial volume						
ADNI Cohort						
Hippocampus	2,134	2646708.94	6.48	0.002*	0.08	0.088
Ventricles	2,134	33816326.80	0.64	0.94	0.12	0.001
Whole brain volume	2,134	9426117372.29	4.21	0.017*	0.16	0.059
Model 3: Dependent variables: Logical Memory delayed recall, Digit Span length, Trails B score and Boston Naming total score, Main effects:CSF sTNFR2, TNFRSF1B SNP, CSF t-tau, Interaction effects: sTNFR2 X TNFRSF1B SNP and sTNFR2 X t-tau, Covariates: age, sex, education, CSF Aβ42/p-tau ratio, APOEε4 status and baseline MMSE						
ADNI Cohort						
Logical memory	2,159	5.22	0.78	0.459	0.038	0.01
Digit span forward length	2,159	8.90	8.89	<0.0001*	0.064	0.099
Digit span backward length	2,159	4.43	4.43	0.036*	0.025	0.041
Trails B-score	2,159	26889.98	3.71	0.027*	0.046	0.045
Category Fluency Animals	2,159	92.64	3.96	0.021*	0.025	0.047
Boston naming test	2,159	22.61	0.84	0.43	0.003	0.01

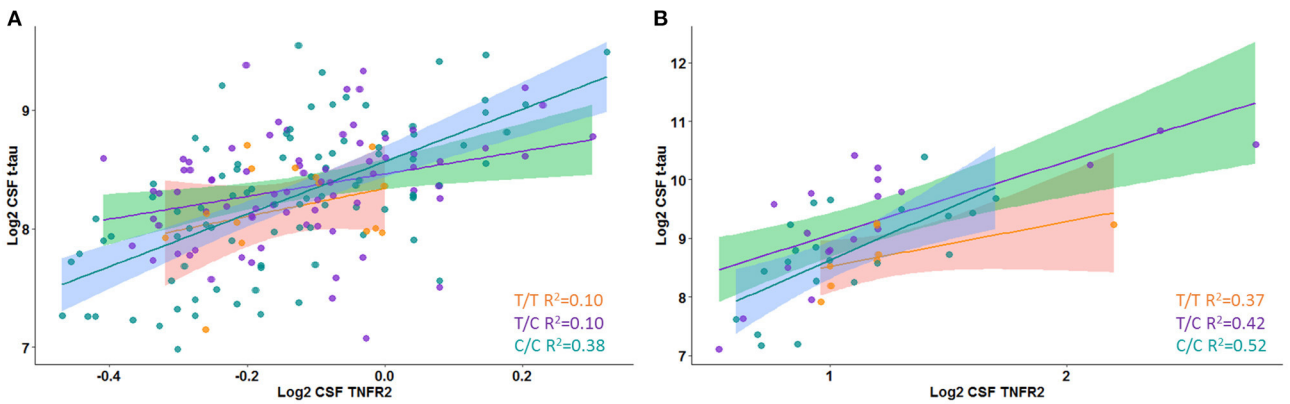

FIGURE 1 | Interaction between rs976881 and soluble tumor necrosis factor receptor 2 (sTNFR2) and the effect on CSF t-tau in Alzheimer's Disease Neuroimaging Initiative (ADNI) and replication cohorts. Regression lines, 95% CI and R^2 values shown for the alleles T/T, T/C, and C/C. **(A)** ADNI cohort. **(B)** Replication cohort.

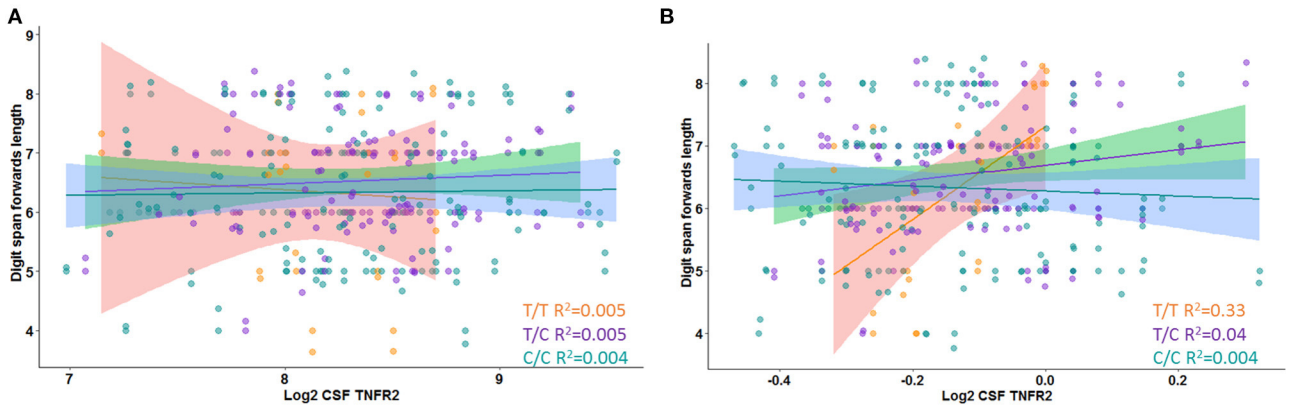

FIGURE 2 | Interaction between rs976881 and soluble tumor necrosis factor receptor 2 (sTNFR2) and between rs976881 and CSF t-tau and the effect on Digit Span Forward score. **(A)** CSF t-tau **(B)** CSF sTNFR2. Regression lines, 95% CI and R^2 values shown for the alleles T/T, T/C, and C/C. Alzheimer's Disease Neuroimaging Initiative (ADNI) data.

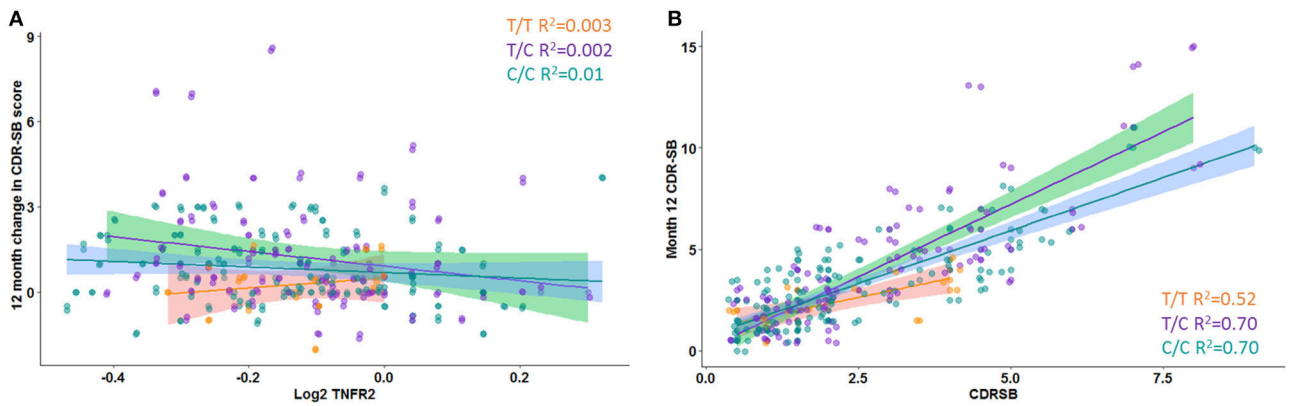

FIGURE 3 | Change in Clinical Dementia Rating Sum of Boxes (CDR-SB) score over time. **(A)** Interaction between rs976881 and sTNFR2 level and the effect on change in CDR-SB score over 1 year. **(B)** Slope of change in score over 1 year. Regression lines, 95% CI and R^2 values shown for the alleles T/T, T/C, and C/C. Alzheimer's Disease Neuroimaging Initiative (ADNI) data.

carriers demonstrated slower rates of CDR-SB score changes in relation to corresponding sTNFR2 levels. Additionally, statistical interaction effects demonstrated that the strength of a linear relationship between sTNFR2 and (1) CSF t-tau and p-tau, (2) MRI whole brain and hippocampal volumes, and (3) Digit Forward test score varied for the rs976881 T/T allele compared to the C/C and T/C alleles. Studies in animal models have demonstrated that TNFR2 plays a protective role against neurodegeneration in the CNS (Marchetti et al., 2004; Fischer et al., 2011; Dong et al., 2016). Taken together, these findings support this genotypic variant is a potential marker of resilience in AD.

sTNFR2 is expressed primarily in immune and endothelial cells. TNFR2 receptor signaling is involved in pro-survival signaling pathways, which activate cellular inhibitors of apoptosis and the NF-κB pathway (Kanehisa et al., 2016). Subsequent downstream activation of PKB/Akt promotes cell survival and proliferation (Fischer et al., 2020). sTNFR2 has previously been found to be highly correlated with CSF t-tau, p-tau, and neuron-specific enolase in MCI-AD (Pillai et al., 2019b), and a similar profile with *TNFRSF1B* activation was noted in a parallel brain transcriptome analysis (Pillai et al., 2019b). It is possible, therefore, that sTNFR2 and *TNFRSF1B* SNPs play a modulating role in regard to clinical outcomes in AD, rather than serving as an AD risk gene that have been the focus of prior genome-wide association studies in AD. This is consistent with prior reports of gene variants related to resilience in AD which suggest that genetic architecture of resilience appears to be distinct from that of clinical AD (Dumitrescu et al., 2020).

Research has shown that rs976881 and rs1061622 are associated with sTNFR2 levels in the periphery, with the rs976881 reference allele (T/T) related to higher sTNFR2 levels than the alternate allele (C/C) (Vistoropsky et al., 2010; Cohen-Woods et al., 2018). In the replication memory clinic cohort in this study, patients with the rs976881 reference allele T/T had lower CSF t-tau and p-tau levels than patients with T/C (given

their corresponding CSF sTNFR2 levels). Previous research has also found that rs976881 T/T is less responsive than T/C to anti-TNFα maintenance therapy (infliximab) in patients with Crohn's disease (Steenholdt et al., 2012). It is therefore likely that among minor allele (T/T) carriers with Crohn's disease, higher levels of TNFR2 counteract TNF-α, making infliximab less effective. In our study, the effect of rs976881 may also be tied to its effect on sTNFR2 levels, with the rs976881 minor allele (T/T) demonstrating a more robust sTNFR2 response than C/C and T/C alleles to CSF t-tau and p-tau levels. Studies have also demonstrated that the minor allele of rs1061622 is also related to sTNFR2 levels and response to anti-TNFα maintenance therapy in patients with Crohn's disease (Medrano et al., 2014). In our study, the significant interaction observed between rs1061622 and sTNFR2 and its effect on CSF t-tau and p-tau in the replication cohort but not in the ADNI cohort suggests differences in the nature of participants recruited, as discussed in further detail later.

In the ADNI cohort, a significant interaction between rs976881 and CSF sTNFR2 was strongest with regards to Digit Span Forwards subtest, but was also noted in Trail Making Part B test, and category fluency test (animals) scores. These measures assess overlapping but distinct cognitive skills, including working memory, complex attention, and speed of information processing, and can be categorized as contributing to domains of attention/executive functioning and processing speed. In this cohort, rs976881 status was also related to the change in CDR-SB score over 1 year. This is consistent with our previous finding that better initial performance on the Digit Span subtest and related working memory task is associated with a slower rate of functional decline on the CDR-SB test over time in patients with AD (Pillai et al., 2014). Additionally, higher levels of pro-cell survival and inflammation resolution chemokines have been found in the human temporal cortex and entorhinal cortex at autopsy among human brains resilient to AD pathology (Barroeta-Espar et al., 2019). These results are reflected in our study, which demonstrated that a significant interaction between rs976881 and CSF sTNFR2 modulated favorable outcomes in three measures related to AD severity: CSF biomarkers of neurodegeneration and tau (CSF p-tau and t-tau), MRI measures (hippocampal and whole brain volumes), and cognitive measures (Digit Span Forward score and CDR-SB score).

Strengths/limitations: The current study demonstrated directional replication by consistently noting a similar directional interaction effect of sTNFR2 and rs976881 on CSF p-tau and t-tau in both the ADNI and replication cohorts. Additionally, the effect of rs976881 on MRI and cognitive outcomes demonstrated in this study is consistent with the known relationship between these biomarkers (CSF p-tau, t-tau, hippocampal volume, and whole brain volumes) and longitudinal cognitive outcomes in the ADNI population. In the ADNI cohort for this study, 4.6% of the variance in CSF t-tau levels was explained by rs976881 and sTNFR2, whereas 11% of the variance in CSF t-tau was attributed to the same variables in the replication cohort. There were some differences between the cohorts, however. First, the positive correlation between the sTNFR2 and neurodegeneration biomarkers are consistent within each cohort but the replication cohort had 2.2 times higher mean levels of p-tau than ADNI (Pillai et al., 2019b). Mean sTNFR2 levels correlating with neurodegeneration biomarkers were also 2.4 times higher in the replication cohort than ADNI. This is likely due to different participant characteristics for the two cohorts; for instance, the replication cohort was a sample of memory clinic patients with a faster rate of disease progression than patients in the ADNI cohort (Pillai et al., 2020). Second, the replication cohort included participants at the MCI stage of AD, whereas the ADNI cohort included patients with MCI and AD dementia. As the replication cohort was not harmonized with the ADNI cohort for all of the neurocognitive variables and MRI volumetric measures, there could not be a close corroboration between the replication cohort and the ADNI cohort for the multiple variables of interest. These results are likely generalizable to MCI and AD dementia subjects with positive AD biomarkers but the fact that patients in both cohorts were predominantly Caucasian and had a higher level of education suggests the need for replication of these results among other racial and ethnic cohorts with a diverse education and socioeconomic backgrounds.

Within the ADNI cohort, MRI hippocampal volumes were noted to have a significant interaction effect between rs976881 and sTNFR2, while logical memory delayed recall scores did not meet significance. One possible reason for this is that the predominance of amnestic patients with MCI despite likely multiple etiologies of amnestic complaints in ADNI cohort, limits our ability to discriminate based on logical memory delayed recall scores alone given normative limits (Nettiksimmons et al., 2014). Additional corroboration of these results in a cohort with recruitment goals different from those of ADNI is therefore warranted. The lack of neuropathologic confirmation of diagnosis also limits our understanding regarding the potential role of mixed pathology on clinical outcomes.

The study is an evaluation of the association between CSF sTNFR level and *TNFRSF1B* SNPS on clinical outcomes, and does not address the mechanistic relationship between them on neurodegeneration. This study further does not provide a comprehensive survey of *TNFRSF1B* SNPs in AD, as we did not analyze other *TNFRSF1B* SNPs reported to be related to sTNFR2 levels (e.g., rs590368). These other SNPs were not included because of the smaller number of participants within ADNI who had these genetic variants and who also had measured levels of sTNFR2 available. Using multiple SNPs may allow researchers to define a stronger *TNFRSF1B* haplotype with regard to sTNFR2 levels, and some variables may become more or less salient considering additional SNP interactions. Independent replication using the same biomarkers used in ADNI would allow for further clarification. Type II errors have to be considered for this study, given the small number of patients within some of the allele groups; smaller effect sizes could therefore have been missed.

The rs976881 T/T reference genotype has been related to higher levels of sTNFR2 previously and activation of TNFR2 signaling has been posited as a promising strategy for AD therapy (Ortí-Casañ et al., 2019). We have now demonstrated that interaction between *TNFRSF1B* gene variant rs976881 and CSF sTNFR2 affects CSF and MRI biomarkers of neurodegeneration,

cognitive profiles, and rate of functional decline over 1 year. These results provide important information regarding the molecular characterization of AD phenotypes and suggest that this genotypic variant could be used as a marker of resilience in AD. Independent confirmation of these results in other cohorts with mutidomain AD biomarkers is warranted.

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: http://adni.loni.usc.edu/, genetic-data.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Cleveland Clinic Institutional Review Board ADNI Individual Site Institutional Review Board. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

JP: obtained funding for research project, organization of research project, execution of research project, design the statistical analysis, execution of statistical analysis, and writing the first draft of manuscript preparation. GB: execution of research project, review and critique of statistical analysis, and review and critique of manuscript preparation. MK: execution of research project and review and critique of manuscript preparation. JB: design the statistical analysis and review and critique of manuscript preparation. CB, WB, and JL: review and critique of statistical analysis and review and critique of manuscript preparation. LB: organization of research project, review and critique of statistical analysis, and review and critique of manuscript preparation. All authors contributed to the article and approved the submitted version.

FUNDING

The Funding was provided by 2018-AARG-592251 Alzheimer's Association, NIA K23AG055685, 1P30 AG062428-01, Keep Memory Alive Foundation and the Jane and Lee Seidman Fund. Research reported in this publication was supported by the National Institute On Aging of the National Institutes of Health under Award Number K23AG055685. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. JP was a program participant of the Research Education Component of the Cleveland Alzheimer's Disease Research Center supported by NIA P30 AG062428. Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI was funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

ACKNOWLEDGMENTS

We thank the patients and families who took part in the replication cohort at Cleveland Clinic Lou Ruvo Center for Brain Health and Megan Griffiths and Dennis Lal for editorial support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2021.638922/full#supplementary-material

REFERENCES

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 270–279. doi: 10.1016/j.jalz.2011.03.008

Arenaza-Urquijo, E. M., and Vemuri, P. (2018). Resistance vs resilience to Alzheimer disease: clarifying terminology for preclinical studies. *Neurology* 90, 695–703. doi: 10.1212/WNL.0000000000005303

Barroeta-Espar, I., Weinstock, L. D., Perez-Nievas, B. G., Meltzer, A. C., Siao Tick Chong, M., Amaral, A. C., et al. (2019). Distinct cytokine profiles in human brains resilient to Alzheimer's pathology. *Neurobiol. Dis.* 121, 327–337. doi: 10.1016/j.nbd.2018.10.009

Beattie, E. C., Stellwagen, D., Morishita, W., Bresnahan, J. C., Ha, B. K., Von Zastrow, M., et al. (2002). Control of synaptic strength by glial TNFalpha. *Science* 295, 2282–2285. doi: 10.1126/science.1067859

Bekris, L. M., Millard, S. P., Galloway, N. M., Vuletic, S., Albers, J. J., Li, G., et al. (2008). Multiple SNPs within and surrounding the apolipoprotein E gene influence cerebrospinal fluid apolipoprotein E protein levels. *J. Alzheimers Dis.* 13, 255–266. doi: 10.3233/JAD-2008-13303

Biffi, A., Anderson, C. D., Desikan, R. S., Sabuncu, M., Cortellini, L., Schmansky, N., et al. (2010). Genetic variation and neuroimaging measures in Alzheimer disease. *Arch. Neurol.* 67, 677–685. doi: 10.1001/archneurol.2010.108

Cohen-Woods, S., Fisher, H. L., Ahmetspahic, D., Douroudis, K., Stacey, D., Hosang, G. M., et al. (2018). Interaction between childhood maltreatment on immunogenetic risk in depression: discovery and replication in clinical case-control samples. *Brain Behav. Immun.* 67, 203–210. doi: 10.1016/j.bbi.2017.08.023

DeBerge, M. P., Ely, K. H., Wright, P. F., Thorp, E. B., and Enelow, R. I. (2015). Shedding of TNF receptor 2 by effector $CD8^+$ T cells by ADAM17 is important for regulating TNF-α availability during influenza infection. *J. Leukoc. Biol.* 98, 423–434. doi: 10.1189/jlb.3A0914-432RR

Deming, Y., Filipello, F., Cignarella, F., Cantoni, C., Hsu, S., Mikesell, R., et al. (2019). The MS4A gene cluster is a key modulator of soluble TREM2 and Alzheimer's disease risk. *Sci. Transl. Med.* 11:eaau2291. doi: 10.1126/scitranslmed.aau2291

Dong, Y., Fischer, R., Naudé P. J., Maier, O., Nyakas, C., Duffey, M., et al. (2016). Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.* 113, 12304–12309. doi: 10.1073/pnas.1605195113

Dubois, B., Feldman, H. H., Jacova, C., Hampel, H., Molinuevo, J. L., Blennow, K., et al. (2014). Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol.* 13, 614–629. doi: 10.1016/S1474-4422(14)70090-0

Dumitrescu, L., Mahoney, E. R., Mukherjee, S., Lee, M. L., Bush, W. S., Engelman, C. D., et al. (2020) Genetic variants and functional pathways associated with resilience to Alzheimer's disease. *Brain* 143, 2561–2575. doi: 10.1093/brain/awaa209

Ellwardt, E., Pramanik, G., Luchtman, D., Novkovic, T., Jubal, E. R., Vogt, J., et al. (2018). Maladaptive cortical hyperactivity upon recovery from experimental autoimmune encephalomyelitis. *Nat. Neurosci.* 21, 1392–1403. doi: 10.1038/s41593-018-0193-2

Fairfax, B. P., Davenport, E. E., Makino, S., Hill, A. V., Vannberg, F. O., and Knight, J. C. (2011). A common haplotype of the TNF receptor 2 gene modulates endotoxin tolerance. *J. Immunol.* 186, 3058–3065. doi: 10.4049/jimmunol.1001791

Fischer, R., Kontermann, R. E., and Pfizenmaier, K. (2020). Selective targeting of TNF receptors as a novel therapeutic approach. *Front. Cell. Dev. Biol.* 8:401. doi: 10.3389/fcell.2020.00401

Fischer, R., Maier, O., Siegemund, M., Wajant, H., Scheurich, P., and Pfizenmaier, K. (2011). A TNF receptor 2 selective agonist rescues human neurons from oxidative stress-induced cell death. *PLoS ONE* 6:e27621. doi: 10.1371/journal.pone.0027621

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state". *A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Glossop, J. R., Dawes, P. T., Nixon, N. B., and Mattey, D. L. (2005). Polymorphism in the tumour necrosis factor receptor II gene is associated with circulating levels of soluble tumour necrosis factor receptors in rheumatoid arthritis. *Arthritis Res. Ther.* 7:R1227–R1234. doi: 10.1186/ar1816

Hohman, T. J., Koran, M. E., Thornton-Wells, T. A., and Alzheimer's Disease Neuroimaging Initiative. (2014). Genetic modification of the relationship between phosphorylated tau and neurodegeneration. *Alzheimers Dement.* 10, 637–645.e1. doi: 10.1016/j.jalz.2013.12.022

Jack, C. R. Jr., Bernstein, M. A., Fox, N. C., Thompson, P., Alexander, G., Harvey, D., et al. (2008). The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. *J. Magn. Reson. Imaging* 27, 685–691. doi: 10.1002/jmri.21049

Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. (2016). KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res.* 44, D457–D462. doi: 10.1093/nar/gkv1070

Kaplan, E., Goodglass, H., and Weintraub, S. (1983). *Boston Naming Test*. Philadelphia, PA: Lea and Febiger.

Kunkle, B. W., Grenier-Boley, B., Sims, R., Bis, J. C., Damotte, V., Naj, A. C., et al. (2019) Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat. Genet.* 51, 414–430. doi: 10.1038/s41588-019-0358-2

Louwersheimer, E., Wolfsgruber, S., Espinosa, A., Lacour, A., Heilmann-Heimbach, S., Alegret, M., et al. (2016). Alzheimer's disease risk variants modulate endophenotypes in mild cognitive impairment. *Alzheimers Dement.* 12, 872–881. doi: 10.1016/j.jalz.2016.01.006

Machiela, M. J., and Chanock, S. J. (2015). LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics* 31, 3555–3557. doi: 10.1093/bioinformatics/btv402

Marchetti, L., Klein, M., Schlett, K., Pfizenmaier, K., and Eisel, U. L. (2004). Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. *J. Biol. Chem.* 279, 32869–32881. doi: 10.1074/jbc.M311766200

Medrano, L. M., Taxonera, C., Márquez, A., Barreiro-de Acosta, M., Gómez-García, M., González-Artacho, C., et al. (2014). Role of TNFRSF1B polymorphisms in the response of Crohn's disease patients to infliximab. *Hum. Immunol.* 75, 71–75. doi: 10.1016/j.humimm.2013.09.017

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 43, 2412–2414. doi: 10.1212/WNL.43.11.2412-a

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., et al. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53, 695–699. doi: 10.1111/j.1532-5415.2005.53221.x

Negash, S., Wilson, R. S., Leurgans, S. E., Wolk, D. A., Schneider, J. A., Buchman, A. S., et al. (2013). Resilient brain aging: characterization of discordance between Alzheimer's disease pathology and cognition. *Curr. Alzheimer Res.* 10, 844–851. doi: 10.2174/15672050113109990157

Nettiksimmons, J., DeCarli, C., Landau, S., Beckett, L., and Alzheimer's Disease Neuroimaging Initiative. (2014). Biological heterogeneity in ADNI amnestic mild cognitive impairment. *Alzheimers Dement.* 10, 511–521.e1. doi: 10.1016/j.jalz.2013.09.003

Ortí-Casañ, N., Wu, Y., Naudé P. J. W., De Deyn, P. P., Zuhorn, I. S., and Eisel, U. L. M. (2019). Targeting TNFR2 as a novel therapeutic strategy for Alzheimer's disease. *Front. Neurosci.* 13:49. doi: 10.3389/fnins.2019.00049

Pape, K., Tamouza, R., Leboyer, M., and Zipp, F. (2019). Immunoneuropsychiatry - novel perspectives on brain disorders. *Nat. Rev. Neurol.* 15, 317–328. doi: 10.1038/s41582-019-0174-4

Perry, V. H., Nicoll, J. A., and Holmes, C. (2010). Microglia in neurodegenerative disease. *Nat. Rev. Neurol.* 6, 193–201. doi: 10.1038/nrneurol.2010.17

Pillai, J. A., Bena, J., Bebek, G., Bekris, L. M., Bonner-Jackson, A., Kou, L., et al. (2020). Inflammatory pathway analytes predicting rapid cognitive decline in MCI stage of Alzheimer's disease. *Ann. Clin. Transl. Neurol.* 7, 1225–1239. doi: 10.1002/acn3.51109

Pillai, J. A., Bonner-Jackson, A., Bekris, L. M., Safar, J., Bena, J., and Leverenz, J. B. (2019a). Highly elevated cerebrospinal fluid total tau level reflects higher likelihood of non-amnestic subtype of Alzheimer's Disease. *J. Alzheimers Dis.* 70, 1051–1058. doi: 10.3233/JAD-190519

Pillai, J. A., Bonner-Jackson, A., Walker, E., Mourany, L., and Cummings, J. L. (2014). Higher working memory predicts slower functional decline in autopsy-confirmed Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 38, 224–233. doi: 10.1159/000362715

Pillai, J. A., Maxwell, S., Bena, J., Bekris, L. M., Rao, S. M., Chance, M., et al. (2019b). Key inflammatory pathway activations in the MCI stage of Alzheimer's disease. *Ann. Clin. Transl. Neurol.* 6, 1248–1262. doi: 10.1002/acn3.50827

Risacher, S. L., Anderson, W. H., Charil, A., Castelluccio, P. F., Shcherbinin, S., et al. (2017). Alzheimer disease brain atrophy subtypes are associated with cognition and rate of decline. *Neurology* 89, 2176–2186. doi: 10.1212/WNL.0000000000004670

RStudio Team. (2020). *RStudio: Integrated Development for R. RStudio*. Boston, MA: PBC. Available online at: http://www.rstudio.com/

Saykin, A. J., Shen, L., Yao, X., Kim, S., Nho, K., Risacher, S. L., et al. (2015). Genetic studies of quantitative MCI and AD phenotypes in ADNI: progress, opportunities, and plans. *Alzheimers Dement*. 11, 792–814. doi: 10.1016/j.jalz.2015.05.009

Shaw, L. M., Hansson, O., Manuilova, E., Masters, C. L., Doecke, J. D., Li, Q. X., et al. (2019). Method comparison study of the Elecsys® β-Amyloid (1-42) CSF assay versus comparator assays and LC-MS/MS. *Clin. Biochem*. 72, 7–14. doi: 10.1016/j.clinbiochem.2019.05.006

Singhal, P., Sharma, U., Hussain, S., Nag, A., and Bharadwaj, M. (2016). Identification of genetic variants in TNF receptor 2 which are associated with the development of cervical carcinoma. *Biomarkers* 21, 665–672. doi: 10.3109/1354750X.2016.1172109

Steenholdt, C., Enevold, C., Ainsworth, M. A., Brynskov, J., Thomsen, O. Ø., and Bendtzen, K. (2012). Genetic polymorphisms of tumour necrosis factor receptor superfamily 1b and fas ligand are associated with clinical efficacy and/or acute severe infusion reactions to infliximab in Crohn's disease. *Aliment. Pharmacol. Ther*. 36, 650–659. doi: 10.1111/apt.12010

Strauss, E., Sherman, E. M. S., and Spreen, O. (2006). *A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary*. 3rd Edn. New York, NY: Oxford University Press.

Therriault, J., Benedet, A. L., Pascoal, T. A., Mathotaarachchi, S., Savard, M., Chamoun, M., et al. (2020). APOEε4 potentiates the relationship between amyloid-β and tau pathologies. *Mol. Psychiatry*. doi: 10.1038/s41380-020-0688-6. [Epub ahead of print].

van der Vlies, A. E., Verwey, N. A., Bouwman, F. H., Blankenstein, M. A., Klein, M., Scheltens, P., et al. (2009). CSF biomarkers in relationship to cognitive profiles in Alzheimer disease. *Neurology* 72, 1056–1061. doi: 10.1212/01.wnl.0000345014.48839.71

Vistoropsky, Y., Ermakov, S., Toliat, M. R., Trofimov, S., Altmüller, J., Malkin, I., et al. (2010). Genetic determinants of circulating levels of tumor necrosis factor receptor II and their association with TNF-RII gene polymorphisms. *Cytokine* 51, 28–34. doi: 10.1016/j.cyto.2010.04.013

Wechsler, D. (1981). *Wechsler Adult Intelligence Scale – Revised*. San Antonio, TX: The Psychological Corporation.

Whitwell, J. L., Dickson, D. W., Murray, M. E., Weigand, S. D., Tosakulwong, N., Senjem, M. L., et al. (2012). Neuroimaging correlates of pathologically defined subtypes of Alzheimer's disease: a case-control study. *Lancet Neurol*. 11, 868–877. doi: 10.1016/S1474-4422(12)70200-4

Wyss-Coray, T., and Rogers, J. (2012). Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. *Cold Spring Harb. Perspect. Med*. 2:a006346. doi: 10.1101/cshperspect.a006346

Conflict of Interest: JP receives research funding from the National Institutes of Health, Alzheimer's Association, and Keep Memory Alive Foundation. CB receives research funding from the National Institutes of Health. WB receives research funding from the National Institutes of Health. JL has received consulting fees from Acadia, Aptnyx, Biogen, Eisai, GE Healthcare, Sanofi, and Takeda and grant support from the Alzheimer's Association, Alzheimer's Drug Discovery Foundation, Biogen, Department of Defense, GE Healthcare, Genzyme/Sanofi, Lewy Body Dementia Association, Michael J Fox Foundation, and National Institute of Health (NIA, NINDS).

The remaining 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.

Copyright © 2021 Pillai, Bebek, Khrestian, Bena, Bergmann, Bush, Leverenz and Bekris. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 17 May 2021
doi: 10.3389/fnagi.2021.665981

Circulating Neurofilament Light Predicts Cognitive Decline in Patients With Post-stroke Subjective Cognitive Impairment

Jian-Hong Wang[1†], Jie Huang[1†], Fu-Qiang Guo[1], Fang Wang[2], Shu Yang[1], Neng-Wei Yu[1], Bo Zheng[3] and Jian Wang[3]*

[1] Department of Neurology, The Affiliated Hospital of University of Electronic Science and Technology, Sichuan Provincial People's Hospital, Chengdu, China, [2] Innovation Center of Nursing Research, West China Hospital, Nursing Key Laboratory of Sichuan Province, Sichuan University, Chengdu, China, [3] Department of Neurology, Ya'an People's Hospital, Ya'an, China

Background: Subjective cognitive impairment (SCI) is common after acute ischemic stroke and adversely affects the quality of life. SCI is associated with an increased risk of developing mild cognitive impairment and dementia. Identifying biomarkers which could predict long-term cognitive outcomes of post-stroke SCI is of importance for early intervention. This study aims to investigate the association between circulating neurofilament light (NfL) and long-term cognitive function in patients with post-stroke SCI.

Methods: This longitudinal study recruited 304 patients with post-stroke SCI, and serum NfL levels were determined at baseline. These patients were followed up for 12 months for the observation of cognitive change. Cognitive performances were assessed by a Chinese version of the Telephone Interview of Cognitive Status-40 (TICS-40) scale.

Results: The patients were divided into a progression group (as determined by decreased TICS-40 scores) and a stable group (as determined by increased or unchanged TICS-40 scores). The progression group had significantly higher serum NfL levels than the stable group at baseline. Serum NfL levels were predictive for longitudinal cognitive decline during follow-up.

Conclusion: These findings imply that circulating NfL could predict the long-term cognitive change of patients with post-stroke SCI.

Keywords: acute ischemic stroke, neurofilament light, biomarker, prognosis, subjective cognitive impairment (SCI)

OPEN ACCESS

Edited by:
Yu-Hui Liu,
Third Military Medical University, China

Reviewed by:
Changming Wen,
Nanyang City Center Hospital, China
Lifei Lian,
Huazhong University of Science and Technology, China

***Correspondence:**
Jian Wang
wangjian0724@126.com

† These authors have contributed equally to this work

Received: *09 February 2021*
Accepted: *01 March 2021*
Published: *17 May 2021*

Citation:
Wang J-H, Huang J, Guo F-Q, Wang F, Yang S, Yu N-W, Zheng B and Wang J (2021) Circulating Neurofilament Light Predicts Cognitive Decline in Patients With Post-stroke Subjective Cognitive Impairment. Front. Aging Neurosci. 13:665981. doi: 10.3389/fnagi.2021.665981

INTRODUCTION

Subjective cognitive impairment (SCI) is a common complaint post-acute ischemic stroke (AIS), with a prevalence estimates ranging between 30 and 90% (Van Rijsbergen et al., 2014, 2019, 2020; Burns et al., 2019). SCI has been reported to be associated with an increased risk of progressing to objective cognitive impairment (OCI), including mild cognitive impairment (MCI) and dementia

(Lee et al., 2020; Liew, 2020). Once the patients advanced to OCI stage, the quality of life would be adversely affected. Therefore, identifying post-stroke SCI individuals that are at risk of developing OCI is important for early intervention for long-term cognitive consequences. However, currently no or few reliable prognostic biomarkers are available to monitor the cognitive change of post-stroke SCI patients.

Neurofilament light (NfL) is a neuron-specific structural protein (Zetterberg, 2016), and NfL concentrations in cerebrospinal fluid could reflect the severity of neuronal damage (Khalil et al., 2018). Circulating NfL is suggested to be a reliable biomarker for monitoring the clinical trajectory of many types of neurodegenerative disease such as Parkinson's disease (Mollenhauer et al., 2020) and Alzheimer's disease (Mattsson et al., 2017). Recent evidence also demonstrated that NfL could serve as a prognostic marker of AIS (Tiedt et al., 2018; Pedersen et al., 2019). However, the predictive effects of circulating NfL for the long-term cognitive change of post-stroke SCI patients are not clear. In this study, we investigated the association between circulating NfL and the cognitive trajectory of post-stroke SCI patients.

SUBJECTS AND METHODS

Patients

Inpatients with AIS from the Department of Neurology, Sichuan Provincial People's Hospital and Ya'an People's Hospital during May 1, 2016 and Dec 31, 2019 were screened for eligibility for this study. The patients were recruited at 1 month after the onset of AIS, when the patients crossed over the acute AIS stage and the symptoms were stable. The patients were included if they fulfil the following criteria: (1) aged 60 years or older, (2) with self-reported cognitive impairment, and this cognitive complaint should be subsequent to an AIS event, (3) no objective cognitive impairment was observed as determined by the Clinical Dementia Rating (CDR) scale, and (4) willing to participate in this study. The subjects were excluded if they have one of the following conditions: (1) have cognitive impairment before AIS onset, (2) have a family history of dementia, (3) have psychiatric disorders, such as schizophrenia, bipolar disorders, depression, etc., before AIS onset, (4) cannot complete the cognitive test due to hearing, language, or communicating disabilities, (5) other severe neurological diseases that may affect circulating NfL levels, such as Parkinson's disease, Alzheimer's disease, traumatic brain injury, etc., and (6) refused to participate in this study. Written consent for participation was obtained from the patients or their legal relatives. This study conformed with the principles of the Declaration of Helsinki and was approved by the Investigational Review Board of the Sichuan Provincial People's Hospital and Ya'an People's Hospital.

Clinical Assessment and Data Collection

At baseline (1 month after stroke onset), the demographic information [including age, sex, education level, body mass index (BMI), and smoking history], medical history (including oral anticoagulants or antiplatelet drug use), and data on comorbidities (including hypertension, diabetes mellitus, hypercholesterolemia, and atrial fibrillation) were collected from the medical records. AIS was diagnosed according to the World Health Organization Multinational Monitoring of Trends and Determinants in Cardiovascular Disease criteria and was verified by magnetic resonance imaging performed within 24 h after symptom onset. The neurological deficits of the patients were examined with the National Institutes of Health Stroke Scale (NIHSS) upon admission (Goldstein and Samsa, 1997), performed by a certified stroke neurologist. AIS subtype was determined with the TOAST criteria.

Cognitive Assessment and Patients' Follow-Up

Assessment of cognitive function was conducted at 1 month after stroke onset. Briefly, to exclude pre-stroke SCI or OCI, cognitive functioning prior to stroke onset was assessed with a Chinese version of the short-form Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE). Patients with a previous diagnosis of cognitive impairment were included based on clinical interview and an average score >4 in IQCODE. This cutoff was previously validated to achieve a high specificity but simultaneously avoiding the exclusion of a large number of patients without prior cognitive impairment (Harrison et al., 2016). As commonly used cognitive testing scales such as the Mini-mental State Examination (MMSE) and Montreal cognitive assessment (MoCa) scales require contact lime functions, a Chinese version of the Telephone Interview of Cognitive Status-40 (TICS-40) was selected to examine the current cognitive status, which was previously validated by others (Fong et al., 2009). The TICS-40 scale used in this study includes 10 variables with a maximum of 40 points. TICS-40 score $\leq$20 was defined as MCI, and a score $\leq$12 was defined as dementia according to a previous study (Fong et al., 2009). Severity of cognitive impairment was examined with the CDR scale. Patients with a CDR score $\geq$ 0.5 were excluded as described above. Patients were followed up for cognitive and dementia severity assessment at 12 months after the first interview. Although the TICS-40 scale was designed as a telephone interview scale, the patients in this study were interviewed face to face. The patients were interviewed by five experienced neurologists who were expert in cognitive examinations. As for the quality control of cognitive tests, 11 subjects with dementia, nine subjects with MCI, and six cognitively normal subjects were included in this study for the inter-rater reliability assessment, generating an interclass correlation coefficient of 0.933, reflecting a relatively high inter-rater reliability.

NfL Concentration Determination and ApoE Genotyping

Blood was sampled at the first face-to-face interview, and serum was separated within 30 min after sampling and stored at $-80°C$ until further analysis. Serum NfL was determined using the single-molecule (Simoa) array according to the manufacturer's instructions (Li et al., 2019). Monoclonal antibodies and purified bovine NfL were used as calibrators.

ApoE genotypes (rs429358 and rs7412) were determined with the restriction fragment length polymorphism method. The PCR reactions were performed with 1 μl DNA sample, 2.0 mM Mg^{2+} (TaKaRa, Japan), 1 × GC-I buffer (TaKaRa, Japan), 1 U HotStarTaq polymerase (Qiagen, Germany), 0.2 mM dNTP (Generay Biotech, China), 2 μM multiple PCR primers (Sangon, China), and ddH_2O in a total volume of 10 μl. The cycling program was the same as mentioned above. The digestion of endonuclease was performed with *Afl*III or *Hae*II (New England Biolabs, United States) for rs429358 or rs7412, respectively. Then, the products were analyzed with ABI3130XL sequencer (Applied Biosystems, United States).

Statistical Analysis

Continuous variables were tested for normality, and if they were normally distributed, independent *t* test was used, but if they were not normally distributed, Mann–Whitney *U* test was used. For categorical data, two-sample tests of proportions were used to compare proportions. A logistic regression model and two linear regression models were utilized to assess the association between circulating NfL levels and cognitive outcomes. The first logistic regression model included disease progression (as indicated by TICS-40 scores ≤20 at the follow-up interview) as the dependent variable, serum NfL levels, age, sex, ApoE ε4 carrier status, education level, BMI, smoking history, antiplatelet drug use, family history of stroke, coexisting disorders including hypertension, diabetes mellitus, hypercholesterolemia, atrial fibrillation, DWI hyperintensity volume, delirium, hemorrhagic transformation, recurrent AIS, infarction locations, post-stroke anxiety, and post-stroke depression, and the baseline TICS-40 score as independent variables. We first fitted univariate models with a single candidate variable at one time. The potential risk factors as determined by a $p < 0.2$ were included in the final multivariate regression models. The final multivariate regression models were adjusted for age and sex, although these two variables were not significant in the univariate models. The second linear regression model included the cognitive status at endpoint as the dependent variable, and the independent variables were the same as described above. The third linear regression model included the change of TICS-40 scores during a 1-year follow-up as the dependent variable. As the variable "antithrombotic drug use" had collinearity with "atrial fibrillation", it was not fitted into these regression models. The receiver operating characteristic (ROC) curve analysis was utilized to test the predictive effects of baseline serum NfL on cognitive outcomes at follow-up. Optimal sensitivity and specificity were determined *via* a non-parametric approach. The Youden index was calculated to determine the cutoff value that maximized the discriminating power of the test. Statistical analyses were conducted using SPSS statistical package, version 24 (IBM SPSS Statistics for Windows, Armonk, NY, United States), and a $p < 0.05$ was regarded as statistically significant.

RESULTS

Demographic Data

This study screened 505 post-stroke SCI patients for eligibility for participation, among which 123 patients refused to participate, 22 patients had pre-stroke cognitive impairment, and 56 patients had co-existing disorders that may affect serum NfL concentrations; thus, 304 patients were finally recruited (**Figure 1**).

We divided the patients into the progression group ($n = 49$) and the stable group ($n = 255$) according to the endpoint

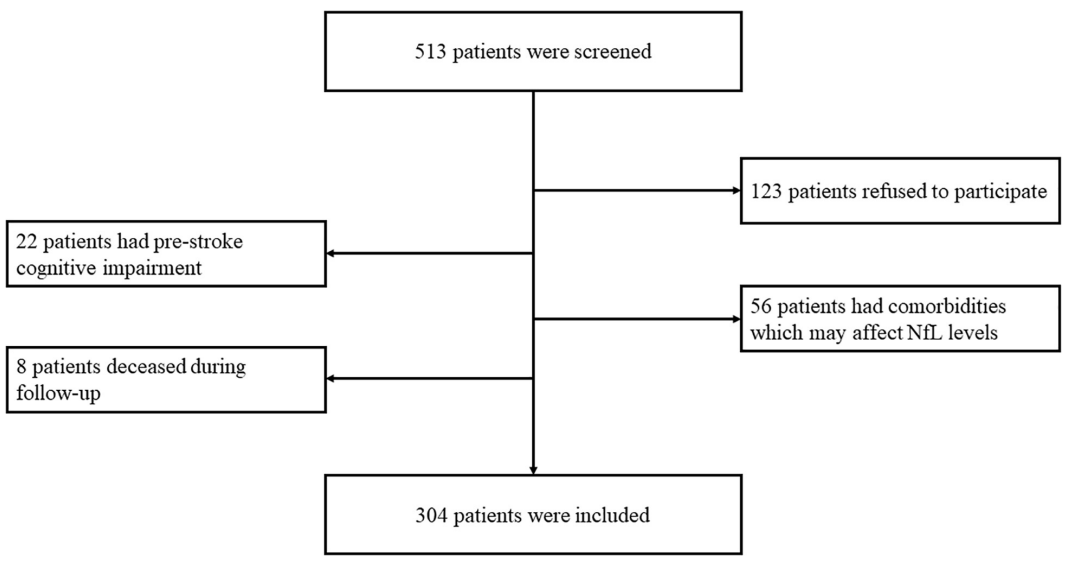

FIGURE 1 | Eligibility and follow-up of patients. In total, 513 patients were screened for eligibility for participation. A total of 123 patients refused to participate, 22 patients had pre-stroke cognitive impairment, 56 patients had comorbidities which may affect serum NfL concentrations, and eight patients were deceased during follow-up. Therefore, 304 patients finally completed the follow-up.

events. Patients with decreased TICS-40 scores during follow-up were allocated into the progression group, and the others were allocated into the stable group. The stable group and the progression group had no significant differences in terms of mean age, frequencies of ApoE ε4 carriers, median education level, median BMI, frequencies of a smoking history, antiplatelet drug use, antithrombotic drug use, a family history of stroke, and frequencies of comorbidities such as hypertension, diabetes mellitus, hypercholesterolemia, and atrial fibrillation. The stable group had significantly lower frequencies of post-stroke anxiety than the progression group. However, the frequencies of post-stroke depression were comparable between the two groups. The two groups also had no significant difference in DWI hyperintensity volume and frequencies of delirium during the acute phase of AIS. No significant difference of infarction site was found between groups. Furthermore, no significant difference was observed in frequencies of hemorrhagic transformation and recurrent AIS between groups (specified in **Table 1**).

TABLE 1 | Demographic data of the subjects.

Variables	Stable group (n = 255)	Progression group (n = 49)	P value
Age, mean (SD)	64.86 (9.37)	65.18 (8.61)	0.318[a]
Female, number (%)	107 (41.96)	23 (46.94)	0.532[b]
ApoE ε4 carriers, number (%)	34 (13.33)	5 (10.20)	0.647[b]
Education, year, median (IQR)	11 (6–15)	9 (4-14)	0.417[c]
BMI, median (IQR)	24.38 (23.07–25.61)	24.49 (23.02–25.40)	0.558[c]
Smoking history, number (%)	19 (7.45)	9 (18.37)	0.027[b]
Antiplatelet drug use, number (%)	35 (13.73)	5 (10.20)	0.647[b]
Antithrombotic drug use, number (%)	15 (5.88)	4 (8.16)	0.523[b]
Family history of stroke, number (%)	15 (5.88)	3 (6.12)	1.000[b]
Comorbidities			
Hypertension, number (%)	86 (33.73)	18 (36.73)	0.743[b]
Diabetes mellitus, number (%)	41 (16.08)	8 (16.33)	1.000[b]
Hypercholesteremia, number (%)	23 (9.02)	5 (10.20)	0.788[b]
Atrial fibrillation, number (%)	15 (5.88)	4 (8.16)	0.523[b]
Post-stoke anxiety, number (%)	17 (6.67)	8 (16.32)	0.031
Post-stroke depression, number (%)	14 (5.49)	5 (10.20)	0.174
DWI hyperintensity volume, ml (SD)	28.30 (9.24)	29.37 (8.02)	0.255[a]
Infarction region[d]			
Cerebral lobe, number (%)	44 (17.25)	8 (16.32)	0.532[b]
Cerebral white matter, number (%)	41 (16.08)	5 (10.20)	0.206[b]
Striatocapsule, number (%)	179 (70.20)	36 (73.47)	0.392[b]
Thalamus, number (%)	8 (3.14)	4 (8.16)	0.110[b]
Cerebellum, number (%)	8 (3.14)	2 (4.08)	0.499[b]
Delirium, number (%)	14 (5.49)	2 (4.08)	1.000[b]
TICS-40 at baseline, median (IQR)	26 (23–29)	29 (23–32)	0.021[c]
TICS-40 at endpoint, median (IQR)	27 (23–30)	15 (10–19)	< 0.001[c]
NIHSS at baseline, median (IQR)	12 (7–17)	12 (5–15)	0.297[c]
NIHSS at endpoint, median (IQR)	6 (2–8)	5 (1.5–7)	0.694[c]
Stroke etiology			
Atherothrombotic, number (%)	218 (85.49)	39 (79.59)	0.287[b]
Cardioembolic, number (%)	15 (5.88)	4 (8.16)	0.523[b]
Lacunar, number (%)	11 (4.31)	6 (12.24)	0.039[b]
Unknown, number (%)	11 (4.31)	0 (0.00)	0.222[b]
Complication			
Hemorrhagic transformation, number (%)	9 (0.04)	1 (0.02)	1.000[b]
Recurrent acute ischemic stroke, number (%)	3 (0.01)	2 (0.04)	0.185[b]

IQR, inter-quartile range; BMI, body mass index; TICS, telephone interview of cognitive status 40; NIHSS, National Institutes of Health Stroke Scale.
[a] Unpaired t test.
[b] Pearson χ^2 test.
[c] Mann–Whitney U test.
[d] It is notable that the infarctions may involve multiple brain regions.

Serum NfL Levels in AIS Patients With Subjective Cognitive Impairment

Serum NfL concentrations at baseline were significantly higher in the progression group (mean ± SD: 75.64 ± 17.22) in comparison with the stable group (mean ± SD: 49.47 ± 24.08, $p < 0.001$, **Figure 2A**). We next conducted a ROC analysis to investigate the diagnostic efficacy of serum NfL for a longitudinal cognitive decline in AIS patients with SCI. Serum NfL levels had a relatively high capacity to differentiate the progression and stable group, with a specificity of 0.906, a sensitivity of 0.653, and an area under the curve of 0.865 at a cutoff value of 79.31 pg/ml (**Figure 2B**).

Association Between Serum NfL Levels and Cognitive Change During Follow-Up

We first utilized a logistic regression model to address the association between serum NfL concentrations at baseline and the progression of cognitive impairment, with the longitudinal cognitive decline (defined as a decreased TICS-40 score during follow-up) as the dependent variable and with demographic variables, medical history, and comorbidities as independent variables. In the univariate analysis, smoking history, post-stroke anxiety, and serum NfL concentrations were found to be significant predictors for a longitudinal cognitive decline. However, in the multivariate analysis, post-stroke anxiety and serum NfL remained to be significant predictors for longitudinal cognitive decline (**Table 2**).

The second linear regression model included TICS-40 scores at follow-up as the dependent variable and demographic variables, medical history, and comorbidities as independent variables. TICS-40 at baseline and serum NfL were significantly associated with TICS-40 scores at endpoint (**Table 3**). The third linear regression model included the change of TICS-40 scores (score at follow-up – score at baseline) during follow-up as the dependent variable and demographic variables, medical history, and comorbidities as independent variables. Similarly, TICS-40 at baseline and serum NfL were significantly associated with the change of TICS-40 scores during follow-up (**Table 4**). This finding demonstrated that patients with lower TICS-40 and higher serum NfL concentrations at baseline were more likely to have longitudinal cognitive decline.

DISCUSSION

The present study investigated the association between baseline circulating NfL levels and the longitudinal cognitive decline in a

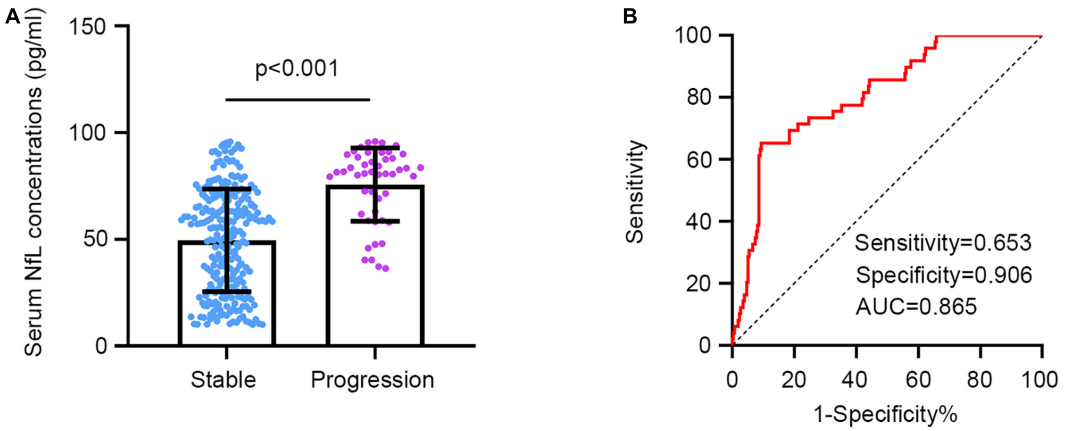

FIGURE 2 | Serum NfL concentrations in patients with and without a longitudinal cognitive decline. **(A)** Serum NfL concentrations are significantly higher in the progression group in comparison with the stable group. Unpaired *t* test. **(B)** Serum NfL can differentiate the progression group from the stable group. Receiver operating characteristic analysis. Cutoff value = 79.31 pg/ml.

TABLE 2 | A logistic regression model to evaluate the risk factors for cognitive decline as indicated by decreased TICS-40 scores in patients with subjective cognitive impairment post-stroke.

Variables	Univariable ORs (95%CI)	*P* value	Multivariable ORs (95%CI)	*P* value
Age, year	1.004 (0.971, 1.038)	0.823	1.005 (0.967, 1.044)	0.806
Sex, male	1.224 (0.662, 2.260)	0.519	1.375 (0.679, 2.781)	0.376
ApoE ε4 carrier status	1.354 (0.502, 3.654)	0.550	0.410 (0.150, 1.121)	0.082
Education, year	0.980 (0.930, 1.033)	0.448		
BMI, kg/m^2	0.928 (0.758, 1.137)	0.473		
Smoking history *vs*. no	0.358 (0.151, 0.846)	0.019		
Antiplatelet drug use *vs*. no	1.400 (0.519, 3.773)	0.506		
Family history of stroke *vs*. no	0.958 (0.267, 3.444)	0.948		
Hypertension *vs*. no	0.876 (0.464 to 1.656)	0.684		
Diabetes mellitus *vs*. no	0.982 (0.429 to 2.247)	0.965		
Hypercholesteremia *vs*. no	0.872 (0.315 to 2.418)	0.793		
Atrial fibrillation *vs*. no	0.703 (0.223 to 2.216)	0.548		
Anxiety *vs*. no	0.366 (0.148, 0.903)	0.029	0.183 (0.059, 0.572)	0.003
Depression *vs*. no	0.511 (0.175, 1.491)	0.219		
DWI hyperintensity volume, ml	1.013 (0.980 to 1.048)	0.449		
Cerebral lobe infarction *vs*. no	1.069 (0.469, 2.437)	0.874		
Cerebral white matter infarction *vs*. no	1.686 (0.631, 4.508)	0.298		
Striatocapsule infarction *vs*. no	0.851 (0.427, 1.693)	0.645		
Thalamus infarction *vs*. no	0.364 (0.105, 1.261)	0.111		
Cerebellum infarction *vs*. no	0.761 (0.157, 3.697)	0.735		
Delirium *vs*. no	1.365 (0.300 to 6.206)	0.687		
Hemorrhagic transformation *vs*. no	1.756 (0.217 to 14.184)	0.597		
Recurrent acute ischemic stroke *vs*. no	0.280 (0.046 to 1.720)	0.169		
TICS-40 at baseline	1.070 (0.983 to 1.165)	0.117		
Serum NfL concentrations, pg/ml	1.061 (1.040 to 1.082)	< 0.001	1.066 (1.044, 1.088)	< 0.001

Dependent variable: cognitive decline as indicated by decreased TICS-40 scores.

cohort of AIS patients with SCI. To our knowledge, this is the first report on the predictive effects of circulating NfL levels for the longitudinal cognitive change in AIS patients with SCI.

SCI is suggested to be accompanied by structural changes in the brain, which might be associated with an increased risk of future OCI (Hu et al., 2019). Therefore, SCI is regarded as a pre-MCI state. SCI occurs in a substantial proportion of patients with AIS, and these subjects are supposed to have increased risks of developing OCI (Sachdev et al., 2014; Van Rijsbergen et al., 2014). Furthermore, only a minor proportion of these OCI subjects belong to Alzheimer's disease type (Sachdev et al., 2014; Van Rijsbergen et al., 2014),

TABLE 3 | A linear regression model to evaluate the risk factors for cognitive impairment as indicated by TICS-40 scores at endpoint in patients with subjective cognitive impairment post-stroke.

Variables	β unadjusted	S.E.	β adjusted	*P* value
Age, year	−0.006	0.033	−0.009	0.865
Sex, male	0.181	0.684	0.015	0.792
ApoE ε4 carrier status	0.015	0.905	0.001	0.987
Education, year	0.083	0.053	0.082	0.123
BMI, kg/m^2	0.054	0.218	0.014	0.806
Smoking history *vs*. no	0.125	1.171	0.006	0.915
Antiplatelet drug use *vs*. no	−0.475	0.898	−0.027	0.597
Family history of stroke *vs*. no	0.187	1.421	0.008	0.895
Hypertension *vs*. no	0.075	0.653	0.006	0.908
Diabetes mellitus *vs*. no	−0.412	0.839	−0.026	0.624
Hypercholesteremia *vs*. no	−0.384	1.276	−0.019	0.764
Atrial fibrillation *vs*. no	1.109	1.318	0.046	0.401
Anxiety *vs*. no	−2.116	1.138	−0.099	0.064
Depression *vs*. no	0.090	1.318	0.004	0.945
DWI hyperintensity volume, ml	−0.080	0.051	−0.123	0.117
Cerebral lobe infarction *vs*. no	1.335	1.715	0.085	0.437
Cerebral white matter infarction *vs*. no	2.149	1.267	0.131	0.091
Striatocapsule infarction *vs*. no	1.618	1.447	0.125	0.264
Thalamus infarction *vs*. no	−1.150	1.584	−0.038	0.469
Cerebellum infarction *vs*. no	0.005	2.066	0.000	0.998
Delirium *vs*. no	1.489	1.328	0.056	0.263
Hemorrhagic transformation *vs*. no	0.937	2.037	0.028	0.646
Recurrent acute ischemic stroke *vs*. no	−3.299	2.390	−0.071	0.169
TICS-40 at baseline	0.742	0.084	0.470	< 0.001
Serum NfL concentrations, pg/ml	−0.066	0.012	−0.279	< 0.001

Dependent variable: cognitive impairment as indicated by TICS-40 scores at endpoint.

TABLE 4 | A linear regression model to evaluate the risk factors for cognitive decline as indicated by a change of TICS-40 scores during follow-up in patients with subjective cognitive impairment post-stroke.

Variables	β unadjusted	S.E.	β adjusted	*P* value
Age, year	−0.006	0.033	−0.010	0.865
Sex, male	0.181	0.684	0.017	0.792
ApoE ε4 carrier status	0.015	0.905	0.001	0.987
Education, year	0.083	0.053	0.091	0.123
BMI, kg/m^2	0.054	0.218	0.015	0.806
Smoking history *vs*. no	0.125	1.171	0.007	0.915
Antiplatelet drug use *vs*. no	−0.475	0.898	−0.030	0.597
Family history of stroke *vs*. no	0.187	1.421	0.008	0.895
Hypertension *vs*. no	0.075	0.653	0.007	0.908
Diabetes mellitus *vs*. no	−0.412	0.839	−0.029	0.624
Hypercholesteremia *vs*. no	−0.384	1.276	−0.021	0.764
Atrial fibrillation *vs*. no	1.109	1.318	0.051	0.401
Anxiety *vs*. no	−2.116	1.138	−0.110	0.064
Depression *vs*. no	0.090	1.318	0.004	0.945
DWI hyperintensity volume, ml	−0.080	0.051	−0.137	0.117
Cerebral lobe infarction *vs*. no	1.335	1.715	0.095	0.437
Cerebral white matter infarction *vs*. no	2.149	1.267	0.145	0.091
Striatocapsule infarction *vs*. no	1.618	1.447	0.139	0.264
Thalamus infarction *vs*. no	−1.150	1.584	−0.042	0.469
Cerebellum infarction *vs*. no	0.005	2.066	0.000	0.998
Delirium *vs*. no	1.489	1.328	0.063	0.263
Hemorrhagic transformation *vs*. no	0.937	2.037	0.032	0.646
Recurrent acute ischemic stroke *vs*. no	−3.299	2.390	−0.079	0.169
TICS-40 at baseline	−0.258	0.084	−0.181	0.002
Serum NfL concentrations, pg/ml	−0.066	0.012	−0.310	<0.001

Dependent variable: cognitive decline as change of TICS-40 scores during follow-up (TICS-40 at baseline – TICS-40 at endpoint).

indicating that vascular mechanisms might also contribute to the conversion from SCI to OCI. Therefore, identifying subjects at a high risk of developing OCI is of importance for early intervention for cognitive decline. However, currently no reliable biomarker, especially blood-based biomarker, is available to determine the long-term risk of developing OCI in patients with post-stroke SCI. We found in the present study that circulating NfL (with a cutoff value of 79.31 pg/ml) can differentiate patients with a longitudinal cognitive decline from those with stable, or improved cognitive functions during follow-up.

In this study, patients with self-reported cognitive impairment post-stroke were screened, and the IQCODE scale was used to exclude pre-stroke cognitive impairment. Furthermore, patients with a CDR score more than 0 were also excluded. These will ensure that the patients were SCI rather than OCI. Patients were recruited and NfL determination was conducted at 1 month after stroke onset to exclude NfL secretion after acute neuronal damage during the acute phase of AIS, as demonstrated by previous studies. Although the patients were interviewed face to face, we used the TICS-40 which is used as a telephone interview scale, instead of MMSE and MoCa. This is because these scales require integrated motor functions, especially functions of the right upper arm. Furthermore, we only included patients aged over 60 years in order to investigate the cognitive change during the 1-year follow-up period, which is actually not long enough for assessment of cognitive change. Indeed among the 304 included subjects, 49 subjects (16.11%) developed OCI during follow-up, which is a relatively a lower prevalence in comparison with reports from previous studies (Van Rijsbergen et al., 2014).

We found in this study that circulating NfL levels were predictive for a longitudinal cognitive decline through three regression models. The first model fitted cognitive decline during follow-up as the dependent variable and found that increased serum NfL levels were associated with a higher risk of OCI. Furthermore, the linear regression models found that lower TICS-40 scores at baseline and higher serum NfL levels were associated with a worse cognitive performance at endpoint and a larger decrease of TICS-40 scores during follow-up. This study failed to figure out the association of other factors, including age and vascular risk factors such as hypertension or diabetes mellitus, with the longitudinal cognitive decline post-stroke, which is not consistent with previous

studies (Jessen et al., 2020; Lee et al., 2020; Liew, 2020). This phenomenon might be attributed to the fact that the subjects in this study had relatively old age and that the follow-up period is not long enough for the observation of the contribution of these factors to cognitive decline. NfL is released from damaged neurons after acute or chronic neuronal injury conditions. It is not clear yet why circulating NfL were still in an increased level, as compared to that in healthy subjects, in AIS recovering stage, during which damaged neurons may have been wiped out. Interestingly, it is suggested that neuronal damage due to chronic inflammation may persist in a very long period after AIS onset (Martin et al., 2018). This could, to some extent, explain the increased NfL levels during the recovery stage of AIS and that increased NfL in this stage could predict long-term cognitive decline.

This study has some limitations. First, no control cohort with SCI but without AIS was included. Therefore, we could not compare the circulating NfL levels between post-stroke SCI subjects and SCI patients without AIS. Second, we could not deny that, for an observational study, the sample size is relatively small; thus, there are some inconsistencies with previous studies, as discussed above. Third, the patients were only followed up for once, thus the dynamic change of NfL during follow-up was not investigated. However, this study identified a potential prognostic biomarker for longitudinal cognitive decline in patients with post-stroke SCI. Patients with increased circulating NfL levels after stroke should be intensively monitored for delayed cognitive decline.

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 author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Investigational Review Board of the Sichuan Provincial People's Hospital and Ya'an People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JW and J-HW designed the study and drafted the manuscript. F-QG and FW collected the samples and patients' information. SY and N-WY participated in the determination of NfL. BZ conducted the statistical analysis. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Science and Technology Ministry of Sichuan Province (2019ZYZF0063 and 2020yj0497).

REFERENCES

Burns, S. P., Mueller, M., Magwood, G., White, B. M., Lackland, D., and Ellis, C. (2019). Racial and ethnic differences in post-stroke subjective cognitive decline exist. *Disabil. Health J.* 12, 87–92. doi: 10.1016/j.dhjo.2018.08.005

Fong, T. G., Fearing, M. A., Jones, R. N., Shi, P., Marcantonio, E. R., Rudolph, J. L., et al. (2009). Telephone interview for cognitive status: Creating a crosswalk with the Mini-Mental State Examination. *Alzheimers Dement* 5, 492–497. doi: 10.1016/j.jalz.2009.02.007

Goldstein, L. B., and Samsa, G. P. (1997). Reliability of the National Institutes of Health Stroke Scale. Extension to non-neurologists in the context of a clinical trial. *Stroke* 28, 307–310. doi: 10.1161/01.str.28.2.307

Harrison, J. K., Stott, D. J., Mcshane, R., Noel-Storr, A. H., Swann-Price, R. S., and Quinn, T. J. (2016). Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) for the early diagnosis of dementia across a variety of healthcare settings. *Cochrane Database Syst. Rev.* 11:CD011333.

Hu, X., Teunissen, C. E., Spottke, A., Heneka, M. T., Duzel, E., Peters, O., et al. (2019). Smaller medial temporal lobe volumes in individuals with subjective cognitive decline and biomarker evidence of Alzheimer's disease-Data from three memory clinic studies. *Alzheimers Dement.* 15, 185–193. doi: 10.1016/j.jalz.2018.09.002

Jessen, F., Amariglio, R. E., Buckley, R. F., Van Der Flier, W. M., Han, Y., Molinuevo, J. L., et al. (2020). The characterisation of subjective cognitive decline. *Lancet Neurol.* 19, 271–278. doi: 10.1016/S1474-4422(19)30368-0

Khalil, M., Teunissen, C. E., Otto, M., Piehl, F., Sormani, M. P., Gattringer, T., et al. (2018). Neurofilaments as biomarkers in neurological disorders. *Nat. Rev. Neurol.* 14, 577–589.

Lee, Y. C., Kang, J. M., Lee, H., Kim, K., Kim, S., Yu, T. Y., et al. (2020). Subjective cognitive decline and subsequent dementia: a nationwide cohort study of 579,710 people aged 66 years in South Korea. *Alzheimers Res. Ther.* 12:52.

Li, Q. F., Dong, Y., Yang, L., Xie, J. J., Ma, Y., Du, Y. C., et al. (2019). Neurofilament light chain is a promising serum biomarker in spinocerebellar ataxia type 3. *Mol. Neurodegener.* 14:39.

Liew, T. M. (2020). Trajectories of subjective cognitive decline, and the risk of mild cognitive impairment and dementia. *Alzheimers Res. Ther.* 12:135.

Martin, A., Domercq, M., and Matute, C. (2018). Inflammation in stroke: the role of cholinergic, purinergic and glutamatergic signaling. *Ther. Adv. Neurol. Disord.* 11:1756286418774267.

Mattsson, N., Andreasson, U., Zetterberg, H., Blennow, K., Alzheimer's Disease, and Neuroimaging, I. (2017). Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurol.* 74, 557–566. doi: 10.1001/jamaneurol.2016.6117

Mollenhauer, B., Dakna, M., Kruse, N., Galasko, D., Foroud, T., Zetterberg, H., et al. (2020). Validation of Serum Neurofilament Light Chain as a Biomarker of Parkinson's Disease Progression. *Mov. Disord.* 35, 1999–2008. doi: 10.1002/mds.28206

Pedersen, A., Stanne, T. M., Nilsson, S., Klasson, S., Rosengren, L., Holmegaard, L., et al. (2019). Circulating neurofilament light in ischemic stroke: temporal profile and outcome prediction. *J. Neurol.* 266, 2796–2806. doi: 10.1007/s00415-019-09477-9

Sachdev, P. S., Lipnicki, D. M., Crawford, J. D., Wen, W., and Brodaty, H. (2014). Progression of cognitive impairment in stroke/TIA patients over 3 years. *J. Neurol. Neurosurg. Psychiatry* 85, 1324–1330. doi: 10.1136/jnnp-2013-306776

Tiedt, S., Duering, M., Barro, C., Kaya, A. G., Boeck, J., Bode, F. J., et al. (2018). Serum neurofilament light: A biomarker of neuroaxonal injury after ischemic stroke. *Neurology* 91, 1338–1347e. doi: 10.1212/WNL.0000000000006282

Van Rijsbergen, M. W., Mark, R. E., De Kort, P. L., and Sitskoorn, M. M. (2014). Subjective cognitive complaints after stroke: a systematic review. *J. Stroke Cerebrovasc. Dis.* 23, 408–420. doi: 10.1016/j.jstrokecerebrovasdis.2013.05.003

Van Rijsbergen, M. W. A., Mark, R. E., Kop, W. J., De Kort, P. L. M., and Sitskoorn, M. M. (2019). Psychological factors and subjective cognitive complaints after stroke: Beyond depression and anxiety. *Neuropsychol. Rehabil.* 29, 1671–1684. doi: 10.1080/09602011.2018.1441720

Van Rijsbergen, M. W. A., Mark, R. E., Kop, W. J., De Kort, P. L. M., and Sitskoorn, M. M. (2020). Course and Predictors of Subjective Cognitive Complaints During the First 12 Months after Stroke. *J. Stroke Cerebrovasc. Dis.* 29:104588. doi: 10.1016/j.jstrokecerebrovasdis.2019.104588

Zetterberg, H. (2016). Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration. *Neuron* 91, 1–3. doi: 10.1016/j.neuron.2016.06.030

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.

Copyright © 2021 Wang, Huang, Guo, Wang, Yang, Yu, Zheng and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 29 June 2021
doi: 10.3389/fnagi.2021.676744

Temporal Ordering of Inflammatory Analytes sTNFR2 and sTREM2 in Relation to Alzheimer's Disease Biomarkers and Clinical Outcomes

Jagan A. Pillai[1,2,3]*, *Maria Khrestian*[4,5], *James Bena*[6], *James B. Leverenz*[1,2,3] and *Lynn M. Bekris*[4,5] *on behalf of the Alzheimer's Disease Neuroimaging Initiative*†

[1] Department of Neurology, Cleveland Clinic, Cleveland, OH, United States, [2] Lou Ruvo Center for Brain Health, Cleveland Clinic, Cleveland, OH, United States, [3] Neurological Institute, Cleveland Clinic, Cleveland, OH, United States, [4] Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH, United States, [5] Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States, [6] Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH, United States

OPEN ACCESS

Edited by:
Jun Tan,
Guizhou Medical University, China

Reviewed by:
Mitsuru Shinohara,
National Center for Geriatrics and Gerontology (NCGG), Japan
Aiko Ishiki,
Tohoku University, Japan

***Correspondence:**
Jagan A. Pillai
pillaij@ccf.org

† Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgment_List.pdf

Received: 05 March 2021
Accepted: 28 May 2021
Published: 29 June 2021

Citation:
Pillai JA, Khrestian M, Bena J, Leverenz JB and Bekris LM (2021) Temporal Ordering of Inflammatory Analytes sTNFR2 and sTREM2 in Relation to Alzheimer's Disease Biomarkers and Clinical Outcomes. Front. Aging Neurosci. 13:676744. doi: 10.3389/fnagi.2021.676744

Inflammatory changes are among the key markers of Alzheimer's disease (AD) related pathological changes. Pro-inflammatory analytes have been related to cognitive decline while others have been related to attenuating neuronal death. Among them, changes in cerebrospinal fluid (CSF) levels of soluble triggering receptor expressed on myeloid cells 2 (sTREM2) and soluble tumor necrosis factor receptor 2 (sTNFR2) have been described as impacting favorable clinical outcomes in AD. We therefore evaluate the effect of CSF sTREM2 and sTNFR2 when taken together on AD biomarkers and longitudinal clinical decline to understand their relative role on impacting AD clinical biomarkers and subsequent clinical outcomes. This longitudinal observational cohort study included 168 amyloid-positive (A+) and p-tau-positive (T+) participants with mild cognitive impairment (MCI) or AD dementia from the Alzheimer's Disease Neuroimaging Initiative (ADNI) with 109 of them having concomitant CSF sTREM2 and sTNFR2 data and 48 A+ T+ participants with MCI from a tertiary memory clinic cohort. An exploratory analysis was performed using data from 86 cognitively normal (CN) participants from ADNI with 72 of them having concomitant CSF AD biomarkers and CSF sTREM2 and sTNFR2 data. General linear models were used to evaluate the effect of sTREM2 and sTNFR2 levels on baseline CSF Aβ42, t-tau, and p-tau, and a linear mixed-effects model was used to assess longitudinal cognitive change after controlling for well-known covariates. Among ADNI A+ T+ MCI and AD dementia participants, CSF sTNFR2 had a stronger association, than CSF sTREM2, with CSF t-tau and p-tau. This was replicated among A+ T+ MCI participants from the memory clinic cohort. On the contrary, among A+ T+ CN participants, CSF sTREM2 explained significant variance in CSF t-tau and p-tau, while CSF sTNFR2 did not. When the effects of CSF sTNFR2 and t-tau on longitudinal cognitive change were taken into account, higher CSF sTREM2 predicted slower cognitive decline in A+ T+ AD dementia participants and faster decline in A+ T+ CN participants. Our results show that given the dynamic changes in sTREM2 and sTNFR2, the clinical impact of these distinct inflammation related biomarkers in tracking AD temporal progression across disease stages are likely to differ.

Keywords: Alzheimer's disease, soluble TREM2, soluble TNFR2, inflammation, MCI, dementia, preclinical AD, ATN classification

BACKGROUND

Genome-wide association studies in Alzheimer's disease (AD) have noted multiple susceptibility loci for late-onset AD related to the innate immune system (Lambert et al., 2013; Van Cauwenberghe et al., 2016). Among these susceptibility loci, the presence of the gene encoding the triggering receptor expressed on myeloid cells 2 (*TREM2*) has been reported to increase the risk of AD development by 2–3-fold (Guerreiro et al., 2013; Jonsson et al., 2013). *TREM2* promotes anti-inflammatory cytokine expression, reduces pro-inflammatory cytokine release, and is involved in osteoclast development and the activation of brain microglia and monocyte-derived dendritic cells (Carmona et al., 2018). *TREM2* is also thought to enhance the rate of phagocytosis and to modulate inflammatory signaling (Gratuze et al., 2018).

These results, amongst animal and *in vitro* models, have prompted researchers to evaluate how *TREM2* may mediate clinical outcomes of interest in AD. However, the results of these studies have been more nuanced with regard to *TREM's* effects in clinical AD. For instance, network analysis of post-mortem AD brain gene expression with the highest connectivity to *TREM2* revealed both anti- and pro-inflammatory gene clusters (Forabosco et al., 2013). Clinical reports regarding cerebrospinal fluid (CSF) levels of sTREM2, a soluble TREM2 protein fragment produced by the cleavage of TREM2, have demonstrated varying levels of this protein in the different stages of AD (Wunderlich et al., 2013). Although most studies have shown that CSF sTREM2 is increased in the presence of AD biomarkers, the results are somewhat inconsistent regarding sTREM2 levels in amyloid-positive (A+) and tau-positive (T+) cognitively normal (CN) individuals (Ewers et al., 2019; Suárez-Calvet et al., 2019), patients with mild cognitive impairment (MCI) (Gispert et al., 2016b; Henjum et al., 2016; Suárez-Calvet et al., 2016b, 2019; Ewers et al., 2019; Knapskog et al., 2020), and those with AD dementia (Gispert et al., 2016b; Piccio et al., 2016; Suárez-Calvet et al., 2016b, 2019). Some studies have demonstrated no differences in sTREM2 levels across the AD spectrum (Gispert et al., 2016a; Henjum et al., 2016; Knapskog et al., 2020), whereas other research has demonstrated decreased levels of sTREM2 in patients with AD dementia, perhaps partially reflecting the variability in clinical symptoms even within the same stage of AD (Kleinberger et al., 2014; Bekris et al., 2018). However, other studies have found that CSF sTREM2 has a dynamic response in the tracking of AD progression (Suárez-Calvet et al., 2016c, 2019; Ma et al., 2020), and a study in patients with MCI or AD dementia who had A+ and T+ biomarkers found that higher concentrations of sTREM2 in CSF were associated with reduced memory decline, lower CSF p-tau levels, and hippocampal shrinkage (Ewers et al., 2019).

A variety of other inflammatory analytes in the CSF are altered in both pre-symptomatic (Janelidze et al., 2018) and subsequent clinical stages of AD (Pillai et al., 2019b). While pro-inflammatory analyte levels in the CSF have been noted to predict AD disease progression (Pillai et al., 2020), other inflammatory markers have been reported to attenuate neuronal death and affect clinical outcomes favorably in AD including sTREM2 (Ewers et al., 2019; Chen et al., 2020; Franzmeier et al., 2020). Recently, our group found that the inflammatory gene, *TNFRSF1B* and related soluble tumor necrosis factor receptor 2 (sTNFR2) CSF levels also relate to favorable clinical outcomes in AD (Pillai et al., 2021). TNFR2 is thought to promote downstream antiapoptotic responses and to play a protective role against neurodegeneration (Fischer et al., 2011; Dong et al., 2016). The effect of TREM2 levels on AD biomarkers and clinical outcomes has garnered significant interest as detailed earlier but there are limited studies of the effects of TNFR2 on AD biomarkers and on clinical outcomes in different stages of AD.

The potential interaction between *TNFRSF1B* and *TREM2* has also been the focus of recent research in animal models. In a study of *TNFRSF1B* conditional knockout mice, lack of TNFR2 activation was found to impair constitutive expression and transcriptional regulation of *TREM2* by soluble TNF (Gao et al., 2017). These findings suggest a complex interplay across the inflammation-related pathways underlying neurodegeneration, making clinical studies of this subject challenging but critical. Elucidating the relationship between sTREM2 and sTNFR2 and the effect of these analytes on AD biomarkers and patient outcomes could help us to make more effective use of these analytes as clinical biomarkers, as well as develop therapeutic strategies that target these inflammation-related pathways.

We therefore sought to evaluate CSF sTREM2 and CSF sTNFR2 levels across the continuum of AD, and to determine the relationship between these analyte levels and AD CSF biomarkers and longitudinal cognitive outcomes. We used data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) research cohort to test whether CSF sTREM2 levels correlate with sTNFR2 among MCI and dementia subjects classified as AD, aggregated Aβ (A+) and aggregated tau (T+), according to the National Institute on Aging and Alzheimer's Association (NIA-AA) AT(N) framework (Jack et al., 2018) and whether CSF sTREM2 levels independent of sTNFR2 levels are associated with the AD biomarkers CSF Aβ42, t-tau, and p-tau. We evaluated the reliability of these results in ADNI by assessing the same variables among A+ T+ MCI participants being treated at a memory clinic. We also sought to evaluate whether CSF sTREM2 levels, independent of sTNFR2, are associated with favorable clinical outcomes in AD. Based on previous reports (Ewers et al., 2019; Franzmeier et al., 2020), we hypothesized that higher concentrations of sTREM2 in CSF would be associated with slower rates of cognitive decline in both the A+ T+ MCI and A+ T+ dementia stages of AD. Finally, we explored the consistency of these results in a smaller cohort of cognitively normal (CN) individuals from the ADNI cohort who met A– T–, A– T+, A+ T–, or A+ T+ criteria.

MATERIALS AND METHODS

Study Cohort: ADNI

The ADNI is a longitudinal multicenter study designed to develop clinical, imaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. ADNI was launched by the National Institute of Aging with additional support from private pharmaceutical companies and non-profit organizations.

The eligibility criteria for the first phase of the ADNI study are described in the ADNI1 protocol (http://adni.loni.usc.edu/methods/documents/). Briefly, eligible participants were aged 55–90 years, had an informant able to provide an independent evaluation of functioning, and spoke either English or Spanish. Participants had completed at least 6 years of education (or had a work history sufficient to exclude intellectual disability). For clinical staging, the categories of CN, MCI, and AD dementia were used (Jack et al., 2011, 2018).

Details regarding the Elecsys method used to measure AD biomarkers in the ADNI cohort are described elsewhere (Shaw et al., 2019). Following the ATN criteria, amyloid deposition (A+) was defined as abnormal values of CSF Aβ1–42, and tau pathology (T+) was defined as abnormal values of CSF p-tau181 (Jack et al., 2017, 2018). Based on previously published cut points in the ADNI sample (Ewers et al., 2019), the criterion for A+ was defined as Aβ1–42 < 976.6 pg/mL; the criterion for T+ was defined as p-tau181 > 21.8 pg/mL. A total of 109 participants in ADNI (MCI, $n = 67$; AD dementia, $n = 42$) met the A+ T+ criteria at baseline and had data on CSF sTREM2 and CSF sTNFR2 (**Table 1**).

CSF sTNFR2 Levels and CSF sTREM2 Levels in ADNI

In ADNI, levels of sTNFR2 are measured in CSF samples using the RBM DiscoveryMAP® v.1.0 panel, which uses a Luminex platform (Myriad Genetics; Salt Lake City, UT). The CSF multiplex data used in this analysis were cleaned and quality controlled based on methodology described in the statistical analysis of the Biomarkers Consortium data primer[1].

The CSF sTREM2 assay used in ADNI is based on the Meso Scale Diagnostics platform and has been described previously (Suárez-Calvet et al., 2016b; Ewers et al., 2019). The CSF sTREM2 values used in this study were corrected based on the values of the four internal standards that were loaded on all plates (variable "MSD_sTREM2CORRECTED" in the ADNI database). Further details regarding the CSF sTREM2 measurements in the ADNI samples, as well as the original data, are available at https://ida.loni.usc.edu.

Cognitive and Functional Measures

The Mini-Mental State Exam (MMSE) (Folstein et al., 1975) and Clinical Dementia Rating–Sum of Boxes (CDR-SB) (Morris, 1993) were used to characterize the degree of baseline cognitive and functional deficits. CDR-SB scores were also evaluated longitudinally to assess cognitive change from baseline.

Study Cohort: Replication Memory Clinic

A cross-sectional replication cohort was created, including 48 participants in the MCI stage of AD (MCI-AD) who were recruited from a specialized memory clinic at Cleveland Clinic (Lou Ruvo Center for Brain Health, Cleveland site). Recruitment details have been described previously (Pillai et al., 2019b, 2020). In brief, consent was obtained from participants to include their CSF, plasma, and DNA samples in the Lou Ruvo Center for Brain Health Aging and Neurodegeneration Biobank (CBH-Biobank), following approval by the local Institutional Review Board.

In these participants, the diagnosis of MCI-AD was confirmed by the presence of CSF Aβ42 and p-tau levels consistent with a diagnosis of AD as the primary etiology; the diagnosis was also confirmed by two neurologists (JP, JL) using published criteria (A+ T+) (Albert et al., 2011). A commercially available test (ADmark® Alzheimer's Evaluation, Athena Diagnostics; Marlborough, MA) was used to measure CSF levels of Aβ42, t-tau, and p-tau. The ADmark® Alzheimer's evaluation uses sandwich Enzyme Linked Immunosorbant Assay (ELISA) kits [Innotest β-amyloid[1–42], Innotest hTAU-Ag, Innotest Phospho-Tau[181P], Innogenetics, Ghent, Belgium]. All participants met the cutoff of Aβ42 ≤ 530 pg/mL, which is consistent with A+ status on the Amyvid TM (Florbetapir F 18 Injection; Eli Lilly and Company; Indianapolis, IN) positron emission tomography used at our center. Participants also met the diagnostic threshold for p-tau per the ADmark test, ≥60 pg/mL consistent with a T+ status. *APOE* status was determined through assessment of blood samples (10 ng per patient) dispensed into 96-well plates for TaqMan (Thermo Fisher Scientific, Waltham, MA) allelic discrimination detection of single nucleotide polymorphisms that discriminate the *APOE* alleles (*rs429358, rs7412*). Polymerase chain reaction (PCR) was performed using a 9700 Gene Amp PCR system (Applied Biosystems, Waltham, MA) and an end-point read in a 7500 Real-Time PCR system (Applied Biosystems).

CSF sTNFR2 and sTREM2 Levels in the Replication Memory Clinic

Details regarding CSF sampling for sTNFR2 have been published previously (Pillai et al., 2021). In brief, CSF was collected and analyzed by an independent laboratory via the validated RBM Multi-Analyte Profile (MAP) platform from Myriad Genetics. The RBM HumanMAP® v.2.0 used in the replication cohort is a subset of the RBM DiscoveryMAP® v.1.0 used in ADNI with the same quality control and thresholding process. The least detectable dose of sTNFR2 was 0.0017 ng/L. Samples were frozen within 15 min of collection, were processed at −70°C (in dry ice), and were continuously maintained at −80°C (in a maximum non-frost-free–type refrigerator). The samples were shipped frozen in a Styrofoam container with sufficient dry ice to maintain the temperature below −70°C for at least 48 h. Samples therefore underwent a single freeze-thaw cycle before analysis.

The CSF sTREM2 assay used in the Bekris lab has been described previously (Bekris et al., 2018). In brief, CSF sTREM2 levels were measured using a Luminex 200 3.1 xPONENT System (EMD Millipore; Chicago, IL) and a custom-designed detection method to capture sTREM2. With this method, a capture antibody bound to MagPlex beads binds sTREM2 (R&D #MAB1828 human TREM2 antibody monoclonal mouse IgG2B Clone #263602; Immunogen His19-Ser174), and a biotinylated antibody with a SAPE conjugate is then used for detection (R&D: #BAF1828; human TREM2 biotinylated antibody; antigen affinity-purified polyclonal goat IgG; Immunogen His19-Ser174).

[1]Biomarkers Consortium Project. Use of Targeted Multiplex Proteomic Strategies to Identify Novel Cerebrospinal Fluid (CSF) Biomarkers in Alzheimer's Disease (AD). Data Primer. Version 28 Dec 2011.

TABLE 1 | Demographics of participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) and replication memory clinic cohorts (participants meeting A+ T+ criteria and concomitant sTREM2 and sTNFR2 data).

Demographic variable	ADNI MCI cohort ($n = 67$)	ADNI AD dementia cohort ($n = 42$)	Replication MCI memory clinic cohort ($n = 48$)	*P*-value[a]
	Mean (SD)	Mean (SD)	Mean (SD)	
Age, y	74.06 (6.99)[1]	74.16 (7.87)[2]	68.10 (7.3)[1,2]	<0.0001
Sex (% female)	41.8%	47.6%	41.7%	0.49
APOEε4 (%)	71.6%	80.9%	77.1%	<0.0001
Patient education, y	15.75 (3.01)	15.17 (3.02)	15.37 (2.87)	0.58
Baseline MMSE score	26.75 (1.76)[3,4]	23.45 (2.00)[3,5]	24.8 (3.1)[4,5]	<0.0001
Baseline CDR-SB score	1.59 (0.9)[6,7]	4.22 (1.51)[6,8]	2.17 (1.2)[7,8]	<0.0001
Log_2 CSF Aβ42[b]	9.21 (0.39)	9.06 (0.45)	8.12 (0.55)	
Log_2 CSF t-tau[b]	8.48 (0.38)	8.50 (0.40)	8.93 (0.92)	
Log_2 CSF p-tau[b]	5.17 (0.43)	5.19 (0.45)	6.27 (0.67)	
Log_2 CSF sTREM2	12.0 (0.69)[9]	12.0 (0.66)[10]	10.26 (0.75)[c,9,10]	<0.0001
Log_2 CSF sTNFR2	−0.12 (0.15)[11]	−0.11 (0.15)[12]	1.15 (0.45)[11,12]	<0.0001
Years of follow up	5.0 (2.5)	3.0 (0.58)	—[d]	<0.0001

AD, Alzheimer's disease; CDR-SB, Clinical Dementia Rating–Sum of Boxes; MCI, mild cognitive impairment; MMSE, Mini-Mental State Exam; SD, standard deviation; sTNFR2, soluble tumor necrosis factor receptor 2; sTREM2, soluble triggering receptor expressed on myeloid cells 2. [a]*P-values from ANOVA for continuous variables and from* χ^2 *tests for categorical variables.* [b]*CSF Aβ, t-tau, and p-tau levels measured in ADNI by Elecsys method and in replication cohort by INNOTEST ELISA.* [c]$n = 42$. [d]*only baseline data analyzed. Tukey HSD Post–hoc test:* [1]*ADNI AD MCI vs. Replication AD MCI: Diff = −5.9600, p = 0.0001.* [2]*ADNI AD dementia vs. Replication AD MCI: Diff = −6.0600, p = 0.0004.* [3]*ADNI AD MCI vs. ADNI AD dementia: Diff = −3.3000, p < 0.0001.* [4]*ADNI AD MCI vs. Replication AD MCI: Diff = −1.9500, p ≤ 0.0001.* [5]*ADNI AD dementia vs. Replication AD MCI: Diff = 1.3500, p = 0.017.* [6]*ADNI AD MCI vs. ADNI AD dementia: Diff = 2.6300, p < 0.0001.* [7]*ADNI AD MCI vs. Replication AD MCI: Diff = 0.5800, p = 0.027.* [8]*ADNI AD dementia vs. Replication AD MCI: Diff = 2.0500, p = 0.00001.* [9]*ADNI AD MCI vs. Replication AD MCI: Diff = −1.7400, p < 0.0001.* [10]*ADNI AD dementia vs. Replication AD MCI: Diff = −1.7400, p < 0.0001.* [11]*ADNI AD MCI vs. Replication AD MCI: Diff = 1.2700, p < 0.0001.* [12]*ADNI AD dementia vs. Replication AD MCI: Diff = 1.2600, p < 0.0001.*

Cognitive and Functional Measures

As in the ADNI cohort, the MMSE and CDR-SB were used to characterize the degree of baseline cognitive and functional deficits in the replication memory clinic cohort. CDR-SB scores were also evaluated longitudinally to assess cognitive change from baseline.

Exploratory Analysis in ADNI CN Participants

CN participants as defined in the ADNIMERGE dataset (downloaded on May 6, 2020) with concomitant data on CSF sTREM2 and CSF sTNFR were included in the exploratory analysis ($n = 72$). Again following the ATN criteria, amyloid deposition (A+) was defined as an abnormal value of CSF Aβ1–42, and tau pathology (T+) was defined as an abnormal value of CSF p-tau181 (Shaw et al., 2019). The cutoff points were as described earlier for the MCI and AD dementia groups (Jack et al., 2017). At baseline, 35 CN participants with sTREM2 and sTNFR2 data were A– T–, 14 were A+ T+, 11 were A+ T–, and 12 were A– T+ (**Supplementary Table 1**).

Additionally, a sensitivity analysis to evaluate the robustness of results was repeated among participants that had data on CSF sTREM2 (including those lacking concomitant CSF sTNFR2 data) and AD biomarkers (A+T+ MCI, $n = 111$, A = T+ AD dementia, $n = 57$) (**Supplementary Table 11**) and 86 cognitively normal participants with CSF sTREM2 values (including those lacking concomitant sTNFR2 data) (**Supplementary Table 12**).

Statistical Analysis

A log (base 2) transformation allowed Pearson correlations to be fit for exploratory univariate analyses for all analytes, and the levels described are therefore dimensionless. Normality of biomarkers was evaluated using Shapiro-Wilk tests and graphical methods. Pearson estimates of correlation and *P*-values were calculated for sTREM2, sTNFR2, and AD biomarkers. All tests were two-tailed, with the significance level set at 0.05. Sensitivity analyses were completed to assess the robustness of the effects, and collinearity between sTNFR2 and sTREM2 was assessed for the dependent variables t-tau, p-tau, and Aβ42. IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY), and R Core Team RStudio (version 1.2.5042) were used for all analyses.

Model 1

To evaluate the effect of sTREM2 and sTNFR2 individually, multivariate general linear models were used to assess the effect of baseline CSF sTREM2 or sTNFR2 on CSF t-tau, p-tau, and Aβ42 levels (dependent variable) after controlling for well-known covariates of age, sex, education years, and *APOEε4* status. Effect size was calculated using partial η^2. F-test for lack-of-fit and residual plots were used to assess the model linearity and fit**.**

Model 2

To evaluate the effect of sTREM2 and sTNFR2 together, multivariate general linear models were used to assess the effect of baseline CSF sTREM2 plus sTNFR2 on CSF t-tau, p-tau, and Aβ42 levels (dependent variable) after controlling for well-known covariates of age, sex, education years, and *APOEε4*

status. The interaction between CSF sTREM2 and sTNFR2 on the AD biomarkers was also assessed for significance. Effect size was calculated using partial η^2. F-test for lack-of-fit and residual plots were used to assess the model linearity and fit.

Model 3

This analysis was performed only for the ADNI cohort, as the replication memory clinic cohort had a maximum follow-up period of only 15 months. To assess whether the effect of baseline sTNFR2 and sTREM2 on future cognitive decline was dependent or independent of the effect on AD biomarker CSF t-tau, linear mixed-effects regression models were applied to the MCI and AD dementia A+ T+ groups. CDR-SB at each visit was the dependent variable. The fixed main effects for sTREM2, sTNFR2, t-tau, and visit number, as well as the interactions between each biomarker and visit number, were evaluated. A random intercept for each patient was included in all models. The visit number was defined as the follow-up duration of the neuropsychological testing in years (with baseline set at zero). Covariates of age, sex, years of education, *APOEε4* status, and CSF Aβ42 were controlled for in each analysis. In addition, 95% confidence intervals (CIs) and Benjamini-Hochberg adjusted false discovery rate (FDR) *P*-values were calculated. Higher order interactions between sTREM2 and sTNFR2 together on longitudinal clinical outcomes were not assessed given challenges in interpretation. A sensitivity analysis was next conducted with and without the covariates and using p-tau instead of t-tau (given the concern for collinearly with both CSF t-tau and p-tau in the model) to evaluate the reliability of these results. Additionally Model 3 (without sTNFR2) results were corroborated in a sensitivity analysis among a larger number of ADNI participants with sTREM2 data alone (demographics in **Supplementary Tables 11**, **12**).

Data Availability

The ADNI data analyzed are available in the ADNI repository, http://adni.loni.usc.edu/.

RESULTS

Demographic details of participants from the ADNI cohort (A+ T+) and the memory clinic replication cohort are presented in **Table 1**. The above two cohorts differed in age, and *APOEε4* status, in addition to biomarker variables and baseline cognitive scores. Between A+T+ CN, MCI and dementia groups in ADNI, only CSF Aβ levels differed between them ($F = 3.91$, $p = 0.023$) but not CSF t-tau, p-tau, sTREM2 and sTNFR2 analytes (**Supplementary Figure 1**).

ADNI and Replication Memory Clinic Cohorts

Among the MCI and AD dementia A+ T+ participants in the ADNI cohort, univariate analysis demonstrated that sTNFR2 and sTREM2 were significantly correlated with each other and with CSF t-tau and p-tau but not with Aβ42. This significant positive correlation between sTNFR2 and sTREM2 was replicated in the memory clinic participants with MCI, but only the sTNFR2 positive correlation with CSF t-tau and p-tau met the significance threshold (**Table 2**).

Models 1 and 2

For ADNI MCI A+ T+ participants, both sTNFR2 and sTREM2 significantly predicted CSF p-tau and t-tau levels but not Aβ42 level (Model 1). The effect sizes were higher for sTNFR2 than for sTREM2. In Model 2, only sTNFR2 significantly predicted CSF p-tau and t-tau levels (**Figure 1**, **Table 3**). For ADNI AD dementia A+ T+ participants, sTNFR2 significantly predicted CSF t-tau levels but not CSF p-tau or Aβ42 levels (Model 1). In Model 2, both analytes failed to meet significance (**Figure 1**, **Table 4**). sTREM2, sTNFR2 interactions on AD biomarker outcomes were checked and were found to be non-significant for both MCI and dementia A+ T+ participants and were removed from model fits.

Among participants in the replication memory clinic cohort, sTNFR2 (but not sTREM2) significantly predicted CSF p-tau and t-tau levels but not CSF Aβ42 level (Model 1). In Model 2, only sTNFR2 significantly predicted CSF p-tau and t-tau levels (**Figure 1**, **Table 5**). sTREM2, sTNFR2 interactions on AD biomarker outcomes in the replication cohort were again non-significant.

Model 3

In ADNI MCI A+ T+ participants, neither CSF sTREM2 nor sTNFR2 levels predicted future cognitive decline on CDR-SB when taking into account CSF p-tau or t-tau levels (**Figure 2**, **Table 6**). In a sensitivity analysis with larger number of participants with sTREM2 and AD biomarkers alone, the above results were again corroborated (**Supplementary Table 13**).

In ADNI AD dementia A+ T+ participants, higher CSF sTREM2 predicted lower future cognitive decline on CDR-SB independent of CSF t-tau levels. With every doubling of CSF sTREM2 levels, the longitudinal change in CDR-SB decreased by 1.3 points ($\beta = -1.33$, $df = 115.2$, $t = -2.27$, $P = 0.006$, $FDR = 0.048$) (**Figure 2**, **Table 7**). In a sensitivity analysis using p-tau instead of t-tau, the same directional trend was observed ($\beta = -1.28$, $df = 114.7$, $t = -2.69$, $P = 0.008$, $FDR = 0.064$). On further evaluating the robustness of this effect among a larger number of participants with sTREM2 and AD biomarkers alone, the significance and directionality of the results were again corroborated (**Supplementary Table 14**).

Exploratory Analysis in ADNI CN Participants

For all ADNI CN participants, all A and T groups combined, sTNFR2 and sTREM2 were correlated ($\rho = 0.487$, $p < 0.0001$). In the A– T– subgroup, sTNFR2 and sTREM2 were modestly correlated with each other ($\rho = 0.35$, $p = 0.036$) and each analyte was significantly related to CSF t-tau (sTNFR2 $\rho = 0.38$, $p = 0.023$ and sTREM2 $\rho = 0.35$, $p = 0.034$). In the A+ T+, A+ T–, and A– T+ subgroups, sTNFR2 and sTREM2 were not significantly correlated with each other. Only sTREM2 was correlated with CSF t-tau and p-tau in the A+ T+ subgroup, whereas only sTNFR2 was significantly correlated with CSF t-tau and p-tau in the A- T+ subgroup (**Supplementary Table 2**).

TABLE 2 | Pearson correlations between sTNFR2/sTREM2 and Aβ42, t-tau, and p-tau for participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) and replication memory clinic cohorts (participants meeting A+ T+ criteria, includes those with concomitant sTREM2 and sTNFR2 data).

Cohort	Analyte	Log_2 sTREM2	Log_2 sTNFR2	Log_2 Aβ42	Log_2 t-tau	Log_2 p-tau
		Correlation (*P*-value)	Correlation (*P*-value)	Correlation (*P*-value)	Correlation (*P*-value)	Correlation (*P*-value)
ADNI MCI ($n = 67$)	sTREM2	1	0.67 (<0.0001)**	0.23 (0.059)	0.31 (0.01)**	0.31 (0.008)**
	sTNFR2	0.61 (<0.0001)**	1	0.072 (0.56)	0.49 (<0.0001)**	0.48 (<0.0001)**
ADNI AD dementia ($n = 42$)	sTREM2	1	0.75 (<0.0001)**	0.23 (0.13)	0.30 (0.052)	0.24 (0.11)
	sTNFR2	0.75 (<0.0001)**	1	0.21 (0.16)	0.31 (0.042)*	0.26 (0.088)
Replication memory clinic ($n = 48$)	sTREM2[a]	1	0.31 (0.042)*	0.058 (0.71)	0.17 (0.25)	0.19 (0.20)
	sTNFR2	0.31 (0.042)*	1	0.27 (0.058)	0.72 (<0.0001)*	0.72 (<0.0001)*

AD, Alzheimer's disease; MCI, mild cognitive impairment; sTNFR 2, soluble tumor necrosis factor receptor 2; sTREM2, soluble triggering receptor expressed on myeloid cells 2. [a]*n = 43.* **P ≤ 0.05. ***P ≤ 0.01 and False Discovery Rate, p = 0.05.*

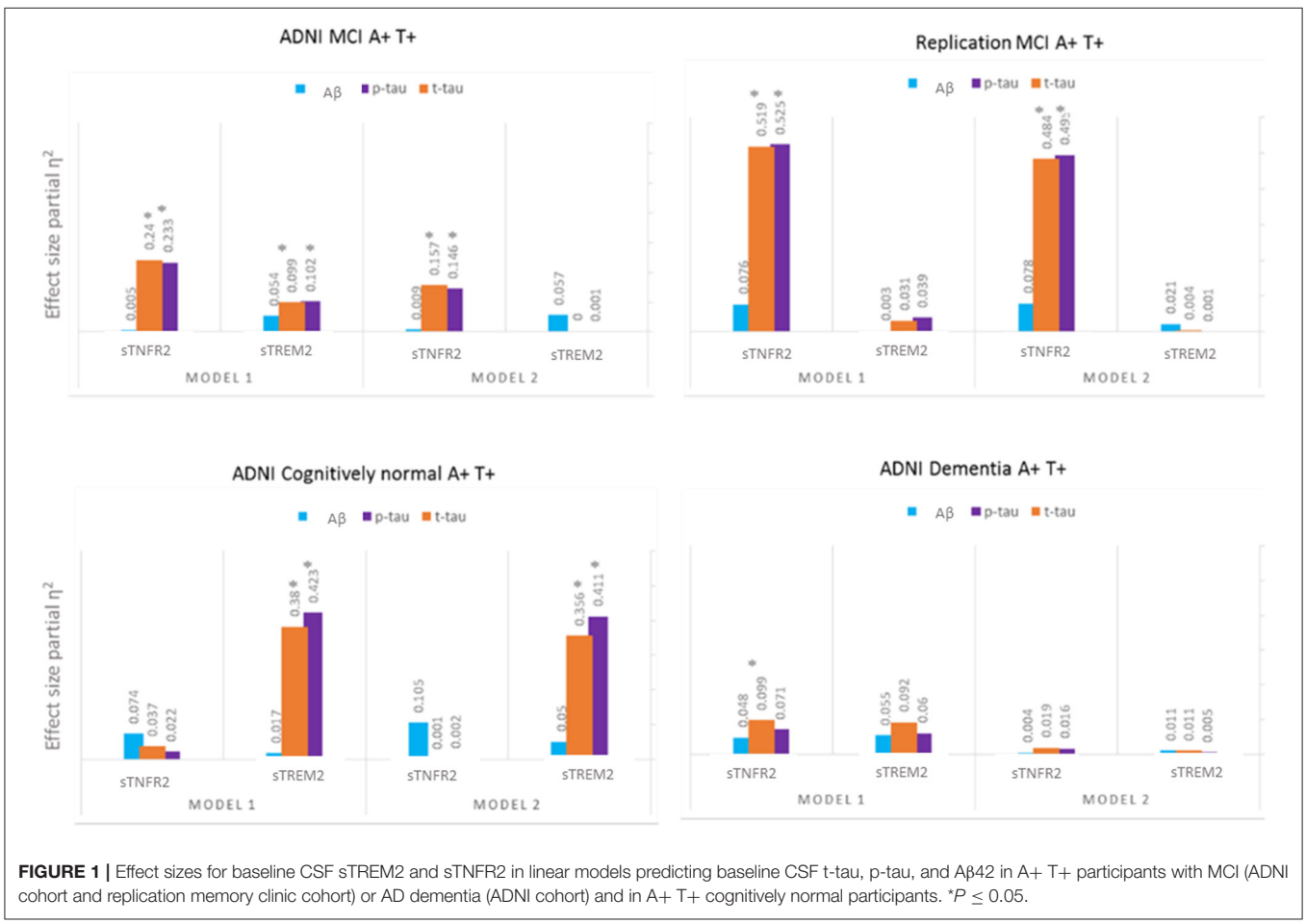

FIGURE 1 | Effect sizes for baseline CSF sTREM2 and sTNFR2 in linear models predicting baseline CSF t-tau, p-tau, and Aβ42 in A+ T+ participants with MCI (ADNI cohort and replication memory clinic cohort) or AD dementia (ADNI cohort) and in A+ T+ cognitively normal participants. *$P \leq 0.05$.

Models 1 and 2

Among A+ T+ CN participants, only sTREM2 predicted CSF t-tau and p-tau levels but not CSF Aβ42 level (Model 1). In Model 2, sTREM2 again significantly predicted CSF p-tau and t-tau levels (**Figure 3**, **Supplementary Table 3**). sTREM2 and sTNFR2 interaction on AD biomarker outcomes among A+ T+ CN participants were again non-significant.

Among A+ T– CN participants, neither analyte significantly predicted CSF p-tau and t-tau levels (**Figure 3**, **Supplementary Table 4**).

TABLE 3 | Key results of the general linear model with CSF Aβ42, t-tau, and p-tau as the dependent variables and covariates: age, sex, education, APOEε4 status, among participants from the Alzheimer's Disease Neuroimaging Initiative cohort with mild cognitive impairment (participants meeting A+ T+ criteria).

Model 1: Main effects: CSF sTNFR2 *OR* sTREM2								
Main effect	**Dependent variable**	**Type III sum of squares**	**df**	**Mean square**	**F**	***P*-value**	**R^2**	**Partial eta squared**
sTNFR2	Aβ42	0.083	1.65	0.054	0.342	0.561	0.005	0.005
	t-tau	2.395	1.65	2.395	20.538	<0.0001**	0.24	0.24
	p-tau	2.858	1.65	2.858	19.711	<0.0001**	0.233	0.233
sTREM2	Aβ42	0.553	1.65	0.553	3.698	0.059	0.054	0.054
	t-tau	0.986	1.65	0.986	7.13	0.01**	0.099	0.099
	p-tau	1.252	1.65	1.252	7.374	0.008**	0.102	0.102
Model 2: Main effects: CSF sTNFR2 *AND* sTREM2								
sTNFR2	Aβ42	0.083	1.64	0.083	0.549	0.461	0.062	0.009
	t-tau	1.411	1.64	1.411	11.921	0.001**	0.24	0.157
	p-tau	1.616	1.64	1.616	10.985	0.002**	0.233	0.146
sTREM2	Aβ42	0.582	1.64	0.582	3.864	0.054	0.062	0.057
	t-tau	0.002	1.64	0.002	0.021	0.886	0.24	0
	p-tau	0.009	1.64	0.009	0.064	0.801	0.233	0.001

*CSF, cerebrospinal fluid; df, degrees of freedom; sTNFR 2, soluble tumor necrosis factor receptor 2; sTREM2, soluble triggering receptor expressed on myeloid cells 2. *P ≤ 0.05. **FDR≤ 0.05.*

TABLE 4 | Key results of the general linear model with CSF Aβ42, t-tau, and p-tau as the dependent variables and covariates: age, sex, education, APOEε4 status, among participants from the Alzheimer's Disease Neuroimaging Initiative cohort with Alzheimer's disease dementia (participants meeting A+ T+ criteria).

Model 1: Main effects: CSF sTNFR2 *OR* sTREM2								
Main effect	**Dependent variable**	**Type III sum of squares**	**df**	**Mean square**	**F**	***P*-value**	**R^2**	**Partial eta squared**
sTNFR2	Aβ42	0.404	1.40	0.404	2.003	0.165	0.048	0.048
	t-tau	0.65	1.40	0.65	4.408	0.042*	0.099	0.099
	p-tau	0.586	1.40	0.586	3.051	0.088	0.071	0.071
sTREM2	Aβ42	0.462	1.40	0.462	2.307	0.137	0.055	0.055
	t-tau	0.599	1.40	0.599	4.029	0.052	0.092	0.092
	p-tau	0.498	1.40	0.498	2.563	0.117	0.06	0.06
Model 2: Main effects: CSF sTNFR2 *AND* sTREM2								
sTNFR2	Aβ42	0.035	1.39	0.035	0.17	0.682	0.059	0.004
	t-tau	0.114	1.39	0.114	0.763	0.388	0.109	0.019
	p-tau	0.126	1.39	0.126	0.642	0.428	0.075	0.016
sTREM2	Aβ42	0.093	1.39	0.093	0.453	0.505	0.059	0.011
	t-tau	0.063	1.39	0.063	0.424	0.519	1.09	0.011
	p-tau	0.038	1.39	0.038	0.193	0.663	0.075	0.005

*CSF, cerebrospinal fluid; df, degrees of freedom; sTNFR 2, soluble tumor necrosis factor receptor 2; sTREM2, soluble triggering receptor expressed on myeloid cells 2. *P ≤ 0.05. **FDR≤ 0.05.*

Among A– T+ CN participants, sTNFR2 predicted t-tau and p-tau levels (Model 1). In Model 2, sTNFR2 significantly predicted CSF t-tau and Aβ42 levels (**Figure 3**, **Supplementary Table 5**).

Among A– T– CN participants alone, the interaction of sTNFR2 and sTREM2 was significant on t-tau and p-tau levels (Model 1) (**Supplementary Table 6**).

Model 3

Among all CN participants combined, neither sTNFR2 nor sTREM2 predicted longitudinal cognitive change after accounting for CSF AD biomarkers (data not presented).

Among A+ T+ CN participants, higher sTREM2 levels predicted more longitudinal cognitive change after accounting for CSF t-tau but this result was not significant after FDR correction ($\beta = 0.94$, df = 21.94, t = 2.43, $P = 0.023$, FDR = 0.092) (**Supplementary Figure 1**, **Supplementary Table 7**). In a sensitivity analysis with a slightly larger number of participants with sTREM2 and AD biomarkers alone, the above results were again corroborated and now met FDR threshold (**Supplementary Table 15**).

Among A– T+, A+ T– and A– T– CN participants, neither sTNFR2 nor sTREM2 predicted longitudinal cognitive change

TABLE 5 | Key results of the general linear model with CSF Aβ42, t-tau, and p-tau as the dependent variables and covariates: age, sex, education, APOEε4 status, among mild cognitive impairment participants from a replication memory clinic cohort (participants meeting A+ T+ criteria).

Main effect	Dependent variable	Type III sum of squares	df	Mean square	F	*P*-value	R^2	Partial eta squared
Model 1: Main effects: CSF sTNFR2 *OR* sTREM2								
sTNFR2	Aβ42	1.093	1.46	1.093	3.775	0.058	0.076	0.076
	t-tau	20.951	1.46	20.951	49.543	<0.0001**	0.519	0.519
	p-tau	11.073	1.46	11.073	50.789	<0.0001**	0.525	0.525
sTREM2	Aβ42	0.035	1.41	0.035	0.139	0.711	0.003	0.003
	t-tau	1.061	1.41	1.061	1.315	0.258	0.031	0.031
	p-tau	0.747	1.41	0.747	1.685	0.202	0.039	0.039
Model 2: Main effects: CSF sTNFR2 *AND* sTREM2								
sTNFR2	Aβ42	0.807	1.40	0.807	3.367	0.074	0.081	0.078
	t-tau	16.004	1.40	16.004	37.482	<0.0001**	0.5	0.484
	p-tau	9.006	1.40	9.006	39.252	<0.0001**	0.515	0.495
sTREM2	Aβ42	0.21	1.40	0.21	0.875	0.355	0.081	0.021
	t-tau	0.071	1.40	0.071	0.167	0.685	0.5	0.004
	p-tau	0.013	1.40	0.013	0.056	0.815	0.515	0.001

*CSF, cerebrospinal fluid; df, degrees of freedom; sTNFR 2, soluble tumor necrosis factor receptor 2; sTREM2, soluble triggering receptor expressed on myeloid cells 2. *P ≤ 0.05. **FDR ≤ 0.05.*

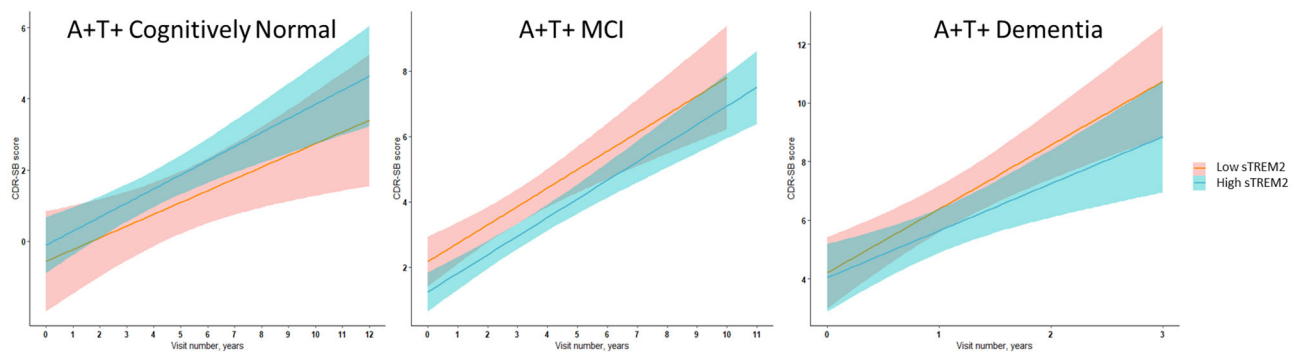

FIGURE 2 | Illustrative linear regression with 95% CI plots for sTREM2 levels at the 25th and 75th percentiles at baseline vs. CDR-SB score longitudinally for A+ T+ participants with MCI stage of AD ($n = 111$) or AD dementia ($n = 57$) and in A+ T+ cognitively normal participants ($n = 16$) also used for sensitivity analyses described in **Supplementary Tables 11–15**.

after accounting for CSF AD biomarkers after FDR correction (**Supplementary Tables 8–10**).

DISCUSSION

This study demonstrates that the CSF levels of sTREM2 and sTNFR2 are dynamic in relation to p-tau and t-tau biomarkers over the temporal stages of AD. A positive correlation was seen between CSF levels of sTREM2 and sTNFR2 in A+ T+ participants with AD dementia (ADNI) or MCI (both cohorts). Among the above participants, sTNFR2 rather than sTREM2 explained most of the variance in relation to CSF t-tau and p-tau, whereas sTREM2 rather than sTNFR2 explained most of the variance in the same biomarkers among A+ T+ CN individuals (ADNI). These results are consistent with previous reports that demonstrated a positive correlation between CSF sTREM2 and t-tau and p-tau in participants with MCI (Heslegrave et al., 2016; Piccio et al., 2016; Suárez-Calvet et al., 2016a,b, 2019), and among preclinical AD with low CSF $A\beta_{42}$ and high total-tau or p-tau levels (Ma et al., 2020) but our results extend previous reports by demonstrating the limited significance of CSF sTREM2 in explaining CSF t-tau and p-tau variance when taking CSF sTNFR2 levels into account among participants in various AD clinical stages (with the exception of A+ T+ CN). There was no significant interaction between sTNFR2 and sTREM2 levels on AD biomarkers in A+T+ CN, MCI and dementia groups but this interaction was significant in the A− T− CN group.

Prior analysis in the ADNI cohort had noted that higher CSF sTREM2 levels at baseline were associated with slower rates of Aβ accumulation as assessed by amyloid PET over 2 years, with the largest effect in the MCI and dementia stages of AD (Ewers et al., 2020). Consistent with this, we note that the effect of CSF

TABLE 6 | Key results of the linear mixed-effects regression model with CDR-SB as the dependent variable among participants with A+ T+ mild cognitive impairment from the Alzheimer's Disease Neuroimaging Initiative cohort (Model 3).

Parameter	Estimate	Standard error	df	t	*P*-value	95% confidence interval	
						Lower bound	Upper bound
Intercept	3.991006	13.69949	107.56	0.291	0.771	−23.165	31.14702
sTREM2	−0.42004	0.632681	107.895	−0.664	0.508	−1.67413	0.834061
sTNFR2	0.829879	3.489585	109.082	0.238	0.812	−6.08631	7.746066
t-tau	0.313797	1.12039	108.769	0.28	0.78	−1.90683	2.534427
Visit number in years	2.761182	2.621731	348.072	1.053	0.293	−2.39525	7.917609
Visit number in years × sTREM2	0.015941	0.120067	360.652	0.133	0.894	−0.22018	0.25206
Visit number in years × sTNFR2	0.129465	0.720227	362.272	0.18	0.857	−1.28689	1.545817
Visit number in years × t-tau	−0.22548	0.238439	365.207	−0.946	0.345	−0.69436	0.243411

CSF sTREM2 × visit number + CSF sTNFR2 × visit number, CSF t-tau × visit number + CSF sTREM2 + CSF sTNFR2 + CSF t-tau + visit number (fixed effect). CDR-SB, Clinical Dementia Rating–Sum of Boxes; CSF, cerebrospinal fluid; df, degrees of freedom; sTNFR 2, soluble tumor necrosis factor receptor 2; sTREM2, soluble triggering receptor expressed on myeloid cells 2.

TABLE 7 | Key results of the linear mixed-effects regression model with CDR-SB as the dependent variable among participants with A+ T+ AD dementia from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort (Model 3).

Parameter	Estimate	Standard error	df	t	*P*-value	95% confidence interval		FDR
						Lower bound	Upper bound	
Intercept	5.76527	14.38224	62.274	0.401	0.69	−22.9819	34.51244	1
sTREM2	0.096417	0.961389	61.919	0.1	0.92	−1.82542	2.018256	0.92
sTNFR2	1.417211	4.269298	61.595	0.332	0.741	−7.11811	9.952532	0.988
t-tau	−0.31314	1.091746	62.298	−0.287	0.775	−2.4953	1.869025	0.885
Visit number in years	9.80077	7.13377	113.013	1.374	0.172	−4.3325	23.93404	0.458
Visit number in years × sTREM2	−1.33428	0.47665	115.165	−2.799	0.006*	−2.27841	−0.39014	0.048*
Visit number in years × sTNFR2	2.457261	2.061383	116.28	1.192	0.236	−1.62547	6.539987	0.472
Visit number in years × t-tau	0.983455	0.56413	113.405	1.743	0.084	−0.13415	2.101055	0.336

*CSF sTREM2 × visit number + CSF sTNFR2 × visit number, CSF t-tau × visit number + CSF sTREM2 + CSF sTNFR2 + CSF t-tau + visit number (fixed effect). CDR-SB, Clinical Dementia Rating–Sum of Boxes; CSF, cerebrospinal fluid; df, degrees of freedom; FDR, false discovery rate; sTNFR 2, soluble tumor necrosis factor receptor 2; sTREM2, soluble triggering receptor expressed on myeloid cells 2. *$P \leq 0.05$.*

sTREM2 on cognitive decline (i.e., higher level of CSF sTREM2 relates to slower longitudinal decline on CDR-SB scores) was significant at the A+ T+ AD dementia stage, possibly subsequent to its effect on Aβ accumulation that appears maximal at the MCI stage and this interestingly was independent of its association with CSF t-tau and p-tau levels. Additionally, we found that sTREM2 levels explain less of the variance in CSF Aβ42 similar to previous reports (Suárez-Calvet et al., 2016a,b, 2019; Ma et al., 2020).

In contrast, CSF sTNFR2 levels were associated with CSF t-tau and p-tau levels in the A+T+ MCI and dementia stages but did not predict rates of cognitive decline on CDR-SB on longitudinal follow up over subsequent years. However, among A+ T+ MCI or AD dementia groups in ADNI, we had previously reported that the interaction between *TNFRSF1B* gene variants and CSF sTNFR2 levels relates to CSF t-tau and p-tau levels and longitudinal cognitive change over 1 year (Pillai et al., 2021). This suggests that the sTNFR2 levels are impacted by both CSF t-tau and p-tau levels and *TNFRSF1B* gene variant status, and mitigate cognitive decline over the short term but do not significantly impact cognitive outcomes over the longer term as the disease continues to evolve.

Our exploratory analysis among CN participants further notes that the relative association of CSF sTREM2 on markers of neuronal injury and neurofibrillary tangles (CSF t-tau and p-tau) is higher in the preclinical stage of AD (A+ T+ CN) than in the A+ T+ MCI or dementia stages. Consistent with this, higher CSF sTREM2 levels that relate to higher CSF t-tau and p-tau levels at the preclinical AD stage was associated with greater cognitive decline, unlike in the dementia stage, in which higher CSF sTREM2 levels were associated with slower cognitive decline (**Figure 2**). These results among CN groups which were limited to CSF measures of Aβ and tau are preliminary given the smaller number of subjects analyzed with both CSF sTREM2 and sTNFR2 data. Although higher levels of CSF sTREM2 do not necessarily relate to cognitive outcomes favorably in the preclinical stage with concurrent increase in CSF t-tau and p-tau in our data, they are still a marker of slower rate of clinical progression longitudinally in later disease stages. This is also supported

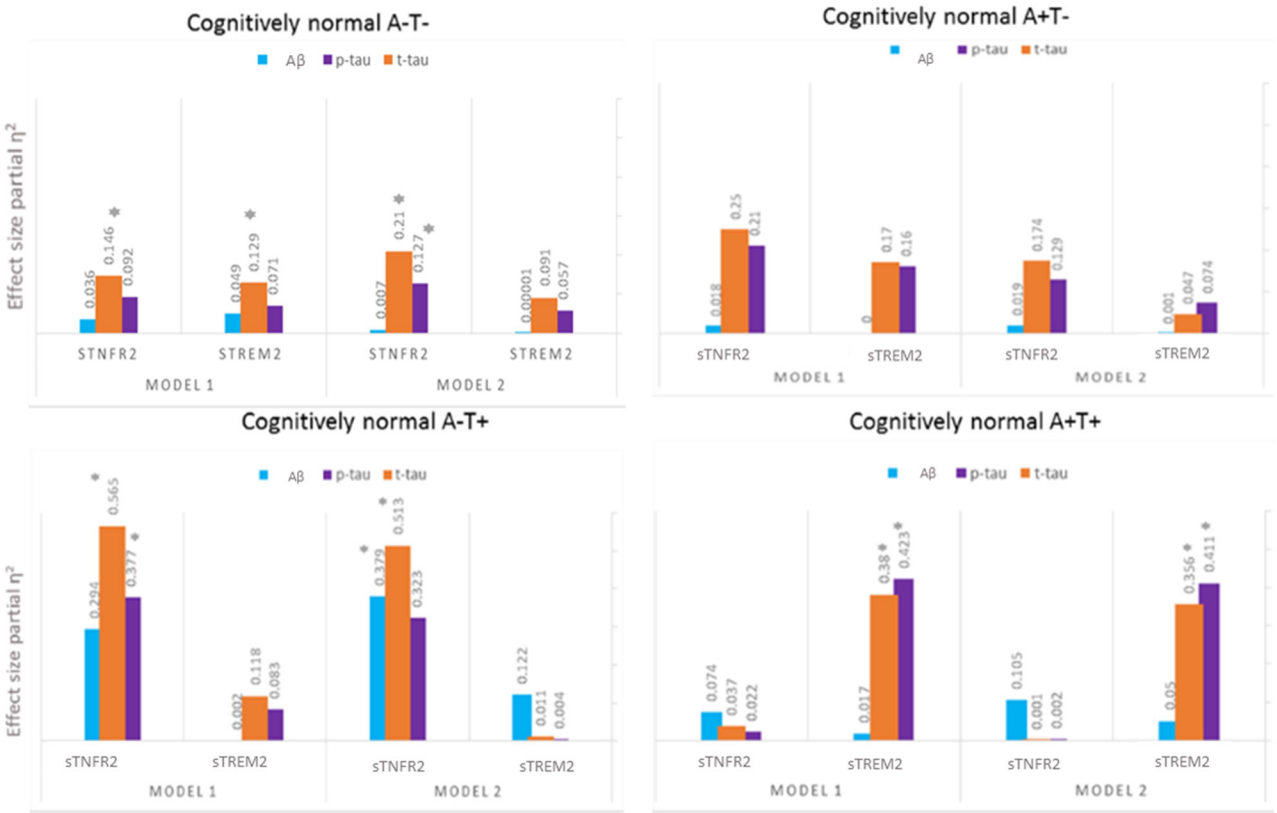

FIGURE 3 | Effect sizes for baseline CSF sTREM2 and TNFR2 in linear models predicting baseline CSF t-tau, p-tau, and Aβ42 among A– T–, A– T+, A+ T–, and A+ T+ cognitively normal participants. *$P \leq 0.05$.

by previous reports (Ewers et al., 2019; Franzmeier et al., 2020).

Taken together our results suggest that there is a temporal window that should be considered for future therapeutic options targeting sTREM2- and sTNFR2-related inflammatory pathways and for the use of CSF sTREM2 in combination with other CSF AD and inflammatory biomarkers in evaluating its clinical significance. The temporal changes in CSF sTREM2 across different AD stages in this study parallels what has been described previously (Suárez-Calvet et al., 2016b, 2019). Based on prior models on the temporal evolution of AD biomarkers (Jack and Holtzman, 2013), **Figure 4** summarizes a hypothetical model based on the key findings of CSF sTREM2- and sTNFR2 relationships to Aβ and tau levels in the different stages of AD, plotting biomarker severity (degree of abnormality) vs. disease stage. The key insight here being that the time course of change among the distinct inflammation related markers (sTREM2, sTNFR2) with the progression of AD stages are very different.

Analysis within the CN subgroups was exploratory given the small number of participants. Nevertheless, we could still delineate some patterns of interest with respect to sTREM2 that needs future validation. First, preclinical AD (CN A+ T+) appears to be a transition state in which the relationship noted between sTREM2, sTNFR2 and CSF t-tau or p-tau in all other stages of AD appears to be reversed, suggesting that in this stage, sTREM2 explains more of the variance of CSF t-tau and p-tau than does sTNFR2. This is consistent with prior results among preclinical AD with low $A\beta_{42}$ and high total-tau or p-tau in the CABLE study (Ma et al., 2020). Within the CN A+ T+ group, higher sTREM2 levels was associated with more cognitive decline (unlike in AD dementia). This finding is consistent with results from the DIAN Study, in which an increase in CSF sTREM2 among autosomal dominant mutation carriers was found to differ from the level in normal controls 5 years before symptom onset, with this increase followed by Aβ, tau, and associated neurodegenerative changes; in advanced stages of the disease in the DIAN cohort, this difference did not reach statistical significance (Suárez-Calvet et al., 2016c). A second pattern we observed in the current study was that higher levels of sTREM2 appear to be related to slower cognitive decline among AD stages in which sTREM2 is not strongly correlated with neurodegeneration markers (i.e., AD dementia), but this was not the case among AD stages that demonstrated a strong correlation between sTREM2 and neurodegeneration markers (CN A+ T+). This suggests that CSF levels of sTREM2 alone are likely not good independent prognosticators of future clinical decline but could reflect a more complex interplay of immune cell

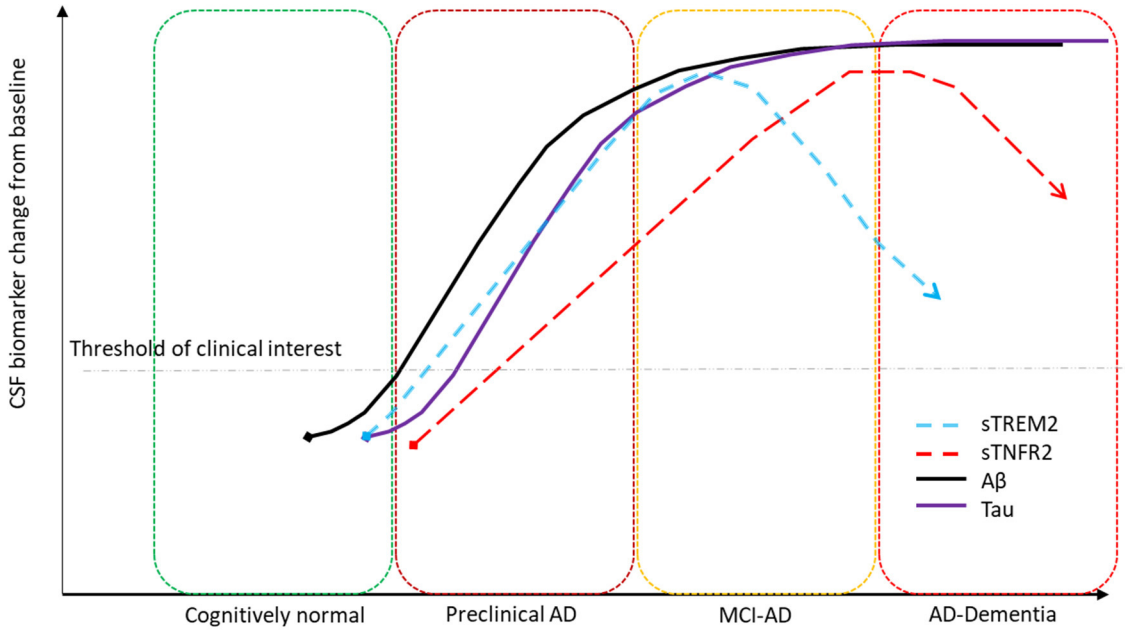

FIGURE 4 | Hypothetical model summarizing key biomarker findings. CSF sTREM2 significantly correlates to CSF p-tau levels in the A+ T+ preclinical and MCI stages of AD and CSF sTNFR2 significantly correlates to CSF p-tau levels in the A+ T+ MCI and dementia stages of AD.

activation in relation to neurodegenerative changes in different AD stages.

The statistical interaction noted between sTNFR2 and sTREM2 among CN A-T- participants shows higher levels of both sTNFR2 and sTREM2 together impact t-tau and p-tau levels more than we would expect if they acted independently, this appears to parallel the reports from animal studies where TNFR2 activation levels were related to transcriptional regulation of TREM2 (Gao et al., 2017). It is possible that the lack of statistical interaction between sTNFR2 and sTREM2 levels on AD biomarkers among A+T+ CN, MCI and dementia suggests altered TNFR2 and TREM2 pathway activations in AD. As the current study is an association study of biomarkers, future studies in AD models are needed to evaluate the mechanistic relationship between TNFR2 and TREM2 in AD.

LIMITATIONS

Biomarker changes alone may not reflect completely the pathophysiological roles of TNFR2 and TREM2 at different AD stages and these should not be interpreted as providing mechanistic insights. Further, all models evaluating CSF sTNFR2 and CSF sTREM2s impact on AD biomarkers relied on crossectional data. Crossectional data do not provide a window into within subject temporal trajectories of CSF sTNFR2 and sTREM2s changes, therefore future longitudinal evaluations of these inflammatory analytes are needed. These study results are most robust for the A+ T+ MCI and dementia stages of AD given the relatively large number of participants and the replication of results across two different A+ T+ MCI cohorts. However, the results in the A+ T+ CN group was consistent with reports from prior studies and therefore provide confidence to our results. Given the limited follow-up (15 months) in the replication cohort, the findings from this group could not be used to corroborate the effects of these analytes longitudinally from ADNI. These findings should also be corroborated in a cohort with different recruitment goals than ADNI, as the ADNI cohort is predominantly White with a high education attainment. We did not screen participants for possible TREM2 mutations, as the likelihood of these mutations is low (Suárez-Calvet et al., 2019). Additionally, lack of neuropathologic confirmation also limits our understanding of the role of mixed pathology.

There were also known differences between the ADNI and the replication cohorts. The replication cohort included a sample of memory clinic participants with a faster rate of disease progression than participants in the ADNI cohort (Pillai et al., 2020). Additionally, the replication cohort also included a few atypical AD participants with highly elevated CSF t-tau levels (Pillai et al., 2019a) and had a higher frequency of *APOE* ε4 carriers, unlike typical amnestic MCI participants in the ADNI cohort. The positive correlation between sTNFR2 and CSF t-tau and p-tau levels was still consistent within each cohort, but the replication cohort had higher mean t-tau and p-tau levels than the ADNI cohort even when the CSF levels were compared using the same measurement technique as previously reported (Pillai et al., 2020). Mean sTNFR2 levels correlating with neurodegeneration biomarkers were therefore much higher in the A+ T+ MCI replication cohort than in the A+ T+ MCI ADNI cohort.

The differences between the ADNI A+ T+ MCI and the replication cohort A+ T+ MCI also gives us pause in

extrapolating the current results to future validation cohorts with different AD clinical and pathological characteristics given the heterogeneity in clinical, biomarker and neuropathology phenotypes of AD (Murray et al., 2011; Pillai et al., 2019a; Suárez-Calvet et al., 2019). The relationship between sTREM2 and CSF t-tau was also stronger in the dementia stage in a prior report by our group (Bekris et al., 2018). Perhaps these differences reflect the variability in clinical symptoms even within the broad stage of AD dementia between cohorts. It is also likely that *APOE* ε4 carrier rates can vary from study to study impacting longitudinal results (Tsuang et al., 1996; Franzmeier et al., 2020). Presence of mixed pathology and the differences in recruitment biases to atypical AD should also be considered when comparisons are made between cohorts in longitudinal outcomes (Dubois et al., 2007; Pillai et al., 2016). It is likely these are some possible reasons behind significant differences in sTREM2 levels reported by prior studies across the AD spectrum (Kleinberger et al., 2014; Gispert et al., 2016b; Henjum et al., 2016; Bekris et al., 2018; Suárez-Calvet et al., 2019; Knapskog et al., 2020).

Type II errors also must be considered in this study given the smaller number of participants within some CN subgroups, as smaller effect sizes could have been missed. It is possible that with a larger sample size among the CN subgroups, some variables of significance could become more salient in the consideration of cognitive and AD biomarker outcomes. Independent replication in larger cohorts using the same biomarkers as those used in ADNI would allow us to clarify this point.

CONCLUSIONS

Our results suggest that the levels of both sTREM2 and sTNFR2 vary dynamically in relation to neurodegenerative biomarkers at different AD stages. This implies that the utility of distinct inflammation related biomarkers in tracking AD temporal progression and their role in predicting clinical outcomes are also expected to differ based on disease stage.

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: http://adni.loni.usc.edu/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Cleveland Clinic. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JP obtained funding, design and conceptualized study, analyzed the data, interpreted the data, and drafted the manuscript for intellectual content. MK analyzed the data and revised the manuscript for intellectual content. JB design of study, interpreted the data, and revised the manuscript for intellectual content. JL interpreted the data and revised the manuscript for intellectual content. LB organized the data, interpreted the data, and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

FUNDING

This project was funded in part by 2014-NIRG-305310 Alzheimer's Association, NIA K23AG055685, R03AG070485, 1P30 AG062428-01, NIH 1R56AG063870-01, Keep Memory Alive Foundation, and the Jane and Lee Seidman Fund. Research reported in this publication was supported by the National Institute on Aging of the National Institutes of Health under Award Number K23AG055685 and R03AG07048. JP was a program participant of the Research Education Component of the Cleveland Alzheimer's Disease Research Center supported by NIA P30 AG062428. Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI was funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd. and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

ACKNOWLEDGMENTS

We thank the participants and families who took part in the replication cohort at Cleveland Clinic Lou Ruvo Center for Brain Health and Megan Griffiths for editorial support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2021.676744/full#supplementary-material

Supplementary Figure 1 | Box plot for CSF levels of log_2 sTREM2, log_2 sTNFR2, log_2 Abeta, log_2 total tau within A+ T+ CN, MCI, and dementia groups. The groups differed only on the mean CSF log_2 Abeta levels ($F = 3.91$, $p = 0.023$) and not on the other analytes.

Supplementary Table 1 | Demographics of cognitively normal participants (includes those with concomitant sTREM2 and sTNFR2 data) in the Alzheimer's Disease Neuroimaging Initiative cohort.

Supplementary Table 2 | Pearson correlations between sTNFR2/sTREM2 and Aβ42, t-tau, and p-tau for cognitively normal participants from the Alzheimer's Disease Neuroimaging Initiative cohort.

Supplementary Table 3 | Key results of the general linear model with CSF Aβ42, t-tau, and p-tau as the dependent variables among cognitively normal A+ T+ participants from the Alzheimer's Disease Neuroimaging Initiative cohort.

Supplementary Table 4 | Key results of the general linear model with CSF Aβ42, t-tau, and p-tau as the dependent variables among cognitively normal A+ T– participants from the Alzheimer's Disease Neuroimaging Initiative cohort.

Supplementary Table 5 | Key results of the general linear model results with CSF Aβ42, t-tau, and p-tau as the dependent variables among cognitively normal A– T+ participants from the Alzheimer's Disease Neuroimaging Initiative cohort.

Supplementary Table 6 | Key results of the general linear model results with CSF Aβ42, t-tau, and p-tau as the dependent variables among cognitively normal A– T– participants from the Alzheimer's Disease Neuroimaging Initiative cohort.

Supplementary Table 7 | Key results of the linear mixed-effects regression model with CDR-SB as the dependent variable among A+ T+ cognitively normal participants from the Alzheimer's Disease Neuroimaging Initiative cohort (Model 3).

Supplementary Table 8 | Key results of the linear mixed-effects regression model with CDR-SB as the dependent variable among CN A– T+ cognitively normal participants from the Alzheimer's Disease Neuroimaging Initiative cohort (Model 3).

Supplementary Table 9 | Key results of the linear mixed-effects regression model with CDR-SB as the dependent variable among A+ T– cognitively normal participants from the Alzheimer's Disease Neuroimaging Initiative cohort (Model 3).

Supplementary Table 10 | Key results of the linear mixed-effects regression model with CDR-SB as the dependent variable among A– T– cognitively normal participants from the Alzheimer's Disease Neuroimaging Initiative cohort (Model 3).

Supplementary Table 11 | Demographics of A+T+ participants with CSF sTREM2 values (includes those without concomitant sTNFR2 data) along with CSF AD biomarkers in the Alzheimer's Disease Neuroimaging Initiative cohort for sensitivity analysis.

Supplementary Table 12 | Demographics of cognitively normal participants with CSF sTREM2 values (includes those without concomitant sTNFR2 data) along with CSF AD biomarkers in the Alzheimer's Disease Neuroimaging Initiative cohort for sensitivity analysis.

Supplementary Table 13 | Sensitivity analysis of linear mixed-effects regression model with CDR-SB as the dependent variable among participants with A+ T+ mild cognitive impairment from the Alzheimer's Disease Neuroimaging Initiative cohort with $n = 111$ with sTREM data alone.

Supplementary Table 14 | Sensitivity analysis of linear mixed-effects regression model with CDR-SB as the dependent variable among participants with A+ T+ dementia from the Alzheimer's Disease Neuroimaging Initiative cohort with $n = 57$ with sTREM data alone.

Supplementary Table 15 | Sensitivity analysis of linear mixed-effects regression model with CDR-SB as the dependent variable among participants with A+ T+ CN from the Alzheimer's Disease Neuroimaging Initiative cohort with $n = 16$ with sTREM data alone.

REFERENCES

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 270–279. doi: 10.1016/j.jalz.2011.03.008

Bekris, L. M., Khrestian, M., Dyne, E., Shao, Y., Pillai, J. A., Rao, S. M., et al. (2018). Soluble TREM2 and biomarkers of central and peripheral inflammation in neurodegenerative disease. *J. Neuroimmunol.* 319, 19–27. doi: 10.1016/j.jneuroim.2018.03.003

Carmona, S., Zahs, K., Wu, E., Dakin, K., Bras, J., and Guerreiro, R. (2018). The role of TREM2 in Alzheimer's disease and other neurodegenerative disorders. *Lancet Neurol.* 17, 721–730. doi: 10.1016/S1474-4422(18)30232-1

Chen, S., Peng, J., Sherchan, P., Ma, Y., Xiang, S., Yan, F., et al. (2020). TREM2 activation attenuates neuroinflammation and neuronal apoptosis via PI3K/Akt pathway after intracerebral hemorrhage in mice. *J. Neuroinflammation.* 17:168. doi: 10.1186/s12974-020-01853-x

Dong, Y., Fischer, R., Naudé, P. J., Maier, O., Nyakas, C., Duffey, M., et al. (2016). Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.* 113, 12304–12309. doi: 10.1073/pnas.1605195113

Dubois, B., Feldman, H. H., Jacova, C., Dekosky, S. T., Barberger-Gateau, P., Cummings, J., et al. (2007). Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 6, 734–746. doi: 10.1016/S1474-4422(07)70178-3

Ewers, M., Biechele, G., Suárez-Calvet, M., Sacher, C., Blume, T., Morenas-Rodriguez, E., et al. (2020). Higher CSF sTREM2 and microglia activation are associated with slower rates of beta-amyloid accumulation. *EMBO Mol. Med.* 12:e12308. doi: 10.15252/emmm.202012308

Ewers, M., Franzmeier, N., Suárez-Calvet, M., Morenas-Rodriguez, E., Caballero, M. A. A., Kleinberger, G., et al. (2019). Alzheimer's disease neuroimaging initiative. Increased soluble TREM2 in cerebrospinal fluid is associated with reduced cognitive and clinical decline in Alzheimer's disease. *Sci. Transl. Med.* 11:eaav6221. doi: 10.1126/scitranslmed.aav6221

Fischer, R., Maier, O., Siegemund, M., Wajant, H., Scheurich, P., and Pfizenmaier, K. (2011). A TNF receptor 2 selective agonist rescues human neurons from oxidative stress-induced cell death. *PLoS ONE* 6:e27621. doi: 10.1371/journal.pone.0027621

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Forabosco, P., Ramasamy, A., Trabzuni, D., Walker, R., Smith, C., Bras, J., et al. (2013). Insights into TREM2 biology by network analysis of human brain gene expression data. *Neurobiol. Aging* 34, 2699–2714. doi: 10.1016/j.neurobiolaging.2013.05.001

Franzmeier, N., Suárez-Calvet, M., Frontzkowski, L., Moore, A., Hohman, T. J., Morenas-Rodriguez, E., et al. (2020). Alzheimer's disease neuroimaging initiative (ADNI). Higher CSF sTREM2 attenuates ApoE4-related risk for cognitive decline and neurodegeneration. *Mol. Neurodegener.* 15:57. doi: 10.1186/s13024-020-00407-2

Gao, H., Danzi, M. C., Choi, C. S., Taherian, M., Dalby-Hansen, C., Ellman, D. G., et al. (2017). Opposing functions of microglial and macrophagic TNFR2 in the pathogenesis of experimental autoimmune encephalomyelitis. *Cell Rep.* 18, 198–212. doi: 10.1016/j.celrep.2016.11.083

Gispert, J. D., Monté, G. C., Suárez-Calvet, M., Falcon, C., Tucholka, A., Rojas, S., et al. (2016a). The APOE ε4 genotype modulates CSF YKL-40 levels and their structural brain correlates in the continuum of Alzheimer's disease but not those of sTREM2. *Alzheimers Dement.* 6, 50–59. doi: 10.1016/j.dadm.2016.12.002

Gispert, J. D., Suárez-Calvet, M., Monté, G. C., Tucholka, A., Falcon, C., Rojas, S., et al. (2016b). Cerebrospinal fluid sTREM2 levels are associated with gray matter volume increases and reduced diffusivity in early Alzheimer's disease. *Alzheimers Dement.* 12, 1259–1272. doi: 10.1016/j.jalz.2016.06.005

Gratuze, M., Leyns, C. E. G., and Holtzman, D. M. (2018). New insights into the role of TREM2 in Alzheimer's disease. *Mol. Neurodegener*. 13:66. doi: 10.1186/s13024-018-0298-9

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., et al. (2013). Alzheimer genetic analysis group. TREM2 variants in Alzheimer's disease. *N. Engl. J. Med*. 368, 117–127. doi: 10.1056/NEJMoa1211851

Henjum, K., Almdahl, I. S., Årskog, V., Minthon, L., Hansson, O., Fladby, T., et al. (2016). Cerebrospinal fluid soluble TREM2 in aging and Alzheimer's disease. *Alzheimers Res. Ther*. 8:17. doi: 10.1186/s13195-016-0182-1

Heslegrave, A., Heywood, W., Paterson, R., Magdalinou, N., Svensson, J., Johansson, P., et al. (2016). Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. *Mol. Neurodegener*. 11:3. doi: 10.1186/s13024-016-0071-x

Jack, C. R. Jr., Albert, M. S., Knopman, D. S., McKhann, G. M., Sperling, R. A., Carrillo, M. C., et al. (2011). Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 7, 257–262. doi: 10.1016/j.jalz.2011.03.004

Jack, C. R. Jr., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., et al. (2018). NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 14, 535–562. doi: 10.1016/j.jalz.2018.02.018

Jack, C. R. Jr., and Holtzman, D. M. (2013). Biomarker modeling of Alzheimer's disease. *Neuron* 80, 1347–1358. doi: 10.1016/j.neuron.2013.12.003

Jack, C. R. Jr., Wiste, H. J., Weigand, S. D., Therneau, T. M., Lowe, V. J., Knopman, D. S., et al. (2017). Defining imaging biomarker cut points for brain aging and Alzheimer's disease. *Alzheimers Dement*. 13, 205–216. doi: 10.1016/j.jalz.2016.08.005

Janelidze, S., Mattsson, N., Stomrud, E., Lindberg, O., Palmqvist, S., Zetterberg, H., et al. (2018). CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. *Neurology* 91, e867–e877 doi: 10.1212/WNL.0000000000006082

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P. V., Snaedal, J., et al. (2013). Variant of TREM2 associated with the risk of Alzheimer's disease. *N. Engl. J. Med*. 368, 107–116. doi: 10.1056/NEJMoa1211103

Kleinberger, G., Yamanishi, Y., Suárez-Calvet, M., Czirr, E., Lohmann, E., Cuyvers, E., et al. (2014). TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci. Transl. Med*. 6:243ra86. doi: 10.1126/scitranslmed.3009093

Knapskog, A. B., Henjum, K., Idland, A. V., Eldholm, R. S., Persson, K., Saltvedt, I., et al. (2020). Cerebrospinal fluid sTREM2 in Alzheimer's disease: comparisons between clinical presentation and AT classification. *Sci. Rep*. 10:15886. doi: 10.1038/s41598-020-72878-8

Lambert, J. C., Ibrahim-Verbaas, C. A., Harold, D., Naj, A. C., Sims, R., Bellenguez, C., et al. (2013). Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat. Genet*. 45, 1452–1458. doi: 10.1038/ng.2802

Ma, L. Z., Tan, L., Bi, Y. L., Shen, X. N., Xu, W., Ma, Y. H., et al. (2020). Dynamic changes of CSF sTREM2 in preclinical Alzheimer's disease: the CABLE study. *Mol. Neurodegener*. 15:25. doi: 10.1186/s13024-020-00374-8

Morris, J. C. (1993). The clinical dementia rating (CDR): current version and scoring rules. *Neurology* 43, 2412–2414. doi: 10.1212/WNL.43.11.2412-a

Murray, M. E., Graff-Radford, N. R., Ross, O. A., Petersen, R. C., Duara, R., and Dickson, D. W. (2011). Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. *Lancet Neurol*. 10, 785–796. doi: 10.1016/S1474-4422(11)70156-9

Piccio, L., Deming, Y., Del-Águila, J. L., Ghezzi, L., Holtzman, D. M., Fagan, A. M., et al. (2016). Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. *Acta Neuropathol*. 131, 925–933. doi: 10.1007/s00401-016-1533-5

Pillai, J. A., Bebek, G., Khrestian, M., Bena, J., Bergmann, C., Bush, W. S., et al. (2021). TNFRSF1B gene variants and related soluble TNFR2 levels impact resilience in Alzheimer's disease. *Front. Aging Neurosci*. 13:638922. doi: 10.3389/fnagi.2021.638922

Pillai, J. A., Bena, J., Bebek, G., Bekris, L. M., Bonner-Jackson, A., Kou, L., et al. (2020). Alzheimer's Disease Neuroimaging Initiative. Inflammatory pathway analytes predicting rapid cognitive decline in MCI stage of Alzheimer's disease. *Ann. Clin. Transl. Neurol*. 7, 1225–1239. doi: 10.1002/acn3.51109

Pillai, J. A., Bonner-Jackson, A., Bekris, L. M., Safar, J., Bena, J., and Leverenz, J. B. (2019a). Highly elevated cerebrospinal fluid total tau level reflects higher likelihood of non-amnestic subtype of Alzheimer's disease. *J. Alzheimers Dis*. 70, 1051–1058. doi: 10.3233/JAD-190519

Pillai, J. A., Butler, R. S., Bonner-Jackson, A., and Leverenz, J. B. (2016). Impact of Alzheimer's disease, lewy body and vascular co-pathologies on clinical transition to dementia in a national autopsy cohort. *Dement. Geriatr. Cogn. Disord*. 42, 106–116. doi: 10.1159/000448243

Pillai, J. A., Maxwell, S., Bena, J., Bekris, L. M., Rao, S. M., Chance, M., et al. (2019b). Alzheimer's disease neuroimaging initiative. Key inflammatory pathway activations in the MCI stage of Alzheimer's disease. *Ann. Clin. Transl. Neurol*. 6, 1248–1262. doi: 10.1002/acn3.50827

Shaw, L. M., Hansson, O., Manuilova, E., Masters, C. L., Doecke, J. D., Li, Q. X., et al. (2019). Method comparison study of the Elecsys® β-Amyloid (1-42) CSF assay versus comparator assays and LC-MS/MS. *Clin. Biochem*. 72, 7–14. doi: 10.1016/j.clinbiochem.2019.05.006

Suárez-Calvet, M., Araque Caballero, M. Á., Kleinberger, G., Bateman, R. J., Fagan, A. M., Morris, J. C., et al. (2016c). Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. *Sci. Transl. Med*. 8:369ra178.

Suárez-Calvet, M., Araque Caballero, M. Á., Kleinberger, G., Bateman, R. J., Fagan, A. M., Morris, J. C., et al. (2016a). Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. *Sci Transl Med*. 8:369ra178.

Suárez-Calvet, M., Kleinberger, G., Araque Caballero, M. Á., Brendel, M., Rominger, A., Alcolea, D., et al. (2016b). sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol. Med*. 8, 466–476. doi: 10.15252/emmm.201506123

Suárez-Calvet, M., Morenas-Rodríguez, E., Kleinberger, G., Schlepckow, K., Araque Caballero, M. Á., Franzmeier, N., et al. (2019). Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related-neurodegeneration but not with amyloid-β pathology. *Mol. Neurodegener*. 14:1. doi: 10.1186/s13024-018-0301-5

Tsuang, D., Kukull, W., Sheppard, L., Barnhart, R. L., Peskind, E., Edland, S. D., et al. (1996). Impact of sample selection on APOE epsilon 4 allele frequency: a comparison of two Alzheimer's disease samples. *J. Am. Geriatr. Soc*. 44, 704–707. doi: 10.1111/j.1532-5415.1996.tb01836.x

Van Cauwenberghe, C., Van Broeckhoven, C., and Sleegers, K. (2016). The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet. Med*. 18, 421–430. doi: 10.1038/gim.2015.117

Wunderlich, P., Glebov, K., Kemmerling, N., Tien, N. T., Neumann, H., and Walter, J. (2013). Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and γ-secretase-dependent intramembranous cleavage. *J. Biol. Chem*. 288, 33027–33036. doi: 10.1074/jbc.M113.517540

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of Interest: JP received research funding from the National Institutes of Health, Alzheimer's Association, and Keep Memory Alive Foundation. JL has received consulting fees from Acadia, Aptnyx, Biogen, Eisai, GE Healthcare, Sanofi, and Takeda and grant support from the Alzheimer's Association, Alzheimer's Drug Discovery Foundation, Biogen, Department of Defense, GE Healthcare, Genzyme/Sanofi, Lewy Body Dementia Association, Michael J. Fox Foundation, and National Institute of Health NIA, NINDS.

The remaining 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.

Copyright © 2021 Pillai, Khrestian, Bena, Leverenz and Bekris. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership