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The Center for Neurodegeneration and Translational Neuroscience: Advances In understanding Alzheimer's and Parkinson's Diseases

CNTN

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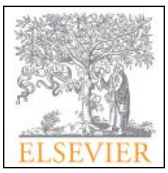

The Center for Neurodegeneration and Translational Neuroscience: Advances in understanding Alzheimer's and Parkinson's Diseases

SPECIAL ISSUE 2018 VOLUME 4

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Alzheimer's & Dementia

Editorial

Neurodegeneration research: Advances in clinical translational neuroscience infrastructure and methods

Biomedical research progress depends on appropriate infrastructure, funding, and expertise. Progress must include both advances in understanding of disease processes and their treatment and in training the next generation of investigators to ensure future progress toward the goals of optimal health, wellness, and dignified longevity (Fig. 1). Brain diseases pose a significant threat to human well-being, limit human capacity and autonomy, and take a great toll on the world economy. In the United States alone, symptomatic Alzheimer's disease (AD) affects 5.3 million, and 2.2 million have other forms of dementia—accounting for 7.5 million affected individuals or 7.4% of the population [1]. Preclinical and prodromal forms of AD—often unrecognized and undiagnosed—nearly double the total number of affected individuals [2]. The elderly are particularly vulnerable to neurodegenerative and vascular brain disorders; AD, Parkinson's disease (PD), dementia with Lewy bodies, vascular cognitive impairment, and mixed vascular–neurodegenerative disorders are all disproportionately common among older individuals [1,3]. Scientific programs aimed at reducing occurrence of diseases that impose a significant burden on society must address neurodegenerative disorders (NDDs). The accompanying suite of articles in *Alzheimer's & Dementia: Translational Research and Clinical Interventions* describes progress in establishing infrastructure and expertise to address the challenges of AD and PD. The role of the National Institute of General Medical Sciences (NIGMS) Institutional Development Awards (IDeAs) in supporting research infrastructure and growth of research expertise is highlighted.

The Lou Ruvo Brain Institute was established in Las Vegas, Nevada, in 2007 with scientific leadership by Zaven Khachaturian, PhD, and operational leadership by Ara Khachaturian. In 2009, the Cleveland Clinic assumed responsibility for the clinical programs of the renamed Lou Ruvo Center for Brain Health (LRCBH) under the guidance of Jeffrey Cummings, MD, ScD [4]. Clinical programs in AD, PD, and multiple sclerosis were initiated with an emphasis on expert clinical care, patient-centered care delivery, broad support for families and caregivers, and clinical trials of experimental medications. A brain-imaging program with expertise in magnetic resonance imaging (MRI) and positron emission tomography (PET) was established.

In 2015, the CCLRCBH established a collaboration with the University of Nevada Las Vegas (UNLV), including the UNLV administration, the leadership of the National Supercomputer Institute, experts in biostatistics, animal model researchers, genomic investigators, and leaders in program assessment. The collaboration focused on translational research in AD and PD and was funded in 2015 with an NIGMS Center of Biomedical Research (COBRE) award (P20GM109025) that inaugurated the Center for Neurodegeneration and Translational Neuroscience (CNTN) as an LRCBH-UNLV collaboration.

The COBRE program is one of the National Institutes of Health (NIHs) IDeA's three major initiatives to establish multidisciplinary research centers in states that historically have had low levels of NIH funding. The IDeA program funds only merit-based, peer-reviewed research that meets NIH research objectives in the 23 IDeA-eligible states and Puerto Rico. The other two IDeA initiatives are the IDeA Networks of Biomedical Research Excellence (INBRE) program and IDeA Clinical and Translational Research (IDeA-CTR) Awards. The COBRE awards can support up to three phases of funding from inauguration of scientific infrastructure, through successful support of investigators, to sustainability of the cores and mission with other sources of support (Fig. 2).

As a COBRE program, CNTN represents a comprehensive blueprint for advancing the careers of junior investigators in the area of NDD and promoting research vital to the nation in resolving the challenges associated with the burgeoning population of NDD patients. Here, research methods and infrastructure needed to advance understanding and treatment of AD and PD are described in the context of the functions of the CNTN.

Funding is needed to fuel AD research, and the sources of funding are described by Dr. Cummings, Dr. Carl Reiber, Vice Provost of UNLV, and Dr. Parvesh Kumar, Vice

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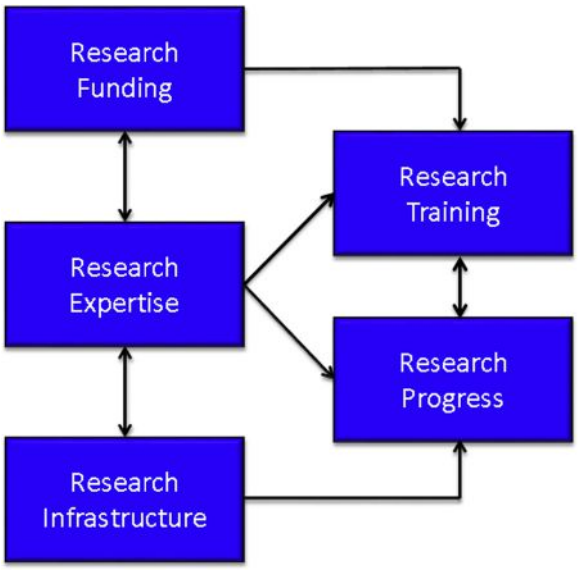

Fig. 1. Research environment consists of expertise, funding, and infrastructure that leads to scientific progress and training.

President for Research of the UNLV School of Medicine and Principal Investigator of the Intermountain West IDeA-CTR Infrastructure Network. They emphasize AD drug development as a critically important and necessary aspect of the AD research enterprise which is required for finding therapies to prevent or delay the onset, slow the progression, or improve the symptoms of AD. In "The Price of Progress", they describe the federal funding, private capital, public-private partnerships that comprise the financial ecosystem necessary to achieve improved therapy.

Scientific systems require assessment and reiterative adjustment to achieve optimal success. The science of research assessment is underdeveloped and has recently come under scrutiny as a high rate of irreproducible observations in both clinical and preclinical research has been documented [5]. The need for rigorous approaches in training, mentoring, and program assessment is described by Dr. Gwen Marchand. She describes novel network analyses that document the growth of collaborations and research networks.

Data science is growing at a rapid rate, and bioinformatics expertise and infrastructure is critical to the success of nearly every scientific enterprise [6]. Capturing clinical data regarding the disease phenotype; integrating clinical, imaging, and biomarker data; making carefully curated data available to many investigators while protecting patient confidentiality; and mining data for new discoveries are all critical aspects of data management described by Dr. Justin Miller and Joe Lombardo. Statistical analyses of data and advancing new means of analyzing natural history and clinical trial data complement data management, and advances in this domain are described by Guogen Shan, PhD.

Longitudinal natural history studies have become increasingly important in defining the expected course of AD and PD, identifying subgroups, and improving differential diagnosis. The Alzheimer's Disease Neuroimaging Initiative, the database of the National Alzheimer's Coordinating Center, and the Parkinson's Progression Markers Initiative are examples of natural history studies that have been highly informative [7–9]. Surprising observations have emerged from the application of new technologies in these longitudinal cohorts. For example, the high number—around 25%—of individuals diagnosed with AD who lack the amyloid plaque pathology needed to support the diagnosis has impacted AD research of all types [10]. The strong correlation between apolipoprotein E ∂-4 genotype and the presence of amyloid plaque has also been an important learning [11]. The structure of the CNTN includes a longitudinal cohort of individuals with AD, PD, or normal cognition. These populations undergo deep phenotyping with neuropsychological and clinical assessments, MRI, and amyloid PET. The structure is unique in including an expanded Alzheimer's Disease Neuroimaging Initiative neuropsychological battery for both AD and PD as well as the normal elderly individuals. The cognitively normal individuals are imaged by amyloid PET to determine if they are amyloid-bearing and at high risk for development of symptomatic AD or not. Dr. Aaron Ritter, Director of the Clinical and Translational Research Core, describes this cohort in the context of other on-going longitudinal studies.

A key translational tool for understanding central nervous system function is brain imaging [12,13]. This biomarker has emerged as a highly effective means of investigating brain structure (MRI measures of cortical thickness), connectivity (diffusion tensor imaging), blood flow (arterial spin labeling), circuitry (functional MRI [fMRI], metabolic

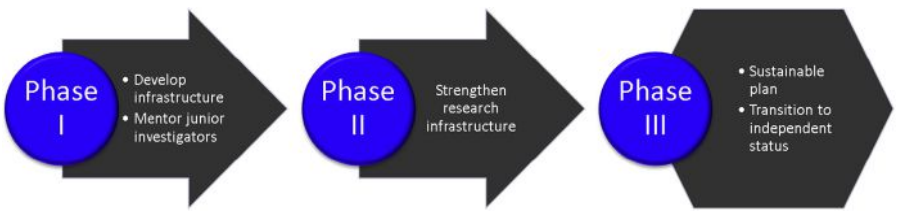

Fig. 2. Key elements supporting current and future research progress. There are two key products of the research enterprise: research progress and training of the next generation of researchers.

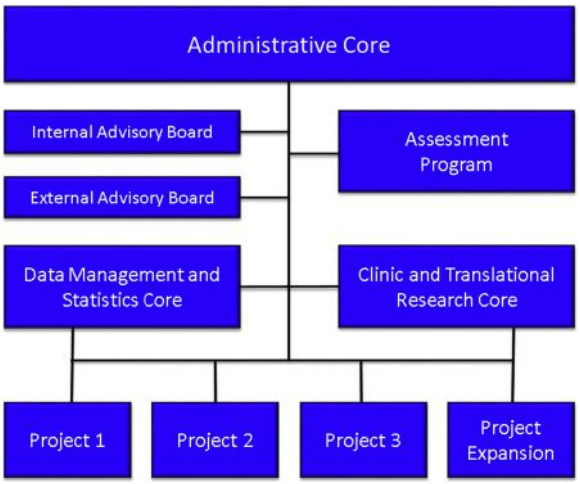

Fig. 3. Structure of the Center for Neurodegeneration and Translational Neuroscience (CNTN). Three cores—administrative, data management and statistics, and clinical and translational research—serve the three projects. The assessment program reiteratively assesses all aspects of the enterprise. The internal and external advisory boards review the center semi-annually and report to the Administrative Core and to the National Institute of General Medical Sciences (NIGMS).

activity (fluorodeoxyglucose PET), molecular composition (amyloid PET and tau PET), and inflammation (microglial PET). The robust repertoire of available imaging techniques of the CNTN and the expert leadership of the LRCBH Brain Imaging Program (Dr. Dietmar Cordes, PhD, Director) allow application of these translational tools to help resolve a wide variety of central nervous system problems. Dr. Cordes describes application of these techniques to AD and PD.

Another key tool to support translational research is collection of biological fluids (plasma or cerebrospinal fluid) for the study of various types of "omic" information including genomics, transcriptomics, proteomics, lipidomics, and metabolomics [14]. Genomics uses advanced sequencing technology and informatics to unravel the human genomic profile in health and disease. Genomic studies can assist in identifying multiple genes pointing to specific pathways in which interventions might be able to impact a network of interacting pathophysiologies [15]. Dr. Martin Schiller, a key UNLV collaborator of the CNTN, discusses genomics as an important means for advancing understanding and treatment of NDD.

The core structure of the CNTN is built to support multiple individual projects led by junior investigators (Fig. 3). Currently, three projects are supported, and more projects will be supported over time as hypotheses are resolved and new investigators are attracted to the center. Project 1, led by Dr. Sarah Banks, PhD, focuses on the use of advanced neuropsychological and brain-imaging techniques to characterize the integrated clinical and biomarker aspects of AD and PD. Inclusion of imaging of brain microglia using GE-180, an experimental microglial ligand, provides unique insight into the relationship of brain inflammation with neuropsychological function in this project [16]. The combination of "cognitive biomarkers" and novel brain-imaging facilitates both the advancement of scientific knowledge and the growth of the investigator. As a junior investigator, Dr. Banks' career will be advanced through her project execution and mentorship with a leading imaging expert, Dr. William Jagust.

Project 2 uses cognitive assessments and fMRI to interrogate freezing and falls in patients with PD. Dr. Brent Bluett leads this program. Preliminary studies impugn executive dysfunction as a key aspect of freezing and falls [17]. Executive function and the underlying cognitive and motor circuits associated with falls will be investigated cross-sectionally and longitudinally in this project. As in project 1, the main goal of project 2 is to advance the career of Dr. Bluett, whose mentor, Dr. Irene Litvan, is a movement-disorder expert.

Project 3 adds an important translational aspect to the CNTN scientific program. Led by Dr. Jefferson Kinney of UNLV, this project uses transgenic animal models to explore the role of γ-aminobutyric acid in the modulation of inflammation in mouse models of AD. Maze learning tasks used in the cognitive assessment of the animal models will be matched with similar virtual maze learning fMRI experiments in project 1 to maximize the translational interchange between the animal and human investigations. Maturation of Dr. Kinney's skills in the use of animal models to study human disorders is guided by mentor, Dr. Bruce Lamb, who has expertise with the role of animal models in AD research [18,19].

The NIGMS has the development of the nation's scientific workforce as a key part of its mission. This includes development of skills in junior investigators [20], attracting an ethnically diverse population of scientific researchers, insuring opportunities for women in science, and advancing scientific infrastructure in states that have historically had limited access to federal funding. The combination of funding, infrastructure, and expertise leads to the key products of new scientific information and the training of new investigators, thereby addressing current problems and creating a sustainable pipeline of researchers for the future (Fig. 1). As CNTN is currently in the phase I of its COBRE-funding cycle (Fig. 2), the center is solidifying the foundation in preparation to compete for the next phase. These IDeAs programs and the COBRE award supporting the CNTN are noteworthy examples of initiatives supporting infrastructure development to advance the scientific and training agenda.

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Jeffrey L. Cummings*
Nadia Fulkerson
Cleveland Clinic Lou Ruvo Center for Brain Health
Las Vegas, NV, USA
*Corresponding author. Tel.: 702-483-6029;
Fax: 702-483-6028.
E-mail address: cumminj@ccf.org

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Alzheimer's & Dementia: Translational Research & Clinical Interventions 4 (2018)

Alzheimer's & Dementia

Featured Article

The price of progress: Funding and financing Alzheimer's disease drug development

Jeffrey Cummings[a,*], Carl Reiber[b], Parvesh Kumar[b]

[a]*Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA*
[b]*University of Nevada, Las Vegas, NV, USA*

Abstract

Introduction: Advancing research and treatment for Alzheimer's disease (AD) and the search for effective treatments depend on a complex financial ecosystem involving federal, state, industry, advocacy, venture capital, and philanthropy funding approaches.

Methods: We conducted an expert review of the literature pertaining to funding and financing of translational research and drug development for AD.

Results: The federal government is the largest public funder of research in AD. The National Institute on Aging, National Institute of Mental Health, National Institute of General Medical Sciences, and National Center for Advancing Translational Science all fund aspects of research in AD drug development. Non-National Institutes of Health federal funding comes from the National Science Foundation, Veterans Administration, Food and Drug Administration, and the Center for Medicare and Medicaid Services. Academic Medical Centers host much of the federally funded basic science research and are increasingly involved in drug development. Funding of the "Valley of Death" involves philanthropy and federal funding through small business programs and private equity from seed capital, angel investors, and venture capital companies. Advocacy groups fund both basic science and clinical trials. The Alzheimer Association is the advocacy organization with the largest research support portfolio relevant to AD drug development. Pharmaceutical companies are the largest supporters of biomedical research worldwide; companies are most interested in late stage de-risked drugs. Drugs progressing into phase II and III are candidates for pharmaceutical industry support through licensing, mergers and acquisitions, and co-development collaborations.

Discussion: Together, the funding and financing entities involved in supporting AD drug development comprise a complex, interactive, dynamic financial ecosystem. Funding source interaction is largely unstructured and available funding is insufficient to meet all demands for new therapies. Novel approaches to funding such as mega-funds have been proposed and more integration of component parts would assist in accelerating drug development.

Keywords: NIH; NIGMS; NCATS; NIMH; NINDS; Venture capital; Advocacy; Philanthropy; Alzheimer's disease; Clinical trials; Pharmaceutical industry; Biotechnology companies; SBIR; STTR

Alzheimer's disease (AD) is increasing in frequency as the world's population ages and poses a major threat to the public health. AD doubles in frequency every 5 years after the age 65, and the number of individuals in the United States with AD dementia is projected to grow from a current 5.5 million to an estimated 14 million by the year 2050 [1,2]. The world's population of AD dementia will increase from 35 million to an astonishing 135 million by 2050 [3]. The

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*Corresponding author. Tel.: 702-483-6029; Fax: 702-722-6584.
E-mail address: cumminj@ccf.org

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corresponding toll in human suffering and socioeconomic costs will be enormous. The identification of milder forms of cognitive impairment and preclinical AD further enlarges considerations regarding the impact of AD on society [2,4,5].

Prevention and treatment of AD by 2025 has been articulated as a goal of the US government and has been endorsed by other countries [6,7]. Prevention and treatment require the development of new treatments that prevent or delay the onset, slow the progression, or improve the symptoms (cognitive, functional, and behavioral) of AD. The failure rate of AD drug development is 99% [8]; the failure rate of the development of disease-modifying therapies for AD is 100%. Despite these discouraging outcomes in drug development programs, the urgent need to address the socioeconomic crisis posed by AD requires that we continue to advance understanding of AD drug development. Lessons learned from AD are likely to generalize to other neurodegenerative disorders (NDDs), given the many similarities in protein aggregation and cell injury across NDD [9]. To advance the research agenda in AD, financial resources are required including funding from government, industry, venture capital, foundations, and philanthropy. Federal research funding programs include the National Institutes of Health (NIH), National Science Foundation (NSF), Food and Drug Administration (FDA), Department of Defense, and Veterans Administration (VA). Private sector funding includes sources in the biopharma industry, venture capital investments, foundations, advocacy organizations, and support from philanthropists. Public-private partnerships have formed to help ameliorate the financial burden to individual entities, and industry collaborations have evolved to de-risk investments [10,11]. Funding and financing resources form a complex financial ecosystem, which is a key to advancing research in AD. Here, we describe major elements of this network of support especially as it pertains to development of new drug treatments for AD.

1. Cost of AD drug development

Total costs of an AD drug development program are estimated at $5.6 billion, and the process takes 13 years from preclinical studies to approval by the FDA [12]. This compares to an estimated cost of cancer treatment development of $793.6 million per agent (assuming 9% cost of capital) [13]. Considering the pharmaceutical industry as a whole bringing a new agent to approval has an estimated cost of $2.8 billion [14]. AD drug development costs substantially exceed most estimates for drugs in other therapeutic areas.

Table 1 shows the average cost and duration of each phase of AD drug development. These figures include the cost of capital and the cost of failures that companies must sustain if they work in the AD drug development arena. The high rate of failure of AD drug development is partly responsible for the high costs of advancing AD drug development [8], but out-of-pocket costs for development of a single AD agent approach $500 million (Table 1). Phase III trials are the most costly part of AD drug development, and pharmaceutical companies are among the few enterprises that can sustain such costs.

Table 1
Cost and duration of each aspect of AD drug development

Stage of process	Duration (months)	Cost (billions)* ($)	Cumulative out-of- pocket costs (at end of each stage) (millions) ($)
Preclinical	50.1	1.65	
Phase I	12.8	1.19	71
Phase II	27.7	1.04	126
Phase III	50.9	1.79	413
FDA	18	0.02	
Total	13.3 years	5.69	

Abbreviations: AD, Alzheimer's disease; FDA, Food and Drug Administration.

*Capitalized and including cost of failures of drug development (from Scott et al, 2014) [12].

2. National Institutes of Health

The principle public funder of research is the US NIH, investing more in health research than any other public enterprise in the world with an annual budget of approximately $34 billion U.S. dollars. The federal budget devoted to NIH has had support from both Republican and Democratic parties. There is a mismatch between the cost of disease to society and the amount of research devoted to it. AD, for example, costs the US society more than $216 billion annually, and it has an NIH budget of $1.8 billion; for every $1 spent on AD, less than 1% of that amount is devoted to research [15,16]. AD has a greater impact on the US economy than cancer or cardiovascular disease [15]; it has a smaller NIH research budget than either of these disorders (cancer – $6.0 billion, cardiovascular disease - $2.2 billion; www.nih.gov).

Neuroscience research at NIH is guided by the Neuroscience Blueprint and within that the NIH Neurotherapeutics Blueprint was launched to create a virtual pharmaceutical company aimed at advancing discovery and development of small molecules to treat Central Nervous System disease including NDDs [17]. The goal was to foster the development of potential therapies in Academic Medical Centers (AMCs) and biotechnology companies and to advance new therapies to clinical trials and potential industry partnership. Once funding is approved, lead discovery teams from the National Institute of Neurological Disease and Stroke work collaboratively and guide the grantee's development program. The lead team assists in bioactivity/efficacy hit-to-lead studies, medicinal chemistry and lead optimization, pharmacokinetics and toxicity, data management, manufacturing and formulation, and phase 1 clinical trials [17].

Within the NIH, the major funding agency for AD research is the National Institute on Aging (NIA). To support

the development of new therapies for AD and related dementias, the NIA funds a trial coordinating center—the Alzheimer Clinical Trial Consortium—that conducts clinical trials on AD and related disorders and advances tools and methods relevant to trials in this population. The NIA provides grant support for promising therapies to be tested with the Alzheimer Clinical Trial Consortium and its trial network. The Alzheimer Clinical Trial Consortium continues the themes of AD clinical trials initiated with the Alzheimer's Disease Cooperative Study [18]. The NIA participates in a public-private partnership—the Alzheimer's Disease Neuroimaging Initiative (ADNI)—funded partially by pharmaceutical companies and NIH whose goal is to simulate a clinical trial and collect data relevant to trial planning. The ADNI studies brain imaging and biomarker changes in longitudinal cohorts of cognitively normal individuals, participants with mild cognitive impairment, and mild AD dementia patients [19]. The ADNI has been very scientifically productive and has produced publically available data relevant to calculating sample sizes needed to power clinical trials, the predictive value of biomarkers and biomarker combinations, and the relationship of biomarkers to clinical measures [20,21]. The ADNI is seen as a model of research acceleration by a public-private partnership [19,22].

The NIA has funded a project to create a Trial-Ready Cohort for Preclinical and Prodromal AD to develop means of enhancing recruitment of participants to clinical trials using electronic means, following them with serial on-line assessments, and creating algorithms that help to predict which among the registrants have positive amyloid scans required for participation in AD clinical trials [23]. Other NIA programs relevant to AD drug development are shown in Table 2.

The National Center for Advancing Translational Science (NCATS) approaches disease states agnostically and emphasizes the development of methods, infrastructure, and collaborations applicable to all human diseases including AD. The NCATS supports both preclinical and clinical aspects of drug development [30]. Resources useful in preclinical drug development are shown in Table 3 (www.ncats.nih.gov).

The NCATS Bridging Interventional Development Gaps program enables research collaborations between individual researchers and NCATS experts to generate preclinical and clinical data through government contracts for use in Investigational New Drug applications to regulatory authorities such as the FDA (www.ncats.nih.gov). Using the Bridging Interventional Development Gap approach, the NIH outsources preclinical studies to contract research organizations (CROs) under the direction of NCATS intramural researchers with expertise in the relevant drug development areas (Table 4).

The NCATS supports clinical translational research and preclinical drug development (Table 5). The NCATS Clinical and Translational Science Awards (CTSAs) form a nationwide collaborative network of clinical trial sites that advance clinical trial training and conduct trials on many disease states [31–33]. The development of a single institutional review board for trials is an example of an initiative led by the NCATS and applied across the NIH to facilitate trials [34].

The NCATS supports federal-pharmaceutical partnerships in the Accelerating Medicines Partnership to develop agents within companies that have repositioning potential [29]. These agents were originally intended for one indication but development was halted. Their mechanism of action suggests that they may be useful in another condition, and the NCATS supports these repositioning efforts in conjunction with the pharmaceutical company and AMC investigators. AD therapies are included in the Accelerating Medicines Partnership [29].

The National Institute of General Medical Sciences (NIGMS) supports research in AD and NDD as well as many other disease states and normal physiology [35]. The NIGMS comprises three scientific divisions including Biophysics, Biomedical Technology, and Computational Biosciences; Genetics and Molecular, Cellular, and Developmental Biology; and Pharmacology, Physiology, and Biological Chemistry and the Center for Research Capacity Building. The NIGMS is responsible for basic science research grants that explore new cellular pathways and new laboratory methods, research training, and diversification of the scientific workforce. The latter includes recruitment and training of an ethnically diversified workforce as well as leadership in funding programs and projects in states that historically have received low levels of NIH funding and have not had an opportunity to develop mature scientific programs, training, and resources [36]. Work force development in states with limited NIH funding is supported by the Institutional Development Award (IDeA) program. The IDeA program includes Clinical Translational Research awards, Center of Biomedical Research Excellence grants, and IDeA Networks of Biomedical Research Excellence [37].

The Center for Neurodegeneration and Translational Neuroscience, a collaboration between the Cleveland Clinic Lou Ruvo Center for Brain Health (LRCBH) [38] and the University of Nevada, Las Vegas, is supported by a Center of Biomedical Research Excellence award and exemplifies the support by the NIGMS of research in AD and NDD. The Center for Neurodegeneration and Translational Neuroscience comprises administrative, data management and statistics, and clinical and translational research cores, as well as projects studying brain imaging and cognitive deficits in AD and Parkinson's disease and animal models of AD (see accompanying articles in this e-book).

Research in AD may be part of the portfolio of other NIH institutes. Research in behavioral issues in AD may be supported by the National Institute of Mental Health. An example of National Institute of Mental Health-funded AD research is the Clinical Antipsychotic Trials of Intervention Effectiveness—Alzheimer's Disease [39,40]. Similarly, a study of ginkgo biloba for prevention of cognitive decline

Table 2
NIA-supported resources relevant to AD drug development

NIA-supported program	Relevance to AD drug development
Alzheimer Clinical Trial Consortium	Conducts clinical trials of AD treatments with an organized network of academic clinical trial sites
Alzheimer's Disease Neuroimaging Initiative	Longitudinal multisite study of biomarkers in preclinical AD, prodromal AD, and mild AD dementia in a simulated trial structure [19–22]
Trial-Ready Cohort for Preclinical and Prodromal AD	Study to identify how best to use innovative technologies to engage participants in clinical trials and predict their biomarker status important for clinical trials [23]; conducted in partnership with GAP
AD Genetics Consortium	Identify genes related to AD risk and progression and indicative of pathways amenable to treatment [24]
National Cell Repository for AD	Repository of biological material derived from AD and other NDD available for study to find disease mechanisms that can be modified by treatment [25]
Dominantly Inherited AD Network	Characterize the natural history of patients with autosomal dominant AD
DIAN-Treatment Unit (DIAN-TU)	Conduct clinical trials in populations of participants with autosomal dominant AD mutations (funded as a partnership with the Alzheimer's Association) [26]
Alzheimer Prevention Initiative	Conducts clinical trials in patients at high genetic risk of developing AD (funded as a public-private partnership with pharmaceutical companies) [26]
Alzheimer's Disease Centers	Network of Centers that collect longitudinal data on AD and conduct AD research
National Alzheimer's Coordinating Center	Monitors, collects, archives, and provides access to data collected by the ADCs [27,28]
Alzheimer's Drug Development Program	Supports therapy development activities including medicinal chemistry, pharmacokinetics, absorption, distribution, metabolism, excretion, toxicology efficacy in animal models, formulation development, chemical synthesis under Good Manufacturing Practices, Investigational New Drug enabling studies and initial phase I clinical testing.
Pilot Clinical Trials for the Spectrum of Alzheimer's Disease and Age-related Cognitive Decline (PAR-18-175)	Funds development and implementation of phase I or II clinical trials of promising pharmacological and nonpharmacological interventions in individuals with age-related cognitive decline and in individuals with AD across the spectrum from pre-symptomatic to more severe stages of disease, as well as to stimulate studies to enhance trial design and methods.
Phase III Clinical Trials for the Spectrum of Alzheimer's Disease and Age-related Cognitive Decline (PAR-18-028)	Funds R01 grant applications that propose to develop and implement phase III clinical trials of promising pharmacological and nonpharmacological interventions in individuals with age-related cognitive decline and across the AD spectrum from presymptomatic to more severe stages of disease.
AD Sequencing Project	Whole genome and whole exome sequencing of genes relevant to AD (discovery and follow-up study)
Molecular Mechanisms of the Vascular Etiology of Alzheimer's Disease Consortium	Supports research to better understand how the vascular system may be involved in the onset and progression of AD and related dementias.
Alzheimer's Preclinical Efficacy Database (AlzPED)	AlzPED provides tg model data across relevant translational criteria data sets such as therapeutic agents and targets. AlzPED is designed to help identify the critical data, design elements, and methodology missing from studies; making them susceptible to misinterpretation, less likely to be reproduced, and reducing their translational value. Through this function, AlzPED is intended to influence the development and implementation of reproducibility strategies, including guidelines for standardized best practices for the rigorous preclinical testing of AD candidate therapeutics.
Accelerating Medicines Partnership-Alzheimer's Disease Target Discovery and Preclinical Validation Project	The goal is to shorten the time between the discovery of potential drug targets and the development of new drugs for AD treatment and prevention by integrating the analyses of large-scale molecular data from human brain samples with network modeling approaches [29].

Abbreviations: AD, Alzheimer's disease; NIA, National Institute on Aging; NDD, neurodegenerative disorders; GAP, Global Alzheimer Platform.

in older adults was supported by the National Center for Complementary and Alternative Medicines [41].

Research funds are accessed through competitive grants that support various types of research (Table 6). The NIH grants include "direct costs" that cover the expenses of the proposed research and "indirect costs" that are provided to the institutions hosting the research to account for research-related expenses not covered by the direct costs including facilities, personnel management, and administration. These indirect costs can comprise up to 60% or more of the total award and have become a major source of revenue for research-intensive institutions [42]. This indirect support is an essential part of the research ecosystem.

In addition to grants, the NIH supports small business initiations through Small Business Innovation Research and Small Business Technology Transfer grants. These grants are a key channel through which discoveries in academic laboratories can be commercialized through small start-up companies that begin the process of product development with the aim of eventually partnering the agent, device, or process for regulatory approval and commercialization. The IDeA program sponsors four Regional Technology Transfer Accelerator Hubs to facilitate development of Small Business Innovation Research and Small Business Technology Transfer applications from IDeA state investigators. The grants show the value of science in stimulating the economy and creating jobs.

The NIH sponsors some large-scale trans-institute programs that address problems applicable to many institutes and populations. The Brain Research through Advancing

Table 3
NCATS resources for preclinical drug development

- Small molecules, compounds, and probes
  - Assay Guidance Manual contains detailed information on developing appropriate assays for high-throughput screening projects
  - Compound management team acquires chemical libraries for small molecule screening
  - Clinical Genomics Center provides access to the NCATS pharmaceutical collection, a publicly available, web-based database with complete information on 2508 drugs approved in the United States and a total of 8969 (as of 2011) agents worldwide that could be repurposed for treatment of human disorders
  - Chemical Genomics Center CurveFit serves as a public, stand-alone, and open-source version of the center's own curve-fitting software, automatically fitting and classifying observed dose-response curves
  - PubChem contains a freely accessible database of small organic molecules and their activities in biological assays
  - Phenotypic Drug Discovery Resource enables access to disease-relevant assays to explore the effects of small molecules on molecular processes
- Biomarkers
  - Biomarkers, Endpoint, and Other Tools Resource hosts an online glossary developed by a FDA and NIH joint committee to clarify terms used in translational science and medical product development
- Informatics tools and information systems
  - Global Ingredient Archival System houses a registration system for the ingredients in medicinal products that makes it easier for stakeholders to exchange information about substances in medicines, supporting scientific research on the use and safety of these products

Abbreviations: FDA, Food and Drug Administration; NIH, National Institutes of Health; NCATS, National Center for Advancing Translational Science.

Innovative Neurotechnologies initiative is one such activity. The Brain Research through Advancing Innovative Neurotechnologies is supported by a partnership of the NIH, NSF, Defense Advanced Research Projects Agency, private foundations, and researchers [43]. The goal of the Brain Research through Advancing Innovative Neurotechnologies is "to accelerate the development and application of innovative technologies to construct a dynamic picture of brain function that integrates neuronal and circuit activity over time and space" [44,45]. Understanding of brain networks in AD will be among the many benefits of this project.

3. Non-NIH federal funding

Non-NIH federal agencies have smaller research budgets and grant portfolios related to AD. These agencies include the NSF, VA, Department of Defense, FDA, Department of Energy Office of Science, National Library of Medicine, and Centers for Medicare and Medicaid Services.

Table 4
Components of the NCATS BrIDGs program

- Synthetic process development
- Scale-up and manufacture of active pharmaceutical ingredients
- Development of analytical methods
- Development of suitable formulations
- Pharmacokinetic/ADME studies, including bioanalytical method transfer and validation
- Range-finding initial toxicology studies
- IND-enabling toxicology studies
- Manufacture of clinical trial supplies
- Product development planning and advice in IND preparation

Abbreviations: NCATS, National Center for Advancing Translational Science; BrIDGs, Bridging Interventional Development Gaps; ADME, absorption, distribution, metabolism, and excretion; IND, investigational new drug application.

The VA funds Geriatric Research, Education, and Clinical Centers that support research projects in AD. The VA projects that approximately 218,000 veterans will be diagnosed with dementia in 2017, an increase of more than 40,000 since 2008 and an urgent cause of concern for how to best meet the needs of aging veterans.

The NSF has grants in Integrative Organismal Systems, Molecular and Cellular Biosciences, and Computational Neurosciences among many areas of investment (www.nsf.gov). Some of these address issues important to understanding AD.

The FDA created the Critical Path Institute (C-Path) which sponsors the Clinical Data Interchange Standards Consortium and the Coalition Against Major Diseases (CAMD). These enterprises develop strategies for data interoperability and for qualification by the FDA of clinical trial assessments and biomarkers. The CAMD led the successful effort to qualify a simulation method of AD clinical trials useful for trial planning [46]. The CAMD also created the CAMD Online Data Repository for AD consisting of standardized placebo group data from 24 AD trials numbering 6500 subjects. The CAMD Online Data Repository for AD represents a unique integrated standardized clinical trial database whose size facilitates a comprehensive understanding of disease heterogeneity and progression [47].

The Centers for Medicare and Medicaid Services funds demonstration projects such as the Imaging Dementia: Evaluating Amyloid Scanning study that is assessing the impact of amyloid imaging on short- and long-term mild cognitive impairment and AD patient outcomes. These data are critical to decide whether amyloid imaging should be reimbursed by the Centers for Medicare and Medicaid Services as part of clinical care. Amyloid imaging is routinely used in clinical trials and Imaging Dementia: Evaluating Amyloid Scanning will help in the translation of trial observations to clinical care.

The Department of Defense has funded imaging research involving positron emission tomography and funds research

Table 5
Clinical drug development resources of NCATS

- Clinical and Translational Science Awards, a network of university-based clinical trial sites
- Accelerated Clinical Trial Agreement, a standardized contract model designed to reduce negotiation time and contracting delays for industry-sponsored multisite clinical studies
- BEST Resource, an online glossary of terms used in translational science and medical product development
- ClinRegs, a public website that helps researchers navigate country-specific regulatory information as they plan and implement clinical trials
- Good Clinical Practice Social and Behavioral Research E-Learning Course, provides training for application of Good Clinical Practice principles to social and behavioral research
- Streamlined, Multisite, Accelerated Resources for Trials IRB Reliance Platform, an umbrella agreement that establishes a harmonized approach for roles and responsibilities of a single institutional review board (IRB) and participating sites
- PhenX Toolkit, well-established, broadly validated measures of phenotypic traits and environmental exposures of interest to investigators in human genomics, epidemiology, and biomedical research
- REDCap, an easy-to-use, freely available tool for clinical study management and data capture
- ResearchMatch, a way to connect people who are trying to find research studies with researchers who are seeking people to participate in their studies

Abbreviations: NCATS, National Center for Advancing Translational Science; BEST, Biomarkers, Endpoint, and Other Tools Resource.

in traumatic brain injury and chronic traumatic encephalopathy relevant to AD.

4. State funding

Some states provide funds for AD centers or AD-related research projects. For example, California funds California Alzheimer's Disease Centers and provides grant support for research projects. Texas funds a Consortium of Alzheimer's Disease Centers, New York supports Centers for Excellence for Alzheimer's Disease, and the Nevada legislature has supported the Cleveland Clinic LRCBH that provides AD and Parkinson's disease care and research in conjunction with the Center for Neurodegeneration and Translational Neuroscience.

5. Academic Medical Centers

AMCs are key to innovation in understanding disease biology, discovery of potential treatment interventions, and initiation of projects that can lead to product commercialization including new drugs for prevention and treatment of AD. AMCs have two main goals: teaching of the next generation of clinicians and biomedical scientists and discovery of new knowledge by their clinical and scientific faculty. In

Table 6
Major NIH grant types (https://grants.nih.gov/grants/funding/ac_search_results.htm)

Grant title	Grant number	Grant description
Research Construction Programs	C06	Research Facilities Construction Grant
Institutional Training and Director Program Projects	DP1	NIH Director's Pioneer Award
Institutional Training and Director Program Projects	DP2	NIH Director's New Innovator Awards
Institutional Training and Director Program Projects	DP4	NIH Director's Pathfinder Award - Multi-Year Funding
Research Career Programs	K12	Physician Scientist Award (Program)
Research Career Programs	K21	Scientist Development Award
Research Career Programs	K23	Mentored Patient-Oriented Research Career Development Award
Research Program Projects and Centers	P20	Exploratory Grants
Research Program Projects and Centers	P30	Center Core Grants
Research Program Projects and Centers	P50	Specialized Center
Research Projects	R01	Research Project
Research Projects	R13	Conference
Research Projects	R21	Exploratory/Developmental Grants
Research Projects	R33	Exploratory/Developmental Grants phase II
Research Projects	R34	Planning Grant
Research Projects	R41/42	Small Business Technology Transfer Grants—phase I and phase II
Research Projects	R43/44	Small Business Innovation Research Grants—phase I and phase II
Research-Related Programs	S06	Minority Biomedical Research Support-MBRS
Research-Related Programs	S11	Minority Biomedical Research Support Thematic Project Grants
Research-Related Programs	S21	Research and Institutional Resources Health Disparities Endowment Grants -Capacity Building
Training Programs	T32	Institutional National Research Service Award
Training Programs	T37	Minority International Research Training Grants (FIC)
Cooperative Agreements	U01	Research Project–Cooperative Agreements

Abbreviation: NIH, National Institutes of Health.

the course of achieving their goals, AMCs deliver care to patients and are part of the health-care system.

Most basic science research conducted at AMCs is funded by the NIH augmented by philanthropists, state funding, and biopharma partnerships. The pharmaceutical industry has downsized its internal research capacities and focused on late stage drug development and commercialization. To insure a steady flow of candidate compounds into their pipelines, many pharmaceutical companies have forged alliances with AMCs [48–54]. They fund AMC investigator research in areas of mutual interest in return for access to information, technology transfer, and commercialization opportunities. AMCs protect the intellectual property of the institution and the investigator through contractual arrangements implemented by Technology Transfer Offices [55,56]. A recent survey identified 78 AMC-based drug discovery centers in the United States with 45 addressing neuropsychiatric and NDD targets [57]. The majority of funding for these centers came from federal sources, but some centers had substantive relationships with for-profit enterprises, mostly pharmaceutical companies.

Investigators in AMCs "spin off" biotechnology start-ups that typically focus on one promising compound, device, or discovery that has commercial potential. The Small Business Innovation Research and Small Business Technology Transfer grants facilitate this process of initiating new biotech start-ups. Angel funds, seed capital, and philanthropy assist AMC faculty in advancing the commercialization process. The spin-off companies are important sources of innovative new drugs. Approximately half of recently approved agents came from small biotechnology companies and AMC laboratories [58–60]. An entrepreneurial spirit is required to bridge the gap between academic culture and attracting private funding in the quest to commercialize a product. Products can be new drugs and treatments but might also be biomarkers with commercial potential or patentable processes that save time or money. Recently, venture capital companies have begun to form relationships directly with AMCs to encourage innovation, support start-ups, and access new products moving toward commercialization.

Fig. 1 provides an overview of how ideas for products originating in AMCs generate financial support, leading to eventual commercialization.

Adjustments are required by AMCs to facilitate drug development by faculty. The AMC conflict of interest policies often impose stringent limitations on academic-industry relationships and have the unintended consequence of hindering the participation of academic investigators in the drug discovery and development process [61,62]. As industries increasingly turn to academic laboratories for target identification and early-stage treatment candidate development and to academic clinics for clinical trial leadership and execution, conflict of interest rules must evolve and be sufficiently flexible to allow AMC investigators to take advantage of the opportunities offered through industry collaboration while limiting influences that may be perceived as inappropriate [49]. Similarly, recognition of the important role of faculty involved in drug development including industry-sponsored research through academic promotion and award of tenure is critical to establishing a culture of drug development in AMCs.

To enhance their role in AD drug discovery and development AMCs need to provide students, residents, fellows, doctoral candidates, and others interested in AD therapeutics with courses, learning experiences, programs, and leadership that will acquaint them with the drug development processes and opportunities. The Stanford SPARK program offers a model of how this can be achieved [63]. SPARK is a hands-on training program in translational research providing guidance and seed funds to teach how to develop and commercialize drugs and diagnostics.

A major threat to AMC-industry collaboration is the lack of reproducibility of many findings reported from academic laboratories. Protocol errors, lack of statistical rigor in data analysis, and inadequate reporting have resulted in poor reproducibility and lack of confidence in research executed in AMC laboratories [64]. Rigorous adherence to conduct and reporting of basic and animal research is necessary to restore confidence in academic laboratory reports and facilitate academic-industry collaborations [65].

6. Biotechnology companies and private equity investment in AD drug development

Biotechnology companies can be defined as venture-backed drug development firms using technological applications centered on biological systems, living organisms, or their derivatives [66]. "Biotech" includes the disciplines of genetics, molecular biology, biochemistry, embryology, and cell biology and is linked to biomaterials, cell therapy, gene therapy, immunotherapy/vaccines, protein therapeutics, and some specialty pharmaceuticals and small-molecule therapeutics [66]. Success in AD drug development will produce a very high return on investment. This possibility attracts venture capital investment to AD research, but the high rate of failure has kept this funding stream small [67]. Venture capital investment in Central Nervous System disease declined 40% in the 2009–2013 period compared with the 2004–2008 period [68]. Angel investors or seed capital providers have high risk tolerance and supply small amounts of money to encourage novel ideas. If the concepts begin to mature and promise to lead to a successful program, venture capital may be attracted to allow more advanced drug development. Venture capital funds are usually raised in "rounds" of stock option sales (rounds A, B, and C) as milestones are reached in the drug development process. Venture capital investors typically want relatively fast turn-around on their investment; exit strategies for venture capital investors include transition of the biotech to partnerships, licensing agreements, co-development or co-marketing agreements, and progression to stock sales and initial public offerings. Venture

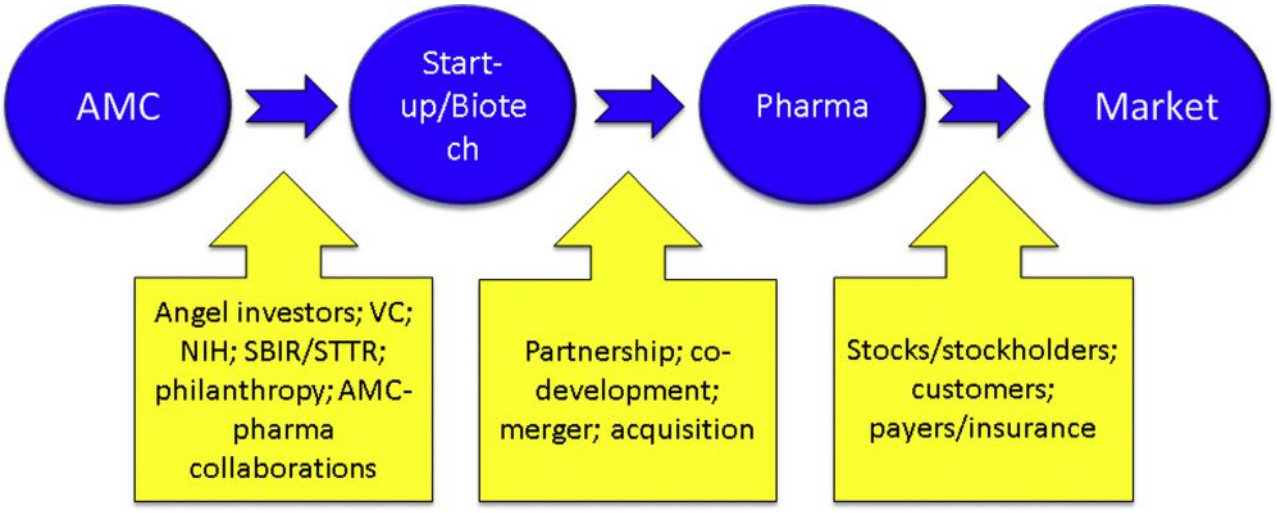

Fig. 1. Financial ecosystem beginning with discovery in an academic medical center (AMC) and progressing through biotechnology to the pharmaceutical industry and eventually to market. Each stage of the process is supported by specific types of capital.

capital investments available specifically to support AD drug development include Dolby Family Ventures and the United Kingdom-based Dementia Discovery Fund. Bill Gates of the Gates Foundation recently contributed $50,000,000 to the Dementia Discovery Fund and is providing $50,000,000 of additional venture capital to encourage AD drug development in the biotechnology sector [69].

Candidate therapies may pass from smaller to larger biotech companies as biotechs seek to strengthen their pipelines, progress toward vertically integrated Central Nervous System companies, or attract investors interested in a broader portfolio. This can be a healthy process allowing drugs to progress in testing before major pharmaceutical companies invest; however, the process also may lead to abuse by passing flawed agents from company to company and attracting capital from enthusiastic but under-informed investors.

7. Advocacy organizations

The Alzheimer Association is the largest private noncorporate funder of AD research. In 2016, the association invested $90 million in research, including $25 million in new project investments and the rest in support of ongoing multi-year commitments [70]. The new project support included $7 million for clinical trials targeting brain inflammation and $4.3 million for the Dominantly Inherited AD Network-Treatment Unit [71] (Table 2).

The Alzheimer Foundation of America and UsAgainstAlzheimer's support advocacy for AD funding and have helped advance the national AD research agenda including maintaining and increasing funding for AD research. UsAgainstAlzheimer's helped advance the Global Alzheimer Platform whose goal is to enhance recruitment and trial conduct to accelerate AD drug development [23].

Advocacy plays a critical role in raising consciousness about AD, referring patients to trials, supporting families, providing research grants, and advocating for increased funding. In some cases, advocacy collaborates directly with laboratories or biotech companies to raise funds for drug development [72,73].

8. Philanthropy

Philanthropists make contributions to advocacy organizations or directly to universities and scientists to support research projects. Many philanthropists are motivated by the experience of AD afflicting a family member, and many family philanthropies have originated with the intent of honoring a family member. Philanthropy plays a critically important role in the AD research ecosystem. Philanthropy often provides seed money for small projects that do not yet have preliminary data that would support a federal grant application. Philanthropy can fund high-risk/high-reward projects that might be too risky to receive funding from other sources such as the NIH.

The Alzheimer's Drug Discovery Foundation (ADDF) is a venture-philanthropy organization that is a key player and innovator in the AD drug discovery and development landscape. The ADDF funds studies in animal models, provides grants to fund animal toxicity testing of promising therapies, and supports early-stage proof-of-concept clinical trials. The venture philanthropy model allows the ADDF to take an ownership position in early-stage companies they fund and re-invest any revenues generated. Venture philanthropy is being more commonly applied as a vehicle for collaboration of foundation and advocacy groups with biotechnology companies [74].

The Cleveland Clinic LRCBH is an AD care and research organization in Las Vegas, Nevada [38]. It is a leader in drug

development and clinical trials. The LRCBH was created and continues to be supported by philanthropists. The LRCBH demonstrates how philanthropy can influence a community to develop resources for AD research, creating a new AD research and drug development enterprise where none existed previously. Once established, philanthropy-based projects can attract federal funding and build clinical trials programs to garner support from other sources. The LRCBH now hosts a Center of Biomedical Research Excellence award from NIGMS as well as other federal funding and biopharma industry support. Multiple funding sources are critical to the sustainability of an AD research organization.

The Cure Alzheimer's Fund and Bright Focus Foundation are two philanthropies that provide grants to AD researchers doing innovative research and have had a substantial influence on research progress.

FasterCures is a disease-agnostic organization promoting information about drug development, convening meetings of drug development stakeholders, and doing analyses of and publishing novel means of advancing drug development (e.g., patient engagement strategies). FasterCures has a Philanthropy Advisory Service that studies disease areas and advises philanthropists on where investments will have maximum impact. The Philanthropy Advisory Service conducted such as analysis for AD [75].

9. Pharmaceutical industry

The pharmaceutical industry is the largest funder of drug discovery and development research in the world, exceeding that of NIH or any other funding organization. Biopharma funds approximately 60% of all annual US research and development activities. The total annual research and development budget for biopharma (biotechnology and pharmaceutical industry) in 2016 was $75 billion [76]. Over 70% of all AD clinical trials are sponsored or co-sponsored by the pharmaceutical industry [77].

Payments from biopharma support much of the AD drug development ecosystem. New agents may be accessed through AMC collaborations, in-house discovery teams, acquisitions of biotechnology companies, mergers with other pharmaceutical companies, in-licensing of promising compounds, and partnering and co-development arrangements. Each of these has corresponding financial support by the pharmaceutical company. Extensive in-house resources and out-sourcing to CROs are needed for each aspect of drug development—toxicity testing, manufacturing, supply line management, site management, recruitment of participants to trials, regulatory affairs, and so on. Outsourcing to CRO's accounted for approximately $20 billion of the 2016 biopharma research and development budget. For global drug development much of the infrastructure must exist in each country in which the company supports research activities [78].

Clinical trial sites are reimbursed for all activities provided to conduct biopharmaceutical trials, including trial site start up, gaining the institutional review board permission, managing the drug supply, advertising for participants to enter the trial, conducting all assessments (imaging, clinical interviews, rating scales, lumbar puncture, and so on), providing all data to the sponsor, and eventually closing the trial and maintaining records for 5 years after trial completion. Indirect payments (usually in the range of 30%–35% of total costs) are provided to the institutions hosting the research program. These payments comprise an important part of the financial infrastructure of many research organizations conducting clinical and translational research. All research must be free of charge to participants.

10. Drug development ecosystem

Fig. 2 summarizes the interactions of the organizations described previously to compose an ecosystem that supports drug development for AD. Advancing new treatments is not the only outcome on which the scientific enterprise is brought to bear, but it is among the most important to citizen-taxpayers who fund aspects of this work, and it serves as an important example of the interaction of the public and private sectors to improve public health.

The NIH is the principle supporter of investigator-initiated research that leads to new targets and potential new interventions. Pharmaceutical companies partner with AMCs to support basic science research as they increasingly divest themselves of in-house research laboratories. Following optimization, the lead agent is tested for efficacy in animal models to determine if effects in an animal model system are supportive of the goals for the molecule. Animal models have not predicted efficacy in humans, but advancing an agent without knowledge of its effects in models would be unwise [79]. Animal model assessments might be financed through NIH funding to AMC investigators, by biotechnology companies, or by pharmaceutical companies.

Once there is sufficient confidence in efficacy at the animal model or test system level, the agent must be assessed for toxicity and the range of doses safe in animals established. Rodent and dog species are commonly used for toxicity assessments. Financing this aspect of drug development can be very difficult and comprises part of the "valley of death," where promising drugs stall because no funding is available for this critically important step in new drug development [22,80,81]. CROs exist to conduct these studies, and other potential sources of support include the NIH NCATS program. Biotechnology companies supported by venture capital can fund this step if the investors are convinced of the return on investment, and venture philanthropy such as the ADDF has supported these studies.

Once safety and efficacy have been shown at the animal level, the drug can be advanced to phase I first-in-human trials. Phase I typically involves healthy volunteers to

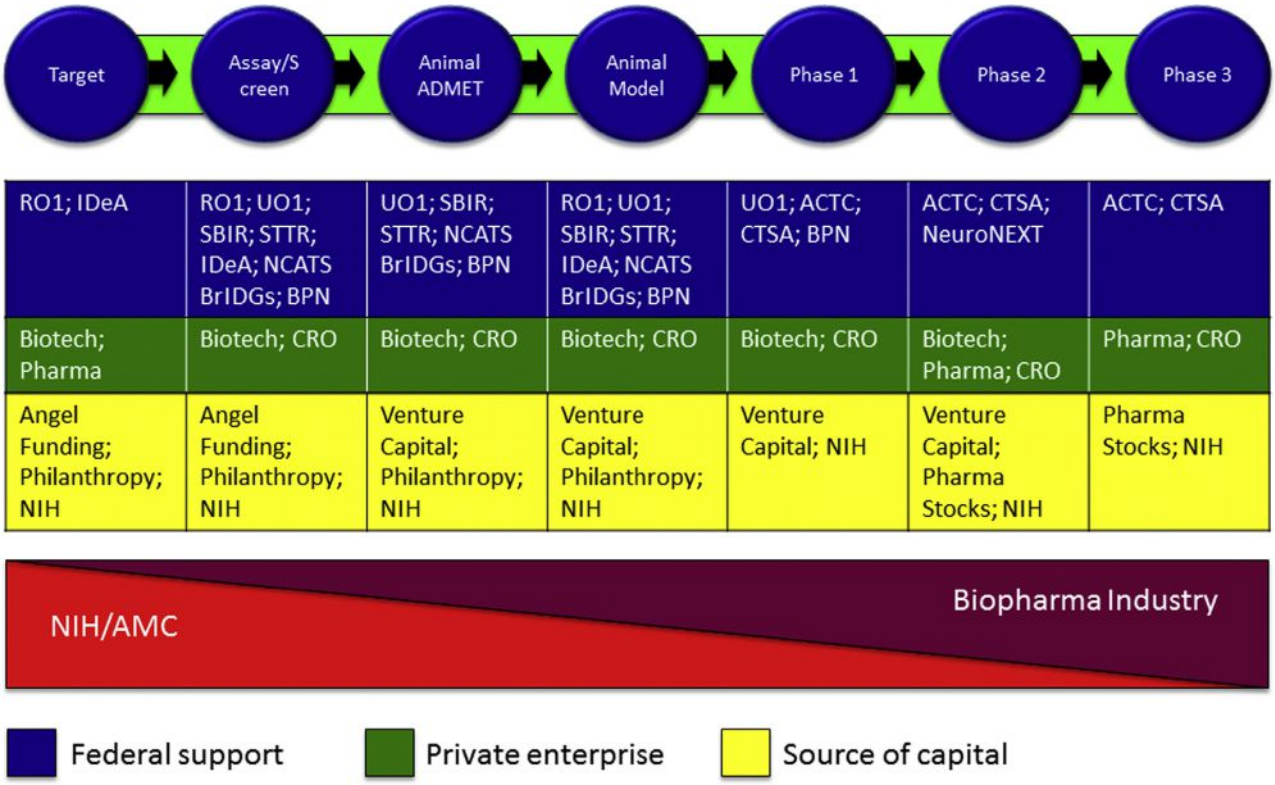

Fig. 2. Drug development ecosystem: phases of drug development and sources of support for each phase. Abbreviations: ACTC, Alzheimer Clinical Trial Consortium; BrIDGs, Bridging Interventional Development Gaps; BPN, Blueprint Neurotherapeutics Network; CRO, Contract Research Organization; CTSA, Clinical and Translational Science Award; IDeA, Institutional Development Award from National Institute of General Medical Sciences (NIGMS); NCATS, National Center for Advancing Translational Science; NIH, National Institutes of Health; SBIR, Small Business Innovation Research; STTR, Small Business Technology Transfer grants.

determine dose, tolerability, and pharmacokinetics of an agent in humans. This phase also faces substantial funding challenges and is part of the valley of death. The NIH may support phase I trials through Clinical and Translational Science Award programs. Biotechnology and pharmaceutical companies may subcontract to CROs to perform the phase I assessments using venture capital or internal budgets generated by sales of other products. Pharmaceutical companies prefer to engage in drug development in late phase II or phase III but sometimes use partnership, in-licensing, acquisition, or co-development strategies earlier in the drug development process if the agent seems very likely to succeed and has a good strategic fit with company objectives. Phase II (learning trials to establish proof-of-concept in patients with AD) is usually financed through biotechnology and pharmaceutical companies, and phase III (confirmatory trials required to advance an agent to regulatory review) is dominated by large pharmaceutical companies although large- and medium-sized biotechnology companies may sometimes advance agents through phase III and to regulatory approval. CROs are typically used to conduct phase II and III trials; some pharmaceutical companies have in-house trial execution capacity. Regulatory review preparation is typically led by in-house regulatory affairs teams, but CROs with regulatory expertise are available to support all or part of this process. Marketing of approved agents to make the new treatment widely accessible to patients is performed by pharmaceutical companies or the large- and mid-sized biotechnology companies that have escorted the drug through phase III trials and regulatory approval.

Ideally, the drug development process will produce products for FDA review that will eventually come to market while also serving as a learning experience to generate new agents as understanding of AD biology progresses. Effective life cycle management of approved agents will extend their use to new populations and new indications (Fig. 3).

11. Innovations in financing translational research

The extreme expense of current drug development for AD is not sustainable (Table 1), discourages companies from working in the AD research arena, dissuades venture capital from investing in AD drug development, and diminishes the opportunity to advance new therapies for patients with AD. Innovation is needed to improve the financial underpinnings of AD drug development and translational research.

Modeling suggests that it will take an estimated $38.4 billion over a decade to deliver a robust pipeline of AD therapeutics [82]. No single investment entity can undertake

Fig. 3. The drug development system envisioned as a cycle that develops new products for FDA review and feeds back to the cycle for improved product development. Abbreviation: FDA, Food and Drug Administration.

such a financial burden; a combination of federal and private equity would allow the development of a mega-fund structure to cover the costs and underwrite AD therapeutic development [83]. This would accommodate a high failure rate and decrease the risk of the investment by distributing the opportunity for success among multiple agents and allowing parallel development of multiple treatment approaches.

Public-private partnerships are an effective means of advancing research by distributing the cost among federal and private sources [10,11,84,85]. This can be especially effective in precompetitive arenas such as biomarker development, disease modeling, and advancing analytics [86]. As noted, the ADNI is an example of a very productive research program jointly funded by the NIH and several pharmaceutical companies.

A novel approach that has emerged involves venture funding approaches adopted by some advocacy groups to directly fund drug development [72].

Crowd funding is another innovation using web-based means of raising funds. This has succeeded in generating small amounts of funding to inaugurate new drug development programs [87,88]. Crowd-sourcing of drug development problems is another innovation using motivational prizes to harness the creativity of web-connected individuals.

Collaboration of two or more pharmaceutical companies is a means of distributing financial risk of AD drug development. Co-development and risk sharing is an increasingly popular strategy. Current examples include collaborative development of a β-site amyloid precursor protein cleaving enzyme inhibitor by Eli Lilly and AstraZeneca and co-development of a β-site amyloid precursor protein cleaving enzyme inhibitor and an anti-amyloid antibody by Eisai and Biogen. The Alzheimer Prevention Initiative is an example of collaboration among NIH, a private institute (Banner Alzheimer Institute), and two pharmaceutical companies [26].

Research centers poised at the interface of health-care systems and academic universities and committed to advancing treatment innovations represent another evolving development that can advance drug development. The Oxford Biomedical Research Center is an example [89].

Funding from the NIH, the Alzheimer's Association, and many other organizations is awarded on a competitive basis with each application scored by scientific peers with funds given primarily on the basis of the rank of the score. An alternative model is used by the Adelson Medical Research Foundation. In this approach, a field-limiting problem is identified by a group of experts, means of solving the problem are posed, and the quality of the proposed solutions reviewed. Skills and resources from several laboratories are usually required to address the identified problem. All participants must agree to collaborate and share data. Once these requirements are fulfilled, all collaborators are funded.

More innovation in financial structures is needed to sustain and accelerate AD drug development. In addition, the ecosystem is relatively unstructured, lacking a comprehensive roadmap for how to optimize and accelerate the process of moving promising treatments through the pipeline. In some cases, promising compounds are not supported while flawed agents find funding and are advanced. The current funding and financing ecosystem is too limited to advance new therapies quickly enough to meet the needs of the burgeoning patient population.

12. Summary

AD research and treatment development requires extensive capital. Funding from federal agencies, state appropriations, private equity, philanthropy, and advocacy is needed to achieve the goal of developing treatments to prevent, delay, slow the progress, or improve the symptoms of AD. Given the high cost of caring for these disorders and the projected increase in the population of those affected, the investment will more than repay itself in decreased costs, market revenue, and improved quality of life for patients.

AD drug development must be accelerated to address the unmet needs of the growing AD population. Greater collaboration among stakeholders, more precompetitive cooperation among industry members, more flexible AMC-industry partnerships, greater investment in basic research to identify viable targets and biomarkers, improved preparation of students for careers in drug discovery and development, more open forums for exchange of ideas about promising compounds, greater risk sharing in the expensive later stages of drug development, and more innovation in drug discovery/development financing can all contribute to finding effective treatments urgently needed by those with or at immanent risk of manifesting AD. The efficiency of drug development must also be improved. Faster

assessment of drugs in nonclinical settings, improved biomarkers to detect effects with smaller sample sizes, and improved conduct of trials can all contribute to decreasing costs of drug development [90,91].

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RESEARCH IN CONTEXT

1. Systematic review: Drug development for Alzheimer's disease (AD) and neurodegenerative disorders (NDD) has a high failure rate and the costs of drug development are very high. These factors combine to reduce interest in AD drug development and discourage investment from venture capital, biotechnology, philanthropy, and pharmaceutical companies in AD therapeutic development. Understanding the financial ecosystem underpinning AD drug development provides insights into this complex process and suggests opportunities for improvement.
2. Interpretation: Drug development typically begins with National Institutes of Health (NIH)-supported basic science research. These investigations might be supported by National Institute on Aging, National Institute of Neurological Disease and Stroke (NINDS), or National Institute of General Medical Sciences (NIGMS). Spinoffs and startups from academic laboratories are financed through small business awards from the NIH, angel funding, or seed monies from philanthropists and donors. Increasing confidence in a drug through toxicity studies and animal efficacy is supported by biotechnology companies and venture capital. As compounds mature into the clinical phase of testing, support from pharmaceutical companies is typical although biotechnology companies and federal agencies can also support advanced drug development.
3. Future directions: AD drug development depends on a complex funding and financing ecosystem. Novel mechanisms for funding drug development are evolving and improvement in the efficacy of drug development funding can accelerate the development of new therapy for patients with AD and other NDD.

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Featured Article

Network-based assessment of collaborative research in neuroscience

Gwen C. Marchand[a,*], Jonathan C. Hilpert[b], Kristine M. Bragg[a], Jeffrey Cummings[c]

[a]*University of Nevada, Las Vegas, College of Education, Center for Research, Evaluation, and Assessment, Department of Educational Psychology and Higher Education, Las Vegas, NV, USA*

[b]*Georgia Southern University, College of Education, Department of Curriculum Foundations and Reading, Evaluation, Assessment, Research, and Learning (EARL) Program, Statesboro, GA, USA*

[c]*Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA*

Abstract

Introduction: The purpose of this study was to describe collaborative research in neuroscience within the context of the Center for Neurodegeneration and Translational Neuroscience (CNTN), a Center of Biomedical Research Excellence supported by the National Institute of General Medical Science. Drawing upon research on the science of team science, this study investigated the way that interactions around research emerged over the course of establishing a new research center. The objectives were to document changes in research activity and describe how human research support infrastructure functioned to support the production of science.

Methods: Social network analyses were used to model coauthorship relationships based on publication histories from baseline (2014) through the current grant year (2017) for key personnel (n = 12), as well as survey data on collaborative engagement among CNTN members (n = 59).

Results: Exponential random graph models indicated that over time, CNTN members were increasingly likely to form coauthorship relationships. Community detection algorithms and brokerage analyses suggested that the CNTN was functioning as intended to support scientific development.

Discussion: Assessment of team science efforts is critical to evaluating and developing appropriate support structures that facilitate successful team science efforts in translational neuroscience.

Keywords: Collaborative research; Neuroscience; Center for Neurodegeneration and Translational, Neuroscience (CNTN); Center of Biomedical Research Excellence (COBRE); National, Institute of General Medical Science (NIGMS)

1. Introduction

Effective assessment of multidisciplinary collaborative research efforts requires the use of assessment strategies that can determine how collaborative research teams are functioning to meet goals, document changes in scholarly productivity, evaluate mentorship relationships, provide early notification of ineffective research supports and structures, identify sources of bottlenecks in information flow, and outline the extent to which resources are being used appropriately [1,2]. In the context of the Center for Neurodegeneration and Translational Neuroscience (CNTN) funded through the National Institute of General Medical Sciences (NIGMS) Centers for Biomedical Research Excellence (COBRE) program, assessment acts to support the development of human capital and research infrastructure necessary for the success of neuroscience research and investigators. The CNTN is reflective of the emerging trend in collaborative, or team, science that has gained ground in biomedical research in part due to the growing evidence that impactful and innovative scientific advances are more likely to result from collaborative science efforts [3–5]. The science of team science, or documenting and evaluating the development and outcomes of collaborative research, has grown into its own robust field, catalyzed by evaluation and assessment policies and recommendations from extramural funding

*Corresponding author. Tel.: 702-895-4303; Fax: 702-895-3253.
E-mail address: gwen.marchand@unlv.edu

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agencies and programs, such as the National Institutes of Health Clinical and Translational Science Awards (CTSA) [6,7]. Although work in this area has used network analytic techniques, for example, documenting the types of networks formed via collaboration [8,9] and productivity metrics of these networks [3,10,11], there remains much to learn from these techniques about how sustainable patterns of collaboration develop to support science.

Funders of biomedical research invest considerable resources into the preparation of emerging medical and academic researchers [12] and development of research infrastructure for neuroscience, which in this case, included human capital for research support. We refer to human capital for research support as teams of individuals who support the production of science. Critical individuals may include, but are not limited to, grant managers, clinical managers and staff, technicians, and students. Individuals such as grant managers and technicians rarely appear in assessments of team science [11] but are often critical to the production of research. Aims of the CNTN include supporting investigators working in human and animal models of neuroscience to produce initial data and assisting investigators in the development of advanced translational neuroscience skills, particularly in the areas of imaging and statistics. For many investigators, lack of research support, infrastructure, and the opportunity to develop advanced skills needed to conduct high-quality research are a detriment to producing scholarly products and grant proposals that are competitive for extramural funds [8,12]. The existence of a robust science infrastructure is critical to facilitating these interactions. This study reports on assessment results of the growth and development in shared authorships among key CNTN members, as well as the functioning of CNTN research support networks designed to support the production of neuroscience research.

1.1. Program evaluation and assessment in collaborative neuroscience research

Within the biomedical sciences, program evaluation research has focused largely on either the impact of scientific research in basic and applied settings, or the collaborative nature of scientific research teams, or the career advancement of investigators [2,13–15]. While no specific set of guiding principles exists solely for the purposes of evaluating scientific research, evaluation research to date has followed guidelines set by the American Evaluation Association broadly intended to cover all kinds of evaluation [16]. In recent years, assessment in government-funded research has grown to play an increasing role in evaluating research quality, reducing costs, and disseminating research credibility to the public [17]. Expenditures from the public purse must increasingly be justified by their measureable impact. Furthermore, a growing presence of translational science–specific evaluation literature [2,18,19] can be attributed to the requirement of a formal evaluation component for all National Institutes of Health CTSA [2].

The CTSA evaluation literature has produced a number of research articles supporting several evaluation designs appropriate for capturing and characterizing the nature of translational research programs [20–23]. Multidisciplinary teams working on biomedical science form and develop in a dynamic manner over time, self-organizing around research topics, specialized skills, and knowledge domains [24]. Studies have demonstrated innovation in describing the complexity of translational teams through various approaches including mixed methods, case study, and network analysis designs [1].

Evaluation may play a critical role in describing interactions within innovative scientific teams. The major challenge for evaluators is appropriately documenting the nature of these interactions to identify patterns that can be used in the service of promoting effective collaborative science. A limiting factor is that little is known about the predictors of successful collaboration, mechanisms that support collaborative researchers' development, or barriers to collaborative success [25,26]. While collaborative teams deliver greater levels of productivity over time and reap the benefits of increased visibility within the scientific community, there are few explanatory models to account for these outcomes [24,27].

1.2. Mapping neuroscience research collaborations

Publication tracking is a commonly accepted form of quantifying research production and has been used to link publishing trajectories with career development [22,26]. Quantity and quality of publications, often measured through journal impact factors and citation indices, are two normative indicators of impact in biomedical fields. Evidence also suggests a trend in high-impact coauthorship relationships in Alzheimer's disease research and related fields [5]. In Alzheimer's disease research, some of the most impactful work has emerged from long-standing collaborations. Collaborative research relationships foster opportunities to share ideas, generate intellectual stimulation, and cross-pollinate skill and knowledge development [28]. Scientific advancement may to some extent rest on scientists' abilities to functionally navigate the processes of forming research teams, effectively work to produce science, and efficiently distribute findings. From this perspective, a third metric of productivity and impact in biomedical research may be the extent to which scientists form and maintain publication and grant relationships.

Developing effective research teams that lead to these publication and grant relationships requires effort, negotiation, and time [8,18,29,30]. Academic faculty and clinical researchers are typically expected to publish research results to advance in their careers. Collaborative research centers and institutions are designed to facilitate

the process of building research teams and should engender scientific collaboration more effectively than that could be generated independently by investigators in neuroscience and other biomedical fields, particularly when investigators are working as specialists without a deep community of institutional peers. Evidence for increased collaborative publications may be one metric by which to measure the success of the center or institute in furthering science. By tracking changes in coauthorship relations over time, potential impact of center or institute structures may become apparent if increased collaboration or different collaboration patterns occur after the onset of the formal research center or institute. Social network analysis (SNA) is a viable technique for identifying scientific collaborative network structures. Introduced at the turn of the 21st century [31–33], SNA has rapidly gained research attention recent years as an emerging best practice for mapping collaboration and producing evidence for effective team science [9–11].

SNA has been applied to document productivity and viability of research teams' collaborative interactions over time, including prediction of interdisciplinary collaboration formation [3,34,35] and cooperative structures and interactions among network members [36–38]. Despite increased SNA investigations into research networks in medical and translational research [3,10,11,34–36,39–43], there are few SNA investigations into collaborative research specifically in neuroscience. Thus, there is relatively little information as to how scientists working in translational neuroscience may form collaborative partnerships that are indicative of successful team science. Publication counts are a conservative measure of productivity and career advancement [8,26]. However, without adequate measures of the quality of collaborative interactions, there may be a failure to accurately understand how these productivity outcomes emerge from collaborative science or how productive collaborations can be encouraged and facilitated [44].

1.3. Research support networks

In this era of increased specialization, the formation of research support networks is critical to conducting high-quality research and developing competitive grant proposals for external funding [8,21,45]. Furthermore, center and institute funding may be maximized when collaborative teams emerge and share resources [29,30]. Collaborative engagement of research support members, such as statisticians and technicians, with lead scientists and the extent to which members of a research community participate across multiple research projects or teams may be indicative of a healthy and sustainable research infrastructure [46]. Effective assessment includes the collection of multiple forms of evidence that can provide insight into the underlying factors that yield collaborative products [28].

1.4. The present study

The purpose of this study was to investigate indicators of research collaboration in translational A&D within an NIGMS-funded research center. The study maximizes the best practices for assessing collaborative research networks by using social network techniques for evaluating changes in scholarly productivity, membership relationships, research supports and structures, and workflow processes. The results contribute to the emerging application of team science in the context of neurodegenerative disease research and NIGMS centers [9,34,35]. Longitudinal, periodic reassessment provides information on the growth and reordering of collaborative networks and provides additional insights into the success of developing a scientific infrastructure. The data presented in this study contribute to understanding the development of emerging collaborative science efforts and smaller scale multidisciplinary research collaboratives in neuroscience. Two primary sets of research questions guided the analytic aspects of the study.

1.5. Research questions

Does being a member of the CNTN increase the likelihood of shared authorship? Does being a member of the CNTN increase the likelihood of sharing authorship with another CNTN member? Does change in network metrics and shared authorship over time indicate the CNTN is having a positive influence on shared authorship between members?

To address these questions, change in publication collaborations (2014 to 2017) were analyzed to determine if and how shared publications among CNTN members increased from baseline through CNTN implementation.

Does the *a priori* defined structure of the CNTN map onto the emergent community network structures of collaborative engagement among CNTN members? What brokerage processes between core CNTN areas drive the emergence of the observed CNTN community structure? Do these brokerage processes align with the predefined roles of CNTN cores areas?

To address these questions, collaborative engagement data were analyzed to examine the emergent community structures in the CNTN and determine how they map onto members' empirically defined roles in the CNTN. A brokerage analysis of the collaborative engagement data was also conducted to determine the process of CNTN workflow. Finally, a multinodal network including persons and their related projects was examined.

1.6. A Multilevel systematic approach to network analysis

Social network analysis was used to answer the study research questions. Social networks are often defined as relations among individuals, or nodes, where the ties between them are referred to as edges [47]. Networks can

also contain relationships between individuals and other abstractions, including projects, objects, or psychological states. An n × n matrix $\boldsymbol{Y}$ is defined, such that Y_{ij} is the value of the relation from node i to node j. Relations can be binary, ordinal, or continuous. Symmetric matrices contain undirected ties, whereas asymmetric matrices contain directed ties. A matrix can be visualized as a graph, or a sociogram that demonstrates relationships among nodes.

Exploratory or descriptive methods are used to summarize the network. Descriptive network measures can exist at the node and network level. Nodes within a network are often described using measures of how central a node is within a network. Measures of centrality can provide information about who brokers information between people or communities within a network and can be useful when making decisions about organization structure and group dynamics [47]. A common measure of centrality in a network is degree. Degree is the number of ties a node has. For an undirected matrix Y, degree for node i is Σj Yij.

The network level is often described using edge count, transitivity, and density metrics. Edge count is a sum of all observed relations in a matrix. Transitivity is the formation of closed triads in a graph, where a loop length 3 is a sequence of nodes (x, y, z,) such that (x, y), (y, z), and (z, x) are edges of the graph. A transitivity index for a given network can be calculated, where the number of observed transitive triads is divided by the number of potential transitive triads. Triads are rare in randomly generated networks and when observed indicate self-organization [48]. Density is of the proportion of observed ties out of all possible ties, calculated as $\sum y/n(n-1)$. Density is a measure of how well connected a graph is and can indicate how well information flows, how much information is being shared, or how well supported individuals are, depending on the nature of ties [3,35,47].

Similarly, network-level structures can be identified, which demonstrate how ties form or how communities of people within a network self-organize during interaction. Exponential random graph modeling (ERGM) comprises a class of models used to inferentially test the formation of ties between actors in a network based on their attribute characteristics [48,49]. ERGMs model the probability of observing network $\boldsymbol{Y}$ given the space of all possible networks Y, calculated by $2^{n(n-1)}$, where n is the number of nodes. From this large distribution of graphs, the probability of observing the number of reciprocated ties and transitive structures in $\boldsymbol{Y}$ can be estimated. Furthermore, emergent community structures can be identified in networks by defining an interconnected topology combining order and randomness [50]. Networks can be decomposed into subcommunities, or sets of highly interconnected nodes. Modularity, then, is an empirically defined, compartmentalized internal structure that indicates the density of connections between nodes within modules and the sparseness of connections between nodes in different modules. High modularity is often interpreted as robustness to external perturbations to a network.

Network processes, such as how information or work flows through a network, can be examined using brokerage analyses [51]. In its most basic form, node v is a broker if for distinct nodes a and b, $a \rightarrow v \rightarrow b$ where a and b are not related. If nodes in a network belong to distinct groups, then group membership may be used to distinguish between different types of brokerage roles. Let $A \rightarrow B \rightarrow C$ describe the two-path relationship at the heart of a brokerage structure. A node from group B brokers the relationship from a node in group A to a node in group C. Gould and Fernandez [51] describe six types of brokerage relationships:

- w_I: Coordinator role; the broker mediates contact between two individuals from his or her own group. Two-path structure: $A \rightarrow A \rightarrow A$
- w_O: Itinerant broker role; the broker mediates contact between two individuals from a single group to which he or she does not belong. Two-path structure: $A \rightarrow B \rightarrow A$
- b_{IO}: Representative role; the broker mediates an incoming contact from an out-group member to an in-group member. Two-path structure: $A \rightarrow B \rightarrow B$
- b_{OI}: Gatekeeper role; the broker mediates an outgoing contact from an in-group member to an out-group member. Two-path structure: $A \rightarrow A \rightarrow B$
- b_O: Liaison role; the broker mediates contact between two individuals from different groups, neither of which is the group to which he or she belongs. Two-path structure: $A \rightarrow B \rightarrow C$
- t: Total (cumulative) brokerage role occupancy (any of the above two paths).

A brokerage score for a given node is the number of ordered pairs having the appropriate group membership brokerage relationship. Aggregate scores can be computed for defined groups within a network as well as at the network level. Expectations and variances of brokerage scores given the size and density of a network can also be computed [52].

Network graphs are often visualized using layout algorithms. These algorithms are specific to the nature of the observed networks. Collaborative human systems (crowds, protests, markets) where people collaborate, cooperate, or interfere are often characterized as small worlds [53–55]. The forced atlas 2 layout algorithm is a practical layout approach that can be used to visualize network data that represent small world phenomena. The forced atlas 2 algorithm is designed to simulate a physical system to spatialize a network. Nodes repulse each other while edges attract their nodes. These competing forces create a movement that converges to a balanced state, where the final configuration can help data interpretation [56]. Conducting network analysis requires collecting specialized forms of data that capture relationships between people. The following section describes the methods used to

collect data that were used to address the research questions using the social network analytic approaches described previously.

2. Methods

The CNTN data were derived from self-reported publications and collaborations from members of the CNTN research collaborative. Because of the relatively small size of the collaborative, the demographic information of those who participated has been withheld to protect member identity. Data for the publication networks were derived from the members' curricula vitae (CV). Key personnel were identified who were likely to lead publishing efforts (N = 15), most of whom provided CV (n = 12). Publications and presentations/abstracts per calendar years spanning the life of the CNTN (2015 to 2017) listed on the CV were used. The 2014 year before the award of the CNTN support was included as a baseline measure. All the authorship information from the publications spanning years members contributed to the CNTN, including baseline, were entered into a data array organized by year and author. These data were manipulated to form adjacency matrices of all coauthors from all years (n = 672), producing an unweighted, nondirected adjacency matrix for each publication year. The attributes for authors were coded as 0 = non-CNTN author and 1 = CNTN author.

Member collaborative engagement data were gathered using an online self-report survey. The survey was designed according to recommendations for best practice [57]. A CNTN census membership list (N = 56) was compiled through a multistep procedure that included document review by CNTN evaluators and subsequent review by key personnel. Members were e-mailed a survey asking them to identify with whom they collaborated to carry out their CNTN duties. Collaborative engagement was defined as, "coordinated activity including conversational interactions, coordinated and supportive behaviors for joint activity and projects, and receiving and giving of feedback guidance or scaffolding. Computer-mediated interactions (i.e., e-mail) should be included." In the survey, members first identified with whom they interacted and then reported the frequency of their engagement with those whom they had identified on a sliding scale of 100, ranging from "almost never" to "daily," with various time intervals specified between. Members were then asked to indicate with whom they collaborated on specific projects: CNTN administrative functions, CNTN technical duties, CNTN-initiated research, and non-CNTN initiated research. Participants could include the names of members who may have been missing from the census list, yielding a final census of 59 individuals. Data were manipulated to form an adjacency matrix of all members, specified by the frequency of their collaborative engagement. A second adjacency matrix was also constructed, such that member relationships to each other and their specific projects could be modeled. Thirty-two CNTN members participated in the survey. Missing data were dealt with by inferring reciprocity (i.e., the matrix was transposed), creating an undirected, valued matrix, with more frequent collaborations represented by higher values. The attributes for member roles were coded according to their CNTN affiliation. These categories included one of three ongoing projects in the CNTN (projects 1–3), the administrative core (i.e., project leadership and assessment teams), the data management and statistics core (i.e., storing the clinical data), the clinical core (i.e., technicians and research-oriented personnel), and an unassigned category (no self-identification with a category).

3. Results

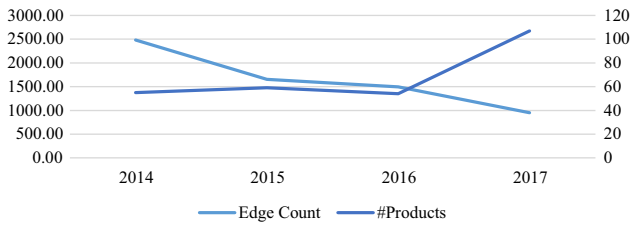
The publication networks and collaborative engagement of CNTN members were examined at the whole network level using network descriptive statistics, including edge count, density, and transitivity. Centrality was calculated for all nodes in the network using degree centrality. Statistics were calculated in R using the package statnet [58].

3.1. Publication network analysis and findings

An ERGM model was fit to CNTN member publication networks (see Table 1 for a summary of results). The ERGM was calculated in R using the package ergm [59]. The model includes an indicator that the author of a publication was a CNTN or a non-CNTN contributor. The indicator corresponds to both a factor effect, or that CNTN member influences tie formation (a shared publication), and a homophily effect, or a dyad covariate that two authors share CNTN membership. Model estimates are presented in

Table 1
Descriptive publication network characteristics by CNTN year

Metric	2014	2015	2016	2017
Edge count	2485.00	1655.00	1497.00	949.00
Transitivity	0.60	0.59	0.70	0.59
Density	0.01	0.01	0.01	0.00
#Connected nodes	290/43%	260/40%	193/29%	196/30%
#Products	55	59	54	107



Abbreviation: CNTN, Center for Neurodegeneration and Translational Neuroscience.

NOTE. Data from 2014 serves as a baseline year, before the CNTN was funded. Node count = 672 for all years. Primary axis scale = edge count. Secondary axis scale = products. Products include publications, presentations, and abstracts.

Table 2
Exponential random graph modeling results for all publication networks

Metric	Est	Std. error	*P*-value
	2014		
Edges	−4.452	0.156	.000
CNTN tie effect	0.579	0.147	.000
CNTN homophily effect	−0.118	0.156	.451
	2015		
Edges	−5.123	0.130	.000
CNTN tie effect	1.081	0.115	.000
CNTN homophily effect	0.077	0.130	.549
	2016		
Edges	−5.466	0.131	.000
CNTN tie effect	1.389	0.113	.000
CNTN homophily effect	0.355	0.131	.007
	2017		
Edges	−6.272	0.100	.000
CNTN tie effect	2.040	0.074	.000
CNTN homophily effect	0.490	0.100	.000

Abbreviation: CNTN, Center for Neurodegeneration and Translational Neuroscience.

NOTE. Node count = 372 for all years.

Table 2. Significant effects are identified if the 95% confidence interval (i.e., ±2 SE) does not contain 0. In all years, CNTN affiliation significantly increased tie formation (which is expected given the publications came from members' CV). Controlling for the CNTN influence on tie formation, in years 2014 and 2015, there are no significant dyad covariate effects for CNTN affiliation. In years 2016 and 2017, there are significant dyad covariate effects for CNTN affiliation, where members of CNTN are more likely to form ties with other CNTN members. Network graphs for CNTN publication networks were visualized using a forced atlas 2 layout algorithm [57] using the open source software Gephi (see Fig. 1) [60]. Isolated nodes were removed from the graph to improve layout.

Visual inspection of the graph suggests increased collaboration of CNTN members during the publication process. The descriptive network statistics indicate that productivity within the publication networks increased over time while the density, edge count, and average degree decreased. Note, the density of these networks is similar to other investigations of research communities [3]. The number of connected components within the network decreased from six in 2014 to two in 2016 and four in 2017, suggesting that network is becoming more connected at the macro level.

3.2. *Collaborative engagement network analysis and findings*

To analyze the community structures within the network, the *a priori* affiliations of CNTN members (top down) were compared to the empirically defined community structures, or graph modularity (bottom up). The network was visualized using a forced atlas 2 layout algorithm [56] using the open-source software Gephi [60]. Isolated nodes were removed from the graph to improve layout. A modularity community detection algorithm [51] was used to analyze the bottom-up, emergent community structures within the network. The visualization was frozen and recolor coded based on the resulting *a posteriori* empirical community structures. Analysis of the percentage of nodes affiliated with structures between graphs provided evidence for the difference between the top-down, *a priori* defined structure of the CNTN and the bottom-up, self-organized community structure that emerged via collaboration engagement (see Fig. 2). Visual inspection of the graphs suggests that while *a priori* structures related to the administrative, data core structures, and research project 3 remained largely intact during community detection, five communities self-organized during member collaborative engagement. To examine the process by which this self-organization occurred, a brokerage analysis was conducted. Brokerage statistics were calculated in R using the package sna [53]. See Table 3 for a summary of results. Results indicated that members affiliated with the administrative core and project 3 brokered coordinated ($A \rightarrow A \rightarrow A$), representative ($A \rightarrow B \rightarrow B$), and gatekeeper processes ($A \rightarrow A \rightarrow B$). Members affiliated with the clinical core and those unassigned to any core brokered the itinerant ($A \rightarrow B \rightarrow A$) and liaison processes ($A \rightarrow B \rightarrow C$).

The visual inspection of the sociograms in Fig. 2 indicated that the members of the CNTN with the greatest centrality were members of the administrative core. The comparison of the *a priori* structures with the empirical community structures suggested that the emergent groups that differed from the *a priori* categories centered around research projects with human populations. The emergent groups each include members from the specific projects, administrative, clinical, and data cores. The brokerage analyses indicate a process by which the relatively intact cores in the *a posteriori* communities were more likely to facilitate internal ties or either providing information that stayed within the receiving group or receiving information from a group that stayed internal. By contrast, the clinical core acted to facilitate more diverse connections, a workflow that facilitates the exchange of information between groups. As the function of the clinical core was to facilitate the production of the research itself, the empirically derived groups would suggest that teams have used this resource effectively.

A second visualization was then constructed, using the adjacency matrix that included relationships between CNTN members and their reported collaborations around project activities, treating the project activities as nodes. Centrality was calculated for the multinodal network and visualization was constructed using the same procedures as described previously (see Fig. 3). Visual inspection of

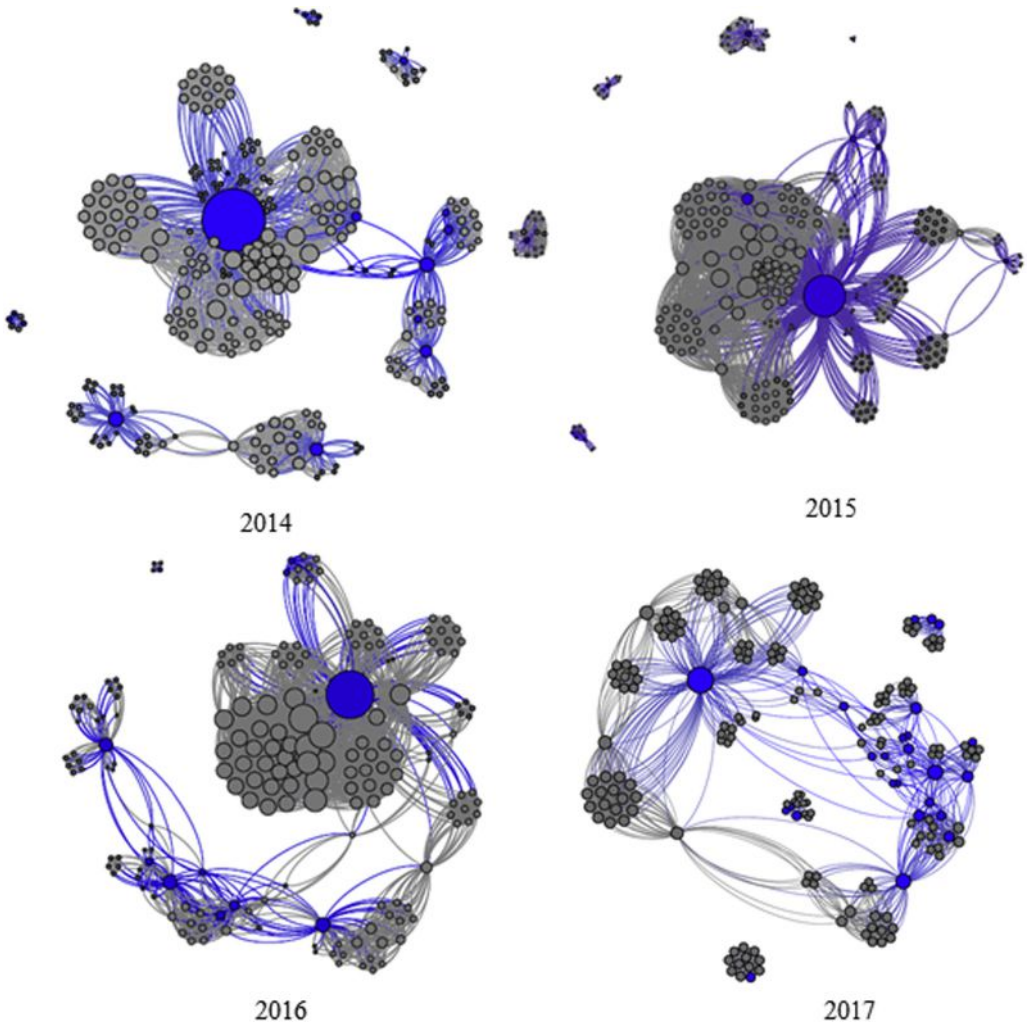


Fig. 1. CNTN member publication network layouts for all study years. Isolated nodes were removed from the analysis and visualizations. Blue nodes = CNTN authors; gray nodes = non-CNTN authors. Modularity (2014 = 0.609; 2015 = 0.748; 2016 = 0.573; 2017 = 0.738); average degree (2014 = 34.6; 2015 = 24.7; 2016 = 31.0; 2017 = 19.35); connected components (2014 = 6; 2015 = 5; 2016 = 2; 2017 = 4). Abbreviation: CNTN, Center for Neurodegeneration and Translational Neuroscience.

the graph indicated that work on multiple projects was at the center of CNTN collaborative engagement. Betweenness scores for project activities were as follows: multiple project = 637.38; non-COBRE project = 346.91; CNTN Admin Project = 330.20; COBRE initiated project = 258.59; CNTN tech project = 191.92.

4. Discussion

The CNTN was initiated in 2015 to support neuroscience research through the development of research infrastructure and investment in emerging scientists. Relatively unique to COBRE-supported centers, the CNTN developed a robust assessment strategy to encourage evidence-based decision making about how the program was functioning to support neuroscience and investigators, as well as regular outcome-based assessments of program influence on metrics used to measure the success of investigators. Contributing to the literature on the application of team science within neuroscience, productivity data were gathered annually to determine change in collaborative authorship patterns over time. Furthermore, in recognition that developing a functioning multidisciplinary and multi-institutional research collaborative is challenging [29], the assessment approach provides information about the processes underlying collaborative group formation in neuroscience through modularity and brokerage analyses of the entire research structure, ranging from research support personnel through the program director.

Similar to other investigations of collaborative research teams initiated after receipt of extramural funding [10], the CNTN shows evidence of increasingly cohesive collaborative relationships among members. The results are suggestive of adaptive change within CNTN publication collaborations. That is, the data suggest that there are growing number of within-CNTN collaborations and a

A Priori Communities				A Posteriori Communities			
ID	Color	Affiliation	%	ID	Color	Community	%
1		Administrative Core	22	1		Module 1	25
2		Clinical Core	12	2		Module 2	24
3		Data Core	15	3		Module 3	22
4		Project 1	22	4		Module 4	15
5		Project 2	10	5		Module 5	14
6		Project 3	14				
7		Unassigned	5				

Fig. 2. CNTN collaborative engagement layout by affiliation and emergent community structure. Node size is proportional to node centrality. Node count = 59; edge count = 685; average degree = 11.61; modularity = 0.326; all statistics apply to both graphs presented. Abbreviation: CNTN, Center for Neurodegeneration and Translational Neuroscience.

pruning of ties that may not be required for current practice, leading to increased productivity. One interpretation of these findings is that the CNTN has been effective in creating opportunities for team science that has direct outcomes relevant to the field (through sharing of research results) as well as careers of emerging scientists (through productive coauthorship relationships).

Furthermore, our study suggests that even early in its implementation, the CNTN appears to be functioning to bring scholars together and support them in developing their scientific agendas through the provision of shared research human capital. The results signaled a natural progression of a newly formed collaborative structure. For example, the non-CNTN project work in which members engage may include collaborative work that predated the CNTN funding or work that allowed for the development of skills (such as imaging or statistical models) while the start-up required before gathering CNTN-specific data unfolded in the first years of the program. Work on multiple projects as the center of CNTN collaborative engagement signals that members are involved in significant cross-talk across predefined organizational structures, using specializations

Table 3
Brokerage analysis for collaborative engagement by CNTN affiliation

ID	Affiliation	Coordinator	Itinerant	Representative	Gatekeeper	Liaison
1	Administrative core	**4.18**	11.45	**17.47**	**17.47**	54.04
2	Clinical core	0.63	**15.44**	8.38	8.38	**71.76**
3	Data core	1.33	14.62	11.30	11.30	66.07
4	Project 1	0.95	15.06	9.87	9.87	68.85
5	Project 2	1.77	14.11	12.66	12.66	63.41
6	Project 3	**4.18**	11.45	**17.47**	**17.47**	54.04
7	Unassigned	0.06	**16.20**	3.54	3.54	**81.25**

Abbreviation: CNTN, Center for Neurodegeneration and Translational Neuroscience.

NOTE. Brokerage scores represent expected values conditional on network size and density. Two highest values per column are in bold. Repeated values represent identical levels of expected brokerage for an affiliation group. Total (any two path) = 104.6 for all affiliations.

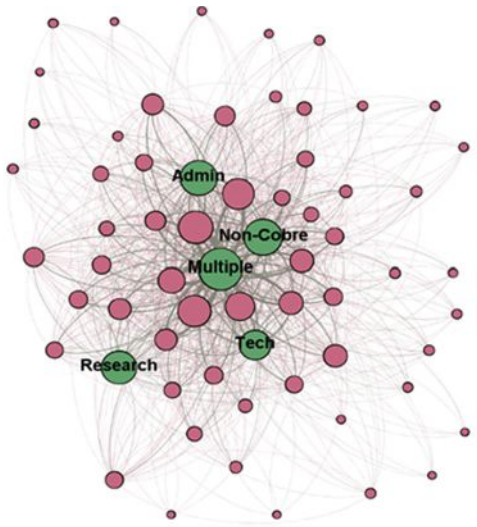


Fig. 3. CNTN multinodal network including persons and project activities. Node size is proportional to network centrality. Pink nodes = persons; green nodes = projects. Node count = 64; edge count = 1101; average degree = 17.20. Abbreviations: CNTN, Center for Neurodegeneration and Translational Neuroscience; Multiple, multiple projects; Non-Cobre, Non–CNTN-related work; Admin, administrative duties; Research, CNTN-related research; Tech, technology- and data-related activities.

of team members and center resources. The study findings support a multitiered, complex structure that includes members coming together as a "team" broadly within the CNTN and in the multiple small worlds that emerge from the collaborative structure.

Continued investigation as the program continues will dive into the conditions that facilitate adaptive team science. Given the community formation and brokerage results from the collaborative engagement survey, it is reasonable to suggest that adequate support cores that coordinate administrative grant functions and provide imaging and statistical expertise function to distribute research demands to enable science to develop more efficiently. Furthermore, the role of the clinical core to coordinate resources among research projects, namely in the form of handling participant enrollment, data entry, and testing, is clearly identifiable by the liaison role the core plays. The clinical core serves as a bridge between members from multiple groups in the CNTN, connecting them with foundational support that enables the conduct of the science itself. As the teams mature, the development of shared norms, language, and expectations should become more integrated into their daily work patterns, potentially resulting in a more efficient time to publication [8] and enhanced proposal funding success.

With regard to the CNTN research project activities, modularity and brokerage analyses suggest that project 3 functions in a distinct way from the other research projects in that the brokerage patterns are similar to those of the administrative core. Notable is that project 3 is not housed at the lead research institution for the CNTN, which could suggest that projects apart from the lead institution may take on more managerial and coordination demands than projects with closer proximity to the research infrastructure. The potential impact of this differential role on collaboration or research productivity cannot be determined from these findings, but future research may be able to gain a more fine-grained understanding of these processes.

4.1. Limitations

Despite the cogent story that took shape around the multiple sources of data, the CNTN is still relatively young in its possible lifecycle and data are limited to date. Furthermore, the productivity metrics were constrained to authorship on publications and presentations/abstracts only. Collaborative partnerships on grant submissions are a key metric to demonstrate success of the CNTN and have been included in other network analyses of productivity. As the CNTN matures, these data are likely to be more robust for inclusion in the productivity analyses. Similarly, the productivity metrics did not account for quality of the publication (e.g., journal impact factor, number of citations). The CNTN is also a relatively small collaborative program. Some of the more complex findings from the initial results, such as the brokerage results, may be an anomaly to the particular nature of the CNTN and may not be informative to other programs engaged in team science. Furthermore, it is possible that types of brokerage, not captured by the existing metrics, may emerge from these partnerships. Finally, given the specific nature of the CNTN, it is not clear if these patterns would replicate in a similarly structured COBRE-funded program or other research center or institute. Without the presence of a control group of personnel without COBRE support, which is not plausible given the contextualized and specialized nature of the scientists, it is important to interpret the study results with caution when considering the effects of the formal research center structure on productivity. However, note that the intent of this study was not to yield generalizable findings but to provide an indication of the types of outcomes and collaborative relationships that might yield outcomes, when infrastructure supports the development of A&D research partnerships.

4.2. Summary

The totality of evidence from this study suggests that the CNTN has been effective in facilitating scientific collaborations in neuroscience. Over time, these collaborations, and those stemming from other centers like the CNTN, may yield high-impact scientific findings and advance the careers of emerging investigators in the field. Evaluation of the structure and function of the CNTN and similar collaboratives is critical for determining how to intentionally create communities that facilitate research engagement and to maximize the impact of these resources for all institutions and members of the collaborative.

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RESEARCH IN CONTEXT

1. Systematic review: The authors conducted a thorough search of the extant literature on assessment of collaboration in large-scale research center programs using traditional databases. The review revealed that network-based approaches to assessment of research collaboration are on the rise, but scarce in the fields of biomedical sciences and Alzheimer's disease.
2. Interpretation: Social network analyses methods found that research collaboration resulting in publications, abstracts, and presentations among research team members supported by a National Institute of General Medical Science Center of Biomedical Research Excellence award increased as the grant matured. A survey of engagement with research suggested that research support for the science was critical to creating a sustainable environment for collaboration to develop.
3. Future directions: Assessment of team science efforts is critical to evaluating and developing appropriate support structures that facilitate successful team science efforts in translational neuroscience.

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Alzheimer's & Dementia

Review Article

Neuroscience learning from longitudinal cohort studies of Alzheimer's disease: Lessons for disease-modifying drug programs and an introduction to the Center for Neurodegeneration and Translational Neuroscience

Aaron Ritter*, Jeffrey Cummings, Christin Nance, Justin B. Miller

Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA

Abstract The development of disease-modifying therapies for Alzheimer's disease is an urgent public health emergency. Recent failures have highlighted the significant challenges faced by drug-development programs. Longitudinal cohort studies are ideal for promoting understanding of this multifactorial, slowly progressive disease. In this section of the special edition, we review several important lessons from longitudinal cohort studies which should be considered in disease-modifying therapy development. In the final section, we introduce the clinical cohort of the Center for Neurodegeneration and Translational Neuroscience. This newly established longitudinal study aims to provide new insights into the neuroimaging and biological marker (biomarkers) correlates of cognitive decline in early Alzheimer's disease and Parkinson's disease (PD).

Keywords: Neuroscience; Longitudinal cohort; Alzheimer's disease; Disease-modifying therapy; Parkinson's disease; clinical trials

1. Introduction

Affecting more than 45 million people worldwide, Alzheimer's disease (AD) is the most common neurodegenerative disease of the central nervous system. The morbidity, mortality, and costs associated with caring for those afflicted by this disease have been well established [1]. With estimates predicting a tripling in prevalence rates by 2050, the search to find disease-modifying therapies (DMTs) has become an urgent global health emergency. Longitudinal cohort studies have been an important source of information regarding the complex chain of events that occur in AD. The insights gleaned from these studies have been used to inform a new generation of increasingly sophisticated clinical trials that have permitted testing of candidate agents earlier in the disease course [2]. Despite significant advances in our understanding of disease, it has been more than 14 years since the last symptomatic agent was approved, and no agent has ever demonstrated disease-modifying effects in clinical trials. The recent spate of high-profile failures [3] has highlighted the challenges for DMT development and thrown into question some of the most fundamental assumptions about AD therapeutics [4].

As part of this special issue introducing the newly established Center for Neurodegeneration and Translational Neuroscience (CNTN), we present five learnings from longitudinal cohort studies and briefly discuss their application in clinical trials. In the final section, we introduce the clinical core of the CNTN. The clinical core of CNTN is a newly established longitudinal cohort study that integrates lessons learned from other cohort studies and brings several new contributions to the field. The following are some among

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*Corresponding author. Tel.: +1 702 701 7981; Fax: +1 702 483 6039.
E-mail address: rittera@ccf.org

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these contributions: (1) an "ADNI approach" to studying cognition in Parkinson's disease (PD); (2) an expanded battery of cognitive testing to better elucidate executive dysfunction in mild cognitive impairment (MCI); (3) positron emission tomography (PET) imaging of microglial activation in the AD and PD disease continuum; and (4) a multimodal recruitment and retention strategy focused on minority recruitment.

2. Longitudinal cohort studies in AD research

Randomized controlled trials (RCTs), which attempt to limit bias and confounding through balanced randomization of carefully selected cohorts, have long been considered the "gold standard" for medical evidence [5]. Any DMT will only be approved based on the results of a well-conducted RCT [2]. The application of RCTs to a slowly progressive disease such as AD is challenging and typically requires enrolling thousands of participants (across hundreds of clinical trial sites) to achieve the requisite statistical power. The degree of complexity required for running large, complicated RCTs has led to a skyrocketing of expenses, and it is now estimated to cost more than $5 billion to bring a DMT to market [6]. It is, therefore, critical that RCTs be informed with a robust knowledge of disease progression and pathogenesis.

Longitudinal cohort studies in AD represent an important resource of information for designing clinical trials. The questions addressed in longitudinal cohort studies of individuals with AD (or at high risk for developing disease) are often different from those of RCTs (regarding, for example, disease trajectory, biomarker evolution, and population-based outcomes) but are no less important. When collected over large periods of time, cohort studies can detect outcomes that appear slowly or inconsistently. These outcomes may not be detected in more narrowly focused clinical trials. Cohort studies, which are often not subject to the same rigorous balanced randomization requirements of RCTs, may also include a wider diversity of participants, more reflective of "typical" rather than "ideal" patient populations [7]. Over the past 3 decades, longitudinal cohort studies have provided key insights into the biological markers (biomarkers), risk factors (environmental and genetic), epidemiology, and disease trajectory of AD.

The Alzheimer's Disease Neuroimaging Initiative (ADNI) serves as a model for conducting longitudinal cohort studies in AD. Launched in 2005, ADNI is a multicenter, longitudinal observational study of cognitive normal elderly, MCI, and early AD [8]. An important contribution of ADNI is its approach to data integrity. Using a study protocol that emphasizes standardized data collection across all clinical sites, ADNI is conducted like a clinical trial but has no intervention. Rigorous adherence to a study protocol improves the reproducibility of data [9]. Now in its third iteration and having expanded to sites all over the world, the ADNI dataset represents a rich repository of multimodal imaging, AD biomarkers, genetics, neuropathology, and neuropsychological testing that is freely and openly shared with collaborators through the ADNI website.

In the following sections, we highlight several lessons learned from both ADNI and other longitudinal cohort studies of AD and consider their impact on DMT development.

2.1. Even at the most experienced academic medical centers, misdiagnosis rates for AD consistently exceed 20%. Eligibility for DMT clinical trials should be confirmed by diagnostic biomarkers

Neuropathology has long been considered the "gold standard" for the diagnosis of AD. The National Alzheimer's Coordinating Center includes a large neuropathology dataset that allows for examination of clinicopathological correlates [10]. An important lesson from the National Alzheimer's Coordinating Center is the significant number of participants who present phenotypically with AD but lack amyloidosis. These individuals are described as having suspected non-Alzheimer pathology (SNAP) [11]. Individuals with SNAP are unlikely to respond to antiamyloid therapies [12]. Looking at a sample of 919 demented subjects, Beach et al. [13] found that a clinical diagnosis of "possible" or "probable" AD was 71% to 87% sensitive and 44% to 71% specific for AD. The authors, furthermore, estimated that the positive predictive value of a clinical diagnosis of AD was 83% (for moderate plaque load, Braak stage III or IV). Although 80% hit rate may appear reasonable, in the context of a clinical trial, this level of misdiagnosis is problematic (again, assuming a poor response rate in non-AD individuals). For example, applied to a trial with a 50% response rate, a 20% misdiagnosis rate would effectively reduce the response rate by 10% [13]. To achieve the same statistical power, recruitment to the trial would need to be doubled. Studies examining misdiagnosis rates in clinical trials have reported even higher numbers, particularly when applied to populations earlier in the AD continuum [14]. These findings are highly supportive that clinical trial populations be enriched by AD diagnostic biomarkers. A recent examination of the AD drug-development pipeline, however, revealed that less than half of phase II and III DMTs used diagnostic biomarkers as entry criteria [15].

2.2. Variability in clinical progression is common in AD, particularly early in the disease continuum. To detect drug-placebo treatment differences, multimodal stratification strategies should be incorporated into the trial design so as to increase the likelihood that participants will progress during the course of the trial

AD is now conceptualized as a clinicobiological entity progressing seamlessly from an asymptomatic high-risk state to MCI and finally ending in dementia. A growing consensus suggests that DMTs must be introduced at a

time point when the pathological processes can still be overcome. Testing therapeutics in participants with minimal (or no) symptoms represents a significant paradigm shift for the field. For the trials to be successful, studies need to be designed to detect significant drug-placebo differences. This requires the selection of participants with a high likelihood of progression during the study. Clinical progression in AD, however, is variable, particularly early in the disease course. Based on clinically diagnosed samples, individuals with MCI progress to dementia at a rate of 10% to 25% per year [16]. A relatively large percentage of these individuals will never convert, and some will even revert back to having normal cognition [17]. Study designers respond to this problem by increasing the trial's statistical power. This means that some clinical trials are expected to enroll thousands of participants over extended periods of time. As a result, new AD studies may now exceed 7 years in length.

Predictive modeling provides a potential alternative solution to this problem. ADNI was specifically designed to validate biomarkers for clinical trials and has driven much of the research on predicting disease trajectory. As no single biomarker or cognitive assessment has demonstrated clear efficacy, investigators have increasingly turned toward multimodal classifiers to inform predictive models. In cognitively normal subjects, combinations of cerebrospinal fluid biomarkers (with cutoff points $<$ 220 pg/mL; Aβ, 42; and $>$61 pg/mL of total tau and 21 pg/mL of phosphorylated tau) predicted cognitive decline and progression to MCI within 3 years [18]. In MCI populations, many predictive models have been developed. An interesting model developed by Barnes et al. was a relatively simple point-based tool used to predict conversion from MCI to AD, incorporating the following elements: (1) the Functional Assessment Questionnaire (2–3 points); (2) magnetic resonance imaging of hippocampal subcortical volume (1 point) and middle temporal lobe thinning (1 point); (3) ADAS-Cog (2–3 points); and (4) the Clock Drawing Test (1 point), the 3-year conversion rate of individuals with a score of 7 to 9 points was 91% [19]. Given the costs associated with recruiting thousands of participants across hundreds of clinical trial sites, it is important that clinical trials begin to integrate predictive models into their designs.

2.3. Executive dysfunction is an important but incompletely understood cognitive characteristic of MCI. Additional measures of cognitive performance should be considered when screening MCI populations to avoid excluding large numbers of candidate participants

The amnestic subtype is commonly used to define MCI in clinical trial populations. To reduce screen failure rates on more expensive biomarker tests, many studies "screen out" potential participants using neuropsychological tests such as the Immediate Memory Section of the Repeatable Battery for the Assessment of Neuropsychological Status or the Free and Cued Selective Reminding Test. Defining MCI solely based on memory performance may prove to be too exclusive. Using cluster analyses to analyze the ADNI dataset, several investigators report that only a percentage of individuals (25.7%–56%) cluster into the amnestic subtype [20–22]. Other MCI clusters include language impaired, visuospatial impaired, and executive dysfunction. An important cluster appears to be those with executive dysfunction (about 1/3 of individuals). This executive dysfunction cluster may represent a valuable population for clinical trials as individuals with both executive dysfunction and elevated levels of cerebrospinal fluid phosphorylated tau exhibit an extremely fast rate of progression from MCI to AD [23]. With the current slate of clinical trials needing more than 20,000 MCI participants to complete recruitment, these cluster analyses from ADNI support the need for a reexamination of clinical trial inclusion guidelines to include more extensive neuropsychological measures, in particular, tests that probe impairments beyond memory functioning [15].

2.4. AD is a multifactorial neurodegenerative disease likely caused by numerous related and parallel biochemical pathways in addition to amyloid plaque and neurofibrillary tangle formation. There is a need to better understand these additional factors involved in disease pathogenesis

Mixed pathologies are common at autopsy in patients diagnosed with AD in the National Alzheimer's Coordinating Center [24]. Common co-occurring pathologies include microinfarcts, white matter lesions, Lewy bodies, and other protein aggregates such as TDP-43 and argyrophillic grains [25]. ADNI includes a group of participants with SNAP—biomarker evidence of neuronal damage without amyloidosis. Because a notable percentage of individuals with plaques and tangles do not manifest dementia, it is possible that the mere presence of amyloid plaques and neurofibrillary tangles alone is not sufficient enough to cause cognitive dysfunction [26]. New research indicates that other metabolic and neuronal processes also play a role. Multiple lines of evidence—increased levels of inflammatory cytokines in AD brains [27], rings of activated microglial cells surrounding amyloid plaques [28], and increased levels of the inflammatory marker YKL40 in the cerebrospinal fluid of AD individuals [29]—point to a key role for neuroinflammation in AD pathogenesis. Given the recent failures of several multibillion-dollar trials testing amyloid-lowering agents, it is imperative that a more integrated understanding of the full diversity of processes involved in AD pathogenesis be integrated and considered when developing DMTs. In this same vein, DMT drug development must also be open to the idea that multiple drug targets may need to be engaged to have a meaningful impact on disease progression. The

recent development of clinical trials testing combination therapies should be embraced as an important trend in AD drug development [30].

2.5. *Clinical trial populations do not accurately represent the diversity of people affected by AD. Clinical trials need to do more to engage underrepresented patient groups*

Longitudinal cohort studies have been key in informing an understanding of the epidemiology of AD. Although the highest incidence rates are seen in North America and Western Europe (10.5 per 1000) [31]—age continues to be the most important risk factor—AD is experienced in all regions of the world. Longitudinal cohort studies have also reported that certain racial and ethnic groups (African-Americans and Hispanics) living in the United States may experience an increased risk for AD compared with both Caucasians and their racial and ethnic counterparts living in their native regions [32]. Despite strong evidence of prevalence across racial and ethnic lines, AD clinical research and clinical trials have traditionally been composed almost entirely of college-educated, Caucasian populations [33]. Low diversity in research studies reduces the generalizability of findings. Barriers to participation in clinical trials for underrepresented patient groups include mistrust of the medical establishment, language, logistical challenges, and lack of cultural sensitivity in recruitment materials [34,35]. To ensure that the findings of clinical trials are broadly generalizable, minority recruitment efforts need to be emphasized, and study designs need to accommodate underrepresented patient group.

3. The clinical core of the CNTN

The CNTN is a newly established biomedical collaboration between the Cleveland Clinic Lou Ruvo Center for Brain Health [36] and the University of Nevada, Las Vegas (UNLV). The CNTN is funded by the NIH/NIGMS through a Center for Biomedical Research Excellence (COBRE) grant. Modeled on two successful federal AD programs—ADNI and the Alzheimer's Disease Coordinating Centers (ADCs)—the clinical core of the CNTN collects longitudinal data on a trial-like cohort of more than 170 research participants with AD, PD, and a cognitively normal control group. Demographic data for the CNTN cohort are presented in Table 1. Similar to ADNI, data collection is standardized through the use of clinical trial-like protocol. The primary focus of the CNTN is to better understand the functional connectivity, neurocognitive correlates, and genetic correlates of cognitive decline in early AD and PD and to develop multimodal predictive models for cognitive decline in both the diseases. As a result, cognitive function is emphasized in participant selection. The AD group consists of participants with MCI and mild-to-moderate AD dementia, whereas the PD group includes participants with normal cognition and MCI (PD-MCI) [37]. Participant eligibility is determined during a screening visit. To ensure performance above floor levels, participants are required to achieve a score of 15 or greater on the Montreal Cognitive Assessment at baseline. After completion of the initial assessments, a panel of clinicians assigns a diagnosis based on the established criteria [38–40].

Table 1
Clinical characteristics of CNTN cohort

	CNTN			
Variables	MCI	AD	PD	Controls
N	54	28	40	52
Age (years)	73	71	66	72
MoCA score	22	19	27	25
MMSE score	25	23	28	28

Abbreviations: CNTN, Center for Neurodegeneration and Translational Neuroscience; MCI, mild cognitive impairment; AD, Alzheimer's disease; PD, Parkinson's disease; MoCA, Montreal Cognitive Assessment; MMSE, Mini Mental Status Examination.

3.1. *Assessments*

Assessments for the CNTN are completed annually and include the following: (1) a structural and functional magnetic resonance imaging; (2) neuropsychological battery; and (3) standardized clinical visit. AD, MCI, and normal controls undergo amyloid PET at baseline. Amyloid PET identifies which participants are on the AD disease continuum and which participants have SNAP. All participants have extensive genetic analysis (genotyping, targeted gene arrays, and whole exome sequencing). Notably, PD participants are scanned before their morning dose of carbidopa/levodopa (in the practically defined OFF state) and one hour after their first dose of the day (practically defined ON state). This allows for exploration of the neuroanatomical networks underlying cognitive decline in PD as well as permitting the effects of dopaminergic therapy on these networks [41].

The neuropsychological test battery is central to the CNTN. A unique feature of the neuropsychological battery is that it integrates ADNI assessments—allowing for direct comparisons with the ADNI dataset—but also expanding the ADNI approach to participants with PD. This will allow for direct comparisons of cognitive decline in these two related neurodegenerative diseases [42]. The CNTN also expands on the ADNI neuropsychological test battery by including several additional neuropsychological measures of executive function (Table 2). This expanded investigation into executive functioning will allow for interrogation of cognitive decline particularly relevant to PD and increasingly recognized as an antecedent to cognitive decline in MCI.

3.2. *Inflammation*

Another aim of the CNTN is to more fully elucidate the role of inflammation in neurodegenerative disease.

Table 2
Neuropsychological assessments included in the CNTN

Category	Clinical assessments
Cognition	Montreal Cognitive Assessment (MoCA)*
	Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog)*
	Mini Mental Status Examination (MMSE)*
	Wide Range Achievement Test (WRAT-4) Reading
	American National Reading Test (ANART)*
	Logical Memory Immediate/Delay Recall Story A*
	Rey Auditory Verbal Learning Test (RAVLT)*
	Brief Visual Memory Test Revised (BVMT-R)
	Digit Span
	Letter-Number Sequencing (WAIS-IV)
	Judgment of Line Orientation (JoLO)
	Color Word Interference Test (D-KEFS)
	Verbal Fluency Test (D-KEFS)*
	Clock Drawing Test*
	Boston Naming Test (BNT)
	Symbol Digit Modalities Oral and Written Test (SMDT)
	Trail Making Test A and B*
Olfactory	**Brief Smell Identification Test (BSIT)**
Functional	**Activities of Daily Living (ADL-Q)**
	Dementia Rating Scale-2 (DRS-2)
	Functional Activities Assessment Questionnaire (FAQ)*
	Clinical Dementia Rating Scale (CDR)*
Neuropsychiatric	Neuropsychiatric Inventory (NPI) and Questionnaire (NPI-Q)*
	Geriatric Depression Scale (GDS)*
	State-Trait Anxiety Inventory (STAI)
Sleep	Epworth Sleepiness Scale
	REM Sleep Behavior Disorder Questionnaire

Abbreviations: CNTN, Center for Neurodegeneration and Translational Neuroscience; ADNI, Alzheimer's Disease Neuroimaging Initiative.
NOTE. Bold assessments indicate those unique to the CNTN.
*Assessments shared with ADNI.

There have been relatively few investigations into the correlations between inflammation and cognitive symptoms in AD or PD. The CNTN's contribution to this area of study will be to probe the relationship between inflammation and neurocognitive testing by using PET ligands related to microglial activation (GE-180). It is hypothesized that differences between amyloid-positive and amyloid-negative participants will provide crucial information about the role of inflammation in cognitive symptomatology.

To improve the generalizability of research findings from the CNTN, recruitment to the CNTN will attempt to match the racial and ethnic composition of the state of Nevada (Table 3). To achieve this goal, the CNTN has developed a comprehensive, multipronged recruitment strategy. A successful element of the recruitment strategy includes the development of a Community Outreach Committee. Consisting of a diverse mix of community leaders from traditionally underrepresented patient groups, this committee meets regularly to shape and guide recruitment efforts. Another

Table 3
Racial and ethnic recruitment goals for the CNTN

Category	Female	Males	Total
Ethnic category			
Hispanic or Latino	5	5	10
Not Hispanic or Latino	65	65	130
Total number of subjects	70	70	140
Racial category			
American Indian/Alaska Native	1	1	2
Asian	2	2	4
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	6	6	12
White	60	60	120
Total	70	70	140

Abbreviations: CNTN, Center for Neurodegeneration and Translational Neuroscience.

novel recruitment strategy is the use of Healthybrains.org [43]. Healthybrains is an interactive, Web-based brain health and clinical trial registry that allows individuals to take active steps in their brain health and learn about clinical trial opportunities. It is free to use and has registered more than 15,000 participants. More than 15% of referrals to the CNTN come from HealthyBrains. Through the first 3 years of its existence, retention to the clinical core remains high (95%). Retention strategies include an annual newsletter to participants and an optional "annual results visit" with the study PI. During the results visit, participants are able to learn the results of selected assessments.

3.3. Data sharing

Data sharing is key to the CNTN's mission. All CNTN data are entered into the study database (OpenClinica) and made available to collaborators through the CNTN website, www.nevadacntn.org. To facilitate the greatest amount of collaboration, data will be provided at several levels of complexity. For example, the database will include a repository of postprocessed imaging data (volumetrics using FreeSurfer) that will permit rapid analysis of more basic questions, whereas the raw images will be made available for investigator seeking to perform complex imaging analyses on the original data.

4. Conclusion

Longitudinal cohort studies have been invaluable tools in increasing our understanding of the pathophysiologic changes that underlie this devastating disease. Lessons learned from cohort studies will need to be incorporated into DMT programs if much-needed new therapies are to be brought to the market. Discussed are five lessons learned from cohort studies that we feel are important to DMT development. Finally, a recently launched cohort study—the clinical core of the CNTN—is introduced. The CNTN integrates lessons learned from other cohort studies and brings several

new contributions to understand early cognitive decline in AD and PD.

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RESEARCH IN CONTEXT

1. Systematic review: Alzheimer's disease (AD) presents variably and progresses slowly. There have been few successes in randomized controlled trials, particularly in trials of disease-modifying agents. Insights from longitudinal cohort studies are informing a new generation of sophisticated clinical trials. We review the findings from several important longitudinal studies of AD and their application to new drug development.
2. Interpretation: Clinical trials need to require diagnostic biomarkers for screening; stratification strategies should be incorporated into clinical trial design; neuropsychological screening for mild cognitive impairment should be expanded; the biological understanding of AD should be expanded beyond amyloid plaques and tau; and clinical trials need to do more to engage underrepresented patient groups.
3. Future directions: We introduce a newly launched longitudinal cohort study, the Center for Neurodegeneration and Translational Neuroscience. By studying AD and Parkinson's disease concurrently, the Center for Neurodegeneration and Translational Neuroscience represents a novel approach to the study of these two important neurodegenerative diseases.

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Alzheimer's & Dementia

Featured Article

Biomedical informatics applications for precision management of neurodegenerative diseases

Justin B. Miller[a,*], Guogen Shan[b], Joseph Lombardo[c], Gustavo Jimenez-Maggoria[d]

[a]Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA

[b]Epidemiology and Biostatistics Program, Department of Environmental and Occupational Health, School of Community Health Sciences, University of Nevada Las Vegas, Las Vegas, NV, USA

[c]National Supercomputing Institute, University of Nevada, Las Vegas, NV, USA

[d]Alzheimer's Therapeutic Research Institute, Keck School of Medicine, University of Southern California, San Diego, CA, USA

Abstract Modern medicine is in the midst of a revolution driven by "big data," rapidly advancing computing power, and broader integration of technology into healthcare. Highly detailed and individualized profiles of both health and disease states are now possible, including biomarkers, genomic profiles, cognitive and behavioral phenotypes, high-frequency assessments, and medical imaging. Although these data are incredibly complex, they can potentially be used to understand multi-determinant causal relationships, elucidate modifiable factors, and ultimately customize treatments based on individual parameters. Especially for neurodegenerative diseases, where an effective therapeutic agent has yet to be discovered, there remains a critical need for an interdisciplinary perspective on data and information management due to the number of unanswered questions. Biomedical informatics is a multidisciplinary field that falls at the intersection of information technology, computer and data science, engineering, and healthcare that will be instrumental for uncovering novel insights into neurodegenerative disease research, including both causal relationships and therapeutic targets and maximizing the utility of both clinical and research data. The present study aims to provide a brief overview of biomedical informatics and how clinical data applications such as clinical decision support tools can be developed to derive new knowledge from the wealth of available data to advance clinical care and scientific research of neurodegenerative diseases in the era of precision medicine.

Keywords: Informatics; Neurodegenerative disease; Biomarker; Clinical decision support; Precision medicine; Evidence-based practice; Alzheimer's disease; Learning healthcare; Machine learning

1. Biomedical informatics applications for precision management of neurodegenerative disease

The practice of medicine is in the midst of a modern-day revolution, driven by "big data," rapidly advancing computing power, and broader integration of technology into health-care service provision. It is anticipated that from 2014 to 2024, health-care information technology job growth is expected to substantially outpace job growth among other industries [1], due in part to the deluge of health data generated by new technologies. These data can create highly detailed and individualized profiles of both health and disease states, which can potentially be used to understand multi-determinant causal relationships, elucidate modifiable factors, and ultimately customize treatments based on individual parameters. In the case of neurodegenerative diseases (NDDs), where many questions are still unanswered and effective therapies have remained elusive, there is a critical need for an interdisciplinary perspective on data and information management to derive novel insights, generate new knowledge, improve care, and facilitate treatment discovery.

*Corresponding author. Tel.: 702-701-7896; Fax: 702-701-7953.
E-mail address: millerj4@ccf.org

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Biomedical informatics (BMIs) is a multidisciplinary field that falls at the intersection of information technology, computer and data science, engineering, and healthcare; this interdisciplinary intersection will play an integral role in the future of medicine. The present study aims to provide a brief overview of BMIs and how novel clinical data applications can be developed to derive new knowledge from existing data to advance clinical care and scientific research of NDD in the era of precision medicine (PM). The generation and organization of big data and its application in healthcare settings depend on a scientific infrastructure that anticipates both the needs of these types of data, as well as, how they may be used. The National Institutes of Health and the National Institute of General Medical Sciences support awards such as the Center for Biomedical Research Excellence grants to support big data infrastructure and advance data science. The Center for Neurodegeneration and Translational Neuroscience is a Center for Biomedical Research Excellence–supported neuroscience enterprise with a Data Management and Statistics Core that serves as a platform for investigating how to apply big data to PM.

1.1. What is PM?

PM has recently been defined as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person." [2]. A primary aim of PM is to link individuals with the best possible treatment for an individual's disease in the hope of improving clinical outcomes, and ultimately, patient health. Effective implementation of PM into clinical practice requires integration of translational research from a diverse array of data sources to ensure that the PM approach is firmly rooted in empirical evidence. Although individualized approaches to clinical care have been present for decades, (e.g., matching blood transfusions or solid organ transplants based on blood type), the wealth of data available in modern medicine, with all its technological advances, moves the potential for truly precise interventions far beyond what has historically been possible. NDDs present significant opportunity for development of PM interventions [3], not only because of the wealth of genetic information now available [4,5] but also because of the concurrent growth in biomarker discovery [6] and the ability to characterize the cognitive and behavioral phenotype in rich detail. Moreover, the historical approaches (e.g., one-size-fits-all treatment) have almost universally failed to uncover an effective therapeutic agent [7], which may in part be due to the incredible diversity in disease manifestations that can result from the same underlying pathology.

1.2. Biomarkers of neurodegeneration for PM

Definitive diagnosis of NDD requires positive identification of the pathologic changes occurring in the brain, which for most NDD begin decades before the onset of observable symptoms. As a result, there is considerable interest in the discovery and validation of reliable biomarkers that could be used to improve diagnostic accuracy, especially early in the disease process before the full clinical syndrome is manifest [8]. At present, body fluid analysis and brain imaging are the two principal sources for biomarker data.

1.3. Fluid biomarkers

Cerebrospinal fluid (CSF) has been a prominent target for discovery of potential biomarkers, given the possibility that it provides molecular insights into pathologic processes within the brain. For example, amyloid β-42 and tau were two of the earliest validated biomarkers in Alzheimer's disease (AD) [9], which has been refined to separate tau into total tau and phosphorylated tau [10,11]. These CSF markers have demonstrated good sensitivity to AD pathology and are widely used in both clinical practice and research. However, limited specificity [12,13] has mitigated their utility as stand-alone diagnostics, especially at preclinical stages [14]. Coupled with the invasive nature of CSF studies, recent efforts have focused on identification of potential biomarkers in peripheral fluids (e.g., saliva, blood).

Though there is considerable appeal in a validated blood test for AD pathology, most efforts to date have not been successful [15]. Recent developments, however, have shown significant promise, with high rates of overall classification accuracy [16]. If replicated and independently validated, the simplicity of a blood test would have significant clinical utility. Once integrated with the clinical history, a blood test with high sensitivity would make an excellent screening tool that could be used to quickly and efficiently rule out the presence of pathology or prompt for additional diagnostic testing.

1.4. Imaging biomarkers

Brain imaging is also a widely used biomarker, including both structural and functional imaging. For example, magnetic resonance imaging can be used to measure both regional (e.g., medial temporal structures in AD; frontal atrophy in frontotemporal dementia) and whole-brain atrophy, both of which can be used to inform differential diagnosis [17]. Several molecular imaging techniques have also been validated for detecting AD pathology (specifically β amyloid), including Pittsburgh compound B [18], and fluorine-18 labeled radiotracers such as florbetapir [19,20], florbetaben, and flutemetamol [21]. Cerebral glucose metabolism has also been widely used (e.g., fluorodeoxyglucose positron emission tomography), both for identification of early AD-related changes [22] and differentiating them from other NDDs (e.g., frontotemporal dementia) [23,24]. Tau imaging is increasingly used to identify the state of tau aggregation in the course of AD [25], which may be particularly beneficial very early in the disease process [26]. The noninvasive, or minimally invasive, nature of

imaging studies makes them among the first biomarkers to be reviewed in the clinical setting. When integrated with additional diagnostic studies such as neuropsychological evaluation or fluid biomarkers, imaging studies can be particularly informative, both for ruling conditions in, and ruling conditions out.

1.5. Genomic data

Genetic information is commonly used to assist clinicians and researchers in guiding differential diagnosis and is also the central component of PM (e.g., pharmacogenomics). Genetic information is especially useful in identifying at-risk individuals long before symptoms emerge. For clinical trials and studies focusing on prevention of disease, this is especially important because it increases the likelihood of including those at highest risk of disease. Although there are specific genes associated with causing early onset AD (e.g., genes encoding amyloid precursor protein and presenilin 1 and 2) and increasing the risk of late onset AD (e.g., the e4 allele of the apolipoprotein E) [27], the development of AD most likely has a polygenic determination [28]. Similarly, several genetic markers have also been associated with idiopathic Parkinson's disease [29], dementia with Lewy bodies [30,31], and frontotemporal dementia [32]. A common theme across diseases is that there is considerable heterogeneity in the possible genetic determinants, most are polygenic disorders, and there is growing evidence of genetic overlap between diseases [28,31,33].

1.6. Cognitive and behavioral markers

Detailed characterization of the clinical presentation, including both the cognitive and behavioral phenotypes are core components of a comprehensive diagnostic work up, whether in clinic or in research participation. At present, active assessments (i.e., an active and intentional approach to measuring cognitive functioning and quantification of behavior) such as cognitive screenings performed in clinic or detailed neuropsychological evaluation are often used both to inform differential diagnosis and to establish baselines of functioning that can be used to monitor disease progression over time. The major advantage of active assessments is the level of control retained by clinicians and researchers over what measures are used, when they are administered, and under what circumstances. Moreover, they can be used to construct a very rich snapshot of functioning that cuts across several domains of ability, and there is an extensive literature base for these measures.

The challenges with active assessments, however, are that not only are they labor intensive, slow, and inefficient [34,35], but most are insensitive to change in the earliest phases of the disease [8], when intervention may be most effective. They are also grossly lacking in temporal resolution, limiting the conclusions that can be drawn at the time of assessment, forcing clinicians and researchers to either estimate previous levels of functioning or make inferences about the pattern of change between assessments (e.g., that decline follows a uniformly linear pattern). The ecological validity of most of these tools is also quite poor, requiring additional inferences about how observed patterns of performance translate to real-world functioning.

One solution is to make better use of technology in the measurement of human behavior via portable and wearable devices (e.g., phones, smart watches, and sensors), which would address many of the limitations of active assessments. Leveraging portable device platforms would foster the development of remote assessments that could reduce the demand on patients and research participants to come to clinic and provide higher frequency data. Portable platforms would also increase the opportunities to collect data and maintain closer contact with patients, which could detect significant changes sooner and allow intervention earlier if needed. In addition, portable technology would also allow for the development and validation of passive data collection (PDC) methods that could be used to automatically "capture" data from everyday behaviors (e.g., embedded background applications, sensors, and so on). These data would generate an incredibly rich characterization of everyday cognition and real-world behavior, and because they are tied directly to an individual through a specific device, the data would be nearly as unique as a genetic sequence.

When integrated with active assessments, these continuous or high-frequency data capture methods that would dramatically enrich the overall cognitive and behavioral profile, and many of the shortcomings associated with traditional active assessments would be mitigated. Although PDC methods would generate a substantial amount of "noise" along with the behavioral data, much of this could be automatically filtered out with concurrent development of purpose-built informatics applications. Moreover, they could be used much in the same ways as traditional biomarkers to detect the presence of underlying NDD. With proper validation and sufficient classification accuracy, continuous data capture also has incredibly high clinical utility and efficiency because they are by definition, computer applications for quantifying everyday behaviors. They do not place significant demands on the local healthcare system and require minimal clinician input once deployed.

1.7. What is BMIs?

BMIs has been defined by the American Medical Informatics Association as "the interdisciplinary field that studies and pursues the effective uses of biomedical data, information, and knowledge for scientific inquiry, problem solving, and decision-making, motivated by efforts to improve human health" [36]. A core emphasis of BMI is on development of technology-driven resources for the storage, access, use, and dissemination of health-care data to derive new information and generate new knowledge. There is currently considerable interest and ongoing work in

comprehension, integration, and usability of diverse data sources in BMI, which aligns closely with NDD and PM. Several distinct, but closely related application areas have been defined by the American Medical Informatics Association. Two of which are particularly important in the study of NDD—translational bioinformatics and clinical research informatics.

Translational bioinformatics is defined as "the development of storage, analytic, and interpretive methods to optimize the transformation of increasingly voluminous biomedical and genomic data into proactive, predictive, preventive, and participatory health." Given the increasing precision with which biomedical data are now being collected, the sheer amount of data that is generated and its complexity, extracting insights that can be used to inform clinical practice is a challenge requiring substantial computing power. However, BMI applications can be developed to detect and translate the deeply embedded patterns within these data into predictive treatments tailored to the individual, ultimately leading to improvement in patient outcomes.

Related to translational bioinformatics and somewhat of a counterpart, clinical research informatics is defined as "the use of informatics in the discovery and management of new knowledge relating to health and disease." Although highly relevant for clinical trials, clinical research informatics also emphasizes the continued utilization of amassed data and secondary use and re-use of data, such as electronic health records and those data aggregated over the course of routine clinical care, for ongoing research purposes (e.g., patient registries, collaborative knowledge-bases). Even though these clinical data are even more complex relative to the highly controlled and well-characterized data amassed in a research setting, they serve as the foundation of the learning health-care system [37]. So, while translational informatics focuses on moving research evidence from bench-to-bedside to support evidence-based practice, clinical research informatics aims to generate complementary practice-based evidence.

1.8. Advancing translational neuroscience research with BMIs

Over the past several decades, it has become increasingly apparent that NDD cannot be reduced to a single determinant and attention has shifted to integration of multidisciplinary data (e.g., biomarker data, imaging data, genetic data, and cognitive and behavioral phenotypes). The major challenge, however, is that these "multi-omic" assessments generate a set of incredibly complex "big" data. When studied at the level of the individual patient, these data can be manually integrated relatively easily, but elucidating patterns within even modest sample sizes quickly exceed what is feasible with manually guided approaches. Efficiently and effectively separating the signal from the noise in such data sets requires dedicated computing power with purpose-built BMIs applications to analyze the data and derive novel insights, which is especially critical for PM.

1.9. Mapping functional networks

NDD is not typified by focal lesions, but rather disruption of complex neuroanatomical networks [38,39], evident even in early disease stages [40]. Computational network analysis is a mathematical modeling approach capable of integrating diverse data sets and mapping distributed networks by identifying shared elements (i.e., nodes) and establishing the relationships between them (i.e., edges). It has been effectively utilized to map both structural and functional connections in the brain (e.g., [41]) and uncover common genetic pathways in AD [42]. With sufficient longitudinal data, the evolution of disease over time can also be modeled [43]. The ability to model and predict disease course is an essential component of PM.

Computational network analysis has also been effectively used to study diseases that may be best characterized as continua instead of distinct categorical states and for syndromes with heterogeneous clinical presentations [44,45]; both of these are characteristic of NDD. The ability of network analyses to integrate highly diverse and complex data sets is a tremendous strength. As data of increasing granularity become widely available, it may soon be possible to uncover precise causal relationships driving NDD, which may lead to the discovery of an effective therapeutic agent.

1.10. Clinical decision support tools

Clinical decisions support (CDS) tools are applications built to deliver filtered information to providers at the point of care to enhance health and health care [46]. In essence, a CDS tool is an application that filters and translates empirical evidence into an immediately usable format to guide clinicians in their clinical decision-making. By integrating patient-specific parameters (e.g., genetics, biomarker profiles), CDS applications can be used to drive PM interventions. CDS applications are not a replacement for clinical judgment, but rather an additional source of information to be integrated with clinical judgment in an effort to formulate personalized treatment recommendations; CDS applications are a means by which evidence-based practice can be realized.

In AD and related NDD, determining specific treatments is not yet an issue because there is not an effective therapeutic agent to slow or reverse the underlying pathologic process; however, there is a considerable need to develop tools capable of empirically deriving individualized risk profiles. Formulating specific diagnoses based on a set of easily observed/assessed clinical characteristics (e.g., biomarkers, behavioral phenotypes) and subsequently developing predictive algorithms of the patient's disease course will be particularly important. In clinical trials, where recruitment is focusing on very early phases of the disease process, it

is critical to study those likely to manifest the disease of interest. Being able to accurately identify those with the highest risk based on individual parameters will increase recruitment accuracy, and therefore, the odds of discovering an effective therapeutic agent.

Machine learning (ML) is a data-driven approach to generating such predictive algorithms using a set of training data that are then validated in independent data sets [47,48]. In NDD, ML could be effectively used to develop statistical models to establish individual risk profiles and make specific diagnoses based on a set of individual clinical characteristics. In supervised learning approaches, researchers identify a specific endpoint or outcome of interest from a set of data and allow the program to find and evaluate the underlying patterns in the remaining data that predict those endpoints. For example, to develop an algorithm for determining *in vivo* amyloid status (i.e., the outcome) based on set of easily observed clinical characteristics (i.e., the predictors), an initial set of training data would be required that includes amyloid status (e.g., florbetapir scan) and potential clinical characteristics of interest (e.g., volumetric neuroimaging, cognitive data). The patterns of performance among the predictors that optimize accurate classification of the output would then be used to generate an algorithm that could be applied to a novel data set containing the same predictors. When provided with performance feedback (i.e., was the classification correct or incorrect?), the algorithms are iteratively refined to reduce error rates, effectively "learning" from experience. While similar classification models can be built using classical statistical methods (e.g., logistic regression), hypothesis-driven approaches require researchers to select predictors of interest based on an *a priori* assumption; in complex data sets, this can be particularly challenging and may not generate the most efficient models.

Even in supervised learning, there is still a need to identify and label a specific output. In highly complex data sets, however, there are likely valuable insights embedded within the data that may be missed using a supervised approach. In unsupervised learning approaches, researchers allow the program to explore the underlying structure of the entire data set without any predetermined endpoint labels; unsupervised learning is especially useful for mining extremely large and complex data sets, dimension reduction, and uncovering patterns of data not readily apparent to researchers through classical hypothesis testing [48,49]. Both supervised and unsupervised learning methods are being used to extract valuable insights and develop predictive algorithms from both data and metadata. When appropriately implemented, classification and predictions derived from hierarchical, or deep, learning methods have demonstrated impressive accuracy comparable with or surpassing human-level accuracy [50–52] and are approaching Bayes error rates.

Historically focused on imaging data, systematically capturing cognitive and behavioral data alongside additional biological data sources (e.g., structural and functional neuroimaging, genetic data, CSF) and subjecting these data to ML as well may lead to identification of precision biomarkers associated with specific cognitive and behavioral phenotypes. The resulting models could be used to generate an empirically derived probability of underlying pathology based solely on the clinical presentation, which has significant utility for both clinical trial recruitment and clinical management. For example, of data of this type might be used to predict evolution of cognitively normal individuals into amyloid-positive states, and thus eligibility for clinical trial participation and eventually for preventative treatment. If integrated into an electronic medical record along with natural language processing application to extract information from clinical notes (e.g., health record phenotyping), classification models could be deployed autonomously, and probabilities updated in real time as new notes are generated. Already validated for use in screening for common conditions (e.g., type 2 diabetes) [53] and with fully automated approaches rivaling manual methods in accuracy [54], extending these applications to NDD to generate CDS tools to guide treatment selection, especially if compounds or therapeutics are developed that have proven efficacy for specific subsets of the disease population, would facilitate the practice of PM.

1.11. Transition from state of wellness to disease

One of the major challenges facing NDD is the blurred boundary between a state of health and one of disease, which in part is due to the difficulty detecting the subtle changes associated with the transition, as well as clear characterization of the earliest disease states. Using a complex network approach, however, it may be possible to identify points of vulnerability (i.e., tipping points) associated with a development of a clinical syndrome following a period of stability (e.g., Hofmann et al, 2016) [55]. In NDD especially, where individual difference factors (e.g., cognitive reserve) can influence the relationship between manifest symptoms and disease burden, being able to identify factors associated with, or even preceding, these pathologic transitions may increase opportunities for intervention. Such approaches have already been used with neuroimaging data [56,57]. High-throughput data streams and user-generated data sources (e.g., wearables) providing behavioral data in real-world settings could then be integrated with clinical data sources (e.g. electronic medical records). ML-derived algorithms could then be utilized to automatically monitor individual data streams and flag patterns of behavioral that suggest an emerging transition from healthy to disease states well before the clinical syndrome emerges.

1.12. Modern database infrastructure

In general, development of CDS tools, whether it be for prediction of disease or for identification of a specific therapeutic agent, has required prohibitively large sample sizes to achieve sufficient stability and reliability. This is especially the case for identifying the very subtle signals in the earliest phases of disease. In the absence of distributed collaborative

networks contributing to a shared data repository, it has been difficult to aggregate samples of this size. Furthermore, when sufficiently powered studies were completed, the data were collected over a predetermined period of time, using prespecified tools, in a restricted sample, to address an *a priori* hypothesis, with highly limited access beyond the original study investigators. On study completion, these data were then typically siloed and ended up sitting idle.

Moving these data sets, however, to cloud-based repositories promotes real-time, open-access data sharing and collaboration, a movement which has been endorsed by federal funding agencies and journal editors [58–60]. Creating a repository to house data collected during the course of routine clinical care alongside data from ongoing molecular and genetic research and clinical trials that are collaboratively aggregated by multiple users would foster

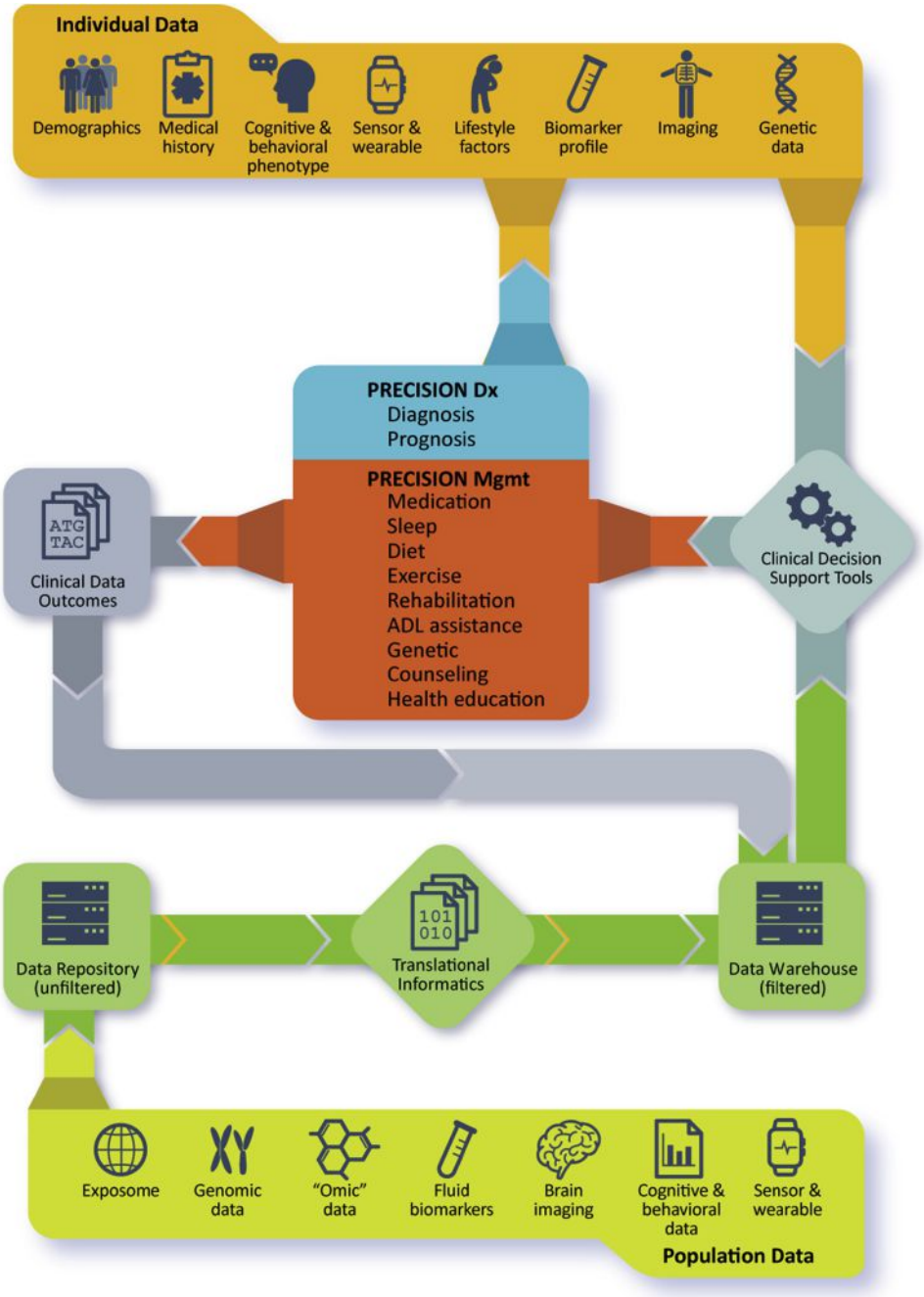

Fig. 1. Precision diagnosis and management of neurodegenerative disease relies on integration of population-level data and individual data derived from multiple sources. Effective utilization will require informatics applications to aggregate and filter data, which can drive development of clinical decision support tools for deployment in clinical settings. Developing tools to systematically capture clinical outcomes at the point of service delivery will facilitate refinement of the clinical decision support tools, creating a learning healthcare system and closing the loop in evidence-based care.

the development of novel measurement tools, establishment of clinical normative data, and facilitation of knowledge discovery via multidisciplinary hypothesis-driven, as well as, data-driven research (e.g., deep learning, data mining, and ML). Providing open access to data will also help address issues of transparency in research, reproducibility of results, and independent replication, especially when paired with simultaneous sharing of software and code [61–63].

Although the costs associated with cloud-based storage were at one point prohibitive for all but the most well-funded studies or larger enterprise organizations, publicly available services such as Amazon's Web Services have brought leading edge technology within reach for most organizations. Ultimately, the utility of research and clinical data are inherently tied to the accessibility of the data, both within studies and across studies and utilization of modern data management can dramatically increase accessibility. Making these resources open-access and built using flexible platforms furthers accessibility and promotes longevity, allowing perpetual use and re-use of the data and integration of new data, maximizing return on financial investments.

2. Conclusion

As the era of personalized medicine continues to evolve and rich individual data sets become increasingly accessible, development of cloud-based repositories that collaboratively aggregate data will rapidly advance basic, translational, and clinical science. Creating dedicated BMIs applications with advanced analytic capability designed identify subtle patterns within those data will bring targeted interventions for NDD based on individually determined risk profiles within reach. Fig. 1 presents a high-level overview, tying these concepts together to show how data from population studies can be aggregated and used to develop clinical decision support tools, which can then be deployed in clinical settings, using individual patient data to guide clinical decision-making. The clinical outcomes resulting from these decisions are then returned to the data set, which are used to refine and improve treatment models and decision support tools, completing the learning health environment. Ultimately, developing comprehensive and integrated data environments will lead to improved patient outcomes and facilitate knowledge discovery, including disease mechanisms, causal factors, and novel therapeutics.

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RESEARCH IN CONTEXT

1. Systematic review: Modern medicine is in the midst of a revolution driven by "big data," rapidly advancing computing power, and broader integration of technology into healthcare. Highly detailed and individualized profiles of both health and disease states are now possible, including biomarkers, genomic profiles, cognitive and behavioral phenotypes, high-frequency assessments, and medical imaging.
2. Interpretation: These individual profiles can be used to understand multi-determinant causal relationships, elucidate modifiable factors, and ultimately customize treatments based on individual parameters, which is especially important for neurodegenerative diseases, where an effective therapeutic agent has yet to be discovered.
3. Future directions: By collaboratively aggregating these data in structured repositories, biomedical informatics can be used to develop clinical applications and decision support tools to derive new knowledge from the wealth of available data to advance clinical care and scientific research of neurodegenerative disease in the era of precision medicine.

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Featured Article

Statistical advances in clinical trials and clinical research

Guogen Shan[a,*], Sarah Banks[b], Justin B. Miller[b], Aaron Ritter[b], Charles Bernick[b], Joseph Lombardo[c], Jeffrey L. Cummings[b]

[a]*Epidemiology and Biostatistics Program, Department of Environmental and Occupational Health School of Community Health Sciences, University of Nevada Las Vegas, Las Vegas, NV, USA*

[b]*Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA*

[c]*National Supercomputing Institute, University of Nevada Las Vegas, Las Vegas, NV, USA*

Abstract

Introduction: New treatments for neurodegenerative disease are urgently needed, and clinical trial methods are an essential component of new drug development. Although a parallel-group study design for neurological disorder clinical trials is commonly used to test the effectiveness of a new treatment as compared to placebo, it does not efficiently use information from the on-going study to increase the success rate of a trial or to stop a trial earlier when the new treatment is indeed ineffective.

Methods: We review some recent advances in designs for clinical trials, including futility designs and adaptive designs.

Results: Futility designs and noninferiority designs are used to test the nonsuperiority and the noninferiority of a new treatment, respectively. We provide some guidance on using these two designs and analyzing data from these studies properly. Adaptive designs are increasingly used in clinical trials to improve the flexibility and efficiency of trials with the potential to reduce resources, time, and costs. We review some typical adaptive designs and new statistical methods to handle the statistical challenges from adaptive designs.

Discussion: Statistical advances in clinical trial designs may be helpful to shorten study length and benefit more patients being treated with a better treatment during the discovery of new therapies for neurological disorders. Advancing statistical underpinnings of neuroscience research is a critical aspect of the core activities supported by the Center of Biomedical Research Excellence award supporting the Center for Neurodegeneration and Translational Neuroscience.

Keywords: Adaptive designs; Clinical trials; Futility design; Neurological disorders; Proper statistical inference

1. Introduction

In clinical trials for neurological disorders, a parallel group study is commonly used to assess the effectiveness of a new treatment as compared to the placebo group [1–4]. Patients are randomized to either the treatment arm(s) or the placebo arm following a prespecified randomization schedule. At the end of the study, the change of the primary outcome from the end to the baseline, calculated from the treatment arm, is compared with that from the placebo arm to make a conclusion whether the new treatment has sufficient activity to move to the next phase for further investigation. The primary outcome to assess the cognitive performance can be measured by established assessment tools, such as the Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog), the Unified Parkinson's Disease Rating Scale (UPDRS), Clinical Dementia Rating, and the Amyotrophic Lateral Sclerosis Functional Rating Scale-revised (ALSFRSr). The commonly used parallel-group design is able to study the

*Corresponding author. Tel.: 702-895-4413; Fax: 702-895-5573.
E-mail address: guogen.shan@unlv.edu

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effectiveness of the new treatment with influential covariates being balanced during the randomization procedure; however, it may not be efficient enough for the purpose of rapidly screening out nonpromising treatments or identifying the most promising treatments [1,5–10].

Futility designs are widely used in early phase neurological disorder trials to screen out new treatments that are highly unlikely to produce successful results [11–15]. Futility designs can be used in a single-arm study with the threshold estimated from historical controls or in a parallel-group study with a nonsuperiority alternative hypothesis [16–18]. The purpose of the futility design is to screen out an unpromising treatment with fewer patients and a much shorter study time period. As compared to the futility design, the commonly used parallel-group study is often used to test the superiority or noninferiority of the new treatment over the placebo. In this article, we review the difference between the futility design and the noninferiority design which is also widely used in clinical trials to test the noninferiority of a new treatment. We also provide some guidance on the proper usage of such designs [19–24].

In recent years, adaptive designs have been introduced and used in trials for neurological disorders to reduce resource use and study length [25–28]. There are a few definitions for an adaptive design. In 2010, the Food and Drug Administration published a draft guidance document on adaptive designs and defined an adaptive design as "a study that includes a prospectively planned opportunity for modification of one or more specified aspects of the study design and hypotheses based on analysis of data (usually interim data) from subjects in the study" [29].

Adaptive designs provide opportunities to modify or change the trial during the study while maintaining the validity and integrity of the trial. These opportunities are prespecified when certain conditions are met. In 2008, Chow and Chang [27] reviewed 10 adaptive designs used in clinical trials, including an adaptive randomization design that allows modification of randomization schedules; a group sequential design that allows early stopping due to futility, efficacy, or both; a sample size re-estimation design allowing sample size adjustment; a pick-the-winner design; an adaptive dose-finding design; a biomarker-adaptive design; an adaptive treatment-switching design; an adaptive seamless design; a hypothesis-adaptive design; and a multiple adaptive design. In this article, we review the following two commonly used adaptive designs in neurological disorder trials. The response-adaptive randomization design uses the patients' responses from the current on-going study to modify the assignment probabilities to each treatment arm, with more patients being treated in the better arms. The response-adaptive randomization design belongs to the adaptive randomization design that also includes treatment-adaptive randomization and covariate-adaptive randomization [27]. The other adaptive design discussed in this article is the adaptive dose-finding design that increases the accuracy of the estimation for the maximum tolerated dose or minimum effective dose [30].

Studies designed by an adaptive method may introduce new challenges in data analysis. It is important that intended statistical analysis should guide the study design [23,31,32]. For this reason, new statistical analysis approaches to analyze the data from adaptive designs properly are also discussed. Review of novel, efficient, and proper statistical approaches in neuroscience research is an important service of the Data Management and Statistics Core of the Center for Neurodegeneration and Translational Neuroscience supported by the Center of Biomedical Research Excellence award from the National Institute of General Medical Sciences.

2. Futility designs

The futility design, also known as the nonsuperiority design, can be used to screen out a new treatment candidate who is not promising for further investigation. It can be implemented in a single-arm study or a parallel group study to investigate the effectiveness of a new experimental treatment. Suppose μ_e and μ_c are the mean of the primary outcome in a new experimental treatment group and in the control group, respectively, in a parallel group study. For a single-arm study, we may use the same notation μ_c to represent the estimated value from historical data. Let $\Delta = \mu_e - \mu_c$ be the difference between the two groups.

For clinical trials in neurology, the primary outcome of interest to measure disease symptoms is often computed from some well-established assessment tools, for example, ADAS-Cog, UPDRS, and ALSFRSr. The change of these measurements from the end to the baseline (post–pre) is often used as the primary outcome, for example, $\mu_e = \mu_{e1} - \mu_{e0}$, where μ_{e1} and μ_{e0} are the outcome of patients from the treatment group at the end and at baseline, respectively. It should be noted that a treatment with a smaller increase (slowing disease progression) or a larger decrease (improving the disease symptoms) in the outcome is considered as a better treatment in some assessment tools (e.g., ADAS-Cog, UPDRS), whereas it is reversed when others are used (e.g., ALSFRSr).

When ADAS-Cog or UPDRS is used to measure the disease symptom, suppose δ_0 is the maximum allowable progression threshold, the statistical hypotheses for the futility design are presented as

$$H_0 : \Delta \leq \delta_0 \text{ against } H_a : \Delta > \delta_0, \tag{1}$$

where δ_0 is a clinically meaningful threshold to measure the disease symptom [33–35]. For example, a clinical trial to assess the effectiveness of coenzyme Q10 and GPI-1485 in Parkinson's Disease (PD) patients [36] was designed as a futility study with $\delta_0 = -3.19$, which is 30% of the total UPDRS change of participants in the placebo group from the Deprenyl and Tocopherol Antioxidant Therapy of

Parkinsonism trial (DATATOP), $\mu_c = 10.65$. This trial is designed as a single-arm futility study with the hypotheses:

$$H_0 : \mu_e - \mu_c \leq -3.19 \text{ against } H_a : \mu_e - \mu_c > -3.19.$$

If the null hypothesis is rejected, we can conclude that the new experimental treatment is not promising for further investigation. The sample size for each arm in this study [36] was calculated as 58 participants per arm to attain 85% power at the significance level of 0.1, with $\mu_e = 7.46$ under the null hypothesis and $\mu_e = 10.65$ under the alternative hypothesis.

When a larger observed outcome represents a better treatment (e.g., the ALSFRSr score), the hypotheses for a two-arm futility design are presented as

$$H_0 : \Delta \geq \delta_0 \text{ against } \Delta < \delta_0, \tag{2}$$

where δ_0 is the minimum worthwhile efficacy, and it is often a positive value. Although the hypotheses can be presented in two different formulae as in Equations (1) and (2) which depend on the direction of the assessment tool, they are statistically identical by reversing one of the assessment tools. Both the alternative hypotheses suggest nonsuperiority of the new experimental treatment as compared to placebo.

3. Noninferiority designs

The aforementioned futility design (also known as the nonsuperiority design) should not be confused with other designs, such as a superiority design or a noninferiority design. We compare these designs to the futility design with the hypotheses in Equation (2) by assuming that a higher score represents a better treatment. The hypotheses of a superiority design or a noninferiority design are expressed as

$$H_0 : \Delta \leq \delta_1 \text{ against } \Delta > \delta_1, \tag{3}$$

where δ_1 is the margin. Let the clinically meaningful estimate of Δ be Δ_{CME}. This estimate is very important in clinical trials to show clinically meaningful improvement by a new treatment. When $\delta_1 > \Delta_{CME}$, Equation (3) represents a superiority design. It becomes a noninferiority design when $\delta_1 < \Delta_{CME}$. In a noninferiority trial, the aim is to show that a new treatment is not much worse than the standard care or not clinically inferior to the standard care. Lesaffre [37] compared the difference between superiority trials and noninferiority trials with two real noninferiority trial examples along with the discussion of the noninferiority margin.

In a randomized clinical trial to investigate the validity and reliability of online delivery of the Lee Silverman Voice Treatment for PD patients with speech and voice disorder [38], the change in sound pressure level (dB-C) after the treatment was the primary outcome. The clinically relevant improvement was estimated as $\Delta_{CME} = 4.5$ dB, with an estimated standard deviation of 2.48 dB. This study was designed as a noninferiority trial to compare the performance with the Lee Silverman Voice Treatment between online and face-to-face administration, with the noninferiority marginal of 2.25 which is half of the estimated clinically relevant improvement, $\delta_1 = 2.25 < \Delta_{CME}$. A sample size of 15 per arm was required to attain 90% power at the significance level of 0.025 for this noninferiority trial.

In another noninferiority study reported by Winblad et al. [39], the rivastigmine capsule was compared with placebo for AD patients by using the ADAS-Cog change from baseline as the primary outcome. The noninferiority margin was set as 1.25 points decrease on the ADAS-Cog, which is half of the estimated treatment difference from other existing studies. This noninferiority margin is considered as the minimum clinically meaningful difference.

In the aforementioned hypotheses for either a futility design or a noninferiority design, the primary outcome is computed as the change from the end to baseline, for example, $\mu_e = \mu_{e1} - \mu_{e0}$. When the primary outcome is measured as the change from baseline to the end (pre–post, e.g., $\mu_e = \mu_{e0} - \mu_{e1}$), the aforementioned hypotheses can still be applied. For example, in a study to confirm the noninferiority of rotigotine to ropinirole for PD patients on concomitant levodopa therapy, the primary outcome was the change of the UPDRS Part III (ON state) sum score from baseline to the end [40]. In this study, a larger observed value (the difference of change between rotigotine and ropinirole) represents a better treatment. For this reason, the hypotheses presented in Equation (3) should be used in this study to assess the noninferiority of rotigotine to ropinirole.

4. Sample size determination and statistical inference

Sample size calculation plays a very important role in clinical trials to ensure a prespecified level of power when type I error rate (α) is controlled. Type I error rate and power are generally computed by using the estimated Δ values under the null and alternative hypotheses, δ_0 and δ_a. Accurate estimates of δ_0 and δ_a would increase the success rate of a trial with the computed sample size adequate to detect the difference between the treatment arms.

The hypotheses discussed in this article are all one-sided; therefore, z_α, instead of $z_{\alpha/2}$, is used in the sample size determination (see Levin [41] for the detailed sample size calculation formula). It should be noted that the sample size calculation provided by Levin [41] is based on asymptotic approaches, which should be used with caution for a study with sample size that is small to medium. For a study with binary outcome (more than 50% decrease in the Inventory of Depressive Symptomatology-Clinician score from baseline in PD [42]), the proper type I error rate should be computed under the null hypothesis (H_0: $\Delta \leq \delta_0$), not just at the boundary of the associated hypothesis space ($\Delta = \delta_0$) [43–45]. In the trial to compare response rates between atomoxetine and placebo [42], we suppose the

null hypothesis is presented as H_0: $p_a \leq p_c$, where p_a and p_c are the response rate for the atomoxetine arm and the placebo arm, respectively. The response rate for the placebo arm is estimated to be 10% from historical data. An exact unconditional approach [46] may be used to calculate the error rate, then the actual type I error rate is computed as

$$TIE(p_a, p_c) = \max_{p_a \leq p_c \leq 10\%} \sum_{T(X_a, X_c) \in \Omega} f(X_a, N_a, p_a) f(X_c, N_c, p_c), \tag{4}$$

where Ω is the rejection region and $f(.)$ is the probability density function of a binomial distribution. The type I error rate should be properly computed over the null space $p_a \leq p_c \leq 10\%$ as in Equation (4). Often, its error rate is computed on the boundary with $p_a = p_c = 10\%$, which is not proper for use without a theoretical proof to show that the error rate occurs at the boundary of the null space [43].

When two proportions are compared, efficient sample size calculation approaches are recommended for use to provide valid sample sizes, such as simulation-based approaches and exact approaches [3,23,47–50]. In addition, the actual type I error rate could be highly affected by the estimated δ_0 [15], and we would encourage researchers to compute and provide sample sizes under multiple possible δ_0 and δ_a scenarios.

At the end of a trial, observed data are analyzed to make statistical inference. Statistical analysis should be intentionally consistent with the design. For a randomized placebo-controlled futility study with the hypotheses given in Equation (1), the null hypothesis is rejected when a large Δ value is observed. The progression threshold δ_0 is the boundary of the one-sided lower confidence interval computed from the observed data. When δ_0 is outside of the interval (δ_0 is less than the lower limit), we have enough evidence to reject the null, and the new experimental treatment is not promising for further investigation. Otherwise, if the computed lower limit is less than δ_0, we fail to reject the null hypothesis. Similarly, the $1 - \alpha$ lower limit of Δ is used in testing the hypotheses in Equation (3), whereas the $1 - \alpha$ upper limit is used for the hypotheses in Equation (2) to make valid statistical inference. The lower or upper limit of δ_0 should be computed properly for different types of data. For a matched-pairs study, a study design that accounts for the matching information should be considered.

5. Adaptive designs

Adaptive designs have been increasingly used in clinical trials to increase the flexibility of a trial by allowing a trial to be stopped earlier for futility when a new treatment is not promising and/or allowing more patients being assigned to a better treatment and so on. The adaptations in a trial have to be prospectively planned to guarantee the validity and integrity of the trial.

An adaptive design provides opportunities for a trial to be modified during the course of the trial; however, it has to be prospectively planned. In other words, any modification of a trial (e.g., adding or dropping a treatment arm) is specified during the planning stage when certain conditions are met. These conditions include the comparison of results based on the observed data from the on-going study. In general, it takes more effort for the research team to prepare an adaptive design than a traditional nonadaptive design. A significant number of simulation studies have to be conducted to investigate all possible outcomes during the planning stage.

In a very recent phase II trial to evaluate the BAN2401 (a monoclonal antibody targeting amyloid protofibrils) for the treatment of AD patients, the response-adaptive randomization model was used in the study design. The probability of the next patient being assigned to one of the treatment arms or the control arm is determined by the probability of that treatment arm being the most effective treatment arm among all arms. The cumulative data of patients from this on-going study are used for calculating these probabilities. An adaptive randomization design allows a trial to assign more patients to better treatment arms, which may lead to imbalances in the sample-size allocation and the distribution of influential covariates across treatment arms. Recently, Saville and Berry [51] proposed using odds ratios to modify the probability in the response-adaptive randomization for each arm to improve the covariate balance. When the primary outcome is binary, a new patient allocation scheme to adjust the covariate imbalance issue during the adaptive randomization procedure has been proposed [52]. New and proper statistical methods are needed to overcome the emergent statistical challenges from the response-adaptive randomization. Alternatively, a study can be designed as a covariate-adaptive randomization to balance the allocation of multiple arms across a set of influential covariates without compromising randomness. That would help reducing the complexity of the final data analysis.

In neurology, adaptive designs are often used in early phases to learn the safety of a new treatment and select the dose for the following trials. In a dose-finding study, there are often a few arms with different dose levels and a placebo arm to estimate the placebo effect. Adaptive methods can be used to stop a dose earlier due to futility, accept a dose due to efficacy, or add a new dose to the study. The aforementioned response-adaptive randomization could be used in conjunction with the adaptive dose-finding design. For example, a phase II trial to evaluate the safety and efficacy of ABT-089 in AD patients was designed by using a Bayesian response-adaptive randomization method to allocate patients to one of the seven arms (six arms of ABT-089 with different doses and placebo) after having at least five patients in each arm [53]. This study was also designed to allow stopping for efficacy or futility based on the conditional power calculated from the on-going study. The objective of this adaptive dose-finding study was to identify the minimum effective dose resulting in at least an average of 1.75-point

ADAS-Cog improvement over placebo. In other studies, the maximal tolerated dose may be the target dose [54].

In addition to the aforementioned adaptive designs, Chow and Chang [27] reviewed other commonly used adaptive design methods in clinical trials: an adaptive seamless phase II/III design, a biomarker-adaptive design, an adaptive treatment-switching design, and so on. More details about these adaptive designs may be found in the literature [1,9,22,24,25,27,55–57].

6. Discussion

The futility design is used in early phase clinical trials to screen out unpromising treatments and save resources for other treatment candidates. By contrast, a futility-stopping boundary is used to drop a treatment arm from a trial or stop a trial earlier if the treatment will not show efficacy based on the observed results. The futility boundary could be defined as the prespecified conditional power or the prespecified confidence limit. They are two different concepts, that is, the futility design is a study design, whereas a futility boundary is a threshold in the study design [41,58].

Adaptive designs are attractive to increase the flexibility of a trial, but they also introduce new statistical challenges to analyze the final observed data properly. The aforementioned imbalance issue on the inferential covariates from the response-adaptive randomization is one of them. When a study is designed by an adaptive approach, the data analysis should align with the study design. For an adaptive two-stage design in which the second-stage sample size depends on the results from the first stage, the data analysis that uses only the final observed data without considering the nature of a two-stage adaptive design is not appropriate [10,59]. For example, a single-arm two-stage adaptive design was used to assess the effectiveness of a new treatment for PD patients with the primary outcome as a binary endpoint. The required sample size for the first stage is $n_1 = 22$ participants, and the second-stage sample size, $n_2(X_1)$, is a function of the number of the responses from the first stage (X1), (e.g., $n_2(X_1) = 35$ when $X_1 = 11$) [59]. At the beginning of the study, the number of responses from the first stage X_1 is unknown. Therefore, the sample space having all possible X_1 from 0 to n_1 should be used in statistical inference, such as P-value calculation and confidence interval calculation.

In practice, it is possible that the final observed sample size is different from that is planned. Then, a statistical approach that incorporates adaptive elements and the observed sample size is valid for data analysis [10,45].

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RESEARCH IN CONTEXT

1. Systematic review: Futility designs are commonly used in early phase neurological disorder trials to screen out new treatments that are highly unlikely to produce successful results. Adaptive designs are increasingly used in drug development to improve the flexibility and efficiency of trials, having the potential to reduce the cost and save sample sizes.
2. Interpretation: A futility design should not be confused with a design that allows a trial to be stopped due to futility. The number of neurological disorder trials designed by adaptive approaches is not as large as expected.
3. Future directions: Adaptive futility designs should be developed for use in trials, and the associated statistical methods for newly developed designs should be proposed to provide proper statistical inference.

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Featured Article

Advances in functional magnetic resonance imaging data analysis methods using Empirical Mode Decomposition to investigate temporal changes in early Parkinson's disease

Dietmar Cordes[a,b,*,1], Xiaowei Zhuang[a,1], Muhammad Kaleem[c], Karthik Sreenivasan[a], Zhengshi Yang[a], Virendra Mishra[a], Sarah J. Banks[a], Brent Bluett[a], Jeffrey L. Cummings[a]

[a]*Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA*
[b]*Departments of Psychology and Neuroscience, University of Colorado, Boulder, CO, USA*
[c]*Department of Electrical Engineering, School of Engineering, University of Management and Technology, Lahore, Pakistan*

Abstract

Introduction: Previous neuroimaging studies of Parkinson's disease (PD) patients have shown changes in whole-brain functional connectivity networks. Whether connectivity changes can be detected in the early stages (first 3 years) of PD by resting-state functional magnetic resonance imaging (fMRI) remains elusive. Research infrastructure including MRI and analytic capabilities is required to investigate this issue. The National Institutes of Health/National Institute of General Medical Sciences Center for Biomedical Research Excellence awards support infrastructure to advance research goals.
Methods: Static and dynamic functional connectivity analyses were conducted on early stage never-medicated PD subjects (N = 18) and matched healthy controls (N = 18) from the Parkinson's Progression Markers Initiative.
Results: Altered static and altered dynamic functional connectivity patterns were found in early PD resting-state fMRI data. Most static networks (with the exception of the default mode network) had a reduction in frequency and energy in specific low-frequency bands. Changes in dynamic networks in PD were associated with a decreased switching rate of brain states.
Discussion: This study demonstrates that in early PD, resting-state fMRI networks show spatial and temporal differences of fMRI signal characteristics. However, the default mode network was not associated with any measurable changes. Furthermore, by incorporating an optimum window size in a dynamic functional connectivity analysis, we found altered whole-brain temporal features in early PD, showing that PD subjects spend significantly more time than healthy controls in a specific brain state. These findings may help in improving diagnosis of early never-medicated PD patients. These key observations emerged in a Center for Biomedical Research Excellence–supported research environment.

Keywords: Resting-state fMRI; Empirical mode decomposition; EMD; Intrinsic mode function; Group ICA; Functional connectivity; PPMI; Parkinson's disease

1. Introduction

Functionally related regions of the resting brain show a high degree of temporal correlation in blood-flow fluctuations, as measured by the blood-oxygenation level-dependent (BOLD) functional magnetic resonance imaging

[1]Both authors contributed equally to this study.
*Corresponding author. Tel.: 1-702-483-6022; Fax: 1-866-372-2720.
E-mail address: cordesd@ccf.org

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(fMRI) signal [1]. Using either seed-based methods or data-driven approaches such as independent component analysis (ICA), brain regions that fluctuate in synchrony and constitute reliable and reproducible functional networks in the human resting brain can be identified [2–6], named resting-state networks. Resting-state networks were assumed to be static in nature in the past, and approximately a dozen of such static networks have been discovered and investigated in relation to how they are impacted by neurodegenerative disorders. However, more recently, it has been shown that resting-state networks are dynamic in character and change on a time scale of several seconds to a minute [7–10]. Analyzing the temporal dynamics of resting-state connectivity provides a more accurate picture of the working brain and can help in the early detection of neurological disorders and in monitoring effects of potential treatments.

Both static and dynamic analysis methods have been applied to study resting-state functional networks in major neurodegenerative diseases, for example, Alzheimer's disease (AD). One of the major brain networks affected in AD is the so-called default mode network (DMN), which is heavily involved in memory formation and retrieval [11]. In normal subjects, the DMN shows functional connections between the posterior cingulate cortex, angular gyrus, hippocampus, and the medial prefrontal cortex. In AD patients, amyloid-beta (Aβ) protein has been found to accumulate in DMN and other regions, which may disrupt connections and lead to the symptoms of memory and cognitive impairment [12–14]. Early Aβ accumulation is associated with reduced static functional connectivity within the DMN and between the DMN and the frontoparietal network (FPN), a network that is involved in attention-demanding tasks [15,16]. The dynamic aspect of the DMN shows significant changes in AD as well [17]. It has been reported that AD subjects spend less time in brain states with strong posterior DMN contributions and more time in states with dorsal medial prefrontal cortex contributions.

Parkinson's disease (PD) is, after AD, the second most common neurodegenerative disorder in the elderly and is characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta with resulting striatal dopaminergic deficiency [18]. Previous neuroimaging studies of PD patients have shown that whole-brain functional networks such as the DMN and networks involving the motor pathway are affected, leading to different functional connectivity patterns when compared to those found in normal controls (NC) [19]. Studies of the temporal characteristics of fMRI resting-state brain networks have also shown abnormal spontaneous low-frequency content in PD [20]. The dynamic aspects of brain networks have been widely studied using electrophysiological recordings. Intraoperative electrophysiological data have shown that the occurrence of motor symptoms in PD is associated with changes in synchronizations within and between brain regions and changes in phase-amplitude coupling between brain regions [21,22]. However, it is not clear if *static* changes in resting-state networks are present in the very early stages (first 3 years) in drug-naïve never-medicated patients with PD. Furthermore, whether changes in temporal dynamics occur in resting-state functional networks in de novo PD subjects is unknown.

In the present study, as part of the National Institutes of Health/National Institute of General Medical Sciences Centers of Biomedical Research Excellence grant to the Center for Neurodegeneration and Translational Neuroscience, we investigated low-frequency BOLD fluctuations of major resting-state networks in early PD using data from the Parkinson's Progression Marker Initiative (www.ppmi-info.org). Previously, frequency-specific analysis of resting-state networks has been carried out using bandpass filtering in which the frequency intervals were specified using information from electrophysiological data [23] or simply by dividing the possible frequency range into equal intervals that were specified by the user [24,25]. An alternative approach toward finding frequency intervals in resting-state data is by Empirical Mode Decomposition (EMD) [26,27]. EMD is a data-adaptive analysis method for studying the naturally occurring frequency bands in time series [28]. EMD can be used, in particular, for nonstationary signals and allows the decomposition of time series into nearly orthogonal modes spanning narrow frequency bands. The oscillatory modes are called intrinsic mode functions (IMFs) and are obtained by a sifting algorithm. The novelty of our EMD approach lies in the adaptive decomposition of fMRI data using EMD and identification of resting-state networks based on energy and period (inverse of frequency) characteristics of IMFs. These novel energy-period relationships of resting-state networks in PD may allow use of imaging biomarkers in characterizing or detecting PD in the early stages of the disease. Early stage identification of PD may improve diagnostic accuracy, enrollment in clinical trials of disease-modifying agents, and allow for more effective treatments.

In a second investigation, we explored the dynamic aspects of functional connectivity using the same data set. In previous research studies, dynamic functional connectivity analysis was carried out mainly by using a sliding-window method, in which pairwise linear correlations among network components are captured in subsequent temporal windows with a fixed window size and further clustered into multiple dynamic functional brain states [29,30]. To find an appropriate window size is challenging because the windows size should be small enough to capture existing temporal transients and large enough to produce stable results [10]. The EMD method, however, provides us an alternative way to compute a time-dependent optimum window size in the sliding-window analysis. IMFs obtained from EMD track local periodic changes of nonstationary time series and an optimum window size can be determined at each time point. We incorporated the optimum window

size in the sliding-window method to explore dynamic functional connectivity within and between the major resting-state networks in early PD.

2. Materials and methods

2.1. Empirical mode decomposition and intrinsic mode functions

EMD is a method defined by an algorithm to decompose any time series, whether nonstationary or nonlinear, into a set of IMFs. This decomposition is based on local characteristics of the time series, which transforms instantaneous amplitude and instantaneous frequency information into meaningful quantities to be computed by the Hilbert transform of the IMFs. Although Fourier and wavelet transforms use preassigned basis functions, the EMD basis functions are data-derived IMFs. The EMD method operates at the scale of one oscillation and is fully data-driven. An IMF represents a simple oscillatory mode but is more general than a harmonic function of one frequency component. An IMF can have variable amplitude and frequency along the time axis.

In general, an IMF is a function that must satisfy two conditions: (1) For the entire time series, the number of extrema and the number of zero crossings must be equal to or can differ at most by one and (2) the mean value of the envelope defined by the local maxima and the envelope defined by the local minima is zero at every time point. A signal $x(t)$ can be decomposed in terms of its K IMFs $f_k(t)$ by

$$x(t) = \sum_{k=1}^{K} f_k(t) + r_K(t) \tag{1}$$

where K is the number of IMFs, $f_k(t)$ is the k-th IMF, and $r_k(t)$ is a small monotone residual (trend) function. The basic algorithm for obtaining the decomposition is an iterative sifting algorithm described in detail in the study by Huang et al. [28]. This iterative process sequentially explores the natural constitutive scales of a time series. The IMF with index 1 (IMF1) contains the highest frequencies, and IMF with index K contains the lowest frequency components. It has been shown that the frequency arrangement in IMFs mimics that of a dyadic filter bank [31]. Instantaneous frequency and amplitude of the IMFs can be computed by extending the signal into the complex plane with the Hilbert transform. In the literature, EMD combined with the Hilbert transform is referred to as the Hilbert-Huang Transform.

2.2. Energy versus period relationship of intrinsic mode functions

The time series in fMRI data are known to contain structured as well as white noise sources. Because the IMFs are basis functions that are derived from the data rather than functions that satisfy given analytic expressions, it is important from a statistical perspective to understand the IMFs of data that contain only noise sources so that IMFs of noisy signals can be compared with IMFs of pure noise data. Comparison with artificial noise data provides a reference standard of results obtained by EMD and allows a statistical significance to be associated to IMFs. Of particular importance is the relationship of the mean energy as a function of the mean inverse of the frequency (mean period) for each IMF. The mean energy per unit time, E_k, of the k-th IMF, $f_k(t)$, is defined by the mean instantaneous squared amplitude of the IMF. This definition leads to

$$E_k = \frac{1}{N\,\Delta t} \sum_{t=1}^{N} f_k(t)^2 \tag{2}$$

where N is the number of data points and Δt is the sampling time which is equal to the TR in fMRI. The mean period, T_k, is defined by the mean value for the inverse of the instantaneous frequency obtained from the Hilbert transform, that is,

$$T_k = \frac{1}{N} \sum_{t=1}^{N} \frac{1}{\nu_k(t)}\ . \tag{3}$$

However, owing to outliers in the estimation of the instantaneous frequency spectrum, especially for frequencies close to zero, Eq. (3) does not provide robust values of T_k. Instead, we determine the density of $\nu_k(t)$ using kernel density estimation (with a Gaussian kernel). For white Gaussian noise, it has been shown that [32]

$$\log(E_k) = 0.12 - 0.934 \log(T_k) \approx -\log(T_k) \tag{4}$$

Thus, $y = \log(E_k)$ as a function of $x = \log(T_k)$ is distributed approximately along the diagonal line $y = -x$ for all IMFs of white noise data.

2.3. Time-dependent window size in dynamic functional connectivity analysis

The time-dependent window size at each time point of two fMRI time series x_1 (t) and x_2 (t) can be computed from the instantaneous period $p_k(t)$ and average energy density E_k of the k-th IMF. The instantaneous periods $p(t)$ capture the local nonstationarity of the original signal [33], and the average energy densities E_k summarize the energy contributions of each IMF to the original signal. A time-dependent period, $T(t)$, for each time course is then determined as an average of p_k (t) weighted by E_k, that is,

$$T(t) = \frac{1}{\sum_k E_k} \sum_k p_k(t) \times E_k. \tag{5}$$

The final time-dependent window size, $T_d(t)$, of x_1 and x_2 to obtain an optimal sliding-window correlation is chosen to be the maximum of $T_1(t)$ and $T_2(t)$ (where $T_1(t)$ and $T_2(t)$ are the time-dependent window sizes of x_1 and x_2, respectively) to ensure that the data in one instantaneous period are included in calculating the correlation coefficient [34]. Thus, $T_d(t)$ captures the local nonstationarity of original

time courses, summarizes different contributions of each IMF to original signals, ensures that the data in one period are included in calculating the correlation coefficient, and therefore is optimum in capturing temporal dynamics between fMRI time series, as compared to a fixed window size. Dynamic functional connectivity analysis is then carried out using a sliding-window approach with this time-dependent window size $T_d(t)$.

2.3.1. Simulation

The simulation aimed at demonstrating that the sliding-window correlation computed with a time-dependent window size can capture local transients and avoid unstable fluctuations, as compared to the correlation values computed using a fixed window size. To provide a specific example, two nonstationary time series

$$y_1 = 0.8\left(1 + 0.25\cos\left(\frac{2\pi}{400}\mathrm{t}\right)\right)\cos\left(0.25\mathrm{t} + 1.25\sin\left(\frac{2\pi}{200}\mathrm{t}\right)\right)$$

and

$$y_2 = 0.6\left(1 + 0.25\sin\left(\frac{2\pi}{400}\mathrm{t}\right)\right)\sin\left(0.25\mathrm{t} + 1.25\cos\left(\frac{2\pi}{200}\mathrm{t}\right)\right)$$

were simulated with a sample rate of 1 second (TR = 1 s) with a static correlation coefficient of −0.02. The dynamic correlations between y_1 and y_2 were calculated and compared using the sliding-window method with two fixed window sizes, 10 TR and 50 TR, as well as the time-dependent window size $T_d(t)$.

2.4. Participants

The data used in this study were obtained from the publicly available anonymized Parkinson's Progression Marker Initiative database [Marek et al., 2011]. We included 18 NCs (14 male (M); age: 64.25 ± 9.78 years (mean ± SD); years of education: 16.72 ± 2.67 years) and 18 newly diagnosed, early stage, and never-medicated PD subjects (10 M; age: 57.11 ± 11.63 years; years of education: 17.00 ± 2.77 years; disease duration: 0.83 ± 0.84 years) in our analysis. A chi-square test was performed to check statistical significance for gender difference between the two groups, and Wilcoxon rank-sum test was performed to check for differences of age and year of education. Differences in age ($P = .06$), gender ($P = .15$), and years of education ($P = .76$) were not significant between the two groups.

Additional information about the distribution of participants with hypercholesterolemia, hypertension, and diabetes was also obtained from the Parkinson's Progression Marker Initiative database and listed in Table 1. A chi-square test was performed to check statistical significant difference for each disease distribution between the PD and NC groups. Differences in the distribution of participants with hypercholesterolemia ($P = .72$), hypertension ($P = .07$), and diabetes (one in the NC group only) were not significant between the two groups.

Table 1
The distribution of participants with hypercholesterolemia, hypertension, and diabetes between the two study groups

Number of subjects has the condition	PD	NC	Differences (*P*-value)
Hypertension	3	8	.07
Hypercholesterolemia	5	6	.72
Diabetes	0	1	N/A

Abbreviations: PD, Parkinson's disease; NC, normal controls.

2.5. MRI data acquisition and preprocessing steps

All subjects underwent resting-state fMRI scans on 3T Siemens scanners. The resting-state fMRI involved an 8 minutes and 24 seconds echo-planar acquisition with 210 time points (TR = 2,400 ms, TE = 25 ms, field of view = 22.4 cm, flip angle = 80°, resolution = 3.3 × 3.3 × 3.3 mm^3, and 40 axial slice). In addition, a T1-weighted structural image was also acquired for each subject (TR = 2,300 ms, TE = 2.98 ms, flip angle = 9°, and voxel size = 1 × 1 × 1 mm^3).

The first 5 time points (12 seconds) were removed to allow the MR signal to achieve T1 equilibrium. Echo-planar data were slice-timing corrected and realigned to the mean echo-planar image in Statistical Parametric Mapping 12 (http://www.fil.ion.ucl.ac.uk/spm/), further coregistered to the subject T1 space, and then normalized to the standard Montreal Neurological Institute-152 2-mm template using Advanced Normalization Tools software (http://stnava.github.io/ANTs/). Six head motion parameters, signals extracted from subjects' white matter and cerebrospinal fluid (3-mm cubes centered at Montreal Neurological Institute [26, −12, 35] and [19, −33, 18]),

Table 2
Number of dynamic states computed from cross-validation and the difference of frequency-of-state alternation between the two groups obtained with the time-dependent window size and fixed window size used in previous studies

Window size	Number of dynamic states	Difference of frequency-of-state alternation between NC and PD (*P* value)
30 s	11	.05
60 s	7	.10
120 s	3	.24
Time-dependent window size (97.75 s ± 41.36 s)	3	.006

Abbreviations: PD, Parkinson's disease; NC, normal controls.

were regressed out from each data set. fMRI data were further spatially smoothed using an 8-mm 3D Gaussian filter.

2.6. Static analysis of resting-state networks

To obtain the spatial resting-state networks, group ICA [35] (based on the FastICA algorithm [36]) was performed by stacking all data in the temporal domain to obtain 30 resting-state networks. Then, spatial regression was used on the networks of the group time series data to obtain the time series signatures for NC and PD. To get more detail on the time signatures of the resting-state networks, we decomposed the corresponding time signatures using EMD into the first 5 IMFs for each spatial resting-state network. These 5 IMFs covered a frequency range from 0.01 Hz to the Nyquist frequency (0.5/TR) of the data. For each IMF, the average instantaneous energy, period, and their standard deviations were computed for NC and PD.

2.7. Dynamic functional connectivity analysis

Dynamic functional connectivity analysis was carried out using a sliding-window approach with the optimum time-dependent window size as determined by EMD. Specifically, whole-brain dynamic functional connectivity was captured by computing the windowed correlation between time courses from every pair of nodes, in which each node is a network component. To obtain network components, another group ICA with 100 components was performed by stacking data from both PD and NC subjects. Seventy-two ICA components were visually identified as network-related components, and the corresponding subject-specific ICA maps and time courses were calculated using dual regression [37]. A voxel-wise comparison was conducted for each of the 72 ICA components. Two sample *t*-tests were performed with age and gender as covariates, and each ICA component was spatially masked with the thresholded group ICA component map.

The connectivity matrices for each subject were then calculated using sliding-window correlations between each pair of nodes with the time-dependent window size $T_d(t)$. The connectivity matrices in each sliding window (size, 72 $\times$ 72) were concatenated in time for each subject and further stacked for both PD and NC subjects. Standard k-means clustering was performed in MATLAB (www.mathworks.com) on the concatenated connectivity matrix from all subjects to estimate dynamic functional states for both the groups. The optimum cluster number K was determined by a leave-one-out cross-validation. Finally, the time spent in each state and the frequency-of-state alternations were calculated for every subject separately and used to compare the temporal dynamics between the PD and NC groups. Two sample t-tests were carried out with age, gender, and the distribution of hypertension as covariates for these comparisons. To compare results with traditional methods which are previously published, the same analyses were also repeated with fixed window sizes of 13 TRs ($\sim$30 seconds), 25 TRs ($\sim$60 seconds), and 50 TRs ($\sim$120 seconds), as suggested in other studies [10,29].

3. Results

3.1. Static functional connectivity analysis in early PD

Fig.1 shows the spatial maps and corresponding temporal IMFs computed for the DMN. A *t*-test showed no significant spatial differences in this network between NC and PD. IMF1 of PD patients shows some variation in the amplitude of the time series signal. All other IMFs have similar characteristics for the same index in NC and PD participants. We calculated the energy and period for each IMF and plotted this information using group-specific markers in a log (energy) versus log (period) diagram. Standard deviations of the markers are indicated by horizontal and vertical lines for log (period) and log (energy), respectively. We found no significant difference in energy or period for any of the IMFs of the DMN for NC versus PD.

In Figs. 2 and 3, we show all six resting-state networks (out of 30) in which the period of the IMF (with the same index) differed by a large effect size (Cohen's $d > 0.8$ [38]) between NC and PD. The obtained networks are the executive control network (ECN), the parietal network (PAR), the cognitive control network (CCN), the prefrontal cortex network (PFC), and the left/right frontoparietal network (lFPN and rFPN). All these networks show spatial and temporal differences. The ECN, CCN, and PFC have reduced activations in PD, whereas the PAR, lFPN, and rFPN have spatially extended activations in PD.

The temporal characteristic of these networks differ; the ECN has increased frequency content (less period) for the very low–frequency band in IMF5, which is in the drift range ($f < 0.01$ Hz), whereas all other networks show a decrease in low frequencies for some of the higher bands (IMFs with index ≤ 4). We found that the period for the same indexed IMF is always larger for PD, for all networks except the ECN, irrespective of the effect size being small (Cohen's $d = 0.2$) or large (Cohen's $d = 0.8$). The amplitude of oscillations of the IMFs as measured by log (energy) is generally smaller for PD for most of the IMFs.

3.2. Dynamic functional connectivity analysis in early PD

3.2.1. Simulation

To illustrate the advantage of using a time-dependent sliding-window size, Fig. 4A shows simulated nonstationary time series y_1 (blue) and y_2 (red). Instantaneous periods of y_1 (dashed blue) and y_2 (dashed red) and the time-dependent window size $T_d(t)$ between y_1 and y_2 (solid green) are plotted in Fig. 4B at every time point. As shown in Fig. 4C, dynamic correlations between y_1 and y_2 calculated with the time-dependent window size (solid green) capture existing

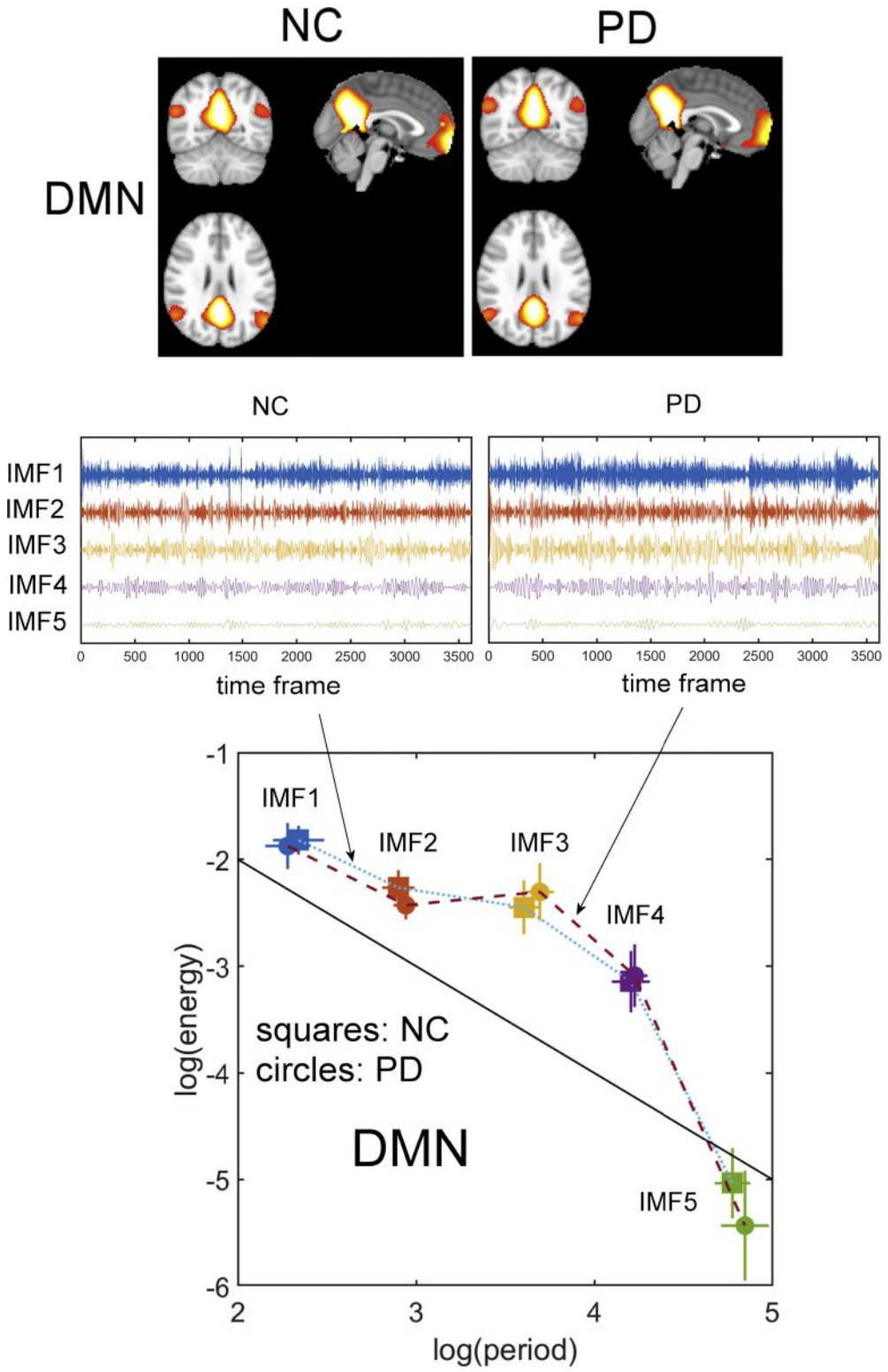

Fig. 1. Top: The spatial DMN determined by group ICA for NC and subjects with Parkinson's disease in the early stages (PD). There are no significant differences in the spatial distribution of this network between NC and PD. Middle: Time signatures of the DMN for NC and for patients with PD were decomposed into five IMFs. Bottom: For each IMF, the average energy and period were calculated and displayed in a log (energy) versus log (period) diagram. The diagonal line indicates the expected mean of Gaussian white noise. Different markers specify the IMF properties for NC (squares, connected by blue dotted line) and for PD (solid circles, connected by red dashed line). The horizontal and vertical bars through the markers indicate the standard deviation in log (period) and log (energy), respectively. Abbreviations: ICA, independent component analysis; DMN, default mode network; NC, normal controls; PD, Parkinson's disease; IMF, intrinsic mode function.

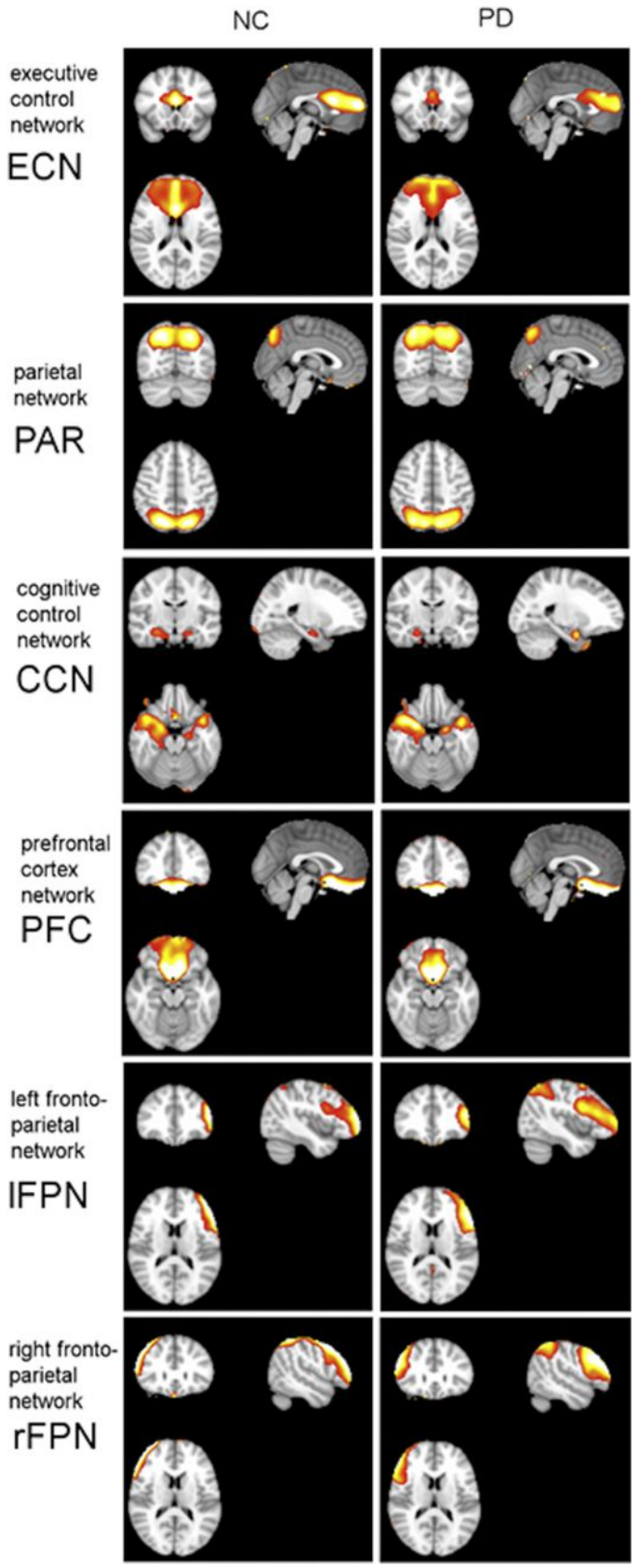

Fig. 2. Six ICA resting-state networks show different spatial patterns for NC and early PD. Note that the ECN and PFC networks show reduced spatial activity, whereas the PAR and FPN show increased activity in PD. The CCN has decreased activity in the hippocampus but increased activity in the inferior temporal lobes in PD. Abbreviations: ICA, independent component analysis; FPN, frontoparietal network; NC, normal controls; ECN, executive control network; PAR, parietal network; CCN, cognitive control network; PFC, prefrontal cortex network; PD, Parkinson's disease.

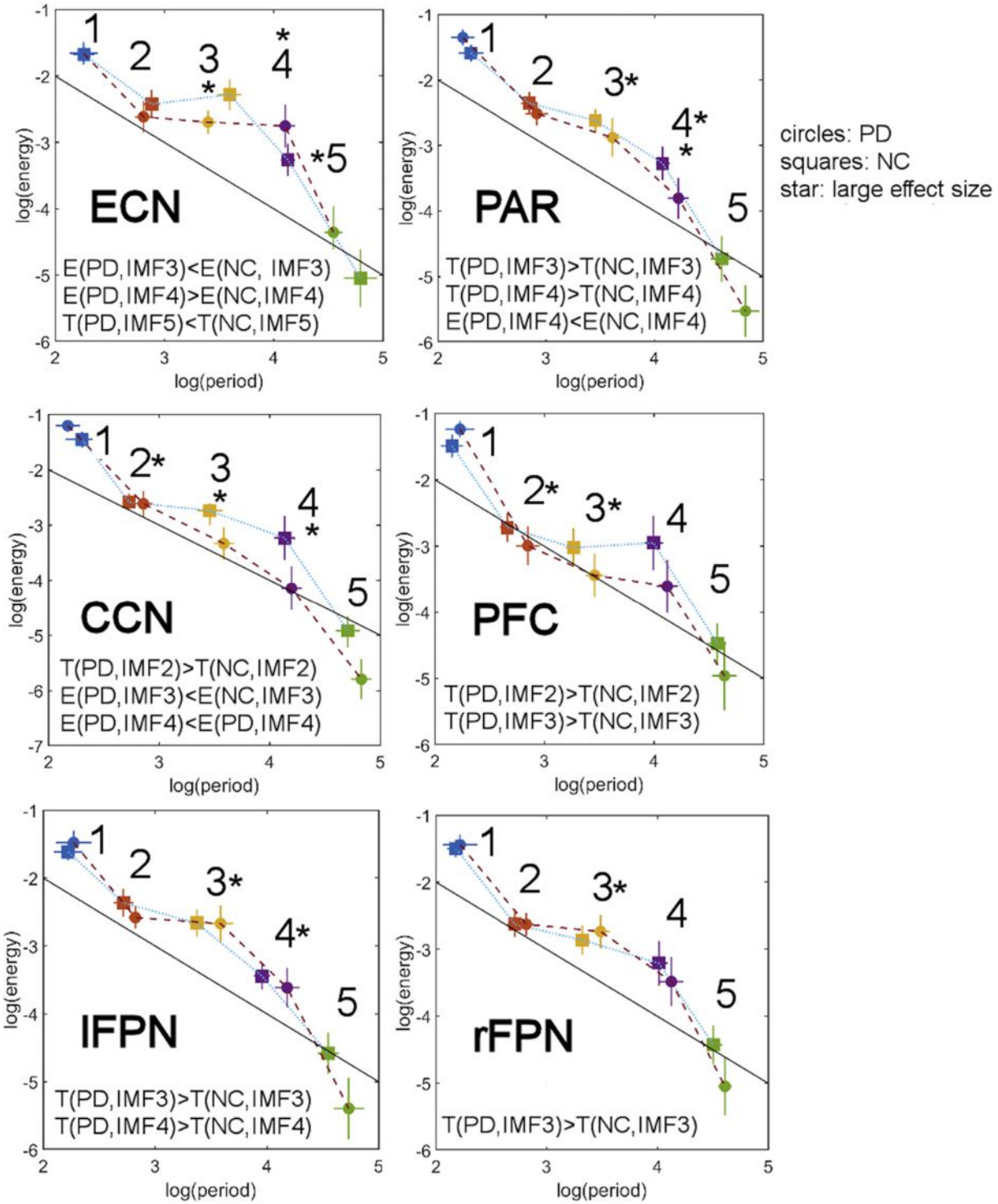

Fig. 3. Different temporal characteristics of IMFs as measured by the log (energy) versus log (period) relationship for the six resting-state networks in Fig. 2. All relationships shown with a "*" indicate a large effect size (Cohen's $d > 0.8$) (either for energy or for period) for specific IMFs. The ECN was the only network found where the period for PD was reduced (in IMF5). For all other networks that show a large effect size between log (period) of PD and NC (i.e., PAR, CCN, PFC, lFPN, rFPN), the mean period is always larger for PD versus NC, indicating that these networks operate at lower frequencies in PD. The letter T in the relational statements indicates the period. Abbreviations: PD, Parkinson's disease; lFPN, left frontoparietal network; rFPN, right frontoparietal network; NC, normal controls; IMFs, intrinsic mode functions; ECN, executive control network; PAR, parietal network; CCN, cognitive control network; PFC, prefrontal cortex network.

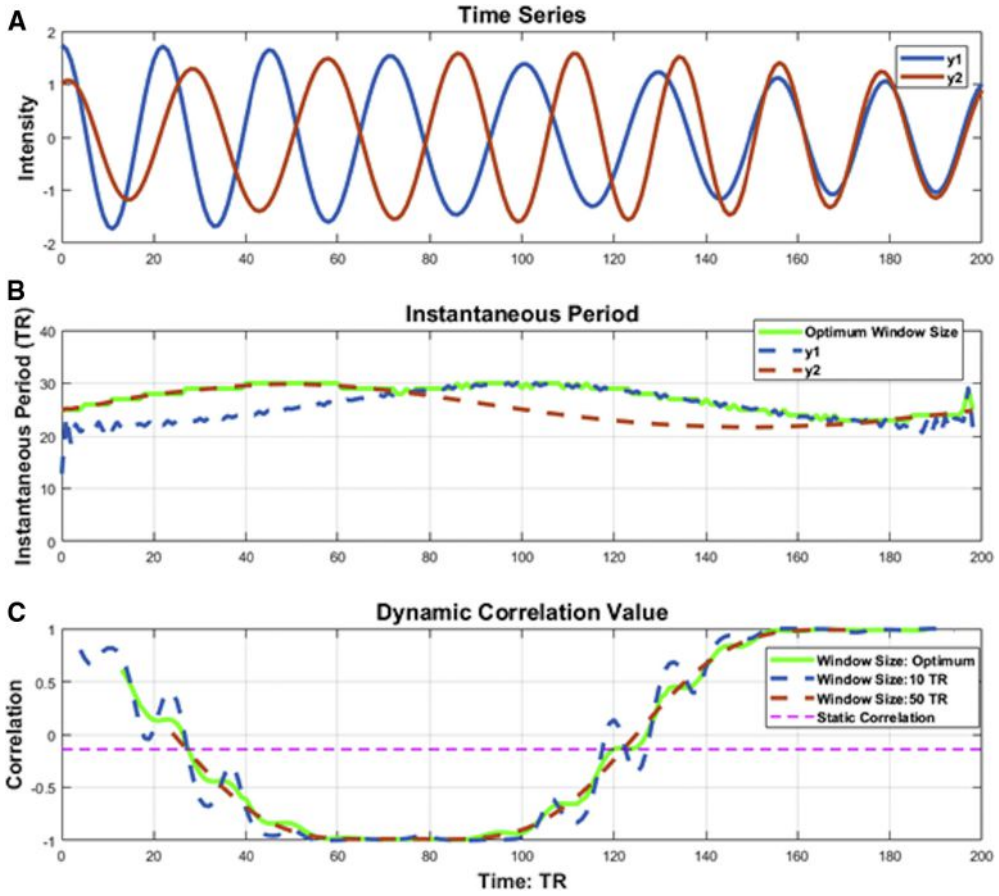

Fig. 4. Simulation. (A) Two nonstationary simulated time series: y_1 (blue) and y_2 (red). (B). Instantaneous period of y_1 (dashed blue) and y_2 (dashed red) and the time-dependent window size (green) at each time point. (C). The dynamic correlation between two synthetic time series was calculated using sliding-window method with different window sizes: 10 TR (dashed blue), 50 TR (dashed red), and time-dependent window size (solid green). The static correlation between these two time series is indicated by the dashed purple line.

local transients without creating unstable fluctuations, as compared to the correlation computed with the fixed window sizes of 10 TRs (10 seconds, dashed blue) or 50 TRs (50 seconds, dashed red line).

3.3. Real fMRI data

A flow chart of the dynamic connectivity analysis is shown in Fig. 5. Eight major resting-state networks, formed by 72 network-related ICA components, were investigated, including the subcortical network, auditory network, sensorimotor network, visual network, CCN, DMN, medial temporal network, and cerebellum network (Fig. 6A). Both the ECN and FPN obtained from the other ICA run with 30 components are combined to be the CCN in the dynamic functional connectivity analysis. A group comparison was conducted for each of the 72 ICA components, and no significant spatial difference was found at a family-wise corrected error rate of $P < .05$. A static correlation matrix between each pair of nodes was converted to Fisher's z statistics and shown in Fig. 6B. Three dynamic functional states are determined from the leave-one-out cross-validation in k-means clustering for both PD and NC subjects (Fig. 7A) with the time-dependent window size. The average window size is listed in Table 2. Fig. 7B and C show that NC subjects spend significantly more time in state II, which has stronger connections, both between and within networks, whereas PD patients tend to stay longer in the more weakly connected functional states I and III. Furthermore, a significant reduced frequency-of-state alternation ($P = .006$) is found in the PD group (Fig. 7D). The same analysis was repeated with a fixed window size of 30 seconds, 60 seconds, and 120 seconds. The number of dynamic states determined by cross-validation and the between-group comparison results of frequency-of-state alternation are listed in Table 2.

4. Discussion

4.1. Static resting-state analysis

In this study, we developed a novel method for the identification of abnormal temporal signatures associated with brain states in early PD using resting-state fMRI data. We used EMD as a data-adaptive method to determine energy and period characteristics of temporal signatures of major

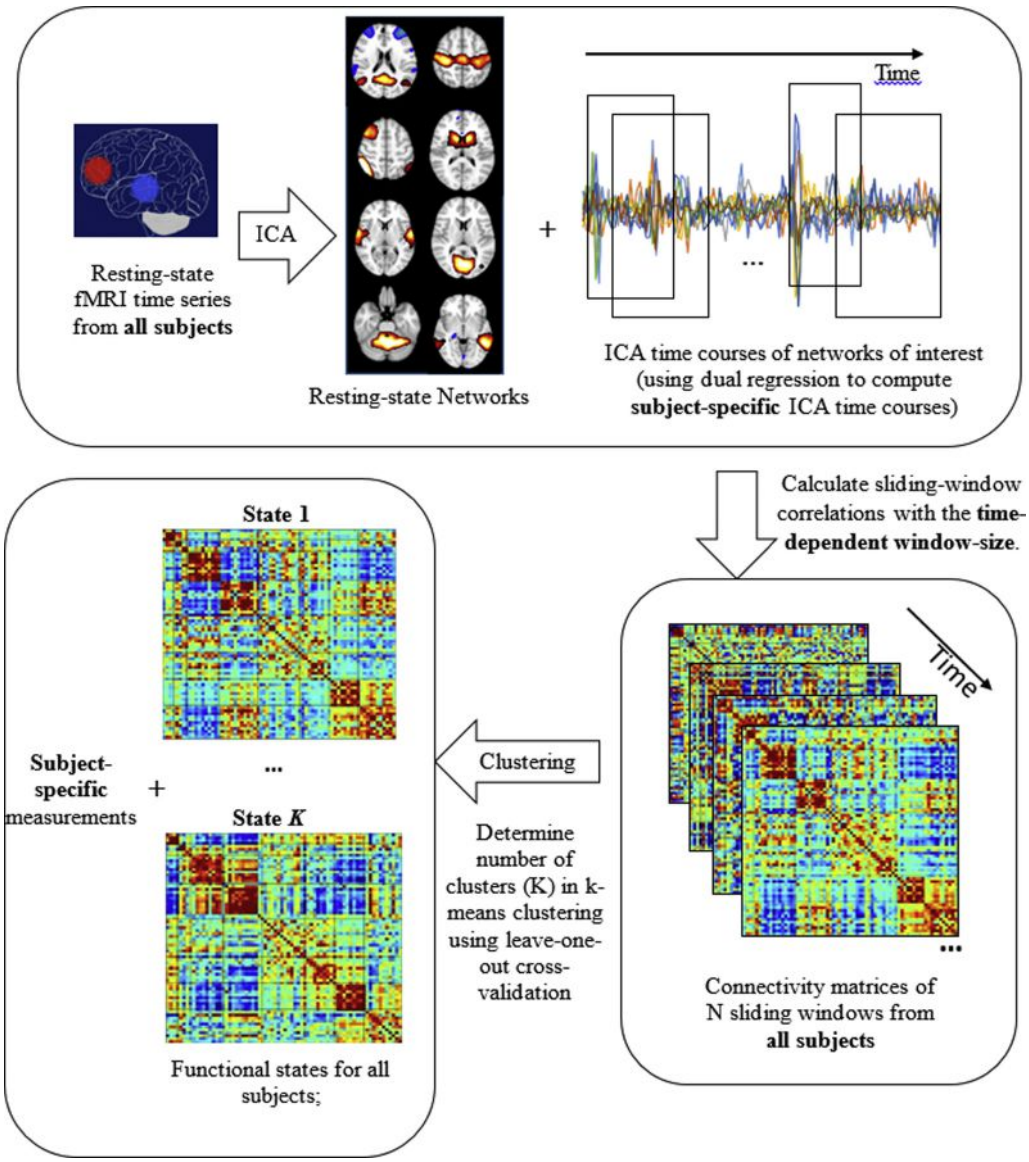

Fig. 5. Whole-brain dynamic functional connectivity analysis flow chart. Abbreviation: ICA, independent component analysis.

cortical networks that were obtained with ICA. The advantage of using EMD is that the temporal signatures can be decomposed into basic modes (namely the IMFs) that are subject-specific, and each basic mode can be characterized by energy density and period content. This analysis is different from a Fourier or wavelet analysis because there are no parameters that need to be adjusted (such as predefined frequency intervals or wavelet types), and the analysis is completely data-driven. We have obtained consistent features of energy and period for all subjects as shown by the small standard deviations about the mean values for average energy and period content. EMD shares a frequency decomposition feature with the discrete wavelet transform in which both methods exhibit a dyadic filter bank decomposition. However, the discrete wavelet transform has a frequency decomposition in the sense of components at different but fixed nonadaptive frequency scales. Using a discrete wavelet transform, energy can be calculated based on wavelet coefficients, and period information from the fixed frequency ranges can be obtained. However, the period information is nonadaptive to individual subject data, and one would obtain the same period information for both PD and NC. An alternate approach would be the use of a continuous wavelet transform, but a relationship between its decomposition level and frequency is not directly defined. For these reasons, EMD is a superior time series analysis method because it determines subject-specific energy densities and periods of fundamental modes.

4.2. Comparison with other studies

FMRI resting-state data have several advantages over other modalities such as fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging to detect characteristic

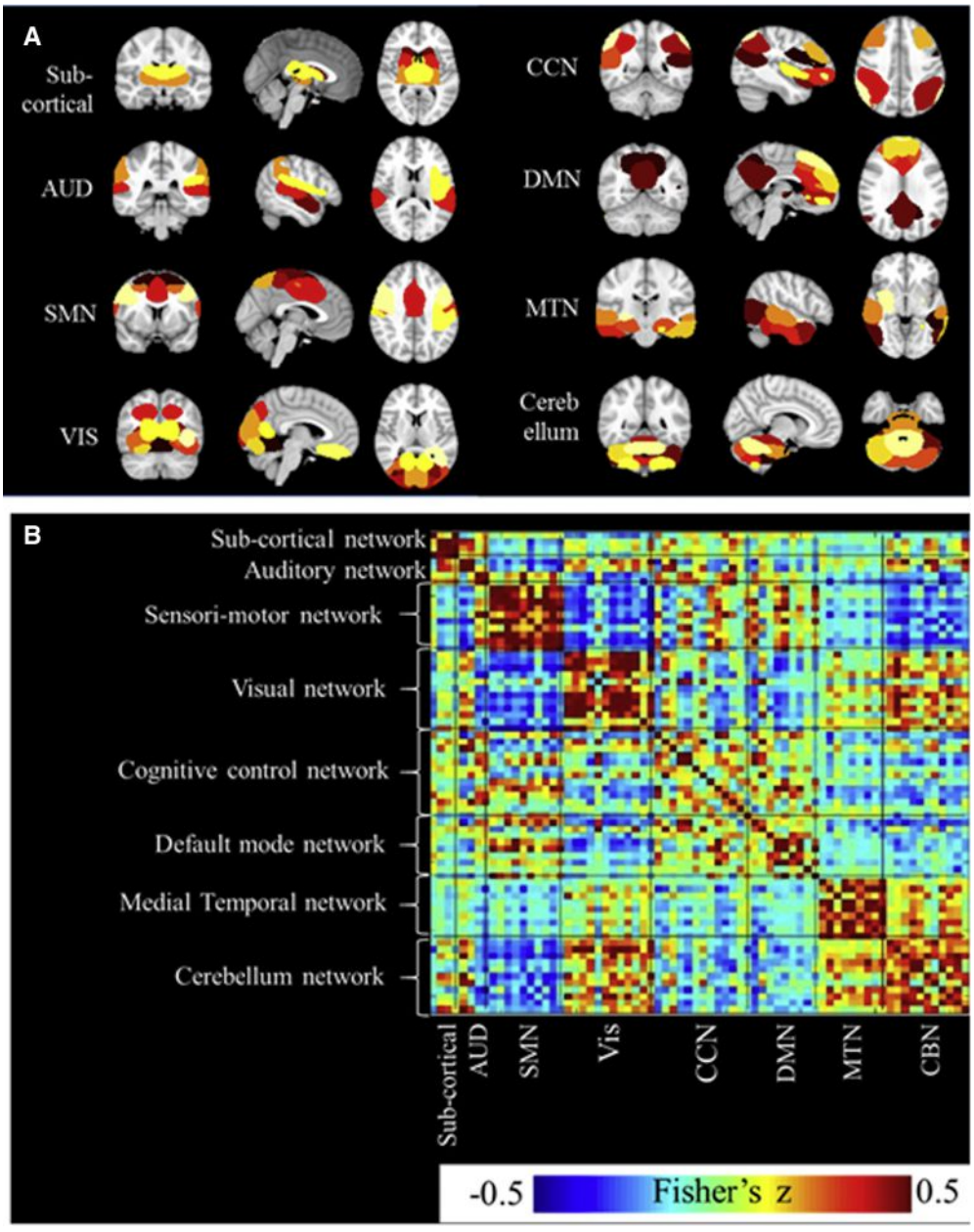

Fig. 6. (A) Spatial components from 72 network-related ICA components. (B) Whole-brain static functional connectivity matrix. Abbreviations: ICA, independent component analysis; AUD, auditory network; SMN, sensorimotor network; Vis, visual network; CCN, cognitive control network; DMN, default mode network; MTN, medial temporal network; CBN, cerebellum network.

features in early PD. In previous studies using FDG-PET imaging in early PD, three disease-specific spatial covariance patterns were found [39], namely the PD motor-related pattern, the PD cognition-related pattern, and the PD tremor-related pattern. Of particular importance is the cognition-related pattern that showed metabolic reductions in preSMA, medial prefrontal cortex, precuneus, and metabolic increases in the cerebellum. Similar results with fMRI have been shown in resting-state data [40]. However, PD-specific resting-state networks were found using a spatial analysis method and not by characteristics of temporal signatures of resting-state as we have proposed here. Furthermore, the spatial covariance pattern was not obtained for never-medicated first-3-year PD patients. For this reason, previous spatial fMRI results are difficult to compare with those of our temporal analysis.

Compared with [^{15}O] H_2O PET, fMRI has greater temporal resolution, slightly greater spatial resolution, and greater sensitivity. However, the BOLD signal is more difficult to relate to dopamine deficiency, and how blood flow, blood volume, and oxygen consumption (which lead to the BOLD signal) are related to neurotransmitters

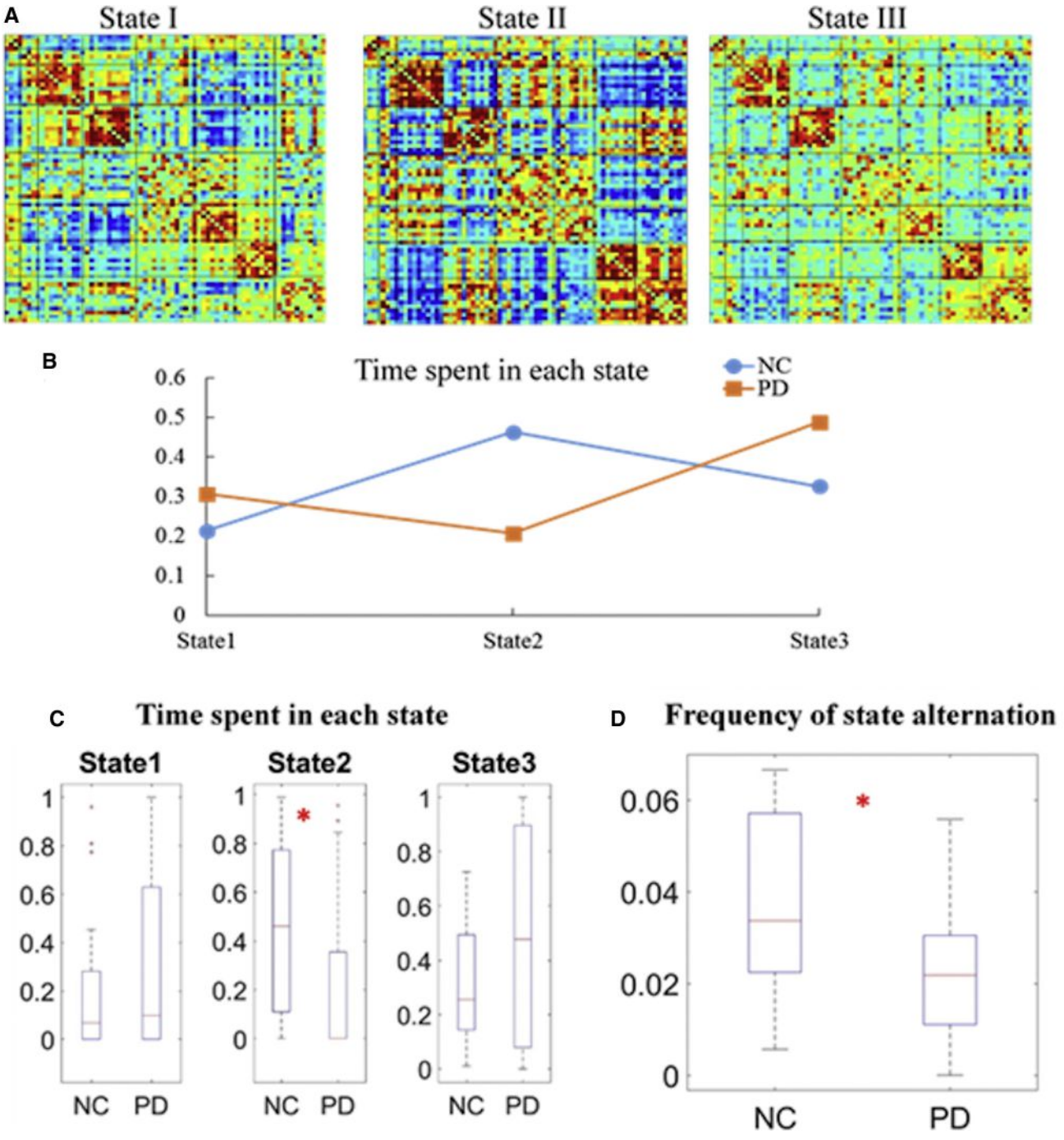

Fig. 7. (A) Three dynamic states were obtained from whole-brain dynamic functional connectivity analysis with the time-dependent window size. (B) Average window fractions of time spent in each state (window in each state/total number of windows) for PD (orange) and NC (blue) groups. (C). Statistical comparisons of window fractions spent in each state between PD and NC groups; $*P < .05$. (D). Statistical comparison of frequency-of-state alternations between PD and NC groups. Abbreviations: PD, Parkinson's disease; NC, normal controls.

such as dopamine is currently unknown. In a recent study, static spatial brain networks obtained by FDG-PET were compared with resting-state fMRI in a larger cohort of healthy nondemented subjects from the Alzheimer's Disease Neuroimaging Initiative database [41]. Most networks obtained showed similar spatial covariance patterns in FDG-PET and fMRI. However, discrepancies were observed for some important networks. For example, some of the anterior-posterior networks (e.g., the DMN and lFPN) could only be partially obtained by FDG-PET. Furthermore, reduced correlations were observed in anterior-posterior correlations in FDG-PET when

compared to BOLD networks, which may indicate different signal mechanisms of metabolism for FDG-PET and BOLD in fMRI. This discrepancy could also arrive due to the different temporal scales of the imaging modalities (seconds for fMRI and minutes for FDG-PET), which lead to different inter-regional couplings [41]. The detection of temporal changes of brain states' dynamic functional connectivity using FDG-PET may not be feasible because of the lower temporal resolution of FDG-PET.

We demonstrated that in early never-medicated PD, there are no spatial or temporal changes detectable in the DMN. However, we found 6 other cortical brain networks that showed spatial and temporal differences of resting-state signal characteristics. These networks are the ECN, PAR, CCN, PFC, lFPN, and rFPN which show overlap with the cognitive resting-state pattern in previous PD studies [39,40]. We have analyzed the first 5 IMFs of the associated temporal profiles in terms of content in average energy density and average period. We found that in early PD, the PAR, CCN, PFC, lFPN, and rFPN networks are driven by reduced frequencies (increased period) for all IMFs in the low-frequency range of less than 0.1 Hz (which is covered by IMFs with k = 2, 3, 4, 5). Several of the IMFs also showed a large effect size for reduced frequency content. In addition, most corresponding energy densities were lower in PD. There was only one network, namely the ECN, with different but not significant characteristics for frequencies above the drift range (f $>$ 0.01 Hz). Overall, most networks in early PD were characterized by a reduction in frequency and energy in specific low-frequency bands of less than 0.1 Hz as determined by EMD. For future studies, it may be interesting to study early PD patients with mild cognitive impairment to see if DMN abnormalities can be detected because mild cognitive impairment occurs in approximately 1 of 4 PD patients.

4.3. Time-dependent window size in dynamic functional connectivity analysis

To date, the sliding-window method with a fixed window size is most commonly used for examining dynamics in resting-state functional connectivity [9,29,30]. Using simulation, we have demonstrated that compared with the fixed window size, the time-dependent window size $T_d(t)$ computed from instantaneous period of IMFs can more precisely capture the local periodic changes without creating unstable fluctuations. This advantage of using time-dependent window size in dynamic functional connectivity analysis is further demonstrated using real fMRI data from PD and NC groups (Table 2). Significant ($P < .01$) reduced frequency-of-state alternations in the PD group is found when a time-dependent window size is used, which is not observed with the fixed window size.

4.4. Altered functional dynamics in PD

In our analysis, three whole-brain functional dynamic states are found for both PD and NC subjects. As shown in Fig.7A, robust within-network functional connectivity is observed in all three states, whereas stronger between-network functional connectivity is observed in state II, as compared to state I and III. Our results indicate that NC subjects stay significantly longer in state II (Fig. 7B), which is consistent with previous findings of increased between-network functional connectivity in healthy aging [42]. The altered dynamics of basal ganglia-cortical circuits in PD subjects have been widely reported using electrophysiological data. Specifically, unmedicated PD subjects exhibit aberrant coherent activity patterns and excessive synchronization of neuronal activities in the basal ganglia-cortical loop, which will in turn affect the neuronal circuits' dynamics [21,43–46]. Using public neuroimaging data, we observe a significant reduced frequency-of-state alternation among the three dynamic functional states, which demonstrates the limited dynamic range of whole-brain functional connectivity in treatment-naive PD subjects.

5. Conclusions

We used EMD to study the energy and period content of IMFs for static resting-state networks in early PD and found a reduction in both IMF frequency content and energy for PAR, CCN, PFC, lFPN, and rFPN. In contrast, ECN showed increased low-frequency content. For the DMN, no spatial or temporal changes were observed. We further studied the dynamic functional connectivity in the same cohort using a sliding-window method with a time-dependent window size computed from IMFs and obtained using EMD. Altered temporal behaviors and reduced whole-brain temporal dynamics were found in early PD subjects.

Acknowledgments

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RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using PubMed. The relevant citations are appropriately cited. Static and dynamic functional connectivity in Parkinson's disease subjects has not been systematically study with Empirical Mode Decomposition.
2. Interpretation: Our findings lead to a characterization of resting-state data in early never-medicated Parkinson's disease patients by providing static and dynamic imaging markers of functional connectivity using Empirical Mode Decomposition.
3. Future directions: The manuscript proposes new techniques to assess static and dynamic functional connectivity using Empirical Mode Decomposition which needs to be further validated in a larger cohort of PD subjects.

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Alzheimer's & Dementia

Review Article

Current understanding of magnetic resonance imaging biomarkers and memory in Alzheimer's disease

Ece Bayram*, Jessica Z. K. Caldwell, Sarah J. Banks

Department of Neurology, Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA

Abstract Alzheimer's disease (AD) is caused by a cascade of changes to brain integrity. Neuroimaging biomarkers are important in diagnosis and monitoring the effects of interventions. As memory impairments are among the first symptoms of AD, the relationship between imaging findings and memory deficits is important in biomarker research. The most established magnetic resonance imaging (MRI) finding is hippocampal atrophy, which is related to memory decline and currently used as a diagnostic criterion for AD. While the medial temporal lobes are impacted early by the spread of neurofibrillary tangles, other networks and regional changes can be found quite early in the progression. Atrophy in several frontal and parietal regions, cortical thinning, and white matter alterations correlate with memory deficits in early AD. Changes in activation and connectivity have been detected by functional MRI (fMRI). Task-based fMRI studies have revealed medial temporal lobe hypoactivation, parietal hyperactivation, and frontal hyperactivation in AD during memory tasks, and activation patterns of these regions are also altered in preclinical and prodromal AD. Resting state fMRI has revealed alterations in default mode network activity related to memory in early AD. These studies are limited in part due to the historic inclusion of patients who had suspected AD but likely did not have the disorder. Modern biomarkers allow for more diagnostic certainty, allowing better understanding of neuroimaging markers in true AD, even in the preclinical stage. Larger patient cohorts, comparison of candidate imaging biomarkers to more established biomarkers, and inclusion of more detailed neuropsychological batteries to assess multiple aspects of memory are needed to better understand the memory deficit in AD and help develop new biomarkers. This article reviews MRI findings related to episodic memory impairments in AD and introduces a new study with multimodal imaging and comprehensive neuropsychiatric evaluation to overcome current limitations.

Keywords: Alzheimer's disease; Dementia; Magnetic resonance imaging; Memory; Biomarker

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder resulting from pathological changes which typically spread through brain networks in a predictable pattern. AD pathology leads to early decline in memory, and some pathology can be detected years before measurable cognitive or functional change. At present, there are no disease-modifying treatments, and symptomatic treatment is limited in efficacy. Trials of novel therapeutics increasingly target the earliest brain changes, when a disease-modifying trajectory could potentially result in reduction or elimination of clinical impact. Memory measures remain an important way of assessing such clinical impact and are required in clinical trials in the United States [1].

Accurate diagnosis of AD was, until recently, confirmed only at autopsy. Today, there are several imaging biomarkers measuring neurodegeneration and amyloid β (Aβ) deposition in the brain to support the diagnosis [2]. Atrophy on

*Corresponding author. Tel.: 702-701-7892; Fax: 782 483 6010.
E-mail address: bayrame@ccf.org

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structural magnetic resonance imaging (MRI), hypometabolism on fluorodeoxyglucose positron emission tomography (FDG-PET), and increased levels of craniospinal fluid (CSF) total and phosphorylated tau are used to assess neurodegeneration. CSF Aβ42 and Aβ PET, on the other hand, are used to assess Aβ pathology. Preclinical studies focus on groups at risk for AD, as defined by the apolipoprotein E (APOE) status or examine cognitively normal control (CNC) performance in the context of other AD biomarkers, such as CSF Aβ. Large, shared, multisite, longitudinal multimodal data sets such as the AD Neuroimaging Initiative and similar studies initiated in Asia, Europe, and Australia allow for widespread exploration of structural and functional magnetic resonance imaging (fMRI) and PET data in addition to clinical, cognitive, and fluid biomarker data across the spectrum of disease. While there are several limitations, these data sets are an important resource in understanding imaging biomarkers in AD.

Memory is a complex construct. AD has an early and specific impact on episodic memory (i.e., the ability to learn and remember new information) [3], which can broadly be subdivided into encoding (or learning), recall, and recognition. Different types of stimuli (e.g., words, faces, and shapes) and memory tests (e.g., single trial and multi-trial presentations, free and prompted recall) can be used to detect deficits in these aspects of memory, and typically used measures often differ between clinical and research settings. Nonetheless, many studies of AD MRI biomarkers and biomarker candidates have included memory measures as correlates or validating factors.

At present, despite exploration of imaging biomarkers for AD, few have become widely accepted and approved for clinical use, and most remain experimental. In this targeted review, we focused on MRI studies. Following the conceptualization of AD as a biological and clinical continuum by Aisen et al [4], we assessed the MRI findings within preclinical (clinically normal individuals with evidence of AD pathology), and clinical (mild cognitive impairment [MCI] or prodromal AD, and AD dementia [ADD]) phases of AD. The transition between these phases is subtle, and individuals may report cognitive decline even when neuropsychological testing does not suggest any impairment. As episodic memory is the first cognitive domain to be affected along the course of AD, we aimed to investigate the association between MRI findings and episodic memory performance specifically. Current diagnostic criteria of MCI (prodromal AD) and ADD are based on clinical history, neuropsychological testing, and neurologic and psychiatric examinations [5,6]. Imaging methods, CSF, and blood tests are used only to support the diagnosis and to exclude other dementia causes. Nevertheless, subtle findings on MRI have been reported years before the onset of clinical symptoms. Thus, imaging findings correlating with the clinical profile may help identify underlying mechanisms and therapeutic targets for the debilitating memory deficit in AD.

2. Structural MRI

Aging is associated with a slow decline in both white matter (WM) and gray matter (GM) volumes, and this atrophy rate is increased in AD [7]. Although GM atrophy has been more frequently assessed in AD, structural MRI approaches also allow for the assessment of cortical thickness, as well as shape and WM alterations. This section will focus on studies investigating the relationship between episodic memory performance and structural changes in GM and WM using different imaging analysis approaches (Table 1). Structural differences between CNC and participants within AD spectrum without any episodic memory associations are beyond the scope of this review and will not be discussed.

2.1. GM changes

Hippocampal atrophy is included in the 2011 National Institute on Aging criteria for ADD and MCI due to AD [3,5]. Before the advent of Aβ PET imaging, hippocampal volumetric changes that can be determined noninvasively and relatively cheaply using MRI were one of the earliest detectable imaging changes in AD. These changes can be quantified using NeuroQuant, an Food and Drug Administration-approved imaging processing tool [42]. Decline in hippocampal volume and thickness has been consistently associated with memory deficits in AD continuum. In preclinical AD, hippocampal and entorhinal cortex volume, and hippocampal and parahippocampal thickness have been associated with verbal memory [9,12,30]. There have also been reported associations between reduced medial temporal lobe (MTL) volume in CNC with AD risk factors and future memory decline [8]. Further along the course of the disease, in MCI and ADD, decline in hippocampal volume and MTL thickness was associated with worsening in verbal memory [13,16,19,21,23–27,29,33–35,39,40]. Although less extensively studied, visual memory has been associated with hippocampal volume in amnestic MCI (aMCI) [39]. Studies of hippocampal subregions revealed that CA1 volume declines within hippocampus were particularly related with recall performance in aMCI and ADD [26,29,37].

With time, GM changes in AD spread outside the MTL. Extratemporal regions implicated in episodic memory decline include the posterior cingulate gyrus (PCG)/precuneus [28,30,31] and middle frontal gyrus [27,28]. Both atrophy and thinning of these regions were associated with memory decline. In MCI patients, who converted to ADD over time, decreased inferior frontal gyrus volume was associated with the verbal memory decline [38], suggesting extratemporal involvement may be predictive of disease progression.

2.2. WM changes

While AD is a disease primarily associated with GM loss, concomitant WM change has a role in cognitive expression.

Table 1
Structural MRI correlates of episodic memory

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Preclinical AD				
Jagust, et al 2006 [8]	60 CNC (6 dementia or MCI converters) (2-year follow-up)	Word list	HC and entorhinal cortex volumetry/ FDG-PET	HC and entorhinal cortex volume predicted delayed recall decline over time.
Lind, et al 2006 [9]	30 APOE ε4 carrier, 30 noncarrier CNC	Word categorization task	HC volumetry	R HC volume negatively correlated with number of false alarms in APOE ε4 carriers.
Westlye, et al 2012 [10]	31 APOE ε4 carrier, 61 noncarrier CNC (3-4 year follow-up)	CVLT-II	Entorhinal cortex, parahippocampal gyrus thickness and WM volumetry, DTI	Entorhinal WM FA positively correlated with memory in the APOE ε4 carriers.
Zhuang, et al 2012 [11]	193 CNC (20 aMCI converters in 2 years)	Logical Memory, RAVLT	VBM, DTI	Lower baseline L parahippocampal cingulum, inferior temporal lobe WM, parahippocampal gyrus, thalamus FA associated with worse verbal memory decline. L parahippocampal gyrus WM was predictive of subsequent memory decline.
Dowell, et al 2016 [12]	21 APOE ε4 carrier, 20 noncarrier young CNC, and 17 APOE ε4 carrier, 20 noncarrier mid-age CNC	Word list	HC and parahippocampus thickness, WM volumetry	Parahippocampal thickness positively correlated with memory in young APOE ε4 carriers.
MCI				
Chetelat, et al 2003 [13]	21 aMCI	Word list	VBM/FDG-PET	HC volume positively correlated with memory.
Stoub, et al 2006 [14]	40 aMCI, 50 CNC	East Boston Story, WMS-R, CERAD-WL	HC and entorhinal cortex volumetry, WM VBM	Entorhinal cortex, HC and total parahippocampal WM were significant predictors of memory.
Goldstein, et al 2009 [15]	14 aMCI, 9 CNC	CERAD-WL, Story A of Logical Memory, BVMT-R	DTI	Temporal and whole brain apparent diffusion coefficient negatively, whole brain FA positively correlated with verbal memory in aMCI.
Wang, et al 2009 [16]	10 MCI (4 ADD converters in 3 years), 12 CNC	CERAD-WL, Logical Memory	HC, parahippocampal gyrus, amygdala volumetry, lobar masking method for frontal, lateral temporal, parietal occipital ROIs/SPECT	MTL volume positively correlated with memory.
Zhuang, et al 2012 [17]	76 aMCI, 51 naMCI, 206 CNC	Logical Memory, RAVLT, Benton Visual Retention Test	HC DBM, fornix DTI	L fornix radial diffusivity negatively correlated with verbal memory.
Meyer, et al 2013 [18]	25 aMCI	CERAD, CANTAB, WMS-R	VBM	Temporal WM volume positively correlated with pattern recognition. Parahippocampal gyrus and L precuneus WM volume positively correlated with story recall.
Fujishima, et al 2014 [19]	186 MCI, 136 CNC	Logical Memory II	Cortical thickness, WMH probability map of the whole brain	L entorhinal cortex thickness positively; WMH volume in the posterior periventricular regions and near the R anterior horn of the lateral ventricle negatively correlated with memory.
Remy, et al 2015 [20]	22 aMCI, 15 CNC	RCFT, DMS48 test	HC volumetry, DTI	L uncinate fasciculus FA positively correlated with recognition.
Peter, et al 2016 [21]	20 MCI, 20 CNC	Verbal Learning and Memory Test	HC and basal forebrain cholinergic system volumetry	HC volume positively correlated with memory.

(*Continued*)

Table 1
Structural MRI correlates of episodic memory (*Continued*)

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Gyebnar, et al 2018 [22]	18 aMCI, 20 naMCI, 27 CNC	CANTAB, RAVLT	Voxel- and ROI-based DTI	Voxel-based: R inferior frontal gyrus pars triangularis FA negatively correlated with visual memory. L parahippocampal gyrus MD negatively correlated with verbal memory. ROI-based: Left cingulum MD negatively correlated with verbal memory, and L stria terminalis/crus of the fornix MD positively correlated with visual memory.
ADD				
Deweer, et al 1995 [23]	18 ADD	WMS, CVLT, Grober and Buschke test	Hippocampal formation, amygdala, caudate nucleus, and ventricle volumetry	Hippocampal formation volume positively correlated with memory.
Kramer, et al 2005 [24]	13 ADD, 11 frontotemporal dementia, 10 semantic dementia, 8 CNC	CVLT	HC, frontal, anterior temporal lobes, and posterior cortex volumetry	HC volume was the only predictor of delayed recall.
Sarazin, et al 2010 [25]	35 ADD	The Free and Cued Selective Reminding Test	VBM, HC volumetry, and three-dimensional hippocampal surface-based shape analysis	VBM: L MTL, and thalamus volume positively correlated with total recall. Automatic hippocampal volumetry: L HC volume positively correlated with total recall. Three-dimensional hippocampal surface-based shape analysis: HC CA1 field volume positively correlated with free and total recall.
Yakushev, et al 2010 [26]	20 ADD, 18 CNC	CERAD	HC volumetry and diffusivity	L body-tail volume positively correlated with recall in ADD. L head diffusivity negatively correlated with delayed verbal recall.
Wolk, et al 2011 [27]	146 ADD	RAVLT, Logical Memory, ADAS-Cog-Word list	Rostral MTL, rostral inferior temporal gyrus, temporal pole, angular gyrus, supramarginal gyrus, superior parietal lobule, precuneus, superior frontal gyrus, inferior frontal sulcus/caudal middle frontal gyrus thickness	HC, MTL, caudal middle frontal gyrus, temporal pole thickness positively correlated with memory.
Irish, et al 2012 [28]	11 ADD, 11 semantic dementia, 10 CNC	Modified version of the past–future task	VBM	R frontal pole, R PCG and precuneus, L inferior temporal and L middle frontal gyri volume positively correlated with past retrieval in ADD and CNC.
Kerchner, et al 2012 [29]	9 ADD	HVLT-R, BVMT-R, Logical Memory	CA1-SP, CA1-SRLM, and entorhinal cortex thickness; DG/CA3 and hippocampal cross-sectional area (proxy for total HC volume) volumetry	CA1- SRLM and entorhinal cortex width, HC volume positively correlated with recall.
Dore, et al 2013 [30]	40 ADD, 93 CNC	CVLT-II, Logical Memory II	HC, temporal lobe, precuneus and PCG thickness combined with a voxel-based approach/PiB PET	R temporal lobe and R precuneus/PCG thickness positively correlated with memory in CNC with high PiB retention. HC thickness positively correlated with memory in both CNC groups.

(*Continued*)

Table 1
Structural MRI correlates of episodic memory (*Continued*)

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Irish, et al 2013 [31]	10 ADD, 10 frontotemporal dementia, 10 CNC	Modified version of the past–future task	VBM	L PCG volume positively correlated with past retrieval in ADD and CNC.
MCI and ADD				
Fellgiebel, et al 2005 [32]	17 aMCI, 25 ADD, 21 CNC	Delayed verbal recall test	DTI	PCG bundle FA positively, MD negatively correlated with memory.
Leube, et al 2008 [33]	21 MCI, 12 ADD, 29 CNC	Verbal learning and memory test	VBM	HC, L perirhinal cortex, L parahippocampal gyrus, L ventral anterior cingulate and R posterior entorhinal cortex, R middle temporal gyrus volume positively correlated with memory.
Sexton, et al 2010 [34]	8 MCI, 7 ADD, 8 CNC	HVLT-R, RCFT	HC volumetry, cingulum and fornix DTI	HC volume, L crus of the fornix FA positively; cingulate gyrus MD negatively correlated with memory.
Molinuevo, et al 2011 [35]	24 aMCI, 27 MCI (ADD converters in 2 years), 31 ADD, 27 CNC	CERAD-recall of constructional praxis, delayed text memory, memory alteration tests	VBM	L lateral, medial, inferior, and R medial, inferior gyri volume positively correlated with memory over time. L medial temporal gyrus positively correlated with delayed text memory.
Bosch, et al 2012 [36]	16 aMCI, 15 ADD, 15 CNC	CERAD-recall of constructional praxis, Grober and Buschke test	DTI	Whole brain FA positively correlated with memory.
Kerchner, et al 2013 [37]	15 aMCI, 11 ADD, 9 young CNC, 18 old CNC	HVLT-R, BVLT-R, Logical Memory	CA1-SP, CA1-SRLM, and entorhinal cortex thickness; DG/CA3 and hippocampal cross-sectional area volumetry	CA1-SRLM width positively correlated with recall in aMCI.
Defrancesco, et al 2014 [38]	14 MCI, 13 MCI (ADD converters), 28 CNC	CERAD-WL, CERAD-figural memory	GM and WM VBM, MD reflected by apparent diffusion coefficient maps	L putamen and inferior frontal gyrus volume positively correlated with verbal memory in ADD converters.
Bonner-Jackson, et al 2015 [39]	82 aMCI, 13 naMCI, 72 other neurological disorders, 34 ADD, 25 CNC	HVLT-R, BVMT-R	HC volumetry	Bilateral HC volume positively correlated with memory. HC volume positively correlated with non-verbal memory in aMCI.
Gomar, et al 2017 [40]	9 aMCI, 9 ADD, 44 CNC	Relational and item-specific encoding task	Entorhinal, perirhinal, parahippocampal cortices thickness, HC volumetry	HC volume, perirhinal and parahippocampal thickness predicted encoding performance.
Reas, et al 2017 [41]	12 MCI, 13 ADD, 31 CNC	WMS-R, CVLT, CERAD	Restriction spectrum imaging in fiber tracts, HC and entorhinal cortex GM; DTI	Fornix, uncinated, inferior fronto-occipital, inferior longitudinal and arcuate fasciculi neurite density positively correlated with recall. HC and entorhinal cortex isotropic free water diffusion negatively correlated with memory.

Abbreviations: MCI, mild cognitive impairment; APOE ε4, apolipoprotein E ε4; CNC, cognitively normal control; aMCI, amnestic mild cognitive impairment; ADD, Alzheimer's disease dementia; naMCI, nonamnestic mild cognitive impairment; CVLT, California Verbal Learning Test; RAVLT, Rey Auditory Verbal Learning Test; WMS-R, Wechsler Memory Scale-Revised; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CERAD-WL, CERAD-Word list; BVMT-R, Brief Visuospatial Memory Test-Revised; CANTAB, Cambridge Neuropsychological Test Automated Battery; RCFT, Rey Complex Figure Test; DMS48, delayed matching to sample-48 items; ADAS-Cog, Alzheimer's Disease Assessment Scale-cognitive subscale; HVLT-R, Hopkins Verbal Learning Test-Revised; HC, hippocampus; FDG-PET, fluorodeoxyglucose positron emission tomography; WM, white matter; DTI, diffusion tensor imaging; VBM, voxel-based morphometry; ROI, region of interest; SPECT, single-photon emission computed tomography; DBM, deformation based morphometry; WMH, white matter hyperintensity; MTL, medial temporal lobe; CA, cornu ammonis; CA1-SP, CA1-stratum pyramidale; CA1-SRLM, CA1-stratum radiatum/stratum lacunosum-moleculare; DG/CA3, dentate gyrus/CA3; PCG, posterior cingulate gyrus; PiB PET, Pittsburgh compound B PET; GM, gray matter; MD, mean diffusivity; R, right; L, left; FA, fractional anisotropy.

Table 2
Task-based fMRI correlates of episodic memory

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Preclinical AD				
Han, et al 2007 [45]	12 APOE ε4 carrier, 13 noncarrier CNC	Word pair association	Whole brain, ROI (HC)/HC volumetry	Increased R anterior cingulate, lingual, middle temporal, middle frontal gyri, PCG, precuneus and cerebellar tonsil activation in APOE ε4 carriers.
Quiroz, et al 2010 [46]	20 Presenilin 1 mutation carriers, 19 noncarrier CNC	Face-name association	Whole brain, ROI (HC)	Increased right anterior HC activation during encoding in presenilin 1 mutation carriers.
Adamson, et al 2011 [47]	10 APOE ε4 carrier, 11 noncarrier CNC	Spatial encoding	ROI (HC)	Reduced HC activation in APOE ε4 carriers.
Erk, et al 2011 [48]	19 subjective memory impairment, 20 CNC	Face-profession association	ROI (HC, DLPFC)	Reduced right HC activation, increased right DLPFC activation in subjective memory impairment group.
Chen, et al 2017 [49]	35 APOE ε4 carrier, 40 noncarrier CNC	Picture encoding	Seed ROI based on group cortical morphology differences and DMN/cortical thickness	Reduced precuneus deactivation, reduced postcentral, precentral, inferior occipital gyri, inferior parietal lobule activation in APOE ε4 carriers.
MCI				
Johnson, et al 2006 [50]	14 MCI, 14 CNC	Picture encoding	Reference group activation	Reduced R HC head and body, L lateral frontal, R inferior temporal lobe activation in MCI during novel pictures. Reduced R PCG/precuneus activation during previously learned items in MCI.
Petrella, 2006 et al [51]	20 aMCI, 20 CNC	Face-name association	Whole brain	Reduced frontal cortex, L cerebellum activation during encoding. Reduced frontal lobe, L HC; increased posterior frontal lobe activation during retrieval.
Heun, et al 2007 [52]	21 MCI, 29 CNC	Verbal encoding	Whole brain	Increased R superior, inferior and L middle frontal gyri activation in MCI.
Kircher, et al 2007 [53]	21 MCI, 29 CNC	Verbal encoding	Whole brain	Increased L HC, medial frontal, postcentral and cingulate gyri activation in MCI
Dannhauser, et al 2008 [54]	10 aMCI, 10 CNC	Verbal encoding	Whole brain	Reduced L ventrolateral PFC activation stretching into premotor cortex in aMCI.
Trivedi, et al 2008 [55]	16 aMCI, 23 CNC	Picture encoding	Whole brain, ROI (frontal cortex, MTL, PCG, inferior parietal cortex)	Reduced inferior frontal, R inferior parietal and parahippocampal cortex activation in aMCI during encoding. Reduced L inferior frontal cortex; increased R HC activation in aMCI during recognition.
Machulda, et al 2009 [56]	19 aMCI, 12 naMCI, 29 CNC	Scene encoding	Whole brain	Reduced temporoparietal and frontal activation in MCI during encoding. Reduced temporoparietal activation in aMCI during recognition.
Mandzia, et al 2009 [57]	14 MCI, 14 CNC	Object and animal encoding	ROI (HC and parahippocampal gyrus)	Reduced L superior and middle temporal, R middle temporal gyri, precuneus, L cuneus, anterior cingulate, R lentiform nucleus, caudate and putamen activation during deep encoding. Reduced L parahippocampal, fusiform, R middle temporal gyri, R inferior frontal, inferior parietal regions, caudate, L cerebellum, middle occipital gyrus and cuneus activation during shallow encoding. Reduced L HC, superior and middle frontal, R lateral inferior and medial frontal gyri, cingulate, L thalamus and middle occipital gyrus activation in during deeply encoded item recognition. Reduced L lentiform nucleus and putamen; increased L fusiform and superior frontal, R cingulate gyri activation during shallowly encoded item recognition.
Clement and Belleville, 2010 [58]	28 MCI, 12 CNC	Word pair association	Whole brain, ROI (HC)	Increased R dorsolateral, ventrolateral PFC, premotor and motor area activation MCI with higher cognition scores. Reduced R occipital lobe and L inferior parietal lobule; increased dorsal L inferior parietal lobule activation in MCI with lower cognition scores. Increased L temporal regions, R precentral gyrus,

(*Continued*)

Table 2
Task-based fMRI correlates of episodic memory (*Continued*)

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
				dorsolateral PFC, L inferior and bilateral superior parietal lobules activation in the MCI with higher cognition scores compared with MCI with lower scores.
Clement, et al 2010 [59]	12 MCI, 10 CNC	Verbal encoding	Whole brain, ROI (HC)	Reduced occipital lobe, R middle and superior temporal gyri, R thalamus, R anterior cingulate, R medial frontal lobe; increased L ventrolateral PFC activation during encoding. Reduced medial frontal lobe; increased premotor area activation during retrieval.
Yassa, et al 2010 [60]	10 aMCI, 10 CNC	Picture encoding	ROI (L CA3/DG, CA1, subiculum, entorhinal cortex)	Increased CA3/DG and reduced entorhinal cortex activation.
Hampstead, et al 2011 [61]	18 aMCI, 16 CNC	Object-location association	Whole brain, ROI (HC)	Reduced ventral and dorsal visual streams, frontal areas, dorsal precuneus, HC, perirhinal cortex, PCG, retrosplenial cortex, thalamus; increased mid-precuneus and L temporoparietal junction activation.
Hanseeuw, et al 2011 [62]	16 aMCI, 16 CNC	Verbal encoding	Whole brain/HC volumetry	HC volume positively correlated with associative memory in aMCI. Reduced L anterior HC activation.
Lenzi, et al 2011 [83]	15 aMCI, 14 CNC	Verbal encoding, Story Recall	Whole brain, ROI (HC, L inferior temporal, R superior temporal gyri)/VBM	Increased R superior temporal gyrus activation. This activation negatively correlated with Story Recall.
Giovanello, et al 2012 [63]	12 aMCI, 12 CNC	Word pair association	Whole brain	Reduced R inferior and superior frontal gyri, increased anterior cingulate and inferior frontal gyrus activation.
Jin, et al 2012 [64]	8 aMCI, 8 CNC	Scene encoding, face-occupation and object-location association	Whole brain, ROI (MTL)	Reduced MTL; increased medial PFC, L precentral and superior motor area activation during scene encoding. Increased L angular gyrus, R cuneus/precuneus activation during face-occupation task. Reduced R Rolandic operculum, insula; increased precentral and postcentral gyri activation during the object-location task.
ADD				
Rombouts, et al 2000 [65]	12 ADD, 10 CNC	Picture encoding	Whole brain	Reduced activation in L HC and bilateral parahippocampal gyrus.
Kato, et al 2001 [66]	7 ADD, 8 young CNC, 8 old CNC	Picture encoding	Whole brain/ hippocampal formation and entorhinal cortex volumetry	Reduced R entorhinal cortex, supramarginal gyrus, prefrontal regions, L anterior inferior temporal lobe activation during encoding. Activations in these regions positively correlated with memory in the overall sample.
Gron, et al 2002 [67]	12 ADD, 12 major depressive disorder patients, 12 CNC	Geometric pattern encoding	Whole brain	Reduced parahippocampal gyrus, HC, temporal cortex, R anterior caudate; increased L middle frontal, R inferior frontal gyri and inferior parietal cortex activation.
Lustig, et al 2003 [68]	23 ADD, 32 young CNC, 27 old CNC	Verbal encoding	ROI (lateral parietotemporal, medial frontal, medial parietal/ PCG, L frontal region)	Reduced medial parietal/PCG deactivation.
Sperling, et al 2003 [69]	7 ADD, 10 young CNC, 10 old CNC	Face-name association	Whole brain/HC volumetry	Reduced hippocampal formation; increased medial parietal cortex, R PCG, superior frontal cortex activation during encoding. Increased superior frontal cortex activation during recall.
Golby, et al 2005 [70]	7 ADD, 7 CNC	Scene encoding	Whole brain, ROI (hippocampal gyrus, parahippocampal gyrus, entorhinal cortex, subiculum,	Reduced MTL, fusiform, lateral occipital activation.

(*Continued*)

Table 2
Task-based fMRI correlates of episodic memory (*Continued*)

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
			fusiform gyrus, calcarine cortex)	
Gould, et al 2005 [71]	12 ADD, 12 CNC	Visuospatial paired association	Whole brain	No differences.
Pariente, et al 2005 [72]	12 ADD, 17 CNC	Face-name association	Whole brain	Increased parietofrontal network activation during encoding. Reduced R HC, increased L parietal lobule and the L inferior frontal gyrus activation during recall.
Remy, et al 2005 [73]	8 ADD, 11 CNC	Verbal encoding	Whole brain/VBM, HC volumetry	Reduced inferior parietal cortex, inferior frontal gyrus, L precentral gyrus, R temporal associative area, L PCG, L perirhinal cortex, and cerebellum; increased medial cerebellum and L middle frontal gyrus activation during encoding. Reduced L inferior frontal and precentral gyri, R lenticular nucleus, R HC and retrosplenial cortex, R inferior parietal cortex, superior temporal gyrus and cerebellum; increased inferior temporal gyrus, L lateral middle and superior frontal gyri activation during recognition.
Gould, et al 2006 [74]	12 ADD, 12 CNC	Visuospatial paired association	Whole brain, ROI (bilateral inferior, middle, superior frontal gyri, medial prefrontal cortex)	Increased L medial and R lateral prefrontal cortex activation during encoding.
Pihlajamaki, et al 2008 [75]	15 ADD, 29 CNC	Face-name association	Whole brain, ROI (HC and medial parietal regions)	Whole brain: Increased middle and inferior prefrontal gyri, L superior parietal lobule, intraparietal sulcus and supramarginal gyrus activation. ROI: Increased L MTL activation. Reduced precuneus, R PCG, L lateral parietal cortex deactivation.
Peters, et al 2009 [76]	16 ADD, 16 CNC	Verbal encoding	Whole brain, ROI (inferior frontal, precentral, middle frontal gyri, insula, posterior parietal, caudate, cerebellum, inferior parietal sulcus, HC, parahippocampus)	Reduced middle frontal, L inferior frontal and transverse temporal gyri, R precuneus activation during encoding. Reduced supplementary motor area, superior frontal, precentral, supramarginal, L postcentral and R middle frontal gyri; increased fusiform gyrus activation during recognition.
MCI and ADD				
Machulda, et al 2003 [77]	9 MCI, 9 ADD, 11 CNC	Picture encoding	ROI (HC, parahippocampal and fusiform gyri)	Reduced MTL activation in MCI and ADD.
Dickerson, et al 2005 [78]	9 MCI, 10 ADD, 10 CNC	Face-name association	ROI (hippocampal formation, entorhinal cortex)/ MTL volumetry	Increased HC activation in MCI. Reduced HC and entorhinal activation in ADD.
Celone, et al 2006 [79]	15 low-CDR MCI, 12 high-CDR MCI, 10 ADD, 15 CNC	Face-name association	Whole brain, ROI (determined by regions contributing significantly to the independent components)	Increased HC and functionally connected neocortical regions activation, increased DMN deactivation in MCI group with low CDR. Reduced HC activation and DMN deactivation in MCI group with high CDR and ADD.
Hamalainen, et al 2007 [80]	14 MCI, 15 ADD, 21 CNC	Picture-name association	Whole brain	Increased thalamus and L ventral visual stream extending to the posterior parahippocampal gyrus and HC activation in MCI.
Petrella, et al 2007 [81]	34 aMCI, 13 ADD, 28 CNC	Face-name association, CVLT	Whole brain	Reduced MTL; increased the posteromedial cortex activation along the spectrum from CNC to ADD. Posteromedial cortex activation magnitude associated with CVLT.

(*Continued*)

Table 2
Task-based fMRI correlates of episodic memory (*Continued*)

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Pihlajamaki and Sperling, 2009 [82]	30 MCI (10 APOE ε4 carriers), 15 ADD (9 APOE ε4 carriers), 29 CNC (8 APOE ε4 carriers)	Face-name association	ROI (PCG, retrosplenial and precuneal regions)	Reduced L precuneus in MCI; bilateral PCG/precuneus deactivation in ADD. Reduced R PCG and bilateral precuneus deactivation in APOE ε4 carrier CNC compared to noncarrier CNC; reduced cuneus deactivation in APOE ε4 carrier ADD compared to noncarrier ADD.

Abbreviations: APOE ε4, apolipoprotein E ε4; CNC, cognitively normal control; MCI, mild cognitive impairment; aMCI, amnestic mild cognitive impairment; naMCI, nonamnestic mild cognitive impairment; ADD, Alzheimer's disease dementia; CDR, Clinical Dementia Rating; CVLT, California Verbal Learning Test; ROI, region of interest; HC, hippocampus; DLPFC, dorsolateral prefrontal cortex; DMN, default mode network; MTL, medial temporal lobe; PCG, posterior cingulate gyrus; DG, dentate gyrus; L, left; R, right; VBM, voxel-based morphometry; PFC, prefrontal cortex.

Diffusion tensor imaging metrics characterizing brain WM integrity are commonly affected in the AD continuum. Increased WM integrity for the whole brain was associated with better memory performance in CNC, MCI, and ADD, suggesting whole brain fractional anisotropy might be an overall marker of severity, rather than a specific measure [15,36].

Genetic status may mediate the relationship between MRI findings and cognition. In APOE ε4 carriers, loss of entorhinal WM integrity was related to worse memory performance [10]. However, other factors such as lower baseline MTL WM integrity have also been identified as predictors of memory decline in CNC converting to aMCI in 2 years [11], which can have the potential to be used as a biomarker for early diagnosis. MTL WM volume and integrity continued to have positive correlations with memory in aMCI and ADD [14,17,18,26,32,34]. Similar to GM changes, which include both MTL and extratemporal regions, precuneus WM volume reduction was also associated with worsened memory in aMCI [18]. Several fasciculi including uncinate, fornix, and cingulum, which are connected to medial temporal regions, were implicated in studies associating fiber density and memory [20,22,41]. In addition to these WM volume and integrity changes, Fujishima et al [19] reported that increased number of WMHs, pointing to increased vascular impairment, in the bilateral periventricular regions was related to worse recall performance in MCI.

Besides these more common MRI techniques, other approaches including diffusion kurtosis imaging, relaxometry, and magnetic transfer imaging may prove to be helpful in investigating WM integrity with high accuracy for whole brain mapping [43,44]. However, the number of studies using these approaches within the AD continuum is currently relatively small.

In summary, structural imaging studies show that hippocampal atrophy, which is closely related to episodic memory performance, is an established neurodegeneration biomarker in AD. Volume and cortical thickness of several additional regions, including PCG and precuneus, require further attention in terms of relationship to memory performance. WM changes, including loss of WM integrity in MTL and fasciculi connected to MTL assessed by formal diffusion tensor imaging metrics and hyperintensities in posterior regions of the brain, were also related to memory decline and should be assessed further in confirmed AD samples.

3. Functional MRI

fMRI is an indirect measure of brain activity relying on blood-oxygen-level dependent response, which is a proxy for neural activation. fMRI can be separated into task-based, when a participant is asked to engage in a task during scanning, or resting state, when the participant is asked to lie still without engaging in a task. In this section, we will summarize studies finding differences between those with preclinical or clinical AD and CNC, either on memory tasks during fMRI, or with resting state fMRI interpreted in relation to memory scores.

3.1. Task-based fMRI

Many studies have implemented task-based fMRI to investigate memory-related activation patterns in AD (Table 2). A variety of tasks have been used, most notably association tasks that pair two different stimuli (e.g., a face and a name). Whereas most studies include verbal stimuli, several studies use nonverbal stimuli (e.g., scene and picture encoding). Results of these studies support and extend the previously mentioned structural MRI findings.

3.1.1. Preclinical AD

Individuals with AD risk exhibit changes in blood-oxygen-level dependent responses even before the onset of memory deficits. These changes are nonlinear, with different activation patterns in MTL and heightened activation in frontal lobes sometimes reported. For example, reduced deactivation of PCG/precuneus [45,49,82], increased frontal activation [45], and altered MTL activation have all been reported, with one study reporting hyperactivation [45] and another reporting hypoactivation in preclinical APOE ε4 carriers [47]. Both presenilin 1 mutation carriers

and individuals with subjective memory impairment had hippocampal hypoactivation [46,48]. Frontal hyperactivation was also observed in individuals with subjective memory impairment [48]. These activation patterns in preclinical AD are suggestive of compensatory mechanisms within these regions which are capable of maintaining normal levels of cognition.

3.1.2. Mild cognitive impairment

Both hypoactivation [50,51,57,59,61,62,64,77] and hyperactivation [53,78,80,83] of the MTL during memory tasks have been reported in MCI. This difference may be a result of the particular memory process being assessed, as suggested by a study by Trivedi et al [55] reporting hypoactivation of parahippocampal cortices during encoding and hyperactivation of hippocampus during recognition in aMCI. A study showing CA3/dentate hyperactivation and entorhinal hypoactivation also suggested that discrepant findings in MTL may be caused by different activation patterns in MTL structures and hippocampal subregions [60]. The discrepancy may also be due to the mixed sample of MCI patients included in the studies. For example, MCI patients with lower dementia score as determined by Clinical Dementia Rating had hippocampal hyperactivation and decreased default mode network (DMN) deactivation, whereas the activation pattern was completely opposite in MCI patients with higher dementia scores [79].

Similar to MTL, while some studies show reduced PCG/precuneus activation [50,61,64], some report hyperactivation or reduced deactivation within these regions [53,81,82,84]. PCG/precuneus is part of the DMN, and hyperactivation of these areas is possibly due to reduced deactivation of the DMN while performing a task. Frontal cortex activation is usually reduced [50,51,54–57,59,61,63] while several studies show hyperactivation in several frontal regions including precentral gyrus [51,52,59,64]. Dividing the MCI sample into two groups depending on cognitive performance, Clement and Belleville [58] revealed that frontal activation during a verbal memory task was decreased in MCI patients with more cognitive decline. Temporoparietal regions are also reported to be affected with some studies showing hypoactivation of these regions during picture or scene encoding tasks [55–57,64] and some reporting hyperactivation [61]. These findings suggest that future studies may benefit from better defined samples instead of including different types of MCI (aMCI and naMCI) patients with various levels of dementia.

3.1.3. Alzheimer's disease dementia

In ADD, MTL hypoactivation [65–67,69,70,72,77–79,81] and PCG/precuneus hyperactivation [69,81] or reduced deactivation [68,75,82] are the most consistent findings. Affected regions are not limited to these more commonly reported areas in the brain. Activation in frontal regions including prefrontal and motor areas were altered during verbal or visual encoding and recognition [66,73,76]. Although results are not consistent, there seems to be a tendency for frontal hyperactivation [67,69,72,74,75].

Overall, task-based fMRI findings suggest that episodic memory tasks lead to MTL hypoactivation, frontal hyperactivation, and reduced PCG/precuneus deactivation in ADD. Although preclinical AD and MCI samples have activation differences within these regions, the results are not consistent yet to provide early diagnosis or disease-tracking biomarker candidates. The discrepancy of the results appear to be caused by inclusion of mixed patient samples, distinct verbal and visual memory tasks, and implementing different analysis methods for imaging. In conclusion, task-based fMRI seems like a promising tool which can detect early changes along the AD continuum requiring further investigations for biomarker research in AD.

3.2. Resting state fMRI

By its nature, resting state fMRI (rsfMRI) does not involve a task, but the connectivity metrics calculated from these data can be used to assess relationships with memory tasks completed outside of the scanner (Table 3). This technique allows the investigation of functional connectivity between two regions and/or within specific networks impaired in AD.

3.2.1. Preclinical AD

In APOE ε4 carriers, verbal memory decline was related to reduced anterior and posterior connectivity as shown by whole brain dynamic functional connectivity [87]. Studies using seed-based analysis reported that verbal memory decline was associated with reduced left medial temporal gyrus; and DMN and executive control network connectivity [85,86]. When episodic memory performance related to structural changes within DMN regions, reduced deactivation shown by task-based fMRI and connectivity decline of this network shown by rsfMRI are considered altogether, this network appears to play a significant role in AD and could be used for early diagnosis.

3.2.2. Clinical AD

The relationship between DMN connectivity reduction and episodic memory decline persisted in MCI [88,93,101,104] and ADD [100,101,104]. Longitudinal studies showed that the progression of memory decline in aMCI was related to the decline of functional connectivity between posterior cingulate cortex and other DMN regions [88], precentral gyrus [99], hippocampal formation [94], and hippocampus subregions [89]. Xie et al investigated the connectivity between regions with atrophy in aMCI and revealed that both atrophy of hippocampus, precuneus, insula, postcentral gyrus, and frontal regions and connectivity reduction between these regions were associated with worse memory performance.

Table 3
rsfMRI correlates of episodic memory

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Preclinical AD				
Goveas, et al 2013 [85]	20 APOE ε4 carrier, 26 noncarrier CNC	RAVLT	Seed-based voxel-wise connectivity analysis (DMN-PCG, ECN-R dorsolateral PFC, Salience network-R orbital anterior insula)/VBM	DMN connectivity positively correlated with memory. ECN: Operculum clusters and R inferior/superior parietal cortex clusters negatively, R inferior temporal gyrus positively correlated with memory.
Matura, et al 2014 [86]	20 APOE ε4 carrier, 43 noncarrier CNC	Word list	Seed-based functional connectivity (L PCG)	L medial temporal gyrus connectivity positively correlated with recognition.
Quevenco, et al 2017 [87]	13 APOE ε4 carrier, 24 noncarrier CNC (2-year follow-up)	Verbal Learning and Memory Test	Whole brain dynamic functional connectivity/PiB PET	Anterior-posterior connectivity positively correlated with memory.
MCI				
Bai, et al 2011 [88]	26 aMCI, 18 CNC (20-month follow-up)	AVLT, RCFT	ICA	Reduced connectivity between PCG/precuneus and mean DMN independent components over time was correlated with episodic memory decline in the aMCI.
Bai, et al 2011 [89]	26 aMCI, 18 CNC (20-month follow-up)	AVLT, RCFT	Seed-based functional connectivity (HC subregions; CA, DG and subiculum)	Reductions in baseline hyperfunctional connectivity between the PCG/precuneus and mean DMN independent components in aMCI were positively correlated with memory decline over time.
Agosta, et al 2012 [90]	12 aMCI, 13 CNC	Babcock Story Recall, RAVLT, RCFT	ICA	No associations.
Liang, et al 2012 [91]	16 MCI, 16 CNC	CVLT	Seed-based functional connectivity (inferior parietal cortex, angular gyrus, supramarginal gyrus)/VBM	Angular gyrus and R precuneus connectivity negatively correlated with CVLT in MCI.
Xie, et al 2012 [92]	30 aMCI, 26 CNC	RAVLT, RCFT	Seed-based functional connectivity (insula subregions)	Intrinsic connectivity of insula positively correlated with memory in aMCI.
Wang, et al 2013 [93]	18 aMCI, 23 euthymic CNC, 16 CNC	CVLT-II	ICA/VBM	DMN connectivity positively correlated with CVLT-II. Positive correlations were most evident in the R HC, R hippocampal gyrus and R thalamus.
Dunn, et al 2014 [94]	24 aMCI, 33 naMCI	RAVLT	Seed-based functional connectivity (DMN-PCG, anteromedial prefrontal cortex; MTL-hippocampal formation, parahippocampal gyrus, retrosplenial cortex, posterior intraparietal lobule, ventromedial PFC; dorsal medial PFC subsystem-dorsomedial PFC, lateral temporal cortex, temporoparietal junction, temporal pole)/HC volumetry	PCG-hippocampal formation connectivity strength positively correlated with retrieval in aMCI.
Jacobs, et al 2015 [95]	18 aMCI, 18 CNC	Verbal word learning task	Seed-based functional connectivity (locus coeruleus)/GM volumetry	R locus coeruleus-L parahippocampal gyrus connectivity positively correlated with memory in aMCI.
Xie, et al 2015 [96]	30 aMCI, 26 CNC	Auditory Verbal Memory Test, RCFT	Seed-based functional connectivity (regions with atrophy in aMCI determined by VBM; bilateral precuneus and insula, L postcentral gyrus, medial frontal gyrus, middle frontal gyrus and HC)	GM volume and intrinsic connectivity positively correlated with memory.
Dillen, et al 2016 [97]	24 aMCI, 27 subjective cognitive impairment, 25 CNC	WMS-IV Logical memory and Design memory, Verbal learning memory test	Seed-based functional connectivity (retrosplenial cortex, PCG)/GM volumetry	Retrosplenial and frontal medial, L lateral occipital cortex connectivities positively correlated with verbal learning in MCI.

(*Continued*)

Table 3
rsfMRI correlates of episodic memory (*Continued*)

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Franzmeier, et al 2017 [98]	44 Aβ + aMCI, 24 Aβ- CNC	RAVLT, ADAS, WMS Logical Memory I and II, MMSE	Seed-based functional connectivity (L frontal cortex)/FDG-PET	At low levels of L frontal cortex connectivity, lower precuneus hypometabolism was associated with worse memory; at high levels of L frontal cortex connectivity, the effect was reduced.
Zhang, et al 2017 [99]	32 aMCI, 40 CNC	AVLT	Seed-based functional connectivity (R PCG)	R PCG connectivity with the L and R central sulci, L precentral gyrus positively correlated with recall in aMCI.
ADD				
Balthazar, et al 2014 [100]	22 ADD, 26 CNC	RAVLT	Seed-based functional connectivity (PCG)	DMN connectivity positively correlated with memory scores in the overall sample.
MCI and ADD				
Binnewijzend, et al 2012 [101]	23 MCI, 39 ADD, 43 CNC (2.8 year follow-up; 7/23 MCI converted to ADD)	RAVLT	ICA	Regional connectivity within the DMN positively correlated with memory.
Pasquini, et al 2015 [102]	22 MCI, 21 ADD, 22 CNC	CERAD-WL	ICA	Local intrinsic functional connectivity of the HC negatively correlated with recall in ADD.
Zhang, et al 2016 [103]	76 aMCI, 19 ADD, 23 CNC	RAVLT, MMSE, ADAS, Logical Memory I and II	Functional connectivity between 18ROIs/Aβ PET, APOE ε4 status	Medial frontal gyrus and parahippocampus functional connectivity negatively correlated with memory in aMCI and ADD.
Contreras, et al 2017 [104]	21 aMCI, 8 ADD, 16 subjective cognitive decline, 13 CNC	CVLT-II	ICA (resting-state network, visual network, DMN and frontoparietal network)	DMN and frontoparietal network connectivity positively correlated with recall.

Abbreviations: APOE ε4, apolipoprotein E ε4; CNC, cognitively normal control; aMCI, amnestic mild cognitive impairment; MCI, mild cognitive impairment; naMCI, nonamnestic mild cognitive impairment; Aβ, amyloidβ; ADD, Alzheimer's disease dementia; RAVLT, Rey Auditory Verbal Learning Test; AVLT, Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; ADAS, Alzheimer's Disease Assessment Scales; MMSE, Mini–Mental State Examination; CERAD-WL, Consortium to Establish a Registry for Alzheimer's Disease-Word list; DMN, default mode network; ECN, executive control network; PCG, posterior cingulate gyrus; R, right; PFC, prefrontal cortex; VBM, voxel-based morphometry; L, left; PiB PET, Pittsburgh compound B positron emission tomography; ICA, independent component analysis; HC, hippocampus; DG, dentate gyrus; MTL, medial temporal lobe; HC, hippocampus; GM, gray matter; FDG-PET, fluorodeoxyglucose positron emission tomography; ROI, region of interest.

Decreased MTL connectivity with locus coeruleus [95], frontal medial cortex, and lateral occipital cortex [97] was associated with worse verbal memory scores. Focusing on insula subregions revealed that increased intrinsic connectivity of insula was also associated with better memory performance [92]. Combining both rsfMRI and FDG-PET approaches, Franzmeier et al [98] revealed an interaction between functional connectivity of frontal cortex and precuneus hypometabolism. With decreased frontal connectivity, precuneus hypometabolism was associated with reduced memory performance, whereas this association was lower at higher levels of frontal connectivity in aMCI. This study suggests that memory performance does not only rely on functional connectivity but also metabolism of DMN regions. Finally, in contrast to findings in aMCI, worse memory performance was associated with increased middle frontal gyrus and parahippocampus connectivity [103], and intrinsic hippocampal connectivity [102] in ADD.

To summarize, rsfMRI findings have revealed that MTL and DMN connectivity changes in AD are related to episodic memory. Reductions in DMN connectivity are closely related to memory decline, whereas MTL connectivity results are not that consistent throughout the AD continuum. Whereas preclinical and prodromal AD samples have reduced connectivity in association with worse memory performance, this pattern in reversed in ADD. Although DMN findings are rather consistent, there is still a need for more studies with sufficient power before rsfMRI can provide a reliable AD biomarker or tracking tool. Future studies may benefit from combining rsfMRI with other imaging techniques, including FDG-PET, and defining patient samples better by supporting the clinical criteria with established structural MRI, PET, and CSF findings.

4. Molecular MRI

Proton magnetic resonance spectroscopy can be used to assess changes in cell-specific metabolites, including choline, creatine, glutamine, glutamate, glutathione, N-acetyl aspartate (NAA), and myo-inositol. Levels of NAA, reflecting neuronal loss or dysfunction, decrease in AD; whereas

Table 4
Molecular MRI correlates of episodic memory

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
MCI				
Didic, et al 2010 [108]	28 aMCI, 28 CNC	DMS48	NAA/MI in hippocampal formation and perirhinal/entorhinal cortices	Anterior subhippocampal cortex and L anterior HC NAA/MI positively correlated with memory in the overall sample.
Duffy, et al 2014 [107]	54 MCI, 41 CNC	RAVLT	GSH in anterior and posterior cingulate	PCG GSH negatively correlated with memory.
ADD				
Chantal, et al 2002 [109]	14 ADD, 14 CNC	CVLT	NAA, Cho, Cr, MI in MTL	L HC NAA positively correlated with memory.
MCI and ADD				
Rami, et al 2007 [110]	27 aMCI, 35 ADD, 27 CNC	Text Memory Test, Wordlist Learning Test, Memory Alteration Test	MI/Cr ratio, NAA in PCG, L temporal pole and L posterior temporoparietal region	L temporal pole MI/Cr ratio negatively correlated with encoding. PCG NAA positively, MI/Cr ratio in all of the regions negatively correlated with memory alteration.
Foy, et al 2011 [111]	21 MCI, 39 ADD, 38 CNC	CERAD	NAA, MI, Cho, Cr + phosphocreatine in HC	NAA positively correlated with memory in MCI and ADD.
Lim, et al 2012 [112]	16 aMCI, 23 ADD, 22 CNC	Seoul Verbal Learning Test, HVLT-R	NAA/Cr ratio in PCG	NAA/Cr positively correlated with memory in the overall sample.
Watanabe, et al 2012 [113]	42 aMCI, 67 ADD, 54 CNC	WMS-R	NAA (N-acetylaspartate and N-acetylaspartylglutamate), MI in HC and PCG	HC NAA positively, MI negatively correlated with memory in the overall sample.
Jahng, et al 2016 [114]	24 aMCI, 24 ADD, 23 young CNC, 24 old CNC	Face-name association	Functional MRS; glutamine and glutamate complex, NAA, Cr, MI in PCG/precuneus	NAA and Cr highest in young CNC, and lowest in AD (AD < aMCI < old CNC < young CNC) during the task.

Abbreviations: aMCI, amnestic mild cognitive impairment; CNC, cognitively normal control; MCI, mild cognitive impairment; ADD, Alzheimer's disease dementia; DMS48, delayed matching to sample-48 items; RAVLT, Rey Auditory Verbal Learning Test; CVLT, California Verbal Learning Test; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; HVLT-R, Hopkins Verbal Learning Test-Revised; WMS-R, Wechsler Memory Scale-Revised; NAA, N-acetylaspartate; MI, myo-inositol; GSH, glutathione; Cho, choline; Cr, creatine; PCG, posterior cingulate gyrus; L, left; HC, hippocampus; MRS; magnetic resonance spectroscopy.

increased myo-inositol levels, reflecting glial cell activation, have been reported in MCI and AD [105,106]. Glutathione is an intracellular antioxidant in the brain and has yet to be extensively studied in AD [107]. In addition, there are only a few studies evaluating the association between these metabolite alterations and memory performance in particular (Table 4).

Levels of NAA in MTL have been consistently reported to have positive associations with verbal memory performance both in MCI and ADD [108,109,111–113]. In addition to the positive correlation between PCG NAA and verbal memory scores [110], NAA within this regions decrease along the AD continuum [114]. Levels of NAA were shown to decrease with age (as shown by the difference between young and old CNCs) and AD progression. Patients with ADD had the lowest NAA and creatine concentration, followed by aMCI patients, whereas young CNCs had the highest concentration in PCG/precuneus. As myo-inositol increases in AD, it also seems to be negatively correlated with verbal memory in MCI and AD [110,113]. These results suggest that increased neuronal dysfunction coupled with glial cell activation play a role in the verbal memory deterioration in MCI and AD. Elevated glutathione levels with decreased memory performance are suggestive of early compensation in MCI [107]. These molecules may prove to be markers to track disease progression with future longitudinal studies investigating the course of the levels of these molecules within specific regions in association with cognitive decline.

5. Arterial spin labeling MRI

Arterial spin labeling MRI measures cerebral blood flow (CBF), which is a more direct evaluation of brain physiology compared with the blood-oxygen-level dependent response measured by fMRI. A small number of studies on this MRI technique reported that the CBF alterations are associated with episodic memory within the AD continuum (Table 5).

Decreases in MTL CBF are detected even in the preclinical phase in individuals with AD risk [115]. Structures of MTL and PCG/precuneus CBF are closely associated with verbal memory performance in this sample of individuals. Individuals with positive Aβ, subjective cognitive decline, and APOE ε4 carriers have a decline in verbal memory performance coupled with increased CBF

Table 5
Arterial spin labeling MRI correlates of episodic memory

Author, year	Study groups	Episodic memory test	Imaging analysis method	Imaging correlates of episodic memory
Preclinical AD				
Fleisher, et al 2009 [115]	13 CNC (positive family history of AD and at least one copy of the APOE ε4; high risk), 10 CNC without these risk factors	Face-name association	CBF and BOLD signal response in MTL	Decreased CBF and BOLD response during encoding in the high risk group.
Bangen, et al 2014 [116]	16 CNC with high, 55 with low vascular risk	CVLT-II	CBF in caudate, thalamus, MTL, posteromedial and frontal cortices	Trend for positive correlation between MTL CBF and memory in high vascular risk group.
Zlatar, et al 2016 [117]	21 APOE ε4 carrier, 38 noncarrier CNC	WMS-R, CVLT-II	Voxel-wise analysis	R anterior cingulate, L HC, parahippocampal gyrus, insula, putamen, middle temporal, supramarginal, R middle and superior temporal gyri CBF negatively correlated with verbal memory in APOE ε4 carriers.
Bangen, et al 2017 [118]	15 Aβ+, 47 Aβ- CNC (florbetapir PET)	AVLT	CBF in HC, PCG, precuneus and postcentral gyrus	HC, PCG and precuneus CBF negatively correlated with recall in Aβ+ CNC.
Hays, et al 2017 [119]	35 subjective cognitive decline, 35 CNC	CVLT-II, WMS-R	Voxel-wise analysis	PCG, corpus callosum, HC, L medial and inferior temporal, fusiform gyri, R inferior frontal gyrus CBF negatively correlated with verbal memory in subjective cognitive decline CNC.
Preclinical AD and MCI				
Bangen, et al 2012 [120]	16 MCI (8 APOE ε4 carriers), 29 CNC (14 APOE ε4 carriers)	Picture encoding	CBF and BOLD signal response in MTL	No CBF or BOLD difference between CNC and MCI; and APOE ε4 carriers and noncarriers.
Wierenga, et al 2012 [121]	20 MCI (9 APOE ε4 carriers), 40 CNC (13 APOE ε4 carriers)	WMS-R, CVLT-II	Whole brain CBF	L parahippocampal and fusiform gyri CBF positively correlated with verbal memory in APOE ε4 carriers.
MCI				
Xu, 2007 et al [122]	12 aMCI, 14 CNC	RAVLT, scene encoding	Voxel-wise analysis	Reduced R precuneus, cuneus and PCG CBF during the task. CBF positively correlated with RAVLT in the overall sample.
Xie, 2016 et al [123]	65 aMCI, 62 CNC	Scene encoding	Voxel-wise analysis and CBF in PCG, precuneus, HC, parahippocampal gyrus	Reduced MTL, temporal pole, precuneus, PCG, L lingual and fusiform gyri, cuneus, superior occipital lobe CBF during the task.

Abbreviations: CNC, cognitively normal control; AD, Alzheimer's disease; APOE ε4, apolipoprotein E ε4; Aβ, amyloidβ; MCI, mild cognitive impairment; aMCI, amnestic mild cognitive impairment; CVLT-II, California Verbal Learning Test-II; WMS-R, Wechsler Memory Scale-Revised; AVLT, Auditory Verbal Learning Test; RAVLT, Rey Auditory Verbal Learning Test; CBF, cerebral blood flow; BOLD, blood-oxygen-level dependent; MTL, medial temporal lobe; HC, hippocampus; PCG, posterior cingulate gyrus; R, right; L, left.

[117–119]. Although there are no directional data regarding this association, this may be suggestive of a compensatory response within these regions aimed toward improving the performance.

In line with other MRI approaches, MCI patients show decreased CBF responses in MTL and PCG/precuneus, which correlate with the verbal memory performance [122,123]. Superior occipital lobe CBF is reduced when tasks demanding visual encoding are used [123].

Owing to diversity of the episodic memory tests used in the current studies, and the small number of studies to date, conclusions about how arterial spin labeling relates to episodic memory across the AD process would be premature. However, results to date suggest that arterial spin labeling magnetic resonance imaging holds a potential to provide biomarkers which can be used in early diagnosis and progression of AD.

6. Limitations and future directions

Existing literature suggests that MRI, widely available in clinical and research settings, may offer several potential biomarkers related to episodic memory impairment in AD. Structural and functional alterations in different regions may increase the predictive value of hippocampal atrophy assessed by MRI for AD diagnosis. As MRI findings

correlate with episodic memory deficits, they have the potential to offer more insight into the etiology of the disease and more utility for tracking progression over time.

Nevertheless, there are several limitations to using MRI in AD. Imaging is expensive, requires skilled staff for acquisition and analysis, and is time consuming. In most of the studies, cohort sizes tend to be small, limiting confidence in results [28,31,65,67,69–74,77–81,124,125]. The existence of large shared data sets such as AD Neuroimaging Initiative mitigates this to some extent and has been extremely useful in better understanding structural aspects of the disease. However, AD Neuroimaging Initiative is also limited in functional imaging data as it includes only rsfMRI and no task-based sequences. In addition, the neuropsychological battery includes only verbal memory testing. This is also true of many clinical research studies that limit our understanding of the relationship between rsfMRI and nonverbal measures. This differs from the task-based literature, where many tasks found to differentiate between AD and other cohorts involve nonverbal stimuli such as faces and scenes.

Another limitation is the use of clinical criteria for probable AD in most of the mentioned studies. For example, only a few used hippocampal atrophy, CSF Aβ, or PET to support the AD diagnosis [20,26,41,73,78,80,90,95,97,98]. Remy et al [20] included hypometabolism assessed by FDG-PET, medial temporal atrophy shown by MRI, and the level of phospho-tau and Aβ-tau index to confirm the AD diagnosis within their patient sample. The rest of the studies included in our review relied only on clinical criteria. Without the integration of supporting biomarkers, the positive predictive value of clinical diagnostic criteria is rather limited with poor negative predictive value [126]. If biomarkers revealing Aβ deposition and neurodegeneration are present at the same time as clinical criteria, likelihood of AD dementia is significantly increased [127]. Thus, whenever possible, these biomarkers should be implemented to reliably define study samples.

Although investigating differences on a whole brain level may help discover other regions implicated in episodic memory performance, these analyses may not be efficient in detecting subtle changes. Compared with region-of-interest analyses, whole brain analyses require spatial blurring and corrections for multiple comparisons leading to decline in power to detect small changes [45]. More powerful analysis methods should be favored in biomarker research to obtain more reliable results.

Moving forward, it seems that multimodal biomarker studies that use both Aβ and/or tau PET ligands and both structural and functional MRI might become more common in AD. Our own research supported by a Center for Biomedical Research Excellence award from the National Institute of General Medical Sciences will use Aβ PET, resting state fMRI, and neuropsychological testing including verbal, nonverbal, and navigational memory techniques in an attempt to fill some of the gaps in the current understanding of AD. Future work building from the current protocol will incorporate task-based fMRI to further understand task-based network connectivity in relation to the Aβ status and neuropsychological performance. Using multimodal imaging and including nonverbal memory tests in addition to verbal tests will expand on previous imaging studies. Navigational tasks used in animal studies are rarely implemented in human research, limiting the translational value of these studies. Thus, by using navigational tasks, we aim to overcome this existing limitation.

7. Conclusions

Several MRI and fMRI metrics, including hippocampal atrophy, hold the potential to become AD biomarkers and may be more relevant to the preclinical stages. However, most imaging studies include only one modality with either verbal or nonverbal memory tasks, which prevent generalized conclusions to be drawn from their findings. Investigating the underlying pathology of AD through the combination of multimodal imaging and extensive neuropsychological evaluation may help in early diagnosis and in testing the effectiveness of novel therapeutics. Longitudinal studies with larger participant samples, where clinical AD diagnosis has been supported by multiple biomarkers, could provide a better understanding of the disease.

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RESEARCH IN CONTEXT

1. Systematic review: Memory impairments are among the most common and early symptoms of Alzheimer's disease (AD). Structural and functional changes assessed by magnetic resonance imaging are related to memory performance.
2. Interpretation: Magnetic resonance imaging findings in AD associated with memory performance can be used as potential biomarkers in the future. However, current conflicting results are probably due to the fact that most studies use limited memory tests in small patient samples with probable AD diagnosis.
3. Future directions: More extensive neuropsychological batteries should be implemented in larger patient groups with multimodal imaging. The diagnosis for AD should be supported by currently available biomarkers to achieve more reliable results.

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Featured Article

Neuroimaging and neuropsychological assessment of freezing of gait in Parkinson's disease

Brent Bluett[a,*], Sarah Banks[a], Dietmar Cordes[a], Ece Bayram[a], Virendra Mishra[a], Jeffrey Cummings[a], Irene Litvan[b]

[a]*Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA*
[b]*Department of Neurosciences, University of California San Diego, La Jolla, CA, USA*

Abstract

Introduction: Freezing of gait (FOG) is a disabling phenomenon characterized by a brief, episodic absence or reduction of forward progression of the feet despite the intention to walk. It is a common cause of falls and mortality in cases with Parkinson's disease (PD). This article reviews neuropsychological and neuroimaging studies to date and introduces a new study of multimodal imaging and cognition in PD-FOG.

Methods: A comprehensive literature search identified studies using neuropsychological evaluation and/or neuroimaging to evaluate PD-FOG.

Results: Several studies have evaluated PD-FOG, but few have combined neuropsychological and comprehensive neuroimaging and none longitudinally.

Discussion: A study using a combined approach longitudinally evaluating cognitive dysfunction and underlying neural networks in FOG is needed. We introduce the framework of a study which demonstrates the use of establishing an infrastructure for studying neurodegenerative disorders using the National Institutes of Health/National Institute of General Medical Science Center of Biomedical Research Excellence grant mechanism.

Keywords: Neuroimaging; Neuropsychology; Gait; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disease characterized by both motor and nonmotor features. Similar to Alzheimer's disease (AD) and other neurodegenerative disorders, PD is caused by misfolding and subsequent accumulation of a brain protein. Accumulation of amyloid and tau proteins occurs in AD patients, whereas accrual of alpha-synuclein protein occurs in PD patients. In both the disorders, buildup of these proteins results in neuronal and synaptic dysfunction, as well as inflammation. The neuronal loss and synaptic dysfunction result in the phenotypic manifestation of symptoms in both the disorders. While cognitive and neuropsychiatric symptoms occur in both AD and PD patients, the cardinal features of PD are motor symptoms including bradykinesia, rest tremor, rigidity, and gait abnormalities including postural instability [1].

PD is the second most common neurodegenerative disorder after AD and is expected to double in prevalence in the next 20 years [2]. Approximately 60% of patients with PD fall each year [3], resulting in significant morbidity, mortality, and direct and indirect medical costs. It is therefore critical to identify modifiable factors that contribute to fall risks.

Freezing of gait (FOG) is one of the most common causes of falls and subsequent morbidity and mortality in PD patient [4]. FOG is a brief, episodic absence or reduction of forward progression of the feet despite the intention to walk [5].

*Corresponding author. Tel.: 702-483-6000; Fax: 866-372-2720.
E-mail address: bluettb@ccf.org

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During these episodes, patients experience a feeling that their feet are "glued" to the floor and are unable to move [4]. During ambulation, four circumstances that most commonly induce FOG are starting to walk, attempting to turn, passing through narrow passages, or nearing the intended destination [6]. FOG is seen in other parkinsonian syndromes and normal pressure hydrocephalus and in patients with microvascular ischemic lesions but is most commonly associated with PD [7].

It is theorized that FOG is a motor manifestation of global dysfunction in the concurrent processing of information across neuronal networks [8]. This is supported by studies showing that FOG is correlated with limited dual-tasking ability [9] and inability to "set-shift" attention among motor, limbic, and cognitive networks [10]. In addition, freezing can occur during speech, handwriting, and other actions aside from gait, suggesting that the dysfunction occurs in generalized neural networks not solely related to ambulation [11].

PD-FOG usually occurs in the "OFF" state (the dopaminergic medication which improves PD symptoms has worn "off" and is not actively effective) but can also occur in the "ON" state (while the dopaminergic medication is effective and actively improving PD symptoms) [12]. Studies of the prevalence of FOG in PD patients indicate that approximately 50% experience FOG, with nearly 60% of these episodes occurring in the "OFF" state and 36%–38% of episodes occurring in the "ON" state [13].

A comprehensive approach to the evaluation of PD-FOG is needed to fully elucidate the underlying mechanisms and pathophysiology of this disabling phenomenon. Studies have evaluated the neuropsychological profile of patients with PD-FOG. Neuroimaging studies have also been performed using functional or structural connectivity to evaluate the networks involved. However, very few studies have combined functional and structural connectivity with neuropsychological evaluation. In addition, longitudinal studies are lacking, especially those that identify PD patients who later develop PD-FOG. This article reviews the current understanding of the neuropsychological profile and neuroimaging features of PD-FOG and discusses how longitudinal evaluation of PD-FOG with multimodal imaging, neuropsychological evaluation, and clinical evaluation can help advance our understanding in hopes of developing effective therapeutic interventions. The research is supported by a National Institutes of Health/National Institute of General Medical Science Center of Biomedical Research Excellence award establishing a Center for Neurodegeneration and Translational Neuroscience shared by the Cleveland Clinic Lou Ruvo Center for Brain Health and the University of Nevada, Las Vegas.

2. Neuropsychologic profile of PD-FOG patients

PD-FOG correlates with cognitive dysfunction in specific domains. Executive dysfunction involving response inhibition, problem solving, divided attention, or switching attention have been implicated [14].

Studies evaluating the neuropsychological deficits in patients with PD-FOG indicate that competing frontostriatal pathways reduce the ability to "set-shift" from one response set to another and may trigger episodes of freezing [10]. One study found deficits in set-shifting, as indicated by poor performance on Trail Making Test B, correlated with PD-FOG. However, there was only a mild correlation between PD-FOG and Trail Making Test A, which focuses more on visuospatial scanning and motor speed [10]. Another study evaluating motor and cognitive determinants identified attention and memory deficits in PD-FOG patients but also found no associated visuospatial deficits [15]. Anxiety is common in patients with PD-FOG and may contribute to the deficits in attentional set-shifting [16].

A study evaluating response inhibition and suppression in PD-FOG patients with the attention network task and Stroop task demonstrated that those with FOG show a deficiency in general conflict-resolution ability compared with those without the deficiency and healthy controls [17]. Another study evaluating executive function in PD-FOG patients found deficiencies in response inhibition correlated with severity of PD-FOG but did not identify significant deficits in set-shifting or updating working memory [18]. Deficiencies in response inhibition in PD-FOG patients are believed to be associated with structural deficits in the right hemisphere's locomotor network involving prefrontal cortical areas [5].

A study of early PD patients with FOG in the "ON" state found frontal dysfunction, as evidenced by decreased total Frontal Assessment Battery scores and phonemic verbal fluency, potentially implicating the dorsolateral prefrontal cortex, anterior cingulate, and left inferior frontal gyrus [19].

Clinical investigations of FOG support the neuropsychological observations of frontal executive dysfunction involving set-shifting of motor programs, deficiencies in attention, and poor response inhibition. FOG may result from an inability to generate normal amplitude in step length, and asking PD-FOG patients to reduce their step length can induce episodes of FOG [20]. Modulating locomotion by changing gait speed, pattern, or direction in obstacle avoidance may also trigger episodes of FOG [21].

2.1. Limitations of neuropsychological studies of PD-FOG

Studies exploring neuropsychological deficits associated with PD-FOG often focus on certain cognitive domains rather than performing a comprehensive evaluation. Therefore, the results are limited to the tests chosen in each study and do not provide a full cognitive profile. In addition, not all PD patients with executive dysfunction develop FOG, which

indicates there are other deficits involved which need to be elucidated. Longitudinal and prospective studies of cognition in PD-FOG patients may better identify the specific deficits involved and should be correlated with imaging findings to determine their relationship to underlying structural defects.

3. Neuroimaging of PD-FOG patients

Neuroimaging studies of PD-FOG have identified abnormalities in connectivity in motor and nonmotor pathways [8,22,23]. Several neuroimaging protocols have been used to evaluate PD-FOG. Most commonly, neuroimaging is performed with the participant in the resting state. However, other protocols involve performing task-based neuroimaging during motor imagery or virtual reality after prolonged walking, while lying supine and simulating walking, and after an intervention (i.e., deep brain stimulation) [24]. This section focuses on magnetic resonance imaging-based functional and structural connectivity analyses of PD-FOG.

3.1. Functional connectivity

3.1.1. Resting-state functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) evaluates functional associations among brain regions by measuring temporal correlations between spatially remote neurophysiological events [25]. Blood oxygen level–dependent signal fluctuations represent areas of brain activity. Anatomically separated but functionally connected regions display a high level of correlated blood oxygen level–dependent signal activity. These reproducible neural networks are called resting-state networks.

fMRI of PD patients (not specific to FOG) shows that levodopa significantly changes the motor and cognitive networks of the corticostriatal pathways, with improvement of motor symptoms due to increased functional connectivity of motor circuits [26]. This partial improvement in circuit function may account for the partial responsiveness of PD-FOG to dopaminergic therapy, although corticostriatal connectivity in PD-FOG in the "OFF" versus "ON" state has not been comprehensively evaluated.

Resting-state fMRI studies in PD-FOG patients have implicated dysfunctional connectivity between cortical and subcortical regions. One study demonstrated reduced functional connectivity in the executive (frontoparietal) and visual (occipitotemporal) networks, and the extent of reduced connectivity correlated with the severity of FOG [27]. Another study showed increased connectivity between the supplemental motor area and mesencephalic locomotor region (MLR), theorized to compensate for decreased connectivity between the supplemental motor area and basal ganglia [28]. A recent study found reduced connectivity of the primary motor cortex and supplementary motor area bilaterally in the sensorimotor network, frontoparietal regions in the default mode network, and occipital cortex in the visual associative network [23].

3.1.2. Task-based fMRI

This technique uses the same acquisition protocol as resting-state fMRI but requires the subject to perform a task, usually in connection with a projection screen instructing the subject of what to do with task buttons to measure the subject's behavioral response.

3.1.3. Actual task

To investigate network-related changes that occur during episodes of FOG, Shine et al. used a virtual reality gait task during a 10-minute fMRI session. The subjects were positioned in the scanner to view a screen on which the virtual reality task was displayed while their feet rested on a pair of foot pedals. During FOG episodes, participants were unable to coordinate foot movements to depress the pedals, which correlated with decreased blood oxygen level–dependent response in sensorimotor areas and subcortical regions (basal ganglia, thalamus, and MLR). The extent of functional changes on fMRI correlated with the severity of FOG [29].

Subsequently, in further studies, the virtual reality paradigm was modified by adding a cognitive interference task (Stroop color task) that allows probing the influence of prefrontal executive function on the occurrence of FOG. Patients with FOG froze more often with increased cognitive load, supporting the notion that FOG is due to functional decoupling between cognitive control networks and motor networks [29].

Using a similar virtual reality paradigm, Gilat et al. investigated FOG when turning versus walking. FOG patients froze more while turning, with increased activation of the visual cortex and inferior frontal regions, implicating the recruitment of a motor-stopping network [30].

3.1.4. Imagined task

Gait planning while imagining walking (without using pedals to advance) through a virtual reality paradigm has been used as an fMRI task in FOG [4]. FOG patients tended to have increased response in the MLR and reduced activity in mesial frontal and posterior parietal regions.

Other fMRI studies using virtual reality tasks simulating FOG have identified increased basal ganglia inhibitory output with subsequent reduction in thalamic and brainstem information processing [31] and abnormal functional connectivity of the pedunculopontine network, mainly affecting the corticopontine-cerebellar pathways as well as visual temporal areas involved in visual processing [32].

3.2. Structural connectivity

Magnetic resonance imaging evaluation of structural connectivity is based on structural associations among and

between different neuronal elements. Morphometric correlation (i.e., voxel-based morphometry) assesses cortical thickness, gray matter volume, and surface area between the brain regions. This allows comparison of the local brain matter (gray matter and white matter) density between groups of subjects based on high-resolution MR images with T1 contrast. True anatomical connectivity analyzes white matter fiber connections between gray matter regions using diffusion tensor imaging (DTI), an MR imaging technique that determines the diffusion properties of water molecules in white matter tracts.

In using VBM, one study found that FOG severity was related to bilateral caudate volumes in the entire cohort, whereas there was significantly reduced gray matter in the left inferior parietal lobe and right angular gyrus in a matched group of those with PD-FOG compared with PD patients without FOG [33]. Another study using VBM found that PD-FOG subjects had specific cortical volume reduction of the posterior parietal cortex, theorized to be an associative area involved in spatial control of motor behavior [34].

One DTI study found reduced structural connectivity between the pedunculopontine nucleus (PPN) and the cerebellum, thalamus, and multiple regions of the frontal cortex [5]. Another DTI study specifically evaluating the PPN found an absence of cerebellar connectivity and increased visibility of the decussation of corticopontine fibers in the anterior pons in PD-FOG patients [35].

3.3. Combined functional and structural connectivity

A study combining VBM and fMRI analysis, using a virtual reality paradigm with motor imagery of gait, found that PD-FOG participants had gray matter atrophy in the MLR, with decreased activity in the mesial frontal and posterior parietal lobes and increased activity in the MLR on fMRI. The increase in activity on fMRI correlated with the severity of FOG episodes [4].

3.4. Studies combining neuropsychological and neuroimaging evaluation in PD-FOG

Several studies using VBM and neuropsychological evaluation have been performed. One found reduced gray matter volume in the left inferior frontal gyrus, precentral gyrus, and inferior parietal gyrus. FOG severity correlated with the extent of frontal executive deficits, as well as bilateral frontal and parietal cortical gray matter volume [36]. A similar study found reduced gray matter volume in the left cuneus, precuneus, lingual gyrus, and posterior cingulate cortex. FOG clinical severity significantly correlated with gray matter loss in posterior cortical regions, and patients with FOG scored lower on tests of frontal lobe function on neuropsychological evaluation [37]. Another study of VBM and neuropsychologic evaluation found PD-FOG subjects had lower cognitive performance in frontal executive and visual-related functions, and the latter correlated with significantly reduced thalamic volumes [38]. A study assessing the neuroanatomical correlation of executive dysfunction and gray matter atrophy in PD-FOG patients found atrophy of the right dorsolateral prefrontal cortex correlated with severity of both executive dysfunction and FOG [39]. Jha et al. found that PD-FOG subjects performed worse than PD patients without FOG on verbal memory, executive functions (including response inhibition, set-shifting, phonemic verbal fluency, and semantic verbal fluency), visuospatial, and attentional domains. These PD-FOG subjects had reduced gray matter volume in the left temporal and right parietal lobe regions (correlating with the neuropsychological findings), as well as reduced gray matter volume in the cerebellum [40].

A study of PD-FOG evaluating dual-task interference, executive function, and structural connectivity of the PPN in PD-FOG patients found attentional deficits correlated with reduced connectivity of the right PPN and reduced go/no-go target accuracy (a measure of response inhibition). The authors proposed that the attentional deficit in PD-FOG patients may be related to structural degeneration of the PPN, with diminished cholinergic input into the basal ganglia and an adverse impact of the deficit of acetylcholine on cognition [41].

3.5. Limitations of neuroimaging studies of PD-FOG

A general limitation of neuroimaging studies is reproducibility, which is supported by the disparate findings of the studies described previously. Abnormalities identified on resting-state fMRI can be construed only as correlated to PD-FOG, rather than causative, as the findings may be unrelated or may reflect compensatory mechanisms. Task-based fMRI is performed while lying supine, which eliminates several important aspects of gait control, most importantly balance. Structural connectivity abnormalities identified may be unrelated to PD-FOG and therefore can be deemed only as associated rather than directly related to this phenomenon. DTI of the PPN is intrinsically difficult to perform because the seed location of the PPN needs to be specified for each subject and may vary among subjects due to the small diameter of the PPN [24]. It is also important to ensure that PD-FOG and PD groups are matched in regard to disease severity when evaluating structural connectivity and to ensure that abnormalities identified are not simply due to a more advanced stage of PD.

4. A longitudinal study of multimodal imaging and cognition in Parkinson's disease FOG

The National Institute of General Medical Science has provided funding to support our study, which seeks to elucidate the underlying pathophysiology of PD-FOG using a combination of multimodal imaging, neuropsychological

Table 1
Neuropsychological tests to be used for analysis in proposed Project 2

Outcome measures	Test	Justification
Overall cognitive outcome measures	Dementia Rating Scale II (DRS) total score	Accurately screens for level of cognitive impairment [42]
	Montreal Cognitive Assessment (MoCA) total score	Global assessment of cognitive function
Key cognitive outcome measures (executive function)	Phonemic verbal fluency	Assessment of mental flexibility and evaluation of frontal lobe function
	Go/no-go task	Measure of response inhibition
	Frontal Assessment Battery	Assesses frontal lobe function by evaluating conceptualization and abstract reasoning, lexical verbal fluency and mental flexibility, motor programming and executive control of action, self-regulation and resistance to interference, inhibitory control, and environmental autonomy
	Trail Making Test A and B	Measures ability to "set-shift"
	Stroop task	Measures mental flexibility (response inhibition/conflict resolution and set-shifting) [26]

testing, and clinical evaluation. This multifaceted approach will help identify the relationship between abnormalities identified by neuropsychological testing and underlying brain network dysfunction specific to FOG. Combining analysis of both functional and structural connectivity provides more insight than using either modality alone. By longitudinally following up PD patients with FOG and evaluating patients with and without dopaminergic medication, we will expand on previous studies and more concretely define which networks are involved in the pathophysiology of the disorder.

All participants are evaluated with the same comprehensive neuropsychological testing battery, with a focus on aspects of executive dysfunction previously found to be affected in PD-FOG patients (Table 1). Anxiety and other nonmotor, noncognitive data pertinent to PD-FOG are being collected. Data points for each test are collected and analyzed between the groups to determine the strength of correlation between imaging and cognitive findings. Creation of a neuropsychological profile of PD-FOG will help identify PD patients at risk of developing FOG, improving early identification of subjects for clinical trials, and potentially allowing for intervention before PD patients develop FOG.

Neuroimaging using functional and structural connectivity is performed. Functional connectivity results are generated by both seed-voxel and independent component analysis. These results are then analyzed to determine between-group functional network differences using mixed-effect models in combination with bootstrapping validation. This approach enables us to examine differences in resting-state connectivity between PD and PD-FOG patients, with and without dopaminergic medication. Connectivity maps generated by the seed-voxel analyses are compared.

Structural connectivity is performed using both probabilistic tractography and tract-based spatial statistics, a method that allows a voxel-wise comparison of fiber tracts. The method is applied to fractional anisotropy maps obtained by fitting a tensor model to the raw diffusion data and aligning the maps into a common space. Then, a mean fractional anisotropy skeleton map is created for each group of subjects, and each subject's aligned fractional anisotropy map is projected onto this skeleton to enable voxel-specific cross-subject statistics (Fig. 1).

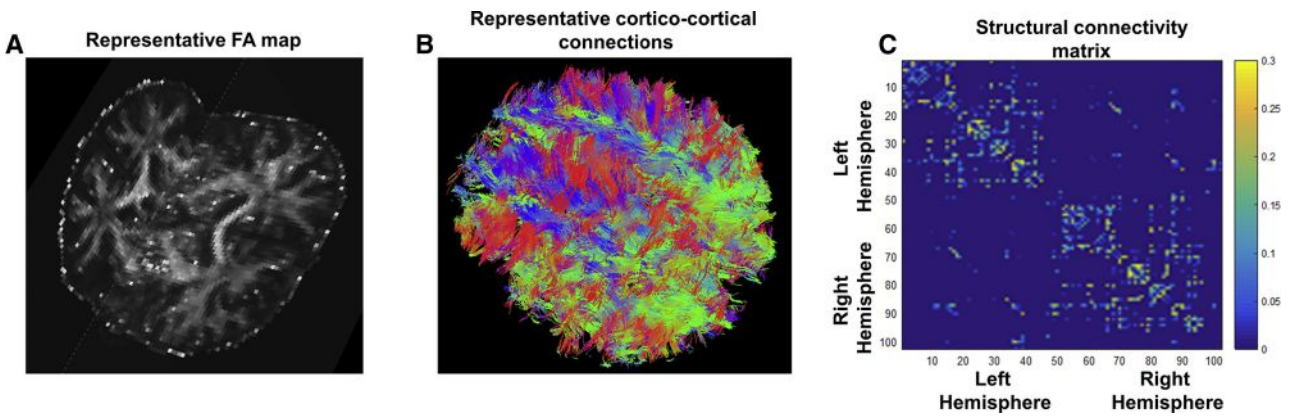

Fig. 1. Structural connectivity analysis. Abbreviation: FA, fractional anisotropy.

The striatal seeds derived from the probabilistic tractography are used in the seed-voxel analysis of functional connectivity. The results of the whole-brain Tract-Based Spatial Statistics analysis identify areas of disrupted white matter integrity. Tractography to and from the TBSS-identified white matter abnormalities are then performed to determine the structural connectivity of these pathologic regions. These results are compared with the functional connectivity data through regression analysis, permitting a combined functional and structural connectivity analysis to be performed in the same patients. This is an important step in development of a combined functional and structural magnetic resonance imaging-based biomarker for PD-FOG.

fMRI will be performed while participants view a modified virtual reality paradigm showing a first person view of the participant walking through a course designed to induce freezing (including starting to walk, multiple turns, walking through a doorway, and attempting to sit upon reaching the final destination). This will enable comparison of resting-state and task-based functional connectivity in PD-FOG patients and to expand upon findings from previous studies.

All PD-FOG, PD patients without FOG, and healthy participants enrolled in the study underwent clinical assessment using the Mini BESTest, Timed Up and Go, and FOG assessments. These tests are validated measures of assessing PD patients at risk for falls, incorporate evaluation of FOG, and assess cognitive deficits in domains associated with FOG [42–44]. PD-FOG and PD patients without FOG perform these assessments (as well as neuroimaging) in the "OFF" state and then 60 to 90 minutes after taking their morning dose of dopaminergic medication, in the "ON" state. Results from the clinical evaluation are combined and compared with findings from the neuropsychologic and neuroimaging data obtained, to acquire a comprehensive profile of PD-FOG patients.

5. Early experimental data

Analysis of preliminary data from our study emphasizes the importance of combining neuroimaging and neuropsychological evaluation of PD-FOG in one study.

First and perhaps most importantly, standard measures used to identify PD-FOG appear to be inaccurate. Of the initial 15 PD patients enrolled in our study, 11 patients self-reported FOG, four patients had FOG during clinical evaluation, and eight patients had FOG according to physical therapy assessment. Almost all studies to date have classified PD-FOG based on self-report and/or clinical evaluation. Self-report of PD-FOG appears to be an overly sensitive measure, whereas clinical evaluation is brief and therefore specific but not sensitive. The validated FOG assessment score used in physical therapy may be the most accurate measure, as participants are observed walking through a course designed to induce FOG, including walking while dual-tasking, through a narrow doorway, and clockwise and counter-clockwise 360° turns. Previous studies of PD-FOG may have erroneous results based on data collection from an improperly stratified cohort. It is possible the foundation of knowledge we have gained from previous studies of PD-FOG may be largely inaccurate.

Preliminary analysis of the effects of levodopa on functional connectivity in PD-FOG patients correlates with the neuropsychological findings. We found greater connectivity of the supramarginal gyrus in both the PD groups in the OFF state than in healthy participants. The supramarginal gyrus is a component of the frontoparietal network that is activated for phonological processing during both language and verbal working memory tasks. Supramarginal gyrus hyperconnectvity essentially normalized in PD without FOG with levodopa but remained abnormal in PD-FOG patients. Neuropsychological testing in the ON state found no differences in executive function between the PD without FOG group and healthy participants. However, the PD-FOG group exhibited executive dysfunction, and frontoparietal functional connectivity was positively correlated with dysfunctional phonological processing. The consistency between neuropsychological and neuroimaging results helps confirm that executive dysfunction is pertinent to the development of PD-FOG and identifies its neuroanatomical structural correlate within the frontoparietal network. This demonstrates how a combined approach using both neuroimaging and neuropsychological analysis can augment interpretation of scientific results in the broad spectrum of neurodegenerative disorders.

6. Summary and conclusion

Numerous studies evaluating PD-FOG have been performed with some overlap in findings, but no unified etiologic or pathophysiologic framework has been identified. A comprehensive approach combining multimodal imaging, neuropsychological evaluation, and clinical findings in a longitudinal study is needed and underway. Evaluations performed in both the OFF and ON state allow us to better understand the role of levodopa therapy in PD-FOG. Elucidating the mechanisms underlying PD-FOG is critical to understanding how pharmacologic and neuroprotective interventions could impact its development. Furthermore, a means of anticipating which PD patients will develop PD-FOG is needed. Ultimately, a better understanding of PD-FOG may promote development of therapeutic modalities to treat this disorder. The neuroscience infrastructure provided by the National Institute of General Medical Science in supporting the Center for Neurodegeneration and Translational Neuroscience supports this important advance in understanding PD and PD-FOG. Improving

our evaluation of phenomena such as PD-FOG provides a guide by which we can investigate phenotypic manifestations of other neurodegenerative disorders, such as AD.

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RESEARCH IN CONTEXT

1. Systematic review: A comprehensive literature search identified studies using neuropsychological evaluation and/or neuroimaging to evaluate freezing of gait in Parkinson's disease.
2. Interpretation: Numerous studies evaluating PD-FOG have been performed with some overlap in findings, but no unified etiologic or pathophysiologic framework has been identified.
3. Future directions: A combined approach of neuropsychological evaluation and innovative neuroimaging to longitudinally evaluate FOG is needed to determine the relationship between the associated cognitive dysfunction and underlying neural networks involved. This review highlights the findings of previous studies and places the current Center for Neurodegeneration and Translational Neuroscience study, "a longitudinal study of multimodal imaging and cognition in Parkinson's disease freezing of gait," in this context.

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Alzheimer's & Dementia

Review Article

Inflammation as a central mechanism in Alzheimer's disease

Jefferson W. Kinney[a,*], Shane M. Bemiller[b], Andrew S. Murtishaw[a], Amanda M. Leisgang[a], Arnold M. Salazar[a], Bruce T. Lamb[b]

[a]Department of Psychology, University of Nevada Las Vegas, Las Vegas, NV, USA
[b]Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN, USA

Abstract Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by cognitive decline and the presence of two core pathologies, amyloid β plaques and neurofibrillary tangles. Over the last decade, the presence of a sustained immune response in the brain has emerged as a third core pathology in AD. The sustained activation of the brain's resident macrophages (microglia) and other immune cells has been demonstrated to exacerbate both amyloid and tau pathology and may serve as a link in the pathogenesis of the disorder. In the following review, we provide an overview of inflammation in AD and a detailed coverage of a number of microglia-related signaling mechanisms that have been implicated in AD. Additional information on microglia signaling and a number of cytokines in AD are also reviewed. We also review the potential connection of risk factors for AD and how they may be related to inflammatory mechanisms.

Keywords: Alzheimer's disease; Inflammation; Microglia; Cytokines; Microglia receptors

1. Overview

Alzheimer's disease (AD) is a neurodegenerative disorder that is the most common cause of dementia and is characterized by the decline in cognitive and function and neuronal loss. AD currently affects over 5 million Americans [1] and is expected to become increasingly prevalent with the rise in life expectancy. It is estimated that by 2050, 13.8 million Americans will be living with AD [2]. The financial burden imposed by AD currently exceeds $230 billion and is expected to reach $1.1 trillion by 2050 [3]. Given the clinical and financial burden associated with AD, the identification of novel mechanisms responsible for pathogenesis, as well as novel therapeutic targets, is urgently needed.

AD is characterized by two core pathologies, the presence of β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs). Aβ pathology arises from the improper cleavage of the amyloid precursor protein (APP) resulting in Aβ monomers that aggregate forming oligomeric Aβ and eventually aggregating into Aβ fibrils and plaques [4]. The function of APP is unknown but is believed to have a role in cell health and growth [5]. Critical aspects of understanding the onset of Aβ pathology rests on knowing the mechanisms of the generation of Aβ monomers, their clearance, and their aggregation into oligomeric Aβ. Normal processing of the APP sequence consists of nonamyloidogenic proteolysis of APP via α-secretase and λ-secretase, producing soluble fragments [6]. When APP is cleaved by λ-secretase and erroneous β-secretase, it leads to insoluble amyloid β peptides that aggregate in the brain to form β-amyloid plaques [4,7–17]. The precise role of Aβ in AD pathology remains an open question as Aβ plaques may accumulate up to 10 years before any observable AD symptoms or diagnosis.

The second core pathology, NFT, arises from the hyperphosphorylation of tau, a microtubule-associated protein that stabilizes microtubules [18–27]. Phosphorylation of tau serves a necessary role in intracellular trafficking to

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*Corresponding author. Tel.: +702-895-4766; Fax: +702-895-0195.
E-mail address: jefferson.kinney@unlv.edu

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remove tau from microtubules, allowing transport, followed by dephosphorylation to return tau to the microtubule [28]. In AD, tau protein is phosphorylated at multiple sites resulting in the removal of tau from the microtubule and causing the collapse of microtubule structures and disruption in a number of cellular processes ranging from protein trafficking to overall cellular morphology [29–31]. In addition, the hyperphosphorylated tau (ptau) aggregates into paired helical fragments that eventually form neurofibrillary tangles [20,24,25,32,33]. The accumulation of ptau tangles and the compromised cellular function leads to loss of neuronal function, and ultimately apoptosis [30].

Despite extensive and productive research investigating the mechanisms responsible for both core pathologies, as well as approaches aimed at the prevention of Aβ plaques and NFT, there remains no treatment that effectively alters either pathology in clinical populations [34]. Furthermore, there exists a considerable gap in the understanding of AD pathogenesis given these two pathological features. As stated previously, patients may exhibit Aβ plaque pathology for up to or greater than a decade before any overt diagnosis of AD [35,36]. For NFT, the overall tangle load is correlated with cognitive decline in AD; however, the appearance of NFT appears to occur before the inauguration of AD pathology in clinical populations and preclinical animal models [37–39]. The combination of the aforementioned gaps in the pathophysiology of AD suggests that other pathological mechanisms may be driving both the onset of the disorder, as well as the progression of the disease.

Over the last 10 years, a third core feature of AD has emerged that may provide insight into AD pathogenesis, as well as provide a link between the other two core pathologies. A number of investigations initially demonstrated that in addition to Aβ plaques and NFT, the brains of patients with AD exhibited evidence of a sustained inflammatory response [40–50]. The inflammatory response has now been observed in multiple studies of postmortem tissues of AD patient samples [51–57] and is routinely observed in preclinical model systems of AD.

Acute inflammation in the brain is a well-established defense against infection, toxins, and injury, but when a disruption in the equilibrium of anti-inflammatory and pro-inflammatory signaling occurs, as seen in AD, it results in chronic inflammation (neuroinflammation) [58–61]. This chronic neuroinflammation is attributed to activated microglia cells and the release of numerous cytokines. The presence of a sustained immune response in the brain is not exclusive to AD. A number of studies have demonstrated elevated markers of inflammation in the brain of patients with Parkinson's disease (PD) [62–66], and traumatic brain injury associated with chronic traumatic encephalopathy (CTE) [67–70], amyotrophic lateral sclerosis (ALS) [70], and Multiple Sclerosis (MS) [71] to name a few key examples. It is increasingly recognized that a sustained immune response is a central feature of neurodegenerative disorders [71–77].

The presence of a sustained inflammatory response in the brain of patients with AD was, at one point, thought to be reactive to the neuronal loss occurring in the disorder. However, substantial body of research has now demonstrated that a persistent immune response in the brain is not only associated with neurodegeneration but it also facilitates and exacerbates both Aβ and NFT pathologies. Furthermore, it has been suggested that the inflammatory response may provide a link between the initial Aβ pathology and the later development of NFT [78–83]. In the succeeding sections, we highlight some of the recent data indicating the role of inflammation in AD, as well as data indicating inflammation may be a central mechanism driving Aβ pathology and progression.

This review highlights the research supported by the National Institutes of General Medical Sciences (NIGMS) through Center for Biomedical Research Excellence (COBRE) awards that develop the national research infrastructure.

2. Inflammation in AD

Many studies now point to the involvement of neuroinflammation playing a fundamental role in the progression of the neuropathological changes that are observed in AD. Since the 1980s, there have been reports of immune-related proteins and cells located within close proximity to β-amyloid plaques [43,84]. Beginning in the 1990s, several large epidemiological and observational studies were published indicating that anti-inflammatory treatments used in diseases, such as rheumatoid arthritis, showed protective qualities against developing AD, demonstrating as much as a 50% reduction in the risk for developing AD in patients who are long-term nonsteroidal anti-inflammatory drug (NSAID) users [77,85–87]. These studies lead to studies utilizing animal transgenic AD models demonstrating that NSAIDs can reduce AD pathology [88]. Human trials of NSAIDS showed variable outcomes with no convincing evidence of benefit using the trial methods of the time [89].

These various epidemiological studies and observational studies serve as the bedrock of support for neuroinflammation playing a major role in developing sAD. Unlike other risk factors and genetic causes of AD, neuroinflammation is not typically thought to be causal on its own but rather a result of one or more of the other AD pathologies or risk factors associated with AD and serves to increase the severity of the disease by exacerbating β-amyloid and tau pathologies [90,91].

Brain inflammation appears to have a dual function, playing a neuroprotective role during an acute-phase response, but becomes detrimental when a chronic response is mounted [92]. Chronically activated microglia release a variety of proinflammatory and toxic products, including

reactive oxygen species, nitric oxide, and cytokines. In deceased patients suffering from recent head trauma, there is an increase in cerebral Aβ deposits 1–3 weeks postinjury, and it has been shown that elevated levels of interleukin 1 (IL-1) are responsible for the increased APP production and Aβ load [93,94]. In addition, elevated levels of IL-1β has been shown to increase the production of other cytokines, including IL-6, which in turn has been shown to stimulate the activation of CDK5, a kinase known to hyperphosphorylate tau [95]. The neuroinflammation observed in AD appears to serve a primary role in exacerbating Aβ burden and tau hyperphosphorylation, suggesting that this dual role could be a leading link between these seemingly disparate core AD pathologies. The mounted immune response via the brain's resident macrophage (microglia) is now a central tenant in the investigation of AD.

2.1. *Microglia*

Microglia are the resident immune cells within the central nervous system (CNS) [96]. In a healthy brain, microglia are in an inactive, "resting" state and are described morphologically as ramified cells with small somas [97,98]. In this state, the cell somas are stationary, while the cell processes extend and retract, surveying their environment and communicating with neurons and other glia cells [99–101]. Overall surveillance of the surrounding neuronal environment is accomplished via a large number of signaling mechanisms [99,102]. This includes surveillance of the local neuronal milieu via numerous receptors for classical neurotransmitters [103], receptors for numerous cytokines and chemokines [104–106], and a number of receptors, such as fractalkine (CX3CR1), that bind ligands constitutively released in healthy neuronal environments [107]. When microglia recognize a threat to the CNS, such as invasion, injury, or disease, it leads to microglial activation, causing a morphological change resulting in retraction of processes, enlargement of the cell, and migration [99,108–111]. Transitioning into an activated state may be triggered by alterations in any number of the aforementioned mechanisms involved in surveillance.

In AD, it is hypothesized that the primary driver of activation of microglia is the presence of Aβ. Activated microglia respond to Aβ resulting in migration to the plaques and phagocytosis of Aβ [108,112,113]. A number of investigations have demonstrated that activated microglia phagocytose Aβ [114–117]; however, these microglia become enlarged and after prolonged periods are no longer able to process Aβ [114,118]. Early in AD pathogenesis, the mounted immune response results in clearance of Aβ and has been demonstrated to exert positive effects on AD-related pathologies in animal models' systems [77,119,120]. However, prolonged activation of the immune response has been demonstrated to result in an exacerbation of AD pathology, likely as a result of sustained activation of microglia in a feed forward loop, referred to as reactive microgliosis. This results in an accumulation of Aβ and sustained pro-inflammatory cytokine singling beginning to damage neurons [118,121,122]. The sustained activation also results in a decrease in microglia efficiency for binding and phagocytosing Aβ and decreases in Aβ degrading enzyme activity of microglia leading, in turn, to a reduced ability to break down the Aβ plaques [123,124]. However, data indicate that the microglial capacity for producing pro-inflammatory cytokines is unaffected [118]. These data demonstrate a unique feature of pathogenesis in that overall clearance of Aβ becomes compromised while immune activation continues simultaneously. The continued release of pro-inflammatory cytokines and associated neurotoxins from microglia serves to exacerbate the neuroinflammation and contribute to neurodegeneration, leading to the activation of yet more microglia.

As the microglia are involved in clearance of Aβ, they release a number of proinflammatory cytokines that recruit additional microglia to plaques [125–127], resulting in a characteristic halo of activated microglia surrounding plaques [112,128]. More recent data indicate that as microglia become less able to clear Aβ, peripheral macrophages may be recruited to Aβ plaque deposition in an effort to clear Aβ [129]. The recruitment of peripheral macrophages into the brain likely exacerbates the effects of sustained inflammation and thus AD pathology. Some of the most compelling data for the importance of inflammation in AD pathogenesis and the regulation of the immune response comes from the recent demonstration that a mutation in the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) confers a greater likelihood of developing AD [129–132]. A rare missense mutation in TREM2 results in a substantial elevated risk of AD [133–136].

3. The role of TREM2 in Alzheimer's disease pathology

Recent identification of a number of genetic variants of *TREM2* has ignited a flurry of research into the mechanistic contributions of this critical innate immune-regulating receptor to the pathogenesis of AD and numerous other neurodegenerative diseases. Original interest in the role of TREM2 and neurodegeneration was generated in the early 2000s, when associations were identified between *TREM2* loss of function mutations and polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), or Nasu-Hakola disease [137–139]. This rare but aggressive neurodegenerative disorder is characterized by abnormal bone cysts and early-onset dementia with profound frontal lobe degeneration and is also associated with mutations in *TYROBP*, the intracellular signaling coreceptor for TREM2 [140–142]. In 2012, two independent studies reported strong associations between

the R47H variant of *TREM2* and late-onset AD [133,136]. The conferral of 2–4.5 fold increased risk of developing late onset AD (LOAD) in carriers of the R47H allele positions *TREM2* as the strongest associated risk gene behind only apolipoprotein E-ε4 (*ApoE4*), and further implicates innate immunity as a key component to the pathogenesis of AD [143,144]. In addition to the R47H variant, the R62H TREM2 mutation has been associated increased risk of LOAD. Using a variety of approaches with cell and rodent models along with human patient tissue and data, great strides have been made to elucidate the mechanistic contributions of TREM2 in the context of AD.

Early studies addressing the contributions of TREM2 to AD pathology utilized the APP/PS1 and 5XFAD mouse models of Aβ pathology along with human AD brain tissues. Initial characterization of TREM2 in AD revealed strongly upregulated protein and transcripts on neuritic plaque-associated macrophages in the brain, but not within microglia or myeloid cells distal to Aβ deposits in amyloid mice and human AD brain tissues [129,145]. Further characterization of these plaque-associated cells using flow cytometry revealed cell surface signatures consistent with peripheral macrophages, including high expression of CD45, Ly6c, and CD11 b, although subsequent studies utilizing parabiosis failed to detect these infiltrates [129,132,146]. Several studies have identified increased *TREM2* expression in human AD blood, further suggesting roles for peripheral TREM2 in modifying disease outcomes [147–149].

Next, the mechanistic roles of TREM2 were explored in the context of amyloid pathology using APP/PS1 and 5XFAD mice, along with various cellular models. Both haploinsufficiency and complete deletion of *Trem2* dramatically reduces the number of plaque-associated macrophages throughout all time points of disease in Aβ-bearing mice [129,150]. Interestingly, this decrease in plaque-associated macrophages reduces hippocampal plaque load at 4-months of age but ultimately exacerbates pathological outcomes by the 8-month time point [146]. This phenomenon was accompanied by decreases in inflammatory cytokines including IL1β, IL6, and TNFα as well as decreased astrocytosis as measured by GFAP and S100β later in mid-late stages of pathology. This suggests that TREM2 alters the inflammatory milieu not only through macrophage-mediated responses but also through alterations in astrocyte activation.

Given the profound loss of plaque-associated macrophages in TREM2-deficient amyloid mice, myeloid cell survival and proliferation were of interest in these models. It has been shown that TREM2-deficient mice have dramatically decreased numbers of proliferating plaque-associated macrophages as measured by proliferation markers Ki67, and BrdU in aging APPPS1 and 5XFAD mice [146,151]. In addition, it was shown by the Colonna lab that TREM2 deficiency results in increased cleaved caspase-3 activation, resulting in enhanced myeloid cell death [151]. Utilizing super resolution microscopy, Yuan et al. further demonstrated that haploinsufficiency of *TREM2* in mice or humans harboring the R47H mutation results in altered plaque compaction with much more diffuse and fibrillar plaques present, and fewer thioflavin S^+ dense core plaques [152]. In this situation, more neurotoxic oligomeric and fibrillar Aβ may be present leading to increased neuronal dystrophy and death. Along with plaque compaction issues, microglia and macrophages lacking TREM2 have been shown to have decreased capacity to phagocytose and clear Aβ, apoptotic cells, as well as other proteins and complexes [131,153,154]. TREM2-deficient myeloid cells also exhibit increased numbers of autophagy-associated phagolysosomes reflective of aberrant mTOR activation resulting in dysregulated metabolic homeostasis within the context of Aβ pathology [155].

More recently, the contributions of TREM2 to tau pathology have been addressed by the Lamb and Holtzman groups. Utilizing hTau mice harboring the entire human tau gene on a complete murine tau knockout background, Bemiller et al. demonstrated that TREM2 deficiency worsens cortical soluble and insoluble tau pathology at 6-months of age [156]. This worsening was accompanied by the presence of morphologically dystrophic microglia and widespread neuronal stress kinase hyperactivation, including within ERK-, JNK-, and GSK3β-associated pathways. The Holtzman group recently reported mitigated neuroinflammation, astrocytosis, and reduced neurodegeneration at advanced ages in the PS19 mouse model of tauopathy, an aggressive model of tauopathy harboring the FTD-associated P301S 4R familial tau mutation [157]. Similar to TREM2 signaling in the context of amyloid pathology, there appear to be disease stage-specific contributions of TREM2, which normally are protective in early stages of disease by facilitating clearance of intracellular and extracellular pathological tau species and damaged neuronal debris, but transformation to becoming pathogenic during neurodegenerative phases of disease where inflammation, astrocytosis, and aberrant synaptic and neuronal engulfment dominate.

The nuanced roles of TREM2 in promoting neurodegeneration can be attributed to three key aspects as currently understood within the context of AD: (1) regulation of phagocytic and autophagic processes; (2) myeloid cell survival and proliferation; and (3) regulation of inflammation. Recent studies by the Amit, Colonna, and Schwartz groups highlighted distinct activation patterns for what have been termed “damage-associated microglia” [158]. In these studies, the authors demonstrate distinct myeloid activation patterns, which initially are independent of TREM2 activation, but later rely on TREM2-dependent pathways to convert to a neurodegenerative transcriptional program altering phagocytosis and lipid metabolism. These studies have further highlighted the disease stage-specific

responses to pathology along with the incredible heterogeneity of cells involved in neurodegenerative processes.

4. Additional microglial receptors

In addition to the TREM2 receptor, a number of other microglia-specific receptors have been explored in the investigation of the exaggerated immune response in AD.

4.1. CX3CR1 and AD

To limit the activated role of microglia under resting, basal conditions in the brain, neurons release a variety of inhibitory factors, including CX3CL1, a chemokine frequently referred to as fractalkine [107]. CX3CL1 consists of a chemokine domain attached to a mucin-rich stalk on the extracellular domain and is synthesized as a membrane-anchored protein that may be cleaved into a soluble form [159,160]. CX3CL1 binds to its obligate receptor, CX3CR1, on the surface of microglia [161]. Originally identified on lymphocytes, CX3CR1 is involved with immune regulation on other tissues, including bone, kidney, and in the cardiovascular system [162–164]. Despite the widespread distribution of CX3L1/CX3CR1 signaling throughout the body, CX3CR1 has been most extensively studied in microglia [165–167], which have an expression of CX3CR1 nearly >1000-fold higher when compared with both peripheral myeloid cells and other CNS cell types, including both neurons and astrocytes [168,169]. Unlike other promiscuous chemokines, CX3L1 is the exclusive ligand for CX3CR1, binding rapidly and with a high affinity [170]. CX3CL1 is a pleiotropic protein involved in the abatement of microglia under basal conditions but also regulates microglial activity by influencing migration and proliferation in injury conditions [171].

Numerous studies demonstrate that CX3CL1 can dose-dependently reduce the expression of nitric oxide, IL-6, and TNFα following stimulation by lipopolysaccharide and suppress neuronal death induced by microglial activation [172,173]. CX3CR1-mediated neuronal signaling to microglia can be disrupted by replacing CX3CR1 with a green fluorescent protein (GFP) reporter gene that leads to systemic inflammation and exacerbated microglial neurotoxicity in a variety of animal models, including PD ALS [165,174].

However, the role of fractalkine signaling in AD pathogenesis appears to be complex and is not well understood. One of the pathogenic hallmarks of AD is aberrant microglial activation, making the CX3CL1-CX3CR1 pathway an ideal candidate for investigations of AD pathophysiology. The effect of CX3CR1 deficiency in mouse models of AD has been discordant, depending on the type of models and methods used. For example, CX3CR1 deletion in a tau transgenic mouse led to increased tau phosphorylation and aggregation, increased microglial activation, and exacerbated deficits in hippocampal-dependent learning [175]. Conversely in amyloidogenic AD models, deleting CX3CR1-attenuated neuronal loss and microglial activation with no impact on amyloidogenesis in 3XTg mice [166] but reduced β-amyloid deposition in both the APP/PS1 and R1.40 mouse models [167].

Studies investigating alterations in CX3CL1-CX3CR1 signaling in humans are rare compared with *in-vitro* and animal studies; however, the emerging human literature suggests that the fractalkine pathway may play an important role in AD pathogenesis. Cortical levels of fractalkine are reduced in the brains of healthy elderly adults with no history of dementia or other neurodegenerative disorders when compared with the brains of healthy, middle-aged adults, suggesting a potential age effect that may be associated with altered surveillance by fractalkine receptors and inflammation [176]. Plasma levels of soluble fractalkine are elevated in patients with both mild cognitive impairment (MCI) and AD [177]. In addition, these same researchers found higher levels of plasma in individuals with mild-moderate AD than in patients with severe AD and the highest levels in MCI patients, which is considered a key turning point from normal aging into AD pathogenesis. Since inflammation often precedes AD pathology, plasma levels of CX3CL1 could potentially be a useful systemic biomarker. Postmortem analyses show modest reductions of CX3CR1 and markedly lower levels of CX3CL1 in the hippocampus of AD brains compared with age-matched nondemented controls [178].

4.2. Alternative receptors on microglia

4.2.1. GABA and $GABA_B$

In the central nervous system, gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter released by neurons. Neurons produce GABA by conversion of glutamate to GABA via glutamate decarboxylases (GADs), which is released in response to neuronal activity. The released GABA binds to a fast-acting chloride channel (GABAA) resulting in rapid hyperpolarization or binding to a receptor coupled to a metabotropic G-protein coupled receptor ($GABA_B$) that results in a slow inhibitory postsynaptic current (IPSC) [179]. As alterations in GABAergic signaling have been reported in AD [180–184], and GABA plays a central role in learning and memory [185,186], there has been interest in its role in AD. GABA also plays a role in regulation of immune signaling [186]. Microglia express $GABA_B$ receptors [187] that are in $G_{i/o}$ subclass of GPCR receptors. Activated microglia exhibit an upregulation of $GABA_B$ receptors compared with resting microglia [187]. GABA and $GABA_B$ also have anti-inflammatory properties. Studies have demonstrated that when GABA is released by astrocytes into the extracellular fluid, it inhibits inflammatory responses of activated microglia and astrocytes

[186,188,189]. GABA has also been demonstrated to decrease the release of pro-inflammatory cytokines, TNFα and IL-6, by activated astrocytes and microglia via $GABA_B$ [186]. In an LPS-induced inflammatory response, activation of the $GABA_B$ receptor reduces the release of IL-12 from microglia [187]. By utilizing baclofen, a $GABA_B$ receptor agonist, intracellular inflammatory pathways were reduced by 40–60% [186]. These data clearly indicate $GABA_B$ on microglia serve an anti-inflammatory role [187] suppressing proinflammatory signaling. In addition, astrocytes synthesize GABA via monoamine oxidase B (MAOB) [190], and it has been demonstrated that reactive astrocytes release large quantities of GABA [186,191] in what is hypothesized to help regulate microglia function. The aforementioned data indicate a pathway that is likely involved in the regulation of microglia function. The expressed $GABA_B$ receptors on microglia likely serve a role in surveillance of neuronal function as GABA released from local synapses bind to $GABA_B$ on microglia to suppress pro-inflammatory signaling in a similar fashion to the fracktalkine ligand and receptor. Furthermore, when microglia and astrocytes move into a more active state, GABA release from astrocytes appears to be a mechanism to regulate and suppress pro-inflammatory signaling in microglia. If GABAergic tone is compromised in AD, this suggests the loss of a mechanism to regulate microglia function. This mechanism is currently the focus of research in our Center of Biomedical Research Excellence (COBRE) award, in particular, the investigation of changes in GABAergic signaling in AD, as well as the role of $GABA_B$ on microglia.

The impact of the TREM2 mutation and other alterations in microglia receptors that alter microglia activation lead to the upregulation of a number of pro- and anti-inflammatory cytokines that regulate the immune response. The evaluation of these cytokines has become a central part of AD inflammation investigations.

4.3. Specific cytokine signaling in AD

4.3.1. Pro-inflammatory signaling: TNF-α

TNF-α is one of the more important proinflammatory cytokines in AD, playing a central role in both initiation and regulation of the cytokine cascade during a response to an inflammatory challenge [41,192]. TNF-α increases vascular endothelial adhesion molecules, allowing leukocytes and immune cells to migrate into areas under duress [193].

TNF-α exerts its biological functions by binding to two main receptors, TNFR1 and TNFR2 [194]. The overexpression of TNFR1 in mouse hippocampal tissue was necessary for the activation of NFκB- and Aβ-induced neuronal apoptosis [195]. Conversely, mice lacking TNFR1 crossed with the APP23 transgenic AD model exhibit reduced plaque deposition, mitigated hippocampal microglial activation, and improved performance in cognitive tasks [196]. High levels of soluble TNFR1 and TNFR2 can be detected in cerebrospinal fluid (CSF) of patients diagnosed with MCI who progress to AD on a 6-year follow-up [197].

Increased levels of TNF-α have been reported in both the brains and plasma of patients with AD [198]. Aβ can directly stimulate microglia production of TNF-α through activation of the transcription factor NFκB [199]. In addition, TNF-α can increase Aβ burden through the upregulation of β-secretase production and increased γ-secretase activity [11,200].

4.3.2. Pro-inflammatory signaling: IL-1β

IL-1β has been described as a "master regulator" within the brain inflammatory cascade due to its integral role in regulating the expression of other proinflammatory cytokines, including TNF-α and IL-6, and that disruptions to IL-1β can delay the onset of neuroinflammation and neurodegeneration [201].

IL-1 is a proinflammatory cytokine that is upregulated early in AD development and is considered crucial for β-amyloid plaque deposition [41]. IL-1β is similarly elevated in both MCI and AD patients compared with controls, suggesting that increased IL-1β production begins early and remains elevated as the disease progresses [202]. Specific IL-1β polymorphisms resulting in higher IL-1β production are linked to increased AD risk [203]. Increased levels of IL-1β have been detected in the prefrontal cortex and hippocampus in brain tissue of patients with AD [204]. IL-1β-mediated actions are through binding to the IL-1 receptor, which is expressed throughout the brain but can be found in greatest concentration in the dentate gyrus and pyramidal cells in the hippocampus, which are key areas in the early development of AD pathology [205].

IL-1β regulates the synthesis of APP, increased APP secretion from glial cells, and amyloidogenic processing of APP through the activation of protein kinase C and increased γ-secretase activity [11,206]. The ability of IL-1β to increase Aβ burden and plaque deposition creates a self-sustaining cycle wherein the increase of Aβ load results in further microglia activation and IL-1β production [207,208].

4.3.3. Pro-inflammatory signaling: IL-6

IL-6 is an important, multifunctional cytokine that can be considered proinflammatory or anti-inflammatory depending on the amount and condition in which it was released [209]. IL-6 is crucial for normal homeostasis of neuronal tissue, and removal of this signaling pathway leads to reduced microglial activation, yet overproduction of IL-6 leads to chronic neuroinflammation and neurodegeneration [210].

Elevated levels of peripheral IL-6 during late midlife have been reported to effectively predict cognitive decline in a 10-year longitudinal study [211]. IL-6 is elevated in

the CSF and serum of AD patients and is considered a significant contributor to neuroinflammation observed in LOAD [212,213].

Studies have demonstrated that IL-6 staining in the hippocampus and cortex is strongly associated with Aβ plaques, which is absent in age-matched controls [214,215]. Aβ has been shown to stimulate the synthesis and release of IL-6 by glial cells [207]. Activation of IL-6 receptors, which show a regional distribution strongest in the hippocampus and cortex, has been shown to enhance APP transcription and expression, as does the IL-6/soluble IL-6 receptor complex, which can be readily found in serum and CSF [216]. IL-6 has also been demonstrated to result in the hyperphosphorylation of several tau epitopes by increasing CDK5 activity via the CDK5 activator p35, potentially serving as an important bridge between the core AD pathologies [95].

4.3.4. Pro-inflammatory signaling: NFκB

The transcription factor NFκB is considered a primary regulator of inflammatory responses by responding to proinflammatory stimuli, such as TNF-α or IL-1 [217]. Activated NFκB is predominately found in neurons and glial cells that are surrounding Aβ plaques and is central to reactive gliosis observed in AD brains [218].

NFκB has been shown to play an important role in regulating β-site APP cleaving enzyme 1 (BACE1) transcription, as numerous NFκB binding sites have been identified near the BACE1 promoter [219]. In addition, Aβ has been shown to stimulate cytokine production through the NFκB-dependent pathway, resulting in a cyclical loop of exacerbating pathology [199].

Both *in vitro* and *in vivo* studies demonstrate that utilizing an NFκB inhibitor, such as 6-amino-4(4-phenoxyphenylethylamino) quinazoline, can reduce TNF-α-induced BACE1 transcription, resulting in lower Aβ burden [196,220]. Certain NSAIDS, such as flurbiprofen and indomethacin, have been shown to reduce NFκB activity, which subsequently results in lower levels of $A\beta_{1–40}$ and $A\beta_{1–42}$ [221,222].

4.3.5. Anti-inflammatory signaling: IL-10

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that is found in healthy brain tissue but is upregulated in patients with AD [223]. There is a correlation between IL-10 levels and the progression of AD, suggesting that IL-10 could serve as a biomarker for AD diagnosis and/or progression. IL-10 is released by both microglia and astrocytes in response to the increase in pro-inflammatory cytokines to attempt to maintain homeostasis in the immune system [224]. IL-10 has been shown to inhibit pro-inflammatory cytokines, including IL-1α, IL-1β, TNF-α, IL-6, and the chemokine MCP-1 [223]. These findings suggest that increases in IL-10 may also be a possible therapeutic target to manage chronic inflammation, though clinical trials involving anti-inflammatory agents have been unsuccessful, and in some studies even exacerbated the disease [225]. Alternative studies have supported this claim, by demonstrating that IL-10 can promote neuroinflammation and cause dysfunction of microglia. In early AD, microglia perform their role of activation, migration, and phagocytosis to alleviate the disease, but as the disease progresses, these functions are inhibited. A study by Guillot-Sestier et al. (2015) suggests that this microglia inhibition is related to IL-10. Il10-deficient APP/PS1 mice (APP/PS1+IL-10−/−) showed a reduction of Aβ in the cerebrum and an increase in the amount of activated microglia surrounding the remaining Aβ, suggesting an increase in microglia migration and phagocytosis [226]. This study also demonstrated that APP/PS1+IL-10−/− mice have reduced synaptic loss and behavioral impairments in comparison with APP/PS1+IL-10+/+ mice. Research also shows that a polymorphism of IL-10 increases the risk of developing AD in some populations [227,228].

4.3.6. Anti-inflammatory signaling: TGF-β1

Transforming growth factor-β (TGF-β) is involved in the regulation of cell growth, cell differentiation, and immunosuppression. Studies have shown that TGF-β is elevated in CSF, serum, and brain microvascular endothelial cells of patients with AD [229,230]. Grammas et al. demonstrates that this increase of TGF-β in endothelial cells also provokes the release of pro-inflammatory cytokines, IL-1β and TGF-α, promoting an inflammatory response [230]. Within this family of cytokines, the most abundant isoform is TGF-β1, which is secreted by astrocytes and is a ligand for receptors found on neurons, astrocytes, and microglia [231]. TGF-β1 is neuroprotective against Aβ production, deposition, and damage, regulates neuroinflammation, and inhibits the pathway for the tau-phosphorylating enzyme, GSK-3β [232]. It is also responsible for causing an increase in the expression of Bcl-2 and Bcl-xl, anti-apoptotic proteins. The quantity of TGF-β1 in plasma has been shown to be decreased in patients with AD [233,234]. When Aβ oligomers are injected into the hippocampus of mice, decrease in synaptic protein levels and atrophy of astrocytic processes occur. However, intracerbroventricular infusion of 10 ng TGF-β1 30 minutes before an injection of 10 pmol Aβ oligomers resulted in reduced levels of the proteins, drebrin, PSD-95, and synaptophysin, and strengthening of astrocytic processes [235]. Also, mice that were intracerebroventricularly injected with Aβ failed to spend more time with a novel object in a novel object recognition test, whereas the mice previously injected with TGF-β1 did not show memory impairments [235]. TGF-β1 deficiency also causes impairment in the TGF-β1/Smad signaling pathway. The disruption of this pathway contributes to the ectopic phosphorylation of Smad2/3, which has been found in the hippocampal cytoplasm of neurons attached to NFT and within Aβ plaques. There is a negative correlation

between TGF-β1 mRNA levels and NFT, suggesting that TGF-β1 deficiency advances tau pathology, causing further impairment of the TGF-β1/Smad pathway [232]. These studies demonstrate how disruption of TGF-β1 signaling contributes to the AD pathogenesis.

The aforementioned studies demonstrate a number of specific roles for inflammatory mechanisms altered in AD, as well as a number of mechanisms that are likely related to AD pathogenesis. Given the very strong relationship between the missense mutation in TREM2 and the risk for developing AD, a number of other known risk factors for AD for which the mechanism underlying the risk is not known have now been hypothesized to be linked to inflammation.

4.4. Relevance of inflammation and risk factors for AD

A number of risk factors have been identified that confer greater risk for developing AD. These include age [236], cardiovascular changes [237], traumatic brain injury [94,238–240], and metabolic disorders such as diabetes. Interestingly, each of the aforementioned risk factors also is associated with an immune response, including in the brain. This has led to hypotheses that elevated inflammation and/or inflammatory signaling may be increasing the risk [241]. As mechanisms are numerous, we have explained one example below in more detail.

4.4.1. Diabetes mellitus (DM), AD, and inflammation

In addition to the aforementioned pathological hallmarks, AD is also characterized by abnormal metabolic changes. Decreased cerebral glucose metabolism is now considered a distinct characteristic of the AD brain [242–244]. The association between T2DM and AD is well established, along with other neurodegenerative diseases, including vascular dementia and PD [245–247]. One of the first key studies, the 1999 Rotterdam Study, found that type-2 diabetes mellitus (T2DM) could double the risk for the development of AD [248]. Since that seminal Rotterdam Study, numerous studies have since substantiated this finding that T2DM nearly doubles the risk for AD [249,250], including that T2DM serves as a useful predictor for the development of AD in a 12-year longitudinal study [251,252].

The defining characteristics of T2DM are impairments in insulin signaling and hyperglycemia [253,254], which appear to be the main contributing factors increasing the risk for AD. Impairments in brain insulin signaling as seen in T2DM has been found to get progressively worse as the pathology advances in patients with AD, corresponding to increased levels of amyloid peptides and, in particular, neuroinflammation [255,256].

A considerable literature has demonstrated that alterations in insulin levels and insulin receptor resistance (in particular in T2DM) within the brain impact survival and function of both neurons and glial cells that are dependent on intact insulin signaling [257,258]. Insulin is even neuroprotective by preventing β-amyloid oligomers from binding within the hippocampus to protect against AD-related synaptic deterioration [259], and the alterations in insulin signaling (including insulin receptor resistance) results in cerebrospinal fluid levels of Aβ being higher in patients with T2DM than nondiabetic controls [260].

Insulin resistance that arises from DM is proposed to manifest from a prolonged, mild state of inflammation occurring within peripheral tissue. Adipose tissue has been shown to recruit macrophage and stimulate the secretion of numerous proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, which are then easily distributed throughout the rest of the body causing systemic inflammation [261–263]. TNF-α is, in turn, a potent inducer of insulin resistance occurring within adipose tissue [261,264].

Numerous studies have demonstrated the correlation between peripheral inflammation and cognitive deficits, particularly with MCI and AD [265,266]. A meta-analysis of 40 studies revealed that peripheral cytokines, including TNF-α, IL-1β, and IL-6, and TGF-β are higher in patients with AD [267]. Whether the peripheral inflammation is arising from adiposity or another source, proinflammatory cytokines can cross the blood-brain barrier, which triggers brain-specific inflammatory responses [268,269]. Systemic inflammation is also a primary cause of damage to the blood-brain barrier, allowing entry of peripheral immune cells into the brain. Decreased blood-brain barrier integrity can be observed in rodents that feed on high-fat, high-energy diets, leading to increased blood-brain barrier permeability, excessive microglial activation, and hippocampal-dependent learning deficits [270–272]. Compromised blood-brain barrier integrity can also allow chronic, low-grade systemic inflammation, as observed in obesity and T2DM, to induce central inflammation.

Microglia are primed in AD brains to be more susceptible to secondary inflammatory insults, resulting in an exacerbated inflammatory response [273]. High-fat chow can be used in the APP/PS1 mouse model to induce systemic inflammation that results in profound neuroinflammation and accelerated AD-pathology, including Aβ and tau hyperphosphorylation, and central insulin resistance [274].

The mechanism of neuronal insulin resistance appears to be similar with Aβ oligomers inducing microglial activation, which in turn release numerous pro-inflammatory cytokines, including TNF-α [275]. Although activation of microglia by Aβ is an adaptive physiological response to reducing Aβ burden through phagocytosis, chronic inflammation leads to exacerbated AD pathology and metabolic abnormalities, which in turn further exacerbate pathogenesis. These data are providing evidence for links between molecular pathways and biochemical abnormalities associated with inflammatory mechanisms shared between AD and DM.

4.4.2. APOE and inflammation

Apolipoprotein (ApoE) is produced primarily peripherally within the liver and by astrocytes within the CNS. While the main roles of ApoE involve cholesterol transport,

regulation of lipid transport, and aid of injury repair within the brain, ApoE plays a role in glucose metabolism [276,277]. Owing to the ApoE-ε4 allele representing the strongest genetic risk factor for late-onset AD (LOAD), present in nearly ∼40% of all patients with AD [278], and the increasing risk that metabolic disturbances play in dementia progression, the links between ApoE-ε4, glucose metabolism, and recently inflammation are important to understanding the shared underlying mechanisms between these risk factors.

A recent investigation has highlighted a particularly important aspect of ApoE risk in AD as it is related to inflammation. Krasemann et. al., (2017) identified a role for ApoE signaling in the regulation of microglia phenotype in response to amyloid β, as well as in other neurodegenerative disorders [279]. Even more compelling is that TREM2 signaling appears to mediate a return to nonpathogenic and reduced phagocytosis of neurons by microglia. This suggests a completely novel interaction between one of the major risk factors for developing AD and microglia function. Additional studies are required to determine the relationship between ApoE and inflammation, but this may serve as another example of the central role of inflammation driving AD pathology. ApoE-ε4 appears to synergistically combine with other AD risk factors, including cardiovascular disease, atherosclerosis, and type-2 diabetes [280]. As each of these other risk factors includes an evoked immune response in the brain the possibility exists that inflammation may be the common thread for increased risk.

5. Summary

The aforementioned sections highlight the central role that investigations of inflammation in AD has taken in the last decade and highlight a number of interrelated mechanisms that may contribute to AD pathogenesis. As the literature demonstrating the role of inflammation in accelerating core AD pathologies increases, so should the investigations of therapeutic approaches targeting the sustained inflammatory response. In addition, extensive investigations in AD patient populations as well as pre-clinical model systems needs to further evaluate microglia signaling cascades and numerous receptors that have not yet been well characterized. Given recent hypothesis that extend beyond the findings of inflammation exacerbating AD pathologies to suggestions that the inflammatory response evoked by Ab may serve to seed the onset of tau pathology, the identification of how the microglia response is regulated and how to modify the response has become an important topic in potential treatments of AD.

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RESEARCH IN CONTEXT

1. Systematic review: Inflammation in Alzheimer's disease (AD) has emerged as a central pathology that likely plays a role in onset and progression of the disease. Numerous investigations have highlighted that the sustained inflammation in the brain accelerates other core pathologies, making inflammatory mechanisms viable targets for therapeutic development as well. In the below review, we highlight a number of the inflammatory mechanisms that have been implicated in AD pathogenesis. We also highlight links between risk factors for AD and potential interactions with inflammatory mechanisms.
2. Interpretation: In the present review we provide coverage of the interactions of inflammatory signaling and the progression of AD. We also discuss a number of possible mechanisms that may account for connections between altered inflammatory signaling and the changes observed in AD.
3. Future directions: This review highlights several emerging mechanisms that may provide a better understanding of AD pathogenesis as well as may serve as novel therapeutic targets for treatment and/or onset of AD.

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Featured Article

Minimotifs dysfunction is pervasive in neurodegenerative disorders

Surbhi Sharma[a,b], Richard J. Young[a,b], Jingchun Chen[a], Xiangning Chen[a,c], Edwin C. Oh[a,d,**], Martin R. Schiller[a,b,d,*]

[a]*Nevada Institute of Personalized Medicine, Las Vegas, NV, USA*
[b]*School of Life Sciences, Las Vegas, NV, USA*
[c]*Department of Psychology, Las Vegas, NV, USA*
[d]*School of Medicine, Las Vegas, NV, USA*

Abstract Minimotifs are modular contiguous peptide sequences in proteins that are important for posttranslational modifications, binding to other molecules, and trafficking to specific subcellular compartments. Some molecular functions of proteins in cellular pathways can be predicted from minimotif consensus sequences identified through experimentation. While a role for minimotifs in regulating signal transduction and gene regulation during disease pathogenesis (such as infectious diseases and cancer) is established, the therapeutic use of minimotif mimetic drugs is limited. In this review, we discuss a general theme identifying a pervasive role of minimotifs in the pathomechanism of neurodegenerative diseases. Beyond their longstanding history in the genetics of familial neurodegeneration, minimotifs are also major players in neurotoxic protein aggregation, aberrant protein trafficking, and epigenetic regulation. Generalizing the importance of minimotifs in neurodegenerative diseases offers a new perspective for the future study of neurodegenerative mechanisms and the investigation of new therapeutics.

Keywords: Minimotif; Posttranslational modification; Trafficking; Binding; Aggregate; Epigenetics; Genetics; Histone code; GWAS

1. Introduction

Minimotifs, also called short linear motifs, are short contiguous peptide sequences or sequence patterns that encode molecular functions. These functions range from the binding of a protein to other proteins and molecules, posttranslational modification (PTM) of a protein, or trafficking of a protein to a subcellular compartment. Two main minimotif databases, Minimotif Miner and the Eukaryotic Linear Motif resource, now house more than one million minimotif instances [1–6]. Recent proteome-wide analysis of minimotifs with 1000 genomes data determined that the vast majority of minimotifs are fixed in humans, suggesting their importance in cellular function [7,8]. At the fundamental level, each minimotif is defined as a sequence or sequence pattern in a source protein and an activity that connects the motif to a target protein. The target protein can be an enzyme catalyzing a PTM, another molecule such as a protein, or a trafficking receptor.

The conservation of most minimotifs and their role in evolution suggests that they might render a significant vulnerability to diseases [5,7–9]. In the first comprehensive review of minimotifs in human diseases in 2007, our group noticed that minimotifs were involved in disease, particularly infectious diseases, and others have expanded upon this observation [5,9,10]. There are at least 35 minimotifs mutated in more than 20 diseases, including both rare and common disorders [11]. These include the three general

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*Corresponding author. Tel.: 702-895-5546; Fax: 702-895-5728.
**Corresponding author. Tel.: 702-895-0509.
E-mail address: edwin.oh@unlv.edu (E.C.O.), martin.schiller@unlv.edu (M.R.S.)

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classes; binding, PTM, and trafficking minimotifs. Several annotations for diseases are listed and described on the Eukaryotic Linear Motif resources website. Approximately 0.1% of missense mutations in the COSMIC somatic cancer mutation database overlap with a minimotif sequence. There are 100s of minimotifs encoded by viruses that are used to hijack host cell processes, several essential for the viral life cycle.

Another line of evidence for the importance of minimotifs in disease is the emergence of Food and Drug Administration (FDA)-approved minimotif mimetic drug therapeutics [11]. For example, protein kinase inhibitor drugs such as Gleevec (imatinib mesylate), Iressa (gefitinib), Sprycel (dasatinib), and Stutnet (sunitinib) among others block phosphorylation of minimotifs and are useful for cancer treatment and immunosuppression [12]. Drugs such as Lotensin (benazepril), Novastan (argatroban), Januvia (sitagliptin), and many HIV protease inhibitors inhibit different proteases that cleave minimotifs and are also minimotif-directed therapeutics. Peptide hormones tend to have core clusters of amino acids that bind to receptors. For example, peptide therapeutics or chemical agonists such as Supprelin LA (histrelin) for the gonadotropin releasing hormone receptor, Byetta (exenatide) for the glucagon-like peptide 1 receptor, Somatuline Depot (lanreotide) for somatostatin receptors, and opiates such as Demerol (meperidine) for opioid receptors mimic the determinants that bind receptors. There are also antiviral drugs to several lipidation enzymes that modify minimotifs, and Aggrastat (tirofiban) is a drug that mimics the Arg-Gly-Asp peptide ligand for integrins.

In this review, by exploring the specific role of minimotifs in neurodegenerative disease (NDs), we consolidate significant evidence that supports our hypothesis that minimotifs have distinct and generalizable functional roles in ND etiology. Minimotifs are the key connections in the cellular molecular network, so it is not surprising that they are vulnerable to pathological dysfunction and that all NDs have multiple dysfunctions of minimotifs. By questioning whether the modification of minimotifs is causal or a consequence of other precipitating events, we recognize both are contributing factors to NDs. From the causal perspective, some of the familial genes in NDs have mutations that disrupt minimotif sources proteins or targets. Minimotifs may also be causal through PTM of the histone code and epigenetics. Other, less direct roles of minimotifs in pathogenesis are through protein trafficking, PTMs, neurotoxic protein aggregation, and protein aggregate clearance.

Because there are approximately 100 NDs known [13], in this review, we have focused on only the major NDs: Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and tauopathies, although where relevant, other NDs are mentioned.

2. Types of minimotifs in neurodegenerative diseases

All three types of minimotifs: binding, modifying, and trafficking, have important roles in neurotoxic aggregate formation and clearance, epigenetics through the histone code, protein trafficking, and PTMs of ND-related proteins (Figs. 1 and 2). Some of the genes with familial inheritance in NDs harbor mutation in minimotifs or enzymes that modify minimotifs, as summarized in Tables 1 and 2 and discussed below.

2.1. PTM minimotifs in neurodegenerative diseases

Previous reviews of NDs summarize the important roles of PTMs, one general category of minimotifs. There are more than 500 types of covalent modifications, with most, if not all proteins in the human proteome having one or more PTMs [74]. These PTMs perturb local and global structure, thereby altering protein binding, trafficking, half-lives, activities, and signaling. Therefore, it is not surprising that several of these PTMs have substantial roles in the pathology of NDs.

2.1.1. Glycosylation/glycation

Half of all proteins in most cell types undergo glycosylation and are N-glycosylated at the minimal consensus sequence Nx[S/T] and/or O-glycosylated on Ser or Thr with no specific sequence determinants [75–77]. Protein glycosylation stabilizes protein structural folds and facilitates protein trafficking, protein quality control, receptor activation, and endocytosis [77,78]. Human mutations causing substitutions at N-glycosylation sites can result in severe disease pathology; for example, a familial mutation encoding a T183A substitution in a NxT glycosylation minimotif in prion protein (PrP) causes Creutzfeldt-Jakob disease (CJD) [11,79–82].

Curiously, there are also several germline mutations immediately juxtaposed to an encoded N-glycosylation consensus sequence: a P504L substitution in WFS1 of Wolframs syndrome, E196K, V180I in PRP (P04156) for CJD, and E196K also for Gerstmann-Straussler disease [83–85]. However, whether these mutations alter glycosylation and influence pathogenesis is not yet clear.

Also unclear is the observation of altered glycosylation in NDs, a likely downstream event or epiphenomena. On a more global scale, proteomic analyses of AD brains revealed altered glycosylation of 131 GlcNacylation sites in 81 proteins. Another global study comparing brains and sera from HD transgenic mice to controls reveals differences in the levels and pattern of glycans [86]. Aberrant glycosylation of an additional ten glycosylated proteins in several NDs (AD, PD, and HD) was summarized previously [87]. Furthermore, acetylcholinesterase is abnormally glycosylated in both CJD and AD [38,87]. Nonenzymatic glycation and aberrant glycosylation of tau are prevalent in AD and FTD [14,44,88,89].

2.1.2. Phosphorylation

Protein phosphorylation of Ser, Thr, or Tyr residues is apparent during the development of disease. During the pathogenesis of NDs, aberrant phosphorylation results in

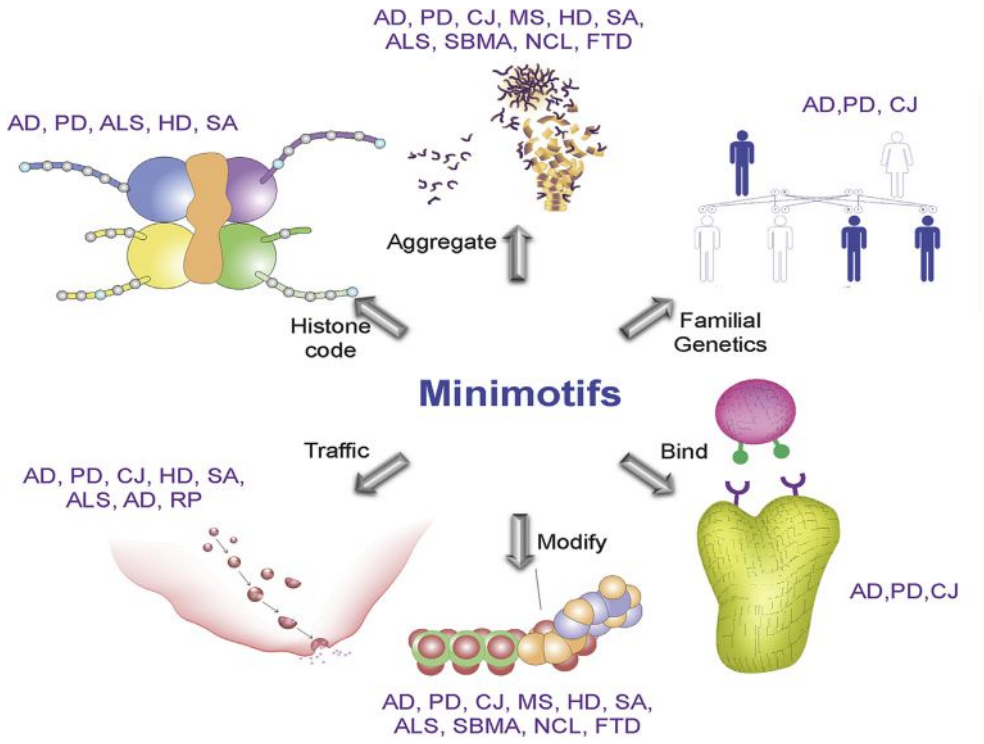

Fig. 1. General functions of minimotifs in NDs. Minimotifs perform multiple functions in NDs. Shown here are a selected functions or cell processes of minimotifs in NDs. Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CJ, Creutzfeldt-Jakob; FTD, frontotemporal dementia; HD, Huntington's disease; NCL, neuronal ceroid lipofuscinosis; PD, Parkinson's disease; RP, retinitis pigmentosa; SBMA, spinal and bulbar muscular atrophy; SA, spinocerebellar ataxia.

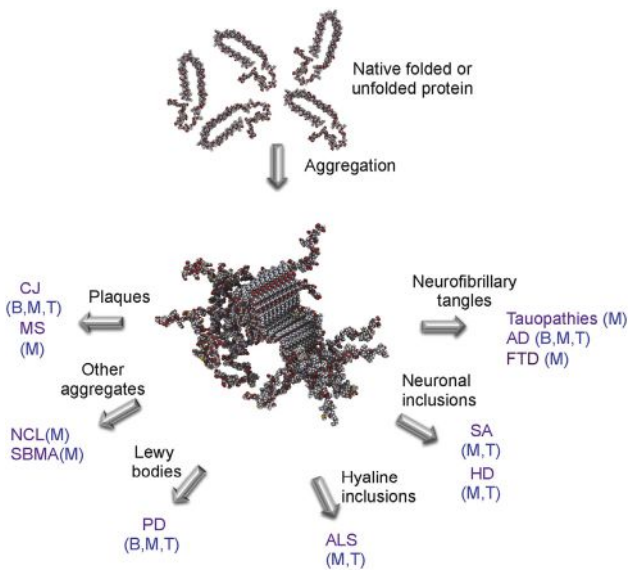

Fig. 2. Minimotifs in neurotoxic protein aggregation. The different types of aggregates in NDs are shown. The types of minimotifs for each aggregate are binding (B), trafficking (T), and posttranslational modification (M). Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CJ, Creutzfeldt-Jakob; FTD, frontotemporal dementia; HD, Huntington's disease; NCL, neuronal ceroid lipofuscinosis; PD, Parkinson's disease; RP, retinitis pigmentosa; SBMA, spinal and bulbar muscular atrophy; SA, spinocerebellar ataxia.

the misfolding and aggregation of neurotoxic proteins. For example, abnormally hyperphosphorylated tau is associated with aggregates in AD and other tauopathies [15]. In addition, more than 40 sites of phosphorylation in tau enhance or reduce its propensity to aggregate with S^{129} being the most prominent [15,90]. The degree of tau phosphorylation differs in AD and FTD, which alters its propensity to aggregate and/or induce toxicity [45,46].

Although the causation and correlation between the phosphorylation and-aggregation of a–synuclein (aSyn), and toxicity of Lewy bodies remain unclear, the phosphorylation of αSyn in Lewy bodies is well established [91,92]. Approximately 90% of αSyn is phosphorylated in Lewy bodies, a hallmark of PD, and other synucleinopathies [91,52,93–97]. Of the 17 potential phosphorylation sites in αSyn, S^{87} is most tightly associated-with synucleinopathies [98]. Several potential reasons for the lack of consistency among different studies are different *in vitro* and *in vivo* comparisons, different efficiencies of kinases, and the balance between the phosphorylation and dephosphorylation of proteins in different model systems.

Both Parkin (*PRKN*) and PTEN-induced putative kinase 1 (*PINK1*) genes are mutated in PD with approximately half of the early onset familial cases caused by *PRKN* [99]. Autoinhibition of Parkin, an E3 ubiquitin ligase, is

Table 1
Neurodegenerative disorder minimotif summary

	Minimotifs			
NDs	Aggregate/gene	Familial genes	Activities	References
Alzheimer's disease (AD)	β-amyloid plaques, neurofibrillary tangles/*APP, MAPT*	*APP, PSEN1, PSEN2, MAPT*	Proteolysis Sumoylation Glycosylation Lipidation Acetylation Methylation Oxidative PTM Ubiquitylation Citrullination Trafficking Binding	[14–28]
Amyotrophic lateral sclerosis (ALS)	Hyaline inclusions/*SOD1*	*SOD1*	Ubiquitylation Sumoylation Phosphorylation Lipidation Oxidation S-nitrosylation S-glutathionylation Trafficking	[19,29–36]
Batten (neuronal ceroid lipofuscinosis)	Aggregation/CSPα aggregation/*DNAJC5*		Lipidation	[37]
Creutzfeldt-Jakob disease (CJD)	Prion (PrP) plaques/*PRNP*	*PRNP*	Glycosylation Trafficking Phosphorylation Acetylation Methylation Ubiquitylation Citrullination Binding	[11,24,38–43]
Frontotemporal dementia/ degeneration (FTD)	Neurofibrillary tangles/*MAPT*	*MAPT*	Phosphorylation Sumoylation Ubiquitylation Glycosylation	[44–48]
Huntington's disease (HD)	Neuronal inclusions/*HTT*	*HTT*	Sumoylation Glycosylation Ubiquitylation Proteolysis Trafficking	[19,49–51]
Parkinson's Disease (PD)	Lewy bodies/*SNCA*	*SNCA* *LRRK2* *GBA* *VPS35* *EIF4G* *DNAJC13* *PRKN*	Proteolysis Sumoylation Glycosylation Lipidation Acetylation Methylation Oxidative PTM Ubiquitylation Phosphorylation Citrullination Trafficking	[16,19,28,52–62]
Spinal and bulbar muscular atrophy (SBMA)	Aggregation/*AR*		Sumoylation Ubiquitylation Phosphorylation of non-aggregating tau	[19,63–66]
Spinocerebellar ataxia (SA)	Neuronal inclusions/*SCA*	*SCA*	Sumoylation Phosphorylation Ubiquitylation Trafficking Proteolysis	[19,50,67–71]

(*Continued*)

Table 1
Neurodegenerative disorder minimotif summary (*Continued*)

	Minimotifs			
NDs	Aggregate/gene	Familial genes	Activities	References
Tauopathies	Neurofibrillary tangles/*MAPT*	*MAPT*	Phosphorylation Glycosylation Acetylation Methylation Ubiquitylation SUMOylation	[72,73]

Abbreviations: PTM, posttranslational modification; CSPα, cysteine-string protein α.

released on its phosphorylation at S^{65} by PINK1 [100,101]. Activated Parkin initiates a downstream ubiquitylation pathway that regulates the quality control of proteins in PD [102].

Phosphorylation is also important for several genes with a familial linkage to NDs. At least part of leucine-rich repeat kinase 2's (LRKK2) pathogenicity in PD is due to its kinase activity and its substrate recognition likely by minimotifs that bind its WD40 domain [103]. Ataxin1 has polyglutamine (polyQ) tracts important for the pathogenesis of spinocerebellar ataxia (SA). Phosphorylation of S^{776} in Ataxin1 at an Akt phosphorylation minimotif increases the cytotoxicity of longer polyQ tracts [67,68]. Phosphorylation likely has a protective role in ALS as a T2D phosphomimetic mutation stabilizes superoxide dismutase (SOD) in the presence of other destabilizing pathogenic mutations [104]. Haplotypes with this variant and a destabilizing SOD variant is one possible explanation for variable penetrance of the pathogenic mutations.

2.1.3. Lipidation

Fatty acid attachment to proteins anchors them to membranes [105]. Some common lipidation PTMs are palmitoylation, N-myristoylation, farnesylation, geranylgeranylation, GPI addition, S-diacylglycerol addition, and prenylation [105]. We do not know of any direct linkage of lipidation with proteins harboring mutations in NDs, suggesting that lipidation is a downstream change. However, several proteins with established roles in ND pathogenesis are covalently linked to fatty acids supporting some role. Palmitoyl acyltransferases (PATs), enzymes that catalyze protein palmitoylation recognize the ΨβxxQP minimotif (Ψ, an aliphatic amino acid; β, a C-β branched amino acid Val, Ile, or Thr) in Huntingtin (HTT) [49,106–111]. ZDHHC17 is a PAT specific for neuronal proteins, including HTT. The reduced palmitoylated HTT in HD implies a reduced activity of PAT for HTT. Palmitoylated HTT has shorter polyQ tracts supporting a protective role against HD. Similar to palmitoylation, myristoylation of HTT is also reduced in HD [112]. One possible explanation is that the expansion of the polyQ tract interferes with this PTM. Palmitoylation of mutant superoxide dismutase 1 (SOD1) and mutant CSPα may play a role in the etiology of familial ALS and neuronal ceroid lipofuscinosis, respectively [29,37,113,114].

In AD, a palmitoylated amyloid precursor protein (APP) is restricted to the Golgi, enhancing its proteolytic processing favoring amyloidogenesis. Several other substrates of PATs associated with AD, PD, and HD were previously reviewed [111].

Other examples of lipidation impacting NDs are a glycosphingolipids. Aglycosphingolipid binding minimotif $[K/H/R]x_{(1-4)}[Y/F]x_{(4-5)}[K/H/R]$, with at least one Gly at $x_{(1-4)}$ is present in αSyn, β-amyloid peptide, and PrP [115,116]. Of the many gangliosides (GM), αSyn

Table 2
Minimotif enzymes, activities, and substrates in familial NDs

	Minimotifs		
ND(s)	Enzymes	Activity	Substrates
AD	PS1, PS2	Proteolysis	APP
ALS/FTD			SOD1
FTD		Phosphorylation	Tau
FTD		Binding	SQSTM1
FTD	TBK1	Phosphorylation	
FTD	VCP, CHMP2B, TARDBP, SQSTM1, DCTN1		
HD	-		HTT
PD	Parkin	Ubiquitylation	α-SP22
PD	LRKK2, PINK1	Phosphorylation	
PD	UCH-L1	Proteolysis	Ub
SMA	UBE1, DYNC1H1	Ubiquitylation	

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; HD, Huntington's disease; PD, Parkinson's disease; SMA, spinal muscular atrophy.

specifically binds GM1 and GM3, inhibiting fibril formation in PD. Conversely, β-amyloid binding to GM1 initiates fibril formation in AD [117,118]. PrP localization to lipid rafts stabilizes its nonpathogenic state. $[L/V]x_{(1-5)}Yx_{(1-5)}[K/R]$ is a cholesterol interaction consensus sequence. αSyn has a VLYVGSK sequence matchin this pattern and upon, binding cholesterol causes αSyn aggregation in neurons [116,119]. A recent review summarizes accumulating evidence for a role for prenylation of small GTPases in Aβ production and secretion in AD [120].

2.1.4. Acetylation and methylation

The role of protein acetylation and methylation in NDs was previously reviewed [121]. These PTMs are common for histones and impact epigenetic inheritance, a topic addressed later in the epigenetics section. Protein acetylation and methylation increase the surface area of, and depolarize Lys and Arg residues, a PTM that generally drives new protein-protein interactions.

Protein acetylation is a reversible attachment of acetyl group to the α-amino group of the N-terminus, ε-amino group of the Lys residues, and many other amino acids [122]. Acetylation of Lys residues in tau inhibits its degradation and induces its aggregation in AD [16,123]. In particular, the K174Q substitution in tau mimics an acetylated Lys state, leading to more severe neuronal atrophy in mice. Conversely, acetylation of α-tubulin, a PTM that increases its stability, is decreased in AD [121]. FTD mice models with the K174Q substituion have similar effects upon tau acetylation [44,47]. αSyn, HTT, β-amyloid, and PrP are all subject to acetylated with either a protective or a detrimental effect on ND pathology [122,124–128].

The ε-amino moiety of Lys can be mono-, di-, or trimethylated, and Arg can be mono- or di-methylated. Methylated residues in tau are located in the microtubule binding repeat region, thus, may block its interactions with microtubules [16]. Tandem mass spectrometry analysis of a human AD brain identified seven monomethylated Lys residues (K^{44}, K^{163}, K^{174}, K^{180}, K^{254}, K^{267}, and K^{290}) in tau and neurofibrillary tangles are immunopositive for methyl-lysine [16]. *In vitro* experiments demonstrate that methylation reduces deimination of Arg residues in myelin basic protein a modification that may sensitize T-cells for autoimmune attack of myelin in multiple sclerosis (MS) [129].

2.1.5. Nitrosylation, glutathionylation, and other oxidative PTMs

There are several types of Cys redox PTMs and corresponding minimotifs in NDs [130]. During oxidative stress, two Cys residues can oxidize creating intermolecular disulfide bridges and protein aggregation. Some Cys thiol groups in SOD1 are oxidized to sulfenic, sulfinic, and sulfonic acids, decreasing the stability of the protein and rendering it prone to aggregation in ALS patients [29–31,131,132]. Similarly, S-glutathionylation of Cys residues in SOD1 induces its aggregation in ALS [32,33].

Aberrant protein S-nitrosylation in several NDs, including ALS, AD, and PD, has been previously reviewed [31,53]. A global analysis of mouse brain identified 31 nitro-tyrosine PTMs, more than half of which have been implicated in PD, AD, and other NDs [17,133,134]. The consensus motif for nitric oxide reaction with proteins is [K/R/H/D/E]C[D/E], where the Cys residues is covalently attached to nitric oxide [135,136]. Protein S-nitrosylation can induce protein misfolding and aggregation [135,137]. Aberrant S-nitrosylation of many proteins (parkin, PDI, DNM1L, CDK5, PRRX2, GAPDH, PTEN, AKT1, MAPK, IKBKP, and XIAP) may contribute to neurodegeneration through multiple pathways [53]. For example, aberrant S-nitrosylation of PDI decreases its enzymatic activity, thereby inhibiting its neuroprotective functions [53].

2.1.6. Ubiquitylation

Many misfolded and damaged proteins are targeted for adenosine triphosphate (ATP)-dependent proteolysis by conjugation with ubiquitin, an 8.5 kDa protein. The role of ubiquitylation in NDs was previously summarized [138]. Proteins that aggregate in NDs tend to be ubiquitylated, likely for initiating targeted protein degradation. In the cerebral cortex of patients with AD, the cerebral spinal fluid of patients with CJD, and αSyn in patients with PD, ubiquitylation of neurofibrillary tangles is increased [18,39,54,139]. Tau, the protein that oligomerizes in these tangles, is ubiquitylated at K^{254} in AD, and the ubiquitylated tau may be blocked by Lys acetylation in FTD [44,123]. A PolyQ tract in Ataxin-1 induces its aggregation in the nucleus and leads to ubiquitylated SA type 1 (SCA-1), inducing cytotoxicity in SA [69]. Ubiquitylated polyQ tracts in HD and other triplet repeat NDs enhance intranuclear protein aggregation in neurons [50,140,141]. Additional PTMs in the polyQ tracts in 12 proteins involved in the pathology of 9 NDs are known [142]. SCF E3 ubiquitin ligase binds survival motor neuron protein through a phosphodegron signal, DSGxx[S/T], where the Ser is phosphorylated [143]. This suggests that oligomerization of the mutant survival motor neuron potentially sequesters the degradation signal and stabilizes survival motor neuron, a potential pathogenic pathway in spinal muscular atrophy [143,144].

2.1.7. Sumoylation

Small Ubiquitin-like Modifiers (SUMOs) are ubiquitin-like proteins covalently attached to other proteins at ΨKXE/D minimotifs. There are four SUMO genes in humans [145]. SUMO normally functions in protein stability, nuclear-cytosolic transport, and transcriptional regulation. Many key proteins in ND pathogenesis are sumoylated and implicated in disease mechanisms [19,146,147]. In AD, sumoylation of APP decreases Aβ production, and sumoylation stabilizes tau by inhibiting its phosphorylation and ubiquitylation [72,147–149]. Similarly, in PD, sumoylation of αSyn inhibits its aggregation [150,151].

The pathogenic fragment of HTT is sumoylated in HD, and SOD aggregation in ALS is also effected [152–155]. Pathogenic mutation of sumoylated residues in valosin-containing protein/p97 inhibits its translocation to the nucleus, the formation of stress granules, reduction of hexamer formation, and eventually, inhibition of its clearance. The amino acid substitutions for these mutants are prevalent in FTDs [48].

2.1.8. Citrullination

Citrullination is an irreversible PTM type of minimotif [156]. Peptidylarginine deiminases deiminate Arg residues in proteins converting this amino acid to citrulline. Because arthritic patients have anti-citrullinated protein antibodies, citrullination may be related to some of the neuroinflammatory aspects of NDs. Abnormal protein citrullination is known for AD, PD, including Lewy bodies, and in prion diseases [157–159]. Autoimmune attack of citrullinated proteins may be prevalent in ND neuroinflammation [160,161]. Although MS is not considered a ND, citrullination of the major myelin component, MBP may play a role in the autoimmune attack of myelin in MS [162]. Deimination depolarizes MBP and is a modification that may trigger the demyelination of axons in MS [163].

2.1.9. Endoproteolysis

Proteolysis is central to the degradation of misfolded proteins by the ubiquitin-proteasome system and autophagy, both driven through minimotifs and central to NDs [164]. The inherent role of endoproteolysis in neurodegeneration was founded in the familial genetics of APP processing to β-amyloid and was recently reviewed [165]. In early onset AD, β-secretase and γ-secretase cleave APP-producing amyloidogenic $A\beta_{40}$ or $A\beta_{42}$ [87,166,167]. Familial mutations in the protease processing minimotif sites in APP cause overproduction of amyloidogenic peptide fragments.

Other NDs have proteolysis minimotifs as well. In patients with PD, familial mutations were identified in UCHL1, a protease that cleaves ubiquitin and colocalizes with Lewy bodies. Furin prohormone convertase cleavage site (KGIQKREA) cleavage of putative type-II single-spanning transmembrane precursor protein (BRI) yields a small C-terminal amyloidogenic peptide fragment [168]. These fragments are present in the amyloid fibrils of patients with Familial British dementia [169–171]. Several proteases cleave HTT at minimotifs producing peptide aggregation and neurotoxic peptides [172–176].

2.2. Trafficking and autophagy minimotifs in neurodegenerative diseases

Minimotifs are important in protein trafficking to and from organelles and are relevant to several NDs [1,177,178]. Defects in endocytic and synaptic vesicle trafficking, Golgi trafficking, retrograde transport, and lysosomal autophagy are apparent in PD and AD [177,179,180]. Trafficking minimotifs are essential for most protein trafficking events, have been previously reviewed, and a few typical examples are presented [180–183]. LRKK2 is a kinase/GTPase with mutations in patients with familial PD [184]. LRRK2 has a WD40 interaction domain that binds to synaptic vesicles and several LRRK2 interactors function in vesicular trafficking [103,185]. Through phosphorylation of S75 in endophilin, LRKK2 plays a role in synaptic vesicle endocytosis in *Drosophila* [186]. LRKK2 is also involved in retrograde trafficking through a functional association with VPS35, another protein mutated in familial PD.

The role of trafficking of APP in AD pathogenesis has recently been reviewed [167]. A KFERQ minimotif at the end of APP binds SCG10 and is essential for trafficking APP to lysosomes for degradation [20,187]. The KDEL receptor binds ER resident proteins containing the KDEL minimotif and returns them to the endoplasmic reticulum [188]. The receptor is redistributed to lysosomes and stimulates autophagy, when aberrant SOD in ALS, αSyn in PD, and HTT in HD are expressed [189].

The cytoplasmic domain of APP contains a YxNPxY sequence, an internalization minimotif that traffics APP to endosomes [21,190]. The same minimotif in APP binds to adaptor proteins, including LDL-Rs, FE65, X11, SNX17, and Dab2, which regulate APP trafficking and thereby regulates β-amyloid production [21].

Mutant ataxin-1 protein has a nuclear localization signal, which is required to mediate toxicity of variants with long polyQ tracts in SAs [67,191]. Mutations in the VxPx> minimotif in rhodopsin cause autosomal dominant retinitis pigmentosa, a progressive ND of photoreceptor neurons [192]. This C-terminal minimotif is essential for trafficking of rhodopsin from the trans-Golgi-network [193]. This minimotif also binds to adenosine diphosphate (ADP)-ribosylation factor 4, a rhodopsin transport carrier, regulating the otherwise bulk flow of proteins to photoreceptor rod outer segments.

Chaperone-mediated autophagy, in addition to ubiquitylation, is another mechanism that maintains protein quality control in NDs [194]. The substrates of autophagy contain a KFERQ-like minimotif that is exposed potentially only after a PTM-induced conformational change. These minimotifs interact with the chaperone complex and are translocated to lysosomes via lysosomal-associated membrane protein 2A, a receptor that triggers autophagy. A VKKDQ minimotif in αSyn targets it for autophagy [55,195]. Mutant αSyn encoding A30P and A53T substitutions may cause familial PD by interacting with lysosomal-associated membrane protein 2A. These mutant proteins fail to translocate into the lysosomal lumen, efficiently stalling the process of autophagy.

Autophagy may be a step in the pathology of PD and HD. Autophagy-targeting minimotifs (QVEVK, KDRVQ) in tau are important in a neuronal cell model of tauopathies [22]. On binding the chaperone complex, Cathepsin L, a lysosomal cysteine protease cleaves mutant tau proteins producing

peptide fragments that aggregate [22]. A more detailed overview of autophagy enumerates eight proteins containing 17 confirmed and putative autophagy-targeting minimotifs in proteins involved in PD etiology [196]. KDRVN and NEIKV minimotifs in HTT are recognized by the autophagy machinery, but mutant HTT with an extended C-terminal polyQ track delays targeting to the lysosomes. This may result in severe HTT aggregation in the neurons of patients with HD [197].

2.3. Binding minimotifs in neurodegenerative diseases

One of the most important activities of minimotifs is for proteins binding other molecules, which is central to many cellular pathways and processes. Interestingly, many types of binding motifs propagate neuronal atrophy in NDs. A linkage analysis of a Turkish kindred identified an autosomal recessive variant encoding a D458V mutation in the C-terminal PDZ binding motif of SANS protein in patients with the atypical Usher syndrome, a neurodegenerative hearing loss syndrome [198,199]. Through an SH3 binding minimotif, RTPPKSP, tau binds the SH3 domains of Fyn and Src nonreceptor tyrosine kinases, which also phosphorylate tau [23,200]. This minimotif is relevant because hyperphosphorylated tau is a hallmark of AD [201]. MED25 binds SH3 domains of Abelson family protein kinases. A mutation encoding the A335V mutation in the proline-rich SH3 binding motif of MED25 decreases binding specificity imparting interactions with a broader range of nonphysiological proteins with SH3 domains. This mutation causes Charcot-Marie-Tooth disease 2B2, a peripheral neuropathy ND [202]. A YENPTY minimotif in APP interacts with PTB domains of Mint, a protein that trafficks and processes APP [203–205]. Ataxin has a RxxSxP 14-3-3 binding minimotif that enhances the toxicity of its polyQ tracts in SA [68].

The GxxxGxxxG glycine zipper minimotif binds cholesterol and has an established role in oligomerization of proteins, including Aβ and PrP [24,206,207]. Cholesterol binding to APP may contribute to amyloidogenesis and AD [24]. In the postsynaptic terminal, β-amyloid recruits PTEN, a lipid phosphatase in a PDZ domain-dependent manner inducing a malfunctional state of amyloids [25]. A PTEN mutant producing a protein lacking its PDZ domain protects against amyloid toxicity.

2.4. Minimotif cooperativity

Minimotif cooperativity is perhaps one of the most understudied aspects of minimotif function and is likely an important factor for understanding molecular dysregulation in NDs. Many proteins, including those associated with NDs, have more than five minimotifs, and there are now numerous examples where one minimotif can induce or create a new minimotif, or compete with other minimotif targets for the same site. Several examples of minimotif cooperativity are mentioned in the other sections, and a few are highlighted here. In PTM minimotifs, certain residues either compete for a particular PTM or induce further modifications. For example, phosphorylation often precedes modifications such as sumoylation and ubiquitylation [92]. In patients with AD, tau phosphorylation follows an initial glycosylation modification [88,208]. When MARK2 phosphorylates tau, it is no longer recognized by E3 ligase, preventing its degradation [209]. Methylation minimotifs in tau are positioned to engage in cross talk with phosphorylation [16].

3. Minimotifs in aggregates

Protein aggregation is common in the etiology of most NDs [210]. β-Amyloid and tau are the major constituents of neuritic plaques and neurofibrillary tangles, respectively. Lewy bodies are intracellular aggregates of αSyn in PD and other synucleinopathies. Proteins with extended polyQ repeats aggregate into nuclear and cytosolic inclusions in HD, SA, and dentatorubral-pallidoluysian atrophy. A hallmark of ALS is cytoplasmic inclusions of SOD1.

Although abnormal protein aggregation in neurodegeneration is well established, the generalized role of minimotifs in aggregation was not previously emphasized. There are several genes with familial inheritance in NDs that encode minimotif target enzymes or their substrates. These genes, minimotifs, and their activities are listed in Table 2. β-Amyloid, the central component of plaques, is ubiquitylated and phosphorylated, PTMs that regulate the stability of plaques [211–213]. Most tauopathies have the common molecular abnormality of hyperphosphorylation of tau, which aggregates into neurotoxic inclusions [214]. Furthermore, there are at least a dozen other PTMs that modulate tau aggregation [72,215]. Lewy bodies are ubiquitylated, sumoylated, phosphorylated, nitrosylated, and also have a unique PTM created by a dopamine radical adduct [150,216,217].

At least 48 PTMs of protein have been identified in NDs, and other neurotoxic proteins with long polyQ tracts have many PTMs [218,219]. Because minimotifs are located on the surfaces of all proteins and cover almost the entire surface of many proteins [220–223], it is not surprising that perturbing minimotifs results in abnormal neurotoxic aggregation. Given their prevalence in neurotoxic aggregate formation and stability, a betting understanding of how minimotifs cooperate to induce and inhibit neurotoxic aggregates would provide a better understanding of ND etiology and open the door for new types of therapeutic intervention. As such, minimotif mimetics should be further considered as targets for reducing aggregation in NDs.

4. Genetics and epigenetics of minimotifs in NDs

Over the past decade, genome-wide association studies (GWASs) have advanced our understanding of the genetic basis of human disease [224]. Similar to other disorders, NDs have a familial component with rare variants of large effect and a more prevalent sporadic component with many common variants of small effect. Given that minimotifs are located at the interface between proteins and are

important constituents of cellular networks, it is no surprise that several of these motifs have been highlighted in GWASs and in the biology of key cell types such as neurons, astrocytes, and microglia. Some of the earliest advances in genetics were derived from genetic linkage and positional cloning in NDs and have led to newer studies relying on genome-wide quantitative trait analyses such as biomarkers or endophenotypes [225].

4.1. Genetics

Through the combination of high-throughput genotyping platforms and next-generation sequencing technologies, disease-causing genetic variants and genetic factors necessary for protection against disease have been discovered at an unprecedented rate. While such technologies have transformed monogenic disease research, several challenges continue to hamper the discovery of actionable genomic variants in complex disorders. For example, variants of unknown significance and an abundance of neutral variants or variants with modest effect size continue to be produced at a staggering rate. As such, leveraging next-generation sequencing for discovery has been contingent on carefully constructed studies of large sample sizes and judicious selection of participants with accurate and detailed phenotyping. Here, we focus on AD and PD as exemplars of NDs, and we also discuss a pervasive role of genetic variants in minimotifs.

4.1.1. Alzheimer's disease

AD is a common ND and a leading cause of dementia with progressive loss of memory, problem-solving skills, and communication. Due to recent improvements in life expectancy, AD is predicted to affect 1 in 85 people globally by 2050 [226]. As a heterogeneous disease, AD is caused by a combination of environmental and genetic factors and current estimates of AD heritability lie between 60-80% [227]. Late-onset AD accounts for more than 95% of all AD cases and is caused by a complex underlying genetic architecture. To date, over 40 GWASs and meta-analyses have identified a few hundred genes and single nucleotide polymorphisms; these include variants in apolipoprotein E (*APOE*) among other notable gene candidates [228]. In contrast to late-onset AD, early onset AD is caused by highly penetrant variants located primarily in three genes with minimotif functions, *APP* (located at 21q21.2), presenilin 1 (*PSEN1*, located at 14q24.3), and presenilin 2 (*PSEN2*, located at 1q42.13) [226]. Structural variants are associated with complex neurological traits; for example, duplication of *APP* causes early-onset AD with cerebral amyloid angiopathy [229,230]. To empower current association studies, new attempts at incorporating quantitative endophenotypes such as age at onset analysis and expression quantitative trait loci are being conducted [231].

Minimotif activities are prevalent in genes associated with AD. On examining disease genes with penetrant variants, we identified at least 11 activities that could be associated with disease: proteolysis, sumoylation, glycosylation, lipidation, acetylation, methylation, oxidative PTM, ubiquitylation, citrullination, trafficking, and binding (Tables 1 and 2). Intriguingly, more than half of all *APP* mutations are located at the secretase proteolytic cleavage sites or the transmembrane domain, a region on exon 16/17 (www.molgen.ua.ac.be/ADMutations). In addition, loss-of-function (LOF) animal and cellular models of AD suggest that such minimotif activities are indeed perturbed. As such, we predict that coding and structural variants that impact minimotif function will be key nodes in ND [232].

4.1.2. Parkinson's disease

As the second most common ND after AD, PD results from the loss of dopaminergic neurons in the midbrain and the accumulation and aggregation of αSyn in Lewy bodies [233,234]. While rare before the age of 60 years, up to 4% of the population at the age of 80 years has PD [235]. Similar to AD, the genetic architecture of PD is complex, and only 5-10% of all patients suffer from an apparent monogenic form of PD [235]. These rare and penetrant variants are located within 19 disease-causing genes such as αSyn (*SNCA*, located at 4q22.1), *LRRK2* (located at 12q12), and acid β-glucosidase (*GBA*, located at 1q22) and segregate with the disease in an autosomal dominant or recessive pattern [236]. GWASs have recently identified more than 40 risk loci, including candidate genes encoding minmotifs that LRRK2 phosphorylates [237].

In a similar minimotif analysis of PD, we note that 11 activities are potentially impacted. These include the following: proteolysis, sumoylation, glycosylation, lipidation, acetylation, methylation, oxidative PTM, ubiquitylation, phosphorylation, citrullination, and trafficking (Tables 1 and 2). To verify the relevance of these predictions, we examined how familial mutations in *LRRK2* might affect minimotif activities (Table 2). Focusing on phosphorylation, we noted that disease-associated mutations or substitutions, Y1699C and I2020T (nonphosphorylated residues), disrupted the phosphorylation of constitutively phosphorylated residues at the amino terminal, resulting in attenuated LRRK2 function [238]. These findings, and additional LOF and gain-of-function (GOF) models, suggest that detailed analyses of minimotif function can identify feedback control mechanisms and novel networks that drive PD biological processes [239].

4.1.3. Microglia and NDs

Microglia are the highly specialized macrophages of the central nervous system, accounting for 10–15% of all cells in the adult brain [240]. Significant advances have been made in understanding the roles of microglia in the development of the brain, such as in neurogenesis, synaptic pruning, surveillance, and homeostasis [241]. In contrast, defining the role of microglia in central nervous system disorders has proven to be more difficult. Given that microglial activation and neuroinflammatory processes play a critical role in the pathogenesis of NDs, biological components linked to

microglial biology have been associated with AD etiology through GWAS; these include the following: *TREM2* (triggering receptor expressed on myeloid cells 2), CD33, *CR1* (complement receptor 1), *ABCA7* (ATP-binding cassette, sub-family A, member 7), *SHIP1* (also known as INPP5D, inositol polyphosphate-5-phosphatase D), *APOE*, *CLU* (clusterin), *CD2AP* (CD2-associated protein), and *EPHA1* (EPH receptor A1) [242–246].

Complementing the human genetic studies, knockout mice models of genes from GWAS have allowed for cause-effect *in vivo* investigations into disease pathogenesis. As expected, a fraction of these genes is implicated in the regulation Aβ accumulation. For example, ApoE- and CLU-deficient APP transgenic mice exhibit earlier and more extensive Aβ deposition compared with control mice [247]. In addition, *CD33* inactivation in *App*/*Psen1* mice reduced Aβ accumulation and plaque burden, whereas *Abca7* deficiency in *App*/*Psen1* and *TgCRND8* mice accelerated Aβ generation [248–250]. Importantly, variants of TREM2, an immunoglobulin-like cell-surface receptor specifically expressed in brain microglia, confer a 2- to 4-fold increased risk for AD [245]. However, exactly how *Trem2* variants confer AD risk is still under investigation. Thus far, the most consistent and striking observation is a strong decrease in microgliosis surrounding Aβ plaques of AD in *Trem2* haploinsufficient and *Trem2* deficient mice; a similar impairment in microgliosis has also been reported in mouse models of prion disease, stroke, and MS, suggesting a critical role for TREM2 in supporting microgliosis in response to pathology in the central nervous system.

A LOF mutation in the lipid sensing function, a minimotif activity of *Trem2* deficient mice may be the underlying mechanism for the loss of microglia proliferation and microglial response to Aβ plaques or reduced infiltration of peripheral macrophages [251,252]. In addition, *Trem2* deficiency leads to exacerbated aggregation of tau in a mouse model of tauopathy [253]. These findings demonstrate that TREM2 has complex multiple roles in regulating Aβ and tau pathologies that may reflect its distinct functions at different stages in AD pathology [254]. These findings also highlight how minimotif activities are present in several proteins relevant to microglial biology.

Minimotifs are a source of functional genetic variation and are important targets in natural selection and evolution [7]. Given their prevalence in NDs, we must consider that common NDs may include a cumulative composition of many minimotif variants that cause network dysfunction. This could provide an explanation for missing heritability in NDs. One exciting possibility is that minimotifs may contribute to the genetic risk of neurodegeneration; however, this will require additional study.

4.2. Epigenetics

Epigenetics refers to inheritance that is not mediated through DNA sequence but through covalent modifications or noncovalent DNA interactions. Recent reviews highlight the emerging role of epigenetics in normal brain function and neurodegeneration [255,256]. Major mechanisms of epigenetics are DNA methylation and PTM of histones/nucleosomes, which is often referred to as the histone code [257]. Minimotifs are the foundation of the histone code. Enzymes that covalently modify minimotifs, including those in histones, are central to AD, PD, ALS, HD, and other polyQ track expansion disorders summarized in recent reviews [258–263].

The role of minimotifs in ND epigenetics is of general interest for ND beyond disease etiology. One emerging area of interest is drugging the enzymes that modify protein components of nucleosomes. Considering the broad role of epigenetics in NDs, there are several drugs such as histone deacetylase inhibitors that are being tested in preclinical investigation and are in different phases of clinical trials [256].

Epigenetics may also explain at least part of the ubiquitous "missing heritability" for common diseases such as the major NDs. Testing the role of epigenetics in NDs may prove difficult without considering the effects of minimotifs. This is because our group has previously reported histone code variability in the population [7]. Approximately 4% of the minimotifs in histone tails have common alleles in humans that are LOF for the PTM. This suggests that the histone code may be wired differently among many people, inferring differing epigenetic responses to the environment [7]. This may be critical to account for in any study that attempts to uncover the epigenetic basis of NDs.

5. Minimotifs as therapeutic targets for NDs

The Therapeutics Peptide Database has no FDA-approved drugs for treating NDs [264]. Despite this lack of success, the investigation of minimotif-directed therapeutics has been, and remains of high interest and potential. Therapeutic interventions based on minimotifs have been discussed in detail for AD, PD, HD, and CJD [265–272]. Since APP was one of the first genes connected with familial NDs and the aggregation of Aβ is a predominate feature of late-onset AD, there has been much interest in inhibiting Aβ production. Inhibitors of α, β, and γ-secretase that inhibit APP processing at minimotifs, Aβ production, and aggregation is still under clinical study as was recently reviewed [273].

Several other minimotif-directed therapeutics are being investigated for reducing aggregation of neurotoxic proteins. One central therapeutic approach, although not directed toward minimotifs, is to lower levels of the neurotoxic protein by antisense oligonucleotide therapy [274,275]. The ubiquitin-proteasome system is strongly associated with NDs and is a target for new therapies with compounds that stimulate proteasome-mediated aggregate degradation or therapeutics that target specific proteins for proteome degradation [138,276]. Peptides such as SNWKWWPGIFD, a

polyQ-binding peptide inhibit HTT aggregation in HD; GAVVT, a peptide in α-Syn that inbibits self aggreation in PD; and a NPxY> PTB domain-interacting minimotif inhibiting APP endocytosis in AD are all being investigated as therapeutics [277–279]. Glycosaminoglycans mimetics are decoy inhibitors of covalent glycosaminoglycan attachment to Aβ, blocking its aggregation [87]. Polyanions such as pentosane polysulfate interfere with aggregation of glycosylated PrP and have shown promise in a preclinical model. Several kinase inhibitors block GSK3β, CDK5, and other tau kinases to reduce tau phosphorylation, and hence their inhibition of aggregation is being investigated for AD and tauopathies [15].

Given the role of neuroinflammation and autophagy in Aβ removal, drugs that enhance amyloid degradation are of growing interest. Many analogs and approaches to target autophagy machinery were previously reviewed [164,197]. Rapamycin is a FDA-approved drug that inhibits the kinase activity of mTOR and improves memory function in model organisms. Preclinical studies of mice show that rapamycin reduces Aβ and tau-associated pathologies by increasing autophagy [87,280,281]. Although not related to minimotifs, lithium also induces autophagy and clearance of HTT, synuclein, and PrP [282].

Three histone deacetylase inhibitors were approved by the FDA for treating various types of cancer [283]. Since NDs have an epigenetic component (addressed earlier), histone deacetylase inhibitors and other drugs targeting epigenetic modifications may be useful for treating NDs. This first test of hypothesis is currently under investigation with a clinical trial testing teatment of AD with ORY-2001. ORY-2001 inhibits histone methylation and reduces cognitive impairment and neuroinflammation in a rodent model.

Although minimotif-targeted drugs are not a major class of therapeutics, as we learn more about minimotifs, we hypothesize that their broad influence on disease will likely become more evident and become useful for treatment of NDs.

6. Conclusion

Minimotifs are short peptide sequences that encode a molecular function. Their role in cancer and infectious diseases has been previously established [9–11]. In this review, we summarize the evidence supporting minimotifs as key regulators of ND pathogenesis and describe numerous examples of causative cases in familial NDs.

Collectively, numerous studies support centrality of minimotifs to NDs, much of which was previously reviewed [15,19,152,157,163,216,276,281,282,284–286]. Our observation of the generalized minimotif pervasiveness in NDs raises several interesting questions. Particularly, why are minimotifs so prevalent in NDs, and can this observation teach us anything more general about ND etiology?

We consider that answers to these questions may arise from the role of minimotifs in protein and larger networks. NDs have an additional layer of complexity when compared with other human diseases. Many diseases are founded in physiological dysfunction of the cellular network that manifests as the emergent network properties of symptoms and even death. This network dysfunction is rooted in dysfunction of the molecular network with cells where minimotifs have an important role. However, networks are more complex in NDs because the symptoms arise from emergent properties of dementia, loss of motor control, and other mental health symptoms. These symptoms are due to breakdown of an additional network, the neuronal network. Cognitive states are considered emergent properties of a neuronal network [287].

Why are minimotifs a point of vulnerability? Thus far, about a million minimotifs have been experimentally verified with large growth anticipated [4]. Minimotifs are the key determinants that enable proteins work together in a network as a basis for emergent properties in the cell such as neurotransmission and neuroinflammation. Most complex networks, including biological networks and the minimotif/protein network, have topologies of cliquish small world networks and have the property of robustness.

Robustness protects the system against immediate shock through adaptive mechanism engineered over time through selection and evolution. Those mutations that target key vulnerabilities in the network cause failure and prenatal lethality.

While the protein/minimotif, cellular, and neuronal networks are evolutionarily tuned for robustness, like any network, they can fail, the failures of interest herein being NDs and death. The study of ND disease etiology has informed us of two general categories of disease failure, familial, and sporadic. Familial ND network failure may arise from hub protein dysfunction [288,289]. Hub proteins are generally highly enriched with minimotifs, and our review consolidates many types of minimotifs involved in familial NDs. Hub nodes can collapse an entire network because of functional overload and poor ability to redistribute information flow [290].

In sporadic NDs, GWAS studies have helped resolve a genetic architecture with the culmination of many genetic variants of small effect size. In this scenario, we suggest that this network dysfunction arises from a dynamic redistribution of flow through the network [289]. The genetic andpolygenic risk scores are measures of reaching a mutational burden threshold and is consistent with this model. While many of the variants are in noncoding regions, disruption of key minimotifs likely contributes to the network dysfunction. Furthermore, the birth of billions of people provides a screen to identify how multiple variants can contribute to breaking network robustness manifested as NDs. This could also explain the higher prevalence of sporadic disease in general.

The role of minimotifs in network dysfunction may also provide an explanation for the slow progression of NDs, often over decades. ND can be considered a cascading failure of networks where several models have been proposed. For late-onset AD, the uncontrolled neuroinflammation

hypothesis proposes a feedback cycle that leads to shutdown of microglial phagocytosis of amyloid plaques [291–293]. This is an example of a cascading failure of a network where the disease manifest once a key threshold is breached with overload of a subnet [294]. Minimotifs are involved in immune responses and autophagy, and are likely part of the network dysfunction [295].

In conclusion, our summarization of NDs shows a pervasive role for many different types of minimotifs in familial mutations, aggregation, the histone code, epigenetics, and biomarkers. Minimotifs are likely a key part of network dysfunction in NDs.

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RESEARCH IN CONTEXT

1. Systematic review: During the course of a literature on the role of minimotifs in neurodegenerative diseases we realized that minimotifs had a central role both in the genetic basis and etiology of general neurodegeneration.
2. Interpretation: Our findings indicate that minimotifs play a central role in NDs with familial mutations altering minimotifs and in the general disease etiology by altering protein trafficking, protein aggregation, PTMs, and epigenetics.
3. Future directions: Our review indicates the role of minimotifs in the pathology of NDs, which can be used to further investigate the underlying mechanisms leading to NDs and for research investigating new therapeutics.

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