Alzheimer's Disease Advisory Board

Ed and Ethel Moore Alzheimer's Disease Research Program

Annual Report February 2022

Ron DeSantis
Governor

Joseph A. Ladapo, MD, PhD
State Surgeon General

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ED AND ETHEL MOORE ALZHEIMER'S DISEASE RESEARCH PROGRAM INTRODUCTION AND OVERVIEW

Alzheimer's disease (AD) is a debilitating brain disease that affects an estimated 6.5 million Americans over the age of 65.[1] Florida is the third most populous state in the nation[2] and, according to the U.S. Census Bureau's latest estimates, has the highest percentage of adults aged 65 and older.[3] In 2020, it was estimated that 580,000 Floridians over the age of 65 were living with AD and this number is projected to reach 720,000 by 2025.[4] AD is the sixth leading cause of death in Florida,[5] and the seventh leading cause of death nationally.[6] The estimated 580,000 Floridians living with AD does not include those under the age of 65, or those living with other forms of dementia.[1]

The progression of AD or other related dementias (ADRD) should be viewed as a continuum, over a lifetime, that may begin even when the brain shows healthy functioning.[1] Over the course of many years, cognitive changes occur in the brain that can eventually result in mild cognitive impairment.[1] Nearly 90% of Americans say that if they were exhibiting confusion and memory loss, they would want to know if the cause of the symptoms was AD.[7] Yet, over half of people aged 45 and older with subjective cognitive decline have not talked with a healthcare provider about their questions, concerns, and fears.[7] Among those whose memory problems were creating functional difficulties, 42% had not shared these issues with a provider.[7]

Missed diagnoses of dementia are more common among Black Americans than White Americans.[1] While Black Americans are two times more likely to have ADRD than White Americans, they are only 34% more likely to receive a diagnosis.[8] Also, both Black Americans and Hispanic Americans with cognitive impairment are less likely than White Americans to say that a doctor has told them they have a "memory-related disease."[8]

Beyond the impact of the disease on the individual, AD also affects family members who often serve as caregivers. Alzheimer's disease can span up to 20 years and is emotionally, physically, and financially challenging to caregivers. The Florida Department of Elder Affairs estimates that there are over 1.2 million Floridians providing loved ones with unpaid care.[2] More than half of these caregivers (54%) provided four to nine years of care, with an additional 17% providing nine or more years of care.[9]

To combat these statistics, the 2014 Florida Legislature created the Ed and Ethel Moore Alzheimer's Disease Research Program (Program) that was signed and enacted by then Governor Rick Scott. Currently, there are 68 active research grants. This Program is managed by the Florida Department of Health. The long-term goals are to:

a) Improve the health of Floridians by researching improved prevention measures, diagnosis methods, treatments, and cures for Alzheimer's disease.

b) Expand the foundation of knowledge relating to the prevention, diagnosis, treatment, and cure for Alzheimer's disease.

c) Stimulate economic activity in the state in areas related to Alzheimer's disease research.

Annually, the Alzheimer's Disease Research Grant Advisory Board submits a fiscal year progress report by February 15, as required by section 381.82, Florida Statutes. With the additional reporting requirements resulting from legislative change effective July 1, 2016, this report provides current findings on the return on investment resulting from the state-supported research grant funding.

ALZHEIMER'S DISEASE RESEARCH GRANT ADVISORY BOARD

The Alzheimer's Disease Research Grant Advisory Board (Advisory Board) is authorized in section 381.82, Florida Statutes, and consists of two gerontologists, two geriatric psychiatrists, two geriatricians, two neuroscientists, and three neurologists.

The Advisory Board advises the State Surgeon General as to the scope of the research program and shall submit recommendations for proposals to be funded to the State Surgeon General by December 15 of each year. Grants and fellowships shall be awarded by the State Surgeon General on the basis of scientific merit after consultation with the Advisory Board. Other responsibilities of the Advisory Board may include, but are not limited to, providing advice on program priorities and emphases; assisting in the development of appropriate linkages to nonacademic entities, such as voluntary organizations, health care delivery institutions, industry, government agencies, and public officials; and developing and providing oversight regarding mechanisms for the dissemination of research results.[10]

Alzheimer's Disease Research Grant Advisory Board Membership

The names and positions of each Alzheimer's Disease Research Grant Advisory Board Member, as of December 15, 2022, are listed below (Biographical Statements or Curriculum Vitae are available upon request):

Gerontologists:
Leilani Doty, PhD, Chair
Retired, Director of the University of Florida Cognitive and Memory Disorder Clinic

The second Gerontologist position is currently vacant.

Geriatric Psychiatrists:
Josepha A. Cheong, MD
Professor of Psychiatry and Neurology, University of Florida
Chief, Consult-Liaison Psychiatry, Malcom Randall Veterans Affairs Medical Center

Uma Suryadevara, MD, FAPA
Assistant Professor of Psychiatry and Program Director,
Geriatric Psychiatry Fellowship Program, College of Medicine, University of Florida

Geriatricians:
Mariana B. Dangiolo, MD
Assistant Professor of Family Medicine and Geriatrics
College of Medicine, University of Central Florida

Niharika Suchak, MBBS, MHS, FACP
Associate Professor, Department of Geriatrics
College of Medicine, Florida State University

Neuroscientists:
Eunsook Yu Lee, PhD
Professor, College of Pharmacy
Florida Agricultural and Mechanical University

Leonard Petrucelli, PhD, Assistant Chair
Chair, Department of Neuroscience and Professor of Neuroscience
Mayo Clinic Jacksonville

Neurologists:
Neill Graff-Radford, MD
Professor of Neurology, Department of Neurology
Mayo Clinic Jacksonville

James Galvin, MD, MPH
Professor
University of Miami Miller School of Medicine

Ruth Henchey, MD
General Neurology
Baptist Hospital and West Florida Hospital

NATIONAL INSTITUTES OF HEALTH (NIH) STATE RANKING IN TOTAL AMOUNT OF ALZHEIMER'S DISEASE RESEARCH FUNDING

Since the inception of the Ed and Ethel Moore Alzheimer's Disease Research Program in 2014, Florida continues to increase federal funding that supports Florida researchers. Florida is currently positioned in the top 8 for federal follow-on funding (Figure 1).[11] Florida is one of two states in the southeastern United States to be ranked in the top 8. This significant increase in federal research dollars can be attributed to the foundational support provided by the Ed and Ethel Moore Alzheimer's Disease Research Program for groundbreaking research and training.

Figure 1: NIH Alzheimer's Disease Research
State Funding and Rankings for Federal Fiscal Year 2022

State	Total Funding	Rank
California	$550,598,678	1
New York	$305,440,824	2
Massachusetts	$284,526,726	3
Pennsylvania	$181,986,861	4
North Carolina	$144,220,313	5
Missouri	$135,317,369	6
Texas	$135,035,354	7
Florida	$124,863,198	8
Illinois	$100,031,381	9
Minnesota	$93,321,652	10
Maryland	$91,890,626	11
Michigan	$83,414,487	12
Arizona	$82,767,577	13
Washington	$60,437,872	14
Georgia	$58,051,664	15
Ohio	$55,134,199	16
Wisconsin	$54,257,192	17
Connecticut	$39,951,095	18
Tennessee	$35,982,778	19
Indiana	$32,948,033	20

Fig.1 NIH Research Funding from the 2022 Fiscal Year Reporting Period: The top twenty ranked states in NIH funding for Alzheimer's disease are displayed. With over $124 million in NIH funding, Florida is ranked eighth in the nation. *Source: National Center for Health Statistics, National Institutes of Health 2022.*

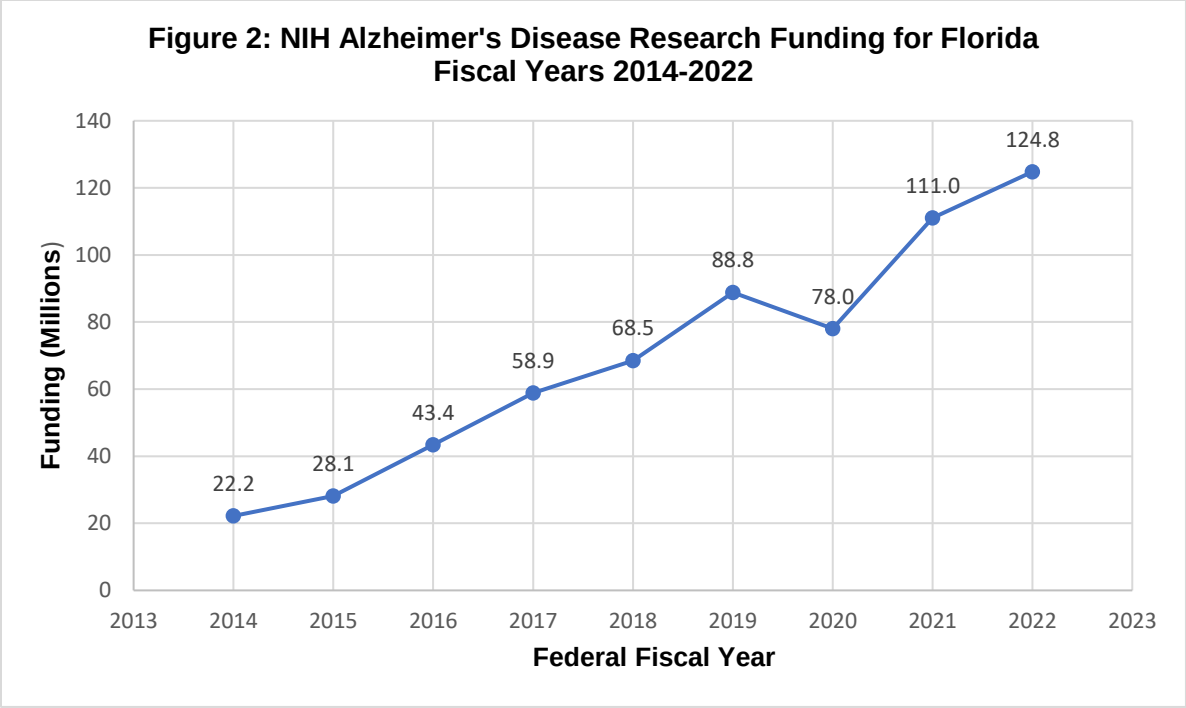

Fig. 2 NIH Research Funding Trends in Florida Fiscal Year 2014-2022: This chart illustrates the recent trends in federal funding for Alzheimer's disease research in the state of Florida. *Source: National Center for Health Statistics, National Institutes of Health 2020.*

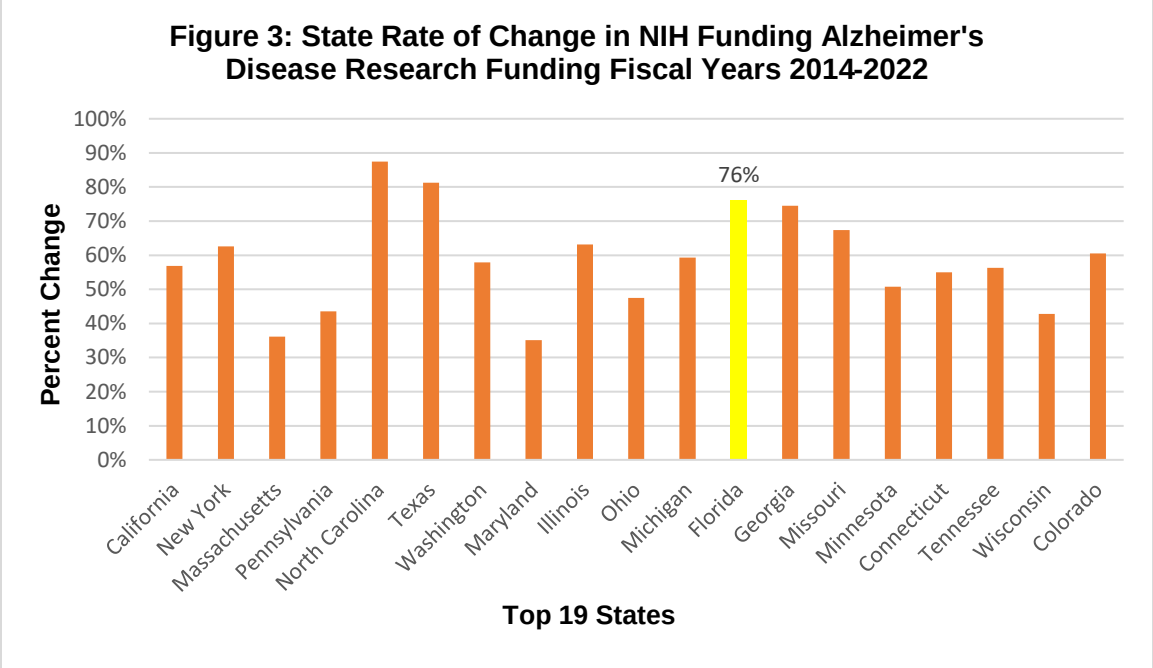

Fig. 3 Change in NIH Research Funding in the Top 19 States Fiscal Years 2014-2022: This graph displays the rate of change in federal Alzheimer's disease research funding for the Top 19 states for fiscal years 2014-2022. Among the Top 10 ranked states in NIH funding for Alzheimer's disease, Florida saw the third highest funding gains since 2014 (76%). *Source: National Center for Health Statistics, National Institutes of Health 2020.*

PROGRESS TOWARD PROGRAMMATIC GOALS

The Ed and Ethel Moore Alzheimer's Disease Research Grant Advisory Board's research agenda emphasizes the creation of intra-state research collaborations to make progress toward Florida becoming the premier state for Alzheimer's disease prevention, diagnosis, treatment, and ultimately, cure for this disease. The research agenda has five research priority areas that are outlined in the Funding Opportunity Announcement and are listed below:

1. The social/behavioral aspects of care, as well as palliative and end of life care for people with Alzheimer's disease.
2. Elucidation of the basic science relating to the disease.
3. Consortium grants between Florida-based institutions to augment established research networks and promote novel networks.
4. Epidemiological studies examining the prevalence, incidence, and risk factors of the disease with priority given to studies examining health disparities.
5. Fellowships aimed at enhancing the workforce of Florida's researchers working on Alzheimer's disease.

In state fiscal year 2021-2022, the legislature provided $5 million for research grants. Appendix A details all newly awarded grants and Appendix B details previously awarded active grants. Appendix B provides information on research progress, follow-on funding, publications, and patents for each previously awarded active grant. Appendix C provides information on research grants that closed within the fiscal year.

RECOMMENDATIONS FROM THE ALZHEIMER'S DISEASE RESEARCH GRANT ADVISORY BOARD TO FURTHER THE MISSION OF THE PROGRAM

During fiscal year 2021-22, 17 grants were awarded. Without this support, the eminent scientific advancements and discoveries in Alzheimer's disease would not be possible.

Statutory change is needed to allow for reimbursement of travel expenses resulting from Advisory Board in-person meetings. Face-to-face communication intensifies the exchange of information to allow for effective strategic planning and in-depth communication about critical research issues. In-person meetings engage full attention, build trust and credibility of the Advisory Board, and strengthen collaboration on ideas from the expertise that may be translated into research priorities for grant applications. These meetings can fuel more and varied planning to hone the Research Agenda and the Funding Opportunity Announcement documents.

The Alzheimer's Disease Research Grant Advisory Board thanks Governor DeSantis and the Florida Legislature for continuous support and for working together to eradicate Alzheimer's disease.

Appendix A
Fiscal Year 2022-2023 Newly Awarded Grants (Announced December 1, 2022)
Funded Fiscal Year 2022-2023

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
23A01	Florida International University	Giri Narasimhan, PhD	99,783.00	01/31/25	No	No	No
23A02	Florida State University	Julia Sheffler, PhD	350,000.00	01/31/27	No	No	No
23A03	Mayo Clinic Jacksonville	Yunjung Jin, PhD	100,000.00	01/31/25	No	No	No
23A04	Mayo Clinic Jacksonville	Wilfried O Rossoll, MSW, MPH, PhD	350,000.00	01/01/25	No	No	No
23A05	Nova Southeastern University	Sibel Antonson, PhD	99,459.00	01/01/25	No	No	No
23A06	University of Central Florida	Sussany Beltran, PhD	99,883.00	01/31/25	No	No	No
23A07	University of Central Florida	Jihe Zhao, PhD	350,000.00	01/31/27	No	No	No
23A08	University of Florida	Stephen Anton, PhD	350,000.00	01/31/25	No	No	No
23A09	University of Florida	Jie Xu, PhD	350,000.00	01/31/26	No	No	No
23A10	University of Miami	David Loewenstein, PhD	349,983.00	01/31/25	No	No	No
23A11	University of Miami	Philip Harvey, PhD	99,345.00	01/30/24	No	No	No
23A12	University of Miami	Oliver Bracko, PhD	350,000.00	01/31/26	No	No	No
23A13	University of Miami	Tatjana Rundek, PhD	100,000.00	01/31/24	No	No	No
23A14	University of Miami	Roger Leblanc DDS, MBA, PhD	52,536.00	01/31/24	No	No	No
23A15	University of Miami	Elizabeth Crocco, PhD	349,102.00	01/31/25	No	No	No
23A16	University of Miami	Rosie Curiel Cid, PhD	349,912.00	01/31/25	No	No	No
23A17	University of Miami	Claes Wahlestedt, PhD	349,995.00	01/31/26	No	No	No
23A18	University of South Florida	Hongdao Meng, MD, PhD	350,000.00	01/31/27	No	No	No

Grant#: 23A01 Microbial DNA in Blood in Alzheimer's Disease Subjects as Potential Biomarkers and the Role of the Microbiome

Principal Investigator: Giri Narasimhan, PhD

Organization: Florida International University

Abstract: Alzheimer's disease (AD) affects an estimated 5.8 million Americans aged 65 and older, with the number expected to triple over the next 30 years. Combining robust causal inferencing methods from Artificial Intelligence (AI) with novel technologies in identifying microbial DNA (mbDNA), neuroinflammatory and AD biomarkers in plasma, researchers will use an interdisciplinary approach to determine if mbDNA could be a causal factor in AD. Microbial DNA with distinct signatures has been found in blood, saliva, and even the brain, emerging as biomarkers in several human diseases. The research team hypothesizes that mbDNA plays a causal role in the late onset of AD (LOAD). This proposal investigates the role of mbDNA in the blood of AD patients by using AI-based causal inferencing techniques. Using blood samples from 50 AD subjects and 50 cognitively healthy older adults obtained from the Florida Alzheimer Disease Research Center, researchers will determine the microbial profile of the mbDNA and the neuroinflammatory markers found in the plasma. While standard differential analysis of the results can identify AD-associated factors, the computational approaches proposed here will allow the conclusions to be elevated from mere associational relationships to causal

relationships. The proposed project is a first step in an exciting new direction. If successful, it will provide in silico evidence for a new hypothesis, i.e., that specific microbes and the metabolites they produce play a role in AD. This transformative project aims to build an integrative biomarker for AD by identifying the microbes represented in the mbDNA and their metabolites that "causally" promote AD pathogenesis. This project has the potential to not only question the basic assumptions about the causes of biological aging, but also to accelerate the progress in developing an integrated, testable biomarker for aging, and in discovering new ways to delay/reverse the aging process. Successful identification of the late-onset AD microbial triggers distinct from normal healthy aging will lead to unraveling the mechanisms of action, suggest innovative diagnostics, pinpoint targeted future experiments to identify mediating metabolites, and most importantly propose novel therapeutic strategies.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A02 Targeting α-synuclein aggregation in Alzheimer's disease and Lewy body dementia

Principal Investigator: Julia Sheffler, PhD

Organization: Florida State University

Abstract: Approximately one-third of Alzheimer's disease (AD) cases are attributable to modifiable risk factors that can be addressed through lifestyle intervention. Ketogenic nutrition (KN) stands out as a well-studied and unique lifestyle intervention that alters cellular energy metabolism and modulates the gut microbiome to reduce AD risk via the gut-brain axis. Specifically, KN has been shown to reduce AD risk through changes in the gut microbiome and microbial metabolites, which influence insulin signaling, β-amyloid production, tau processing, and AD-associated inflammation, mitochondrial function, and oxidative stress. KN involves very low intake of carbohydrates, adequate protein, and a higher caloric percentage of fat intake, which causes a metabolic shift from using glucose to ketone bodies and fatty acids for energy. Further, KN may directly target neurobiological mechanisms associated with the development of ADRDs, such as defects in mitochondrial function, tau levels, insulin signaling, inflammation, and dysfunctional glucose and lipid metabolism, as well as cognitive functioning and mood. KN's influence on the gut-brain axis makes it especially valuable for targeting early biological risks of ADRDs. Further, researchers recently demonstrated how components of a Mediterranean diet can be combined with KN to provide high quality sources of antioxidants and omega-3 and -6 fatty acids and how this Mediterranean-KN (MKN) improves neurocognitive markers in subjects with mild cognitive impairment (MCI) via modulating the gut microbiome. However, MKN requires clear and guided education to ensure it is used in a safe and effective manner, especially for older adults who may have some memory impairment and are at higher risk for AD. In particular, diverse rural communities may require additional intervention tailoring due to disparities in AD risk and unique socio-geographical factors that may impede adherence to nutrition and other lifestyle interventions. Further, the benefits of MKN have only been

examined in resource-rich and relatively controlled and structured research environments. The research team developed a MKN adherence intervention that is ready for community translation, and researchers are ideally positioned to enroll and evaluate a diverse rural sample in surrounding North Florida counties, which are largely rural and have the highest percent black population in Florida. Further, the research team has experience and expertise in collecting and analyzing biological data (e.g., microbiome, metabolome, and cardiometabolic risks) on the effects of MKN for individuals with MCI and elevated AD risk. Thus, the project aims to evaluate how a scalable MKN intervention beneficially modulates the gut-microbiome-brain axis in underserved rural older adults with MCI. Aim 1: To scale-up the Mediterranean-Ketogenic Nutrition intervention for testing in predominantly Black and African-American rural communities in North Florida. First, researchers will evaluate the feasibility and acceptability of adapting the nutrition program for these underserved communities. Next, the researchers will examine whether the modifications produce high adherence to MKN, comparing rates among individuals with MCI and cognitively normal (CN) older adults. Aim 2: To evaluate the effects of Mediterranean-Ketogenic nutrition on novel gut-brain axis markers of AD pathogenesis in individuals with MCI compared to CN older adults.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A03 Targeting α-synuclein aggregation in Alzheimer's disease and Lewy body dementia

Principal Investigator: Wilfried O. Rossoll, PhD

Organization: Mayo Clinic Jacksonville

Abstract: Accumulating studies show that Lewy pathology is one of the most common co-pathologies in Alzheimer's disease (AD). Importantly, these additional co-pathologies lower the age of onset and accelerate the progression of AD. The ε4 allele of apolipoprotein E gene (APOE4), the strongest genetic risk factor for AD, is also the most replicated genetic risk factor for Lewy body dementia (LBD), suggesting that AD and LBD share a common APOE-mediated pathogenic mechanism. α-synuclein (α-SYN), encoded by SNCA gene, is the primary component of Lewy pathology such as Lewy bodies (LB) and Lewy neurites (LN), which drives neurodegeneration. More than half of AD brains present with concomitant Lewy pathology at autopsy, suggesting that α-SYN aggregation is a regulated event in AD pathogenesis. The misfolding and aggregation of α-SYN involves a mechanism of seeding and nucleation in which initial seeds of α-SYN recruit other soluble monomers that assemble to form aggregates. The recent work has shown that α-SYN seeds are detected in postmortem AD brains even without detectable Lewy pathology. Moreover, the amplified α-SYN aggregates from AD brains were toxic to human induced pluripotent stem cells (iPSC)-derived neurons. Therefore, the researchers hypothesize that the seeding and aggregation of a-SYN is one of the key events in the pathogenesis of both AD and LBD, and targeting this pathway has therapeutic potential to prevent or delay the disease onset and progression. In this proposal, the researchers aim to

develop a novel synucleinopathy model system using human iPSC-derived cerebral organoids and neurons, and to discover potential drug candidates to inhibit α-SYN aggregation using this model. Specifically, in Aim 1, researchers will generate cerebral organoids and neurons using the iPSC lines from individuals carrying SNCA triplication and from normal controls. The α-SYN pathological features such as α-SYN phosphorylation and aggregation will be assessed in these organoids and neurons. To understand the molecular mechanisms related to α-SYN pathology in this model and the relevance to human diseases, researchers will perform single cell RNA sequencing (scRNA-seq) with organoids at different times. Single nuclei RNA sequencing (snRNA-seq) will also be performed using the postmortem AD, LBD, and SNCA multiplication human brains to compare the similarities and differences of the molecular pathways between human brains and organoids. In Aim 2, researchers will screen FDA-approved drug library to identify drug candidates that can inhibit α-SYN aggregation using the real-time quaking induced conversion (RT-QuIC) assay. The α-SYN seeds from human AD and LBD brains will be used to screen the candidates. The drug candidates will then be used to treat the organoids, and their effects on α-SYN pathology will be evaluated. Functional assays will be performed to validate the efficacy of drug candidates on alleviating the pathological pathways related to α-SYN aggregation. Overall, this project will enable the discovery of potential drugs that can inhibit α-SYN seeding activity using human iPSC-derived models, providing a great opportunity to identify and develop highly applicable therapeutic strategies targeting α-SYN aggregation for AD and LBD.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A04 Targeting α-synuclein aggregation in Alzheimer's disease and Lewy body dementia

Principal Investigator: Yunjung Jin, PhD

Organization: Mayo Clinic Jacksonville

Abstract: One of the defining pathological hallmarks of Alzheimer's disease (AD) is the accumulation of insoluble aggregates of the microtubule-binding protein tau inside neuronal cells in the brain. The close correlation of these inclusions, called neurofibrillary tangles (NFTs), with observed clinical symptoms and disease progression makes them a promising target for AD therapy development. There is urgent need for novel therapeutic approaches that can target these toxic tau aggregates to improve symptoms or alter the disease course even at later stages of disease. Work from several labs has shown that tau aggregates associate with components of the nuclear pore complex, called nucleoporins, causing a dysfunction of nucleocytoplasmic transport. Recently, the research lab has discovered that specific members of the nuclear import receptor protein family (importins) can strongly reduce the aggregation and toxicity of various disease-causing proteins, including pathological tau. Based on findings, researchers have identified a novel non-canonical role for importins as potent molecular chaperones that are recruited by nucleoporins into pathological protein aggregates, where they

act to reverse the aberrant aggregation and restore their normal nuclear localization and cellular functions, suggesting a promising new strategy for therapeutic intervention in AD and related disorders. To test this hypothesis, the objectives are to use cutting edge in vitro and in vivo methods to gain a detailed mechanistic understanding of how importins restore solubility, proper localization, and normal function of tau; how importins reduce neurodegeneration; how importin dysfunction contributes to human disease; and how researchers can use this knowledge of importin functions to develop therapeutic strategies for AD. The specific aims are: (i) to determine how importins restore proper tau solubility in vitro, using a combination of advanced neuronal cell culture models of AD and biochemical characterization of tau aggregation; (ii) to identify how importins reduce tau-dependent neurodegeneration in tissue culture and animal models ex vivo and in vivo, employing organotypic brain slice cultures and somatic brain transgenesis with adeno-associated viral vectors (AAVs) in tauopathy mouse models, and (iii) to clarify the relationship of importins and nucleoporins to tau pathology in autopsy brain tissue. Successful outcome of this project will establish the role of importins in regulating tau pathology, which will be critical for developing new therapeutic strategies to target aberrant protein aggregation in AD.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A05 Effect of an Alternative Dental Intervention in Stabilizing Oral Health and Slowing Cognitive Decline for the Patients with Alzheimer's Disease

Principal Investigator: Sibel Antonson, PhD

Organization: Nova Southeastern University

Abstract: Quality, consistency, and predictability of long-term oral healthcare (OH) significantly improves quality of life (QOL) for patients with Alzheimer's disease (PAD), and also reduces the burden on the healthcare system by preventing chronic dental diseases, such as periodontitis and caries. These diseases result in devastating outcomes leading to painful inflammatory and degenerative oral diseases. It is also shown that there is a link between periodontal disease and the initiation and progression of AD. Also, a 24% increase in PAD population is expected by 2025 in Florida. Therefore, it is critical to develop a new intervention strategy that will contribute to the provision of a sustainable and achievable OH maintenance program, thereby reducing this significant risk factor, slowing cognitive decline for PAD, and increasing QOL for PAD. PAD have multiple OH problems due to loss of manual dexterity, daily OH routines, and xerogenic medications. Training caregivers in OH implementation has been successful, however, long-term maintenance as well as sustainability of consistency has been a challenge. Along with the administration of daily OH, topical chlorhexidine (CHX) is frequently used in dentistry as an antibacterial agent to control periodontal disease, and for caries control in high-caries risk patients. It requires patients' cooperation to swish the CHX solution in their mouth for 30 seconds, then spit out the solution, twice daily. Compliance is low, even for healthy patients due to application time, unpleasant taste, and staining of the dental hard tissues. There is an

alternative delivery system in varnish form that can be applied by a dentist or dental professional, and this eliminates the need for patient compliance. Also, fluoride varnish can be applied along with the CHX to provide an additional benefit to reduce caries lesions. The objective of this study is to assess if CHX and fluoride application in a varnish form can provide a sustainable improvement in PADs' periodontal and caries status. Additionally, patients' cognitive status will be assessed to test if there is a correlation between the maintenance of OH and the rate of cognitive decline. In partnership with the Alzheimer's Association Florida Region (AAFR), researchers will enroll 250 PAD for a 6-month OH intervention. Participants will have a series of cognitive assessments, a dental examination, and saliva collection to investigate the base protein levels to identify AD biomarkers, prior to being randomly assigned into 3 groups: 1) a group which will receive debridement, toothbrushes, and care instructions for their teeth; 2) a group receiving additional CHX and fluoride varnishes; and 3) a control group that will not have dental health intervention. At 3 and 6 months, the same cognitive, biological, and dental health tests will be conducted. If evidence indicates that the newly developed dental health intervention is successful, groups 1 and 3 will be given the opportunity to receive it. This work aligns with FDOH's priority of developing treatments for PAD through interprofessional partnerships. The research team expects to establish an implementable, sustainable OH protocol to prevent periodontal disease and caries, and slow cognitive decline for the PAD. In partnership with the AAFR, a larger community outreach effort will be performed to increase dental health in the Florida PAD population based on successful findings from this work.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A06 Adapting the Advanced Dementia Prognostic Tool for High-Medicaid Nursing Homes (ADePT-NH)

Principal Investigator: Susanny Beltran, PhD

Organization: University of Central Florida

Abstract: Almost two-thirds of dementia patients die in nursing homes (NH), where end-of-life care is often of poor quality. Hospice has been shown to benefit residents dying with dementia and their family, yet use of the Medicare hospice benefit remains low, especially in high-Medicaid NHs that host high proportions of Black and Hispanic residents. To qualify for the Medicare hospice benefit, a patient must be certified by two physicians as having a medical prognosis of ≤6 months. However, prognostication is challenging in dementia given long and unpredictable disease trajectories. The Advanced Dementia Prognostic Tool (ADePT), which can be used online to calculate 6-month mortality risk, has shown better discrimination in predicting 6-month mortality compared to standard hospice guidelines. This low-resource tool can be incorporated into NH practice to standardize the process of identifying dementia residents appropriate for hospice referrals. However, ADePT has been tested with predominantly White samples, and its incorporation into NH care planning practices has not been tested in high-Medicaid NHs, specifically. Nursing homes are reputed to be challenging

environments for implementation and adoption, and research shows that planning the intervention collaboratively is key to successful implementation in this setting. Thus, the goal of this exploratory, community-engaged study is to develop, and pilot test, the integration of ADePT into routine care planning practice in high-Medicaid NHs.Year one, focus groups and semi-structured interviews will be conducted with a random sample of high-Medicaid NH stakeholders across Central Florida, to explore current practice processes for identifying hospice-eligible dementia residents, perceived barriers, and enablers to implementing ADePT. Barriers and enablers will be explored qualitatively as well as quantitatively through secondary analysis of resident and facility characteristics. In this stage, researchers will also obtain recommendations for the adaptation of the intervention for this setting (Aim 1). Year two, researchers will conduct a small pilot study to examine feasibility and acceptability of the intervention (Aim 2). This study aims to develop a novel practice process to reduce variations in practices that disproportionately limit access to hospice care among racial/ethnic minority NH dementia residents. As such, it aligns with the Florida State Health Improvement Plan priority of enhancing supports for Floridians with dementia and advances the Ed and Ethel Moore Alzheimer's Disease Research Program Priority Area 1 (Focus Area 1.3.3 Palliative and End-of-Life Care) focus on care processes that improve the lives of people with dementia. Specifically, the researchers explore the feasibility of an intervention to improve care transitions to hospice (NIH Stage I). The study also furthers the National Alzheimer's Project Act's goal of enhancing care quality and efficiency (Strategy 2.F- Effective Transitions), as well as its specific call to address dementia health disparities among racial/ethnic populations. The study is informed by previous research demonstrating the need to address variability in referrals as a key barrier to timely introduction of hospice in NHs and focuses on working with stakeholders to develop an intervention feasible in high-Medicaid/low resource NHs. Findings will result in a refined intervention to be tested for efficacy (NIH Stage II).

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A07 Role of KLF8 in Cerebral Neuroprotection

Principal Investigator: Jihe Zhao, PhD

Organization: University of Central Florida

Abstract: Alzheimer's disease (AD) and related dementias claim the lives of approximately 30% population at age 60 and above. Two-thirds of the cases are AD. More than 50 million people around the globe and 6 million Americans including about 600,000 Floridians are living with AD alone. In the US alone, the annual cost of providing care is estimated to be 300 billions of dollars each year and will surpass 1.1 trillion by 2050 if better therapies remain unavailable due to the lack of knowledge about the pathological mechanisms. Therefore, it is urgent to understand the disease mechanisms better for developing early diagnosis and effective treatment in order to save lives for both patients and the healthcare system. The pathohistological hallmarks of AD are the plaques, inter-neuronal amyloid β protein (Aβ), and

neurofibrillary tangles, intra-neuronal accumulation of hyperphosphorylated tau. However, current therapies targeting the reduction of Aβ production or abnormal tau phosphorylation are not effective, indicating that they may not directly cause AD. Thus, further investigating the cellular and molecular mechanisms of AD underlying its relationship with the pathology of Ab and tau is needed. In the current proposal, researchers focus on KLF8, a transcription factor reduced in the AD brain. Although KLF8 turns on and off many genes critical for learning and memory in the adult brain, its role in AD pathology has not been investigated due to the lack of proper animal models. The research team developed novel mouse models where researchers can regulate KLF8 expression in the neurons. The preliminary data demonstrated that the loss of KLF8 caused the neuronal death and cognitive decline. The data also showed that the loss of KLF8 caused dysregulation of genes critical for maintaining cognitive function and a normal brain environment, preventing abnormal tau phosphorylation. These results strongly suggest a role of KLF8 for in the protection of neurons and loss of KLF8 function in the cells may involve the pathological progression of AD. The research team proposes to investigate the role of KLF8 in tau pathology in AD by crossbreeding the novel mouse models, which allows researchers to regulate KLF8 expression level and tau transgenic mouse model for AD. Completion of this project will significantly advance understanding of the mechanisms underlying AD pathology and provide new targets for AD therapy.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A08 Fasting to Provide Energy Needed to Help Adults in Need of Cognitive Enhancement

Principal Investigator: Stephen Anton, PhD

Organization: University of Florida

Abstract: The unprecedented growth of the aging population has been accompanied by an increase in the number of individuals living with chronic metabolic and neurocognitive disease conditions, including obesity, metabolic syndrome, and Alzheimer's disease and Related Disorders (ADRD). This has created an urgent need for interventions that can preserve older adult's cognitive and physical function and maintain their ability to live independently. To date, there is no conclusive evidence supporting the efficacy of an intervention to prevent or reverse cognitive decline or the dementia process in older persons. The rate of cognitive and functional decline among older adults, however, has been found to be highly influenced by biological and metabolic changes during aging which are largely affected by lifestyle factors, namely dietary intake, physical activity, and sleep. The transformative discovery that interventions targeting the fundamental biology of human aging have the potential to delay, if not prevent, the onset of aging-associated conditions, such as ADRD, has heightened interest in geroprotective approaches that can improve cognitive and physical function, and thereby extend health-span. The research team's work and others have shown that intermittent fasting regimens can have positive effects on biological, metabolic, and functional parameters in middle-age and older

adults. Such interventions may also help align circadian rhythms through lifestyle cues, such as meal timing, and therefore help regulate metabolic processes including autophagy. For these reasons, there is increasing scientific interest in further exploring the biological and functional effects, as well as the feasibility and safety of popular types of intermittent fasting regimens, such as time restricted eating, in older adults. The scientific premise of this proposal is that a time-restricted eating intervention can target the cellular and metabolic alterations that underlie age-related metabolic and neurocognitive disease conditions, and thereby extend health-span in the growing population of at older adults at risk for cognitive decline. To test the central hypothesis, the proposed randomized controlled study will randomly assign overweight, older adults with self-reported cognitive difficulties to either a time restricted eating intervention or a successful aging comparison group for a 24-week period. In the time restricted eating intervention, participants will be asked to fast for a target of 16 hours per day throughout the intervention period. In the successful aging educational intervention, participants will attend weekly lectures on health topics unrelated to diet. It is hypothesized that the time-restricted eating regimen will improve cognitive and physical function, sleep quality, mood states, and quality of life, as well as reduce known biomarkers of neuroinflammation.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A09 Utilizing Data from the Electronic Health Record to Understand the Progression Pathway of Alzheimer's Disease and Related Dementia

Principal Investigator: Jie Xu, PhD

Organization: University of Florida

Abstract: Alzheimer's disease (AD) and AD-related dementia (AD/ADRD) – the 6th leading cause of death in the United States – is a devastating aging-related neurodegenerative diseases affecting 6.5 million Americans aged 65 and older based on the recently updated report, "2022 Alzheimer's disease facts and figures". Enrolling and retaining AD patients for clinical trials has been challenging. Most available data-driven studies on AD progression, risk factors, and endophenotypes use cohort study data and apply stricter selection criteria in participant enrollment, thus, limiting the generalization of findings to real-world patients. Identification of clinically meaningful AD/ADRD subphenotypes, which are subgroups of patients with coherent clinical characteristics, is critical for improved understanding of the underlying disease mechanisms and informing precision medicine. Identification of clinically meaningful AD/ADRD subphenotypes, which are subgroups of patients with coherent clinical characteristics, is critical for improved understanding of the underlying disease mechanisms and informing precision medicine.On the other hand, different hospitals own the electronic health records (EHR) of different patient populations (i.e., lead to between-site heterogeneity of the clusters observed); a comprehensive understanding of the "clusters" would require researchers to "study" a large enough population pooling EHRs from diverse hospitals together; nevertheless, these EHRs are difficult to share across hospitals because of their sensitive

nature. This creates a big barrier to developing effective analytical approaches that are generalizable, which need diverse, "big data." OneFlorida+, including multiple sites, offers unique opportunities to mimic multiple institutions and generate real-world evidence (RWE) that will have direct translational impacts on AD/ADRD. In the past, RWD such as EHRs have limited use for AD/ADRD subtyping and were primarily used only for "hypothesis refinement" due to a number of key methodological gaps: (1) the lack of validated computable phenotyping (CP) and natural language processing (NLP) algorithms and tools that can accurately define the study populations, extract key relevant patient characteristics and meaningful outcomes (e.g., MMSE scores to determine severity), (2) the lack of integration with different model structures to link the data from different sites, (3) the lack of consideration on the heterogeneity of the disease (i.e., AD/ADRD subtypes), and (4) the lack of recognition of the inherent biases in RWD and the need of applying causal inference principles.In this project, using historical data collected at primary care provider (PCP) visits and stored in the EHR's relational database, researchers will (1) develop computable phenotyping algorithms and tools to support AD/ADRD subtyping, (2) advance the use of EHR data to build a reliable federated learning framework that can accommodate the heterogeneous model structure for generating AD/ADRD progression subtypes across sites, (3) uncover differences in AD/ADRD subtypes through applying a causal principled ML framework, (4) identify risk factors (e.g., clinical, and SDoH) contributing to AD/ADRD patient's subtypes, and (5) make suggestions on potential subtype-specific modifiable risk factors and treatment plans or patient care.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A10 Innovative Cognitive, Plasma-Based and Extra-Cellular Free-Water as Early Biomarkers of AD

Principal Investigator: David Loewenstein, PhD

Organization: University of Miami

Abstract: This application combines novel and state of the art methods for the detection of the earliest stages of Alzheimer's disease (AD). Increasing evidence indicates that it is not a single biomarker but a combination of several biomarkers that will best characterize risk to develop AD and Alzheimer's Disease and Related Disorders (ADRD). However, the rapid translation of these biomarkers into the clinical setting will require a combination of accessible technologies along with cost-effective clinical and cognitive outcomes that can reliably detect AD/ADRD during its earliest stages, and also have prognostic value. The research team has been at the forefront of developing innovative culture-fair Cognitive Challenge Tests (CCTs) which have been successfully validated by the investigative team and other national and international investigators. These CCTs have been related to multiple biomarkers of AD and neurodegeneration including amyloid burden on PET in older adults with preclinical and prodromal AD, and related to glucose metabolism deficits on FDG PET, as well as functional dysconnectivity on functional MRI (fMRI). CCTs have also been able to predict progression from

PreMCI to MCI and aMCI to dementia. New pilot data suggest that CCTs are associated with a sensitive biomarker of early neurodegeneration on MRI which is extracellular free-water (FW) diffusion in the hippocampus and tracts linking the entorhinal cortex and Nucleus Basalis of Meynert. FW has shown to be more sensitive to AD neurodegeneration than volumetric MRI in AD prone regions. The knowledge gained thus far has led to increased precision regarding which precise cognitive deficits map onto AD biomarkers, thus, the researchers have expanded upon and refined these CCT measures to better characterize: a) deficits specific to AD in recovering from proactive semantic interference, b) deficits in semantic inhibitory control; c) new innovative measures of delayed semantic source memory; and d) new and improved semantic paired associate measures that assess the unbinding of previously learned associations. This proposal examines the extent to which novel refinements of the CCTs, as compared to standard measures used in the field are related to emerging biomarkers of AD including extracellular free-water, especially in persons with early-stage MCI (eMCI) who are at greater risk for AD. The focus is on the earliest stages of MCI, as well as the use of powerful plasma-based biomarkers of AD using SiMoA such as P-tau217, P-tau181, and AB42/40 are 1000-fold more sensitive than standard ELISA assays in measuring AD pathology. Other plasma-based markers of neurodegeneration including Glial Fibrillary Acidic Protein (GFAP) and Neurofilament Light Chain (NfL) will be examined. This proposal is further strengthened by considerations of relevant sociodemographic factors, vascular burden on MRI, cerebrovascular risk factors, inflammatory markers (IL-6, IL-10), genetics, and metabolic risk markers such as Hemoglobin A1C (HbA1c).

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A11 Postdoctoral Fellowship in Neuropsychology and Cognitive Neuroscience

Principal Investigator: Phillip Harvey, PhD

Organization: University of Florida

Abstract: The early detection of Alzheimer's disease (AD) is of critical importance as the population ages, and emerging clinician scientists are required to have knowledge about early cognitive changes and the associated biological markers that render risk of progression. Clinical researchers across medicine need to acquire a set of core competencies and skills to effectively contribute to the ever-evolving field of AD research. Moreover, leadership, team science, study management, data management and grant writing are only a few of the many pillars that must be developed and nurtured during the training years to prepare future investigators for an independent career. The group of investigators at the Center for Cognitive Neuroscience and Aging (CNSA) and the 1Florida Alzheimer's Disease Research Center (ADRC) have extensive expertise and a consistent track record in training the next generation of neuropsychologists, cognitive neuroscientists and geriatric specialists. The graduates develop advanced skills in clinical, cognitive and functional assessment, research methods, grant writing, test development, and cognitive remediation in a diverse cohort of older Floridians who are at-risk

for developing of neurodegenerative disorders. Central to the research is the use of a state-of-the-art, multimodal suite of biological markers of AD pathology that serve as an excellent platform to train emerging investigators about genetics, plasma-based biomarkers, cerebrospinal fluid markers, and various neuroimaging modalities. This fellowship will emphasize upon the integration of biological markers in cognitive aging research. The proposed one-year postdoctoral research fellowship will offer specialty training in AD and AD related disorders to an individual with predoctoral training in neuropsychology and cognitive neuroscience. The diverse population that is served at the CNSA consists of older adults from historically underrepresented backgrounds. This provides the trainee with unique experience to work on important cross-cultural issues that are considered a national priority to ensure that early detection efforts and cognitive testing procedures are generalizable to the nation's growing diverse population. The current NIH-funded research portfolio at the CNSA including the 1Florida ADRC Center of Excellence awarded by the National Institutes of Health/National Institute on Aging will serve as the training environment. The mentorship team is highly experienced and integrated and has a longstanding history of training postdoctoral fellows. Primary Mentor, Dr. Philip Harvey has decades of experience in educating and preparing postdoctoral trainees and early-stage investigators and has been an active Ed and Ethel Moore Fellowship Mentor. Dr. Harvey would serve as the Primary Mentor of this award given his expertise related to cognition and aging. Inclusion of underrepresented minority populations is a central focus of the UM CNSA and this aspect of training is essential for patient -centered clinical research. Candidates will be expected to generate an independent project that can be piloted in the Center and submit a training award or other grant application upon completion of the postdoctoral fellowship year.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A12 Novel Behavioral and Neural Markers of Alzheimer'S Disease Progression: A Case for Visual Orienting

Principal Investigator: Oliver Bracko, PhD

Organization: University of Miami

Abstract: There is growing evidence that vascular dysfunctions are risk factors for Alzheimer's disease initiation and progression. Overall, vascular pathologies such as large and small infarcts, microbleeds, cerebrovascular disease, and white matter disease are prevalent in older persons and increase the risk for Alzheimer's disease and cognitive impairment. Until today there is no cure for Alzheimer's disease, and new research directions are needed to develop novel treatment strategies. Brain blood flow reductions of 10-30% are a known symptom of Alzheimer's disease, seen in patients and mouse models, and likely impact cognitive impairment but remain poorly understood. The researchers have recently shown that white blood cells can get stuck in the smallest vessels, the capillaries, and block brain blood flow and contribute to Alzheimer's pathology; however, it is not understood what drives these white blood

cells to plug capillaries. Researchers will investigate if platelets, which typically regulate hemostasis and thrombosis, are hyperreactive in Alzheimer's. Normally platelets respond very quickly to injury sites, interact with circulating neutrophils, and initiate wound healing. However in Alzheimer's disease, these platelets are hyperreactive and continuously drive vascular inflammation. Surprisingly, hyperreactive platelets correlate with cognitive impairment, indicating a pivotal role in disease.Using in vivo two-photon microscopy, researchers will investigate platelet dynamics in the microvasculature of Alzheimer's disease mice after a laser-induced hemorrhage (bleeding) and in blocked capillaries. Researchers will simultaneously label platelets, neutrophils, and blood vessels to visualize injured or blocked capillaries in the cortex of control and Alzheimer's disease mice. Measurements for vascular pathologies, such as blood-brain barrier leakage, neutrophil behaviors, inflammatory receptor expressions, capillary blockages, and brain blood flow reductions, are imaged before, during, and after the injury or a blocked capillary. Furthermore, researchers will analyze the whole blood for changes in cell numbers, cytokines, chemokines, and amyloid-beta. These factors are known to impact the injured microvasculature and inflammatory processes in the brain and periphery. After imaging, the brain will be harvested, and the vascular network map is used to identify the injured and stalled capillaries. Lastly, hypoxia (low oxygen), inflammation, and other markers of vascular damage will be studied to confirm the obtained results. This project will give crucial insights into neutrophil-platelet dynamics and the interactions before and after a capillary injury and a capillary blocking event. This proposal will establish the first insight into platelet dynamics in Alzheimer's disease and the interaction of platelets with neutrophils and blood vessel cells (endothelial cells). In the future, using the tools demonstrated in this project, the researchers will manipulate the interaction between platelets and neutrophils to reduce vascular inflammation and increase brain blood flow to improve functional outcomes in Alzheimer's disease mice. Finally, the research team aim to transfer platelets from patients with Alzheimer's disease in control mice, proving their critical contribution to microvascular damage. This data will shed light on an understudied blood cell type and its role in Alzheimer's disease. Understanding the function of hyperreactive platelets could open up new therapeutic routes that have not yet been explored.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A13 TRANSlational Fellowship Opportunity for Research on Multimorbidity in Alzheimer's Disease: TRANSFORM-AD

Principal Investigator: Tatjana Rundek, PhD

Organization: University of Miami

Abstract: The program invests in the development of the TRANSlational Fellowship Opportunity for Research on Multimorbidity in Alzheimer's Disease: TRANSFORM-AD, a one-year program for one fellow (MD or PhD) at the University of Miami and leverages the experience in research training programs for over two decades. The overall goal of the

TRANSFORM-AD program is to increase the number of diverse AD/ADRD investigators-leaders in cross-disciplinary clinical translational research, who can effectively and rapidly translate, implement, and disseminate discoveries to practice and community, and to address the special health challenges and health disparities of the diverse AD/ADRD patients researchers serve through team science and collaborations with community partners and diverse health care stakeholders. Researchers will prepare TRANSFORM-AD Trainee for research independence in AD/ADRD research by providing the requisite knowledge and skills in biology of brain aging and AD/ADRD, multimorbidity, health disparity, behavioral research, data science, drug discovery, intervention, and implementation science for rigorous and cutting-edge AD/ADRD research. There is increasing evidence that multimorbidity may dramatically alter the risk and course of AD/ADRD. The TRANSFORM-AD training program will provide specific patient-oriented research training that stimulates novel research into understanding of multimorbidities in AD/ADRD. The TRANSFORM-AD training program will provide (1) specific AD/ADRD content education curriculum that incorporates multimobidity, deleterious factors as well as protective factors such as physical exercise and healthy sleep hygiene, neuroimaging and biomarkers; (2) general research skills and tools, and (3) immersion into research focused on cerebrovascular factors, and multimorbidities (stroke, MI, AFib, heart failure) that accelerate cognitive decline, including immersion in the two FL DOH studies (The Florida IMAGINE Study of AD Risk; and The Florida VIP study of AD Risk). In addition, Trainee will receive novel training in dissemination and implementation science to learn skills and tools on how to disseminate research findings into valuable information to benefit patients, caregivers, and the community. The program is specifically designed to address health disparities in AD/ADRD for the rapidly growing aging and diverse population of south Florida.TRANSFORM-AD Fellow will be integrated into the highly successful research training programs including, NIA 1FL ADRC (OneFlorida Alzheimer's Disease Research Center), the CTSI (Clinical Translational Research Institute) KL2 program, MSCTI (Master of Science in Clinical Translational Investigation), and NIH T32s. PI of this proposal serves as Director of these training programs. In addition, Fellow will have clinical rotations with the cognitive neurologists at the Cognitive Division in Neurology and at the Alzheimer's Disease Initiative Memory clinic funded by the state of Florida.TRANSFORM-AD Fellow will be selected from a large pool of eligible applicants in the neurology, psychiatry, psychology, neuropathology, and neuroscience programs with a strong interest in brain and cognitive aging, multimorbidities, quality of life for aging adults and caregivers, and novel therapeutic approaches in AD/ADRD. By the end of this fellowship, Trainee will apply for an NIH K or K-like grant, which will be one of the most important deliverables of the TRANSFORM-AD program.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A14 Nanoarchitectonics of Carbon Dots as a Combinational Drugs Multi Target Systems For Treating Progression of Alzheimer's Disease

Principal Investigator: Roger Leblanc, MD, PhD

Organization: University of Miami

Abstract: Majority of the existing therapies are used as symptomatic treatments in Alzheimer's Disease (AD) and deal with one principal neuropathological hallmark at a time. Therefore, single modality of "One-molecule-one-target" strategies creates severe limitations for treating AD. On the contrary, restoring neurotransmitter levels by combined combinatorial inhibition of cholinesterases, and regulation of glutamate production, in conjunction with strategies to counter tau protein and beta-amyloid plaque accumulation, would constitute a therapeutically robust, multitarget approach. However, combination of the multiple drugs without any carrier is strictly limited by the chemical structure of the drugs and conjugation techniques. In addition, each of these drugs have an active site related to their efficiency towards AD. Therefore, combination of these drugs just by themselves terribly reduces their treatment effects. Traditional nanomaterials such as liposomes, dendrimers and metal-based particle have been applied for the treatment of AD as the nanocarriers. However, it has been shown that there are main challenges for the use of these traditional nanomaterials due to their poor water solubility, high toxicity to the healthy tissue, deprived particle stability, and most importantly the lack of ability to cross the blood-brain barrier (BBB). As a novel nanomaterial discovered in 2000, Carbon Dots (CDs) have exhibited various properties. Most significantly, it was constantly observed that CDs could inhibit the formation of APP, Aβ, Aβ fibrils, and tau protein aggregates by diverse surface interactions. Additionally, CDs won't induce the generation of ROS in the dark, so the use of CDs won't cause oxidative stress or inflammation to the brain. Furthermore, CDs are promising drug nanocarriers considering their biocompatibility, abundant surface functional groups, high surface area-to-volume ratio, small size, excellent photoluminescence, and most importantly the ability to cross BBB. In this study, researchers plan to benefit from abundant surface functional groups of CDs as "Combination-drugs-multi-targets" agents to combat AD. Briefly, CDs will be conjugated three different ligands to address the inhibition of cholinesterases, regulation of glutamate productions, up-regulation of nerve growth, tau protein and beta-amyloid plaque accumulations. These three drugs are tramiprosate, huperzine A and memantine. Among these drugs, tramiprosate binds to amyloid to prevent its conversion to beta-sheets and subsequent aggregation. In addition to the symptomatic, cognitive-enhancing effect via inhibition of AChE, several recent studies have reported that huperzine A has "non-cholinergic" effects on AD. Finally, memantine is meant to prevent excess glutamate from killing the nerve cells, without disturbing the normal transmission of nerve signals. Currently, there has been neither an ongoing nor finished investigation which incorporates four principal hallmarks of AD in addition with a premise to upregulate the neuronal cell growth. Therefore, this study holds great premises as a powerful nanomedicine and drug nanocarrier with the capability to deliver three separate drugs across the BBB to inhibit the cholinesterases, glutamate productions, tau protein and beta-amyloid plaque accumulations while upregulating the nerve growth with higher efficacy than one molecule one target drug delivery system.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A15 Building an Expanded Registry for African Americans At-Risk for Alzheimer's disease and Related Dementias

Principal Investigator: Elizabeth Crocco, PhD

Organization: University of Miami

Abstract: A critical gap in Alzheimer's disease (AD) and Alzheimer's disease related disorders (ADRD) clinical research is the vast under-under-representation of Black/African American (AA) older adults. Despite the pandemic, researchers have been able to develop an African American Registry in which over 100 participants have been enrolled and the research team intends to meet the original goal of 120 participants during the awarded no-cost extension period. Researchers have conducted extensive baseline cognitive, neurological, and medical evaluations, have obtained comprehensive blood panels including fasting glucose, Hemoglobin A1C, and complete lipid panel assessing comprehensive cardiovascular and metabolic risk factors as well as lifestyle measures including social and structural determinants of health. Importantly, over 70 percent of participants in the Registry have enrolled in NIH studies such as the 1Florida ADRC and several NIH funded longitudinal studies of aging. The research team's vast outreach and community partnerships with stakeholders in the African American Community make this proposal even more feasible, and researchers have an unprecedented opportunity to do more. In this proposal to expand the Registry, the research team will recruit an additional 150 AA Registry participants to obtain plasma biomarkers that were not collected initially such as plasma-based biomarkers including p-tau217, plasma-based neurodegenerative biomarkers (Glial Fibrillary Acidic Protein [GFAP] and Neurofilament Light Chain [NfL]) and inflammatory markers. The data obtained for this registry will better characterize and expand upon the current database, that is flourishing. Promising plasma-based markers represent invaluable information that the institution (University of Miami) and other Florida-based AD/ADRD investigators can access to further advance knowledge and answer critical scientific questions in understudied African American older adults. Ultimately, the important data collected will be a resource for investigators seeking to close the gap of health disparities among African American older adults.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A16 Deep Phenotyping of African American Older Adults at Risk for Alzheimer's disease

Principal Investigator: Rosie Curiel Cid, PhD, MSW

Organization: University of Miami

Abstract: A critical gap in Alzheimer's disease (AD) and Alzheimer's disease related disorders (ADRD) clinical research is the vast under-under-representation of Black/African American (AA) older adults. AD/ADRD is more prevalent in AA individuals relative to white individuals of

European ancestry. Early detection is critical for clinical trials aiming to develop optimal therapeutics. Therefore, there is a need to include and deeply phenotype AAs using novel cognitive and biomarker assessments that consider the multiple co-morbidities identified in this population. Study location is one of the most important enrollment barriers for AA older adults. This research proposal leverages the expertise in home-based assessment to evaluate clinical and neuropsychological status. The research team will provide door-to-door transportation for MRI studies to facilitate and support the engagement of AA older adults. Other novel innovative aspects include: a) the use of the well-validated cognitive semantic interference test that is highly related to biomarkers of AD and which have shown to be effective for use in AA older adults with and without Mild Cognitive Impairment (MCI); b) use of promising blood-based biomarkers (BBM) of AD and neurodegeneration that leverage extremely sensitive single molecule array (SiMoA) technology to detect specific proteins in the plasma; c) comparison of BBM with neurodegenerative changes on MRI using sensitive imaging techniques including extracellular free water; d) examining comorbidities, researchers will obtain sensitive measures of cerebrovascular, metabolic and genetic risk by measuring physical, clinical, blood-based, and neuroimaging variables; e) social determinants of health will also be assessed. This unique deep phenotyping of 100 AA older adults will allow the research team to gain the critical preliminary data needed to apply for extramural funding to further study and longitudinally follow this invaluable cohort of persons who have a disproportionate risk for AD/ADRD.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 23A17 Selective RNA targeting of mTORC 1 and 2 to ameliorate Alzheimer's disease pathogenesis

Principal Investigator: Claes Wahlestedt, PhD

Organization: University of Miami

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disorder where aging is the biggest risk factor. It has been well described that inhibition of the kinase, mechanistic target of rapamycin (mTOR), improves age-related pathologies including AD. However, mTOR exists as a complex and exerts differential functions depending on its binding partners. Regulatory-associated protein of mTOR (raptor) is a component of mTOR complex 1 (mTORC1) while rapamycin-insensitive companion of mTOR (rictor) is a component of mTORC2. Suppression of mTORC1 has yielded positive outcomes for AD-related measures (decreased amyloid beta and tau deposition, improved brain insulin sensitivity, and improved cognitive function) and upregulating mTORC2 via rictor also resulted in positive outcomes in AD models, suggesting an increased ratio of mTORC2 to mTORC1 is desirable for AD. Commonly used small molecule inhibitors of mTOR do not discriminate between mTORC1 and mTORC2, as mTOR is a component of both. Furthermore, small molecules targeting unique binding partners of either complex lack specificity and tend to have pleiotropic effects. The research team proposes to inhibit mTORC1 and upregulate mTORC2 using RNA therapeutics targeting the sense transcript

of raptor and the natural antisense transcript (NAT) of rictor for degradation. Researchers hypothesize that increasing the mTORC2/1 ratio with highly selective RNA therapeutics which bind their targets based on sequence complementarity will decrease both amyloid beta accumulation and tau phosphorylation (hallmarks of AD), as well as improve other AD-related cellular functions in a cell type specific manner, with minimal off-target effects. Researchers plan on targeting Raptor and the NAT for Rictor in vitro and in vivo. The preliminary data indicate that targeting the NAT of a target with RNA therapeutics results in increased upregulation of the target. Researchers have successfully identified the sequences needed to efficiently target both Rictor and Raptor. The overall therapeutic approach is built on technologies developed in the Wahlestedt lab over the past two decades. Moreover, some of the specifics of the present application are covered in a pending patent application from the University of Miami. The team has successfully used RNA therapeutic techniques in the past and participated in projects that are now at the clinical stage. Notably, the research team has published unique work relating to RNA mediated gene upregulation (e.g., Science 2005: Nature Rev Drug Discovery, in press 2022). Additionally, the research team has successfully published on pre-clinical drug discovery in AD mouse models and human cells. Innovation and Significance: Increasing the expression of a target gene has traditionally been very difficult. It is biochemically easier indeed to block the activity of a target than to increase it. The work has shown that by targeting NATs, the research team can successfully increase the expression of a target gene. To date, nobody has successfully targeted the NAT of Rictor to mitigate AD pathology, while also targeting the mTOR pathway (one of the most validated aging pathways). Data from the proposed work has the potential to yield a novel therapeutic strategy for AD and to promote healthy aging.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant #: 23A18 Group Music Intervention to Reduce Agitation in Memory Care: A Cluster Randomized Trial

Principal Investigator: Hongdao Meng, MD, PhD

Organization: University of South Florida

Abstract: Alzheimer's Disease and related dementias (ADRD) affect more than 42% (400,000) of residents in assisted living (AL) communities. Up to 90% of persons with ADRD experience neuropsychiatric symptoms (NPS, e.g., agitation, anxiety, and apathy) during the disease progression. NPS are associated with functional decline, poor quality of life, and contributes to resistance to care in AL. Current evidence suggests that participating in music activities improves mood and reduces NPS among persons living with dementia (PLWD). However, there is a lack of evidence-based music interventions that can be disseminated widely in memory care units in ALs. The lack of such interventions remains a key barrier to improving the quality of life for the growing PLWD population. With the support from the Ed and Ethel Moore Alzheimer's Disease Research Program's Pilot Program (#9AZ28, PI: Meng), the USF team demonstrated preliminary feasibility and acceptability of the University of South Florida Group Music

Intervention ("USF-GMI") in two memory care communities. The USF-GMI program consisted of 12 group sessions of music video for reminiscence delivered over four weeks (50-minute per session, three sessions per week). The proposed research project will build on the early success of the pilot study by refining the USF-GMI program, developing staff-training program, and pilot test the resulting program in a cluster-randomized trial (CRT). The specific aims of the proposed pilot CRT are to: 1) Conduct qualitative interviews with key stakeholders (AL administrators and activity directors, and dementia care training experts) in three ALs to obtain their assessment of the USF-GMI program and confirm their priorities, needs, constraints, and other determinants of implementation to adapt the program for the current trial; 2) Refine the protocol and produce a customized training program based on the standard operating procedures and training manuals developed in the pilot study for the training of memory care staff; 3) Pilot test the resulting refined intervention program as implemented by AL staff in reducing agitation among residents with ADRD. The research team will enroll six AL communities with memory care units in the Tampa Bay area and conduct a cluster-randomized trial. ALs will be randomized into USF-GMI intervention or usual care groups. The primary outcome variable will be neuropsychiatric symptoms (NPS), as measured by the Cohen-Mansfield Agitation Inventory (CMAI). The secondary outcome will be care staff strain and psychotropic medication use. Qualitative methods will also be used to gain an in-depth understanding of the personal (e.g., motivation, perceived benefits), structural (e.g., space, equipment, and staffing), and program (protocol, training, and technical support) factors that will aid in the interpretation of the trial findings and the scaling of the intervention in a future efficacy trial. Successful completion of the project will support a five-year R01 application to the National Institute on Aging (NIA) to conduct a Stage III cluster-randomized efficacy trial. The proposed research is expected to provide consumers, ALs, researchers, and policymakers with important new information on a staff-delivered, low-cost group music intervention to reduce NPS and improve the wellbeing of residents with dementia in memory care.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix B
Fiscal Year 2022-2023 Newly Awarded Grants (Announced December 1, 2022)
Funded Fiscal Year 2021-2022

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
22A01	Florida Atlantic University	Randy Blakely, PhD	349,819.00	03/31/24	Yes	No	No
22A02	Florida Atlantic University	Lisa Wiese, PhD, MSN, RN, GERO-BC, PHNA-BC, CNE	250,000.00	03/31/26	No	No	No
22A03	Florida State University	Robert Tomko Jr., PhD	100,000.00	03/31/24	No	No	No
22A04	Mayo Clinic Jacksonville	Yang You, PhD	100,000.00	04/01/24	No	No	No
22A05	Mayo Clinic Jacksonville	Shunsuke Koga, MD	100,000.00	04/01/24	No	No	No
22A06	Mayo Clinic Jacksonville	Mariet Allen, PhD	100,000.00	03/31/23	No	No	No
22A07	Mayo Clinic Jacksonville	Fabienne Fiesel, PhD	350,000.00	03/31/26	No	No	No
22A08	Mayo Clinic Jacksonville	Yasuteru Inoue, MD, PhD	100,000.00	03/31/24	No	No	No
22A09	Mayo Clinic Jacksonville	Nilufer Ertekin-Taner MD, PhD	350,000.00	03/31/24	No	No	No
22A10	University of Central Florida	Nichole Lighthall, PhD	742,833.00	03/31/26	No	Yes	Yes
22A11	University of Florida	Jeremy Grant, PhD	99,569.00	06/30/24	No	No	No
22A12	University of Florida	Adam Barnas, PhD	100,000.00	03/31/24	No	No	No
22A13	University of Florida	Jada Lewis, PhD	350,000.00	03/31/25	Yes	No	No
22A14	University of Miami	Claes Wahlestedt, MD, PhD	349,981.00	03/31/24	No	No	No
22A15	University of Miami	Karen Nuytemans, PhD	350,000.00	03/31/24	No	No	No
22A16	University of Miami	Philip Harvey, PhD	99,887.00	03/31/23	No	No	No
22A17	University of South Florida	Hariom Yadav, PhD	743,661.00	03/31/26	No	Yes	No

Grant#: 22A01 In Vivo Functional Analysis of MBLAC1: A Novel Genetic Risk Factor In Alzheimer's Disease With Therapeutic Potential

Principal Investigator: Randy Blakely, PhD

Organization: Florida Atlantic University

Abstract: Significant progress has been achieved in understanding the pathophysiological basis of Alzheimer's disease (AD) through the identification and study of rare, functional mutations in familial forms of the disease. Recently, the research team discovered an unstudied gene in *C. elegans*, termed swip-10 whose function in non-neuronal (glial) cells supports the health and signaling of nearby neurons *in vivo*. Current work with the model supports a "two-hit model" for the contribution of swip-10 to neuronal health, whereby mutation of the gene disrupts copper homeostasis, leading to altered mitochondrial metabolism and oxidative stress, leading to an over-excitation that can drive neurons toward degeneration. Following this discovery, the team identified the gene Metallo-β-lactamase Domain-Containing Protein 1 (MBLAC1) as the likely swip-10 homolog in man and have published evidence that a loss of function mutation introduced into the mouse form of the gene produces metabolic stress in *vivo*. Recently, Genome-Wide Association Studies (GWAS) identified MBLAC1 as a risk factor for AD with comorbid cardiovascular disease. Expression of MBLAC1 was found to be reduced in postmortem AD brain, compared to healthy, age-matched controls. The team hypothesized that the contribution of MBLAC1 to forms of AD with comorbid heart disease may reflect the intense

energy demands characteristic of brain and heart. Importantly, a recent genetic study of the impact of a hereditary Presenilin1 (PSEN1) mutation linked to AD has revealed conspicuous changes in MBLAC1 gene expression in the brain, suggesting that MBLAC1 may function in a critical pathway that contributes to the pathophysiology of known AD mutations, further justifying an intense analysis of the function of MBLAC1 *in vivo* and evaluation of its possible targeting for novel AD therapeutics. Importantly, the team recently demonstrated that MBLAC1 protein is the major, if not sole target in the mouse brain for the FDA approved drug, ceftriaxone, a molecule reported by others to have neuroprotective activity, including in AD models. The proposal uses an iterative approach, utilizing both *C. elegens* and mouse models, to more deeply characterize the biological mechanisms underlying the neuroprotective potential of MBLAC1 and its pharmacological targeting, including studies of the impact of MBLAC1 on the AD features seen in mutant mice expressing well known AD mutations (e.g. PSEN1, Amyloid Precursor Protein (APP)), studies that may lead to desperately needed therapeutic options for AD and its comorbidities. From preliminary studies, the lab predicted that the experiments supported by this project would reveal deficits in copper homeostasis, which leads to age-dependent brain disease.

The team's progress has demonstrated that:

1) Using the copper selective dye CF4, that nematode swip-10 deleted worms show a significant reduction in Cu+, a form of copper that is required for energy production and reduced oxidative stress.

2) Using primary cultures of glial cells (astrocytes) from wildtype and MBLAC1 deleted mice, that these important cells for maintaining brain neurons, is diminished just like worms lacking swip-10.

Follow on Funding: None at the time of reporting.

Collaborations: The team is collaborating with Dr. Christopher Chang at the California Institute of Technology who is supplying the project with a fluorescent probe specific for reduced copper (Cu+). Dr. Chang is also providing mass spectrometry analyses of copper levels in whole worms and mouse tissues and fluids. No funds are derived from the project and no students are involved in Dr. Chang's efforts. One of the team's graduate students is supported by the project and is directly involved in the collaboration. The lab is also in collaboration with Dr. Gary Miller at Columbia University who is analyzing the *C. elegans* metabolome in WT and swip-10 mutant worms. No funds are derived from the project and no students are involved in Dr. Chang's efforts. One of the team's graduate students is supported by the project and is directly involved in the collaboration.

Journals: None at the time of reporting.

Patents: Inventions Declared: Targeting MBLAC1 for the Therapeutic Modulation of Copper Homeostasis
Patents Filed: Compositions and Methods Targeting SWIP-10 and MBLAC1 for the Therapeutic Modulation of Copper Homeostasis
Application Number: 63/37

Grant#: 22A02 Optimizing Rural Community Health Through Interdisciplinary Dementia Detection and Care (ORCHID)

Principal Investigator: Lisa Wiese, PhD, MSN, RN, GERO-BC, PHNA-BC, CNE

Organization: Florida Atlantic University

Abstract: One in three older adults die with Alzheimer's disease; it kills more people than prostate and breast cancers combined (Alzheimer's Association, 2021). In Florida, with the second-largest percentage of persons age 65 and older nationally, earlier detection of Alzheimer's disease and related dementias (ADRD) is imperative. Early dementia diagnoses provide opportunities for modifying behaviors e.g. smoking, managing chronic conditions that contribute to ADRD risk, and initiating medications, which are more effective earlier in the disease process. Earlier detection also provides critical time to connect patients with community resources, and diminish caregiver burden. Although the primary care setting is well-suited for dementia screening due to long-standing relationships between providers and patients, low screening rates by providers reflect a combination of factors. These factors include unfamiliarity with updated cognitive assessment tools, lack of access to updated prevention and treatment protocols, and inaccurate perceptions that nothing can be done to help families facing a dementia diagnosis. This dementia detection and management gap is more severe in Medically Underserved Areas, such as rural Florida's racially/ethnically diverse "Glades" region. The Ed and Ethel Moore Alzheimer's Disease Research Program plays a vital role in enabling the research team to address these disparities in dementia care in rural south Florida. The consortium will investigate the hypothesis that creating a circle of community-based engagement among local academic, clinical, and community resources will increase rates of dementia diagnosis and care management in a rural, racially/ethnically diverse underserved community. A multidisciplinary team of nursing students and faculty (Palm Beach State College), adult geriatric nurse practitioners (AGNP), and dementia researchers (Florida Atlantic University and University of Miami) will coordinate study activities with local providers and stakeholders. The long-range goal is to decrease costly preventable hospital admissions and early institutionalization. Specifically, nursing students will be trained to provide ADRD education and conduct cognitive screenings at senior housing facilities and faith-based organizations. Each student will be partnered with a community health worker, including local faith-based health educators. Positive screenings will be followed with visits by AGNPs in the research participant's dwelling or at the area hospital for cognitive assessments, who will communicate results to the resident's local provider. The providers will be offered a dementia diagnosis and management educational intervention based on a previously successful model (University of Miami), which includes guidelines for disclosing dementia diagnoses. Provider office staff will be offered an educational program using the new FDOH ADRD Resource Guide. To address caregiver burden, community health workers will complete the circle by assisting provider offices to connect patients and families to the Lake Okeechobee Rural Health Network—a coalition of mental health and social workers, health insurance experts, day services, and advanced care planners. Results will be compared with control and comparison provider or office staff groups who do not receive education and support. Applying this community-based participatory research design will facilitate translation of the findings back to caregivers, providers, and community agencies.

Follow on Funding: None at the time of reporting.

Collaborations: This consortium grant is for collaborating with the postsecondary educational institutions of the University of Miami and Palm Beach State College.The majority of recent the collaboration concerned Palm Beach State College. Students were invited to join in the (voluntary) education and screenings conducted in the summer in the target population with great success. Over two dozen students completed the training and 23 participated in the community health education and screening events. Nine students requested to continue in the Fall with Aim two work. This involvement is recognized by Palm Beach State College as a means of fulfilling their community health clinical hours (Nine students are the maximum allowed in one clinical group). These students are completing their CITI training in the month of October 2022, under the direction and guidance of Professor Joanne Pulido, the grant consortium Primary Investigator (PI). The students are poised to begin the actual work of following residents and coordinating their provider visits and contacts with resources for the remainder of the semester.In addition, as noted above, the research team has collaborated with Lakeside Hospital to provide a major health fair event on November 5, 2022, with 10 different local agencies listed below, and transportation to the health fair by the local shuttle bus company (Go Glades).

1. Diabetes Coalition of Palm Beach and Martin County
2. Delta Sigma Theta (African American sorority)
3. AmeriHealth Caritas Florida
4. Lake Okeechobee Rural Health Network
5. Health Care District, Palm Beach County

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A03 Engineering Genetically Encoded Nanomachines to Block or Reverse Accumulation of toxic Amyloid Beta and Tau Oligomers

Principal Investigator: Robert Tomko Jr., PhD

Organization: Florida State University

Abstract: The goal of the work funded under this award is to engineer biological "nanomachines" that selectively cut up two toxic proteins that help drive Alzheimer's disease (AD): tau and amyloid beta (Aβ). A second goal is to test whether introduction of the most promising nanomachines into neurons can protect them from the toxicity of tau and Aβ. If successful, these nanomachines will serve as prototypes for a new form of biological therapy (or prophylaxis) for AD. This award was activated on April 1, 2022. In the six months since activation, the research team have established proof-of-principle for the concept originally proposed in this award; namely, that protein-specific nanomachines can, in fact, be developed. This was demonstrated using a model protein that is more amenable to experimentation than are tau or Aβ. Importantly, the research team has shown that destruction of this model protein is dependent on each component of the engineered nanomachine, and that "bystander" proteins are not errantly destroyed by these machines as collateral damage. After comparing five different nanomachines, each engineered from different starting chassis, the most effective one was selected for further development. The research team is now producing tau- and Aβ-selective nanomachines based upon the most effective design identified from their proof-of-

principle experiments. The research team will evaluate the ability of these machines to destroy the toxic forms of tau and Aβ using a test-tube model, and will begin to move the experiments into cultured neuronal cells as originally described.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A04 Investigation of Therapeutic Effects of Neutral Sphingomyelinase Inhibitors on Tau Propagation In Different APOE Genotypic AD Patient-Derived iPSC organoids

Principal Investigator: Yang You, PhD

Organization: Mayo Clinic Jacksonville

Abstract: One of the hallmarks in Alzheimer's disease (AD) is the abnormal accumulation of tau proteins, known as tau tangles, in the brain. These tangled proteins tend to form harmful clumps in one region and spread to the others as seeds. Numerous studies indicate that tau accumulation would cause cell death and impair brain functions. In addition, the strongest AD risk factor Apolipoprotein E (APOE)4, which is expressed in more than half of AD patients, was reported to markedly exacerbate tau-mediated neurodegeneration in a mouse model of tauopathy. Extracellular vesicles (EVs) are cell-derived small membranous vesicles, which have gathered great interest in studying AD with the capability of transferring pathogenic molecules including Tau. Therefore, blocking of EV biogenesis is potentially considered as a therapeutic target for AD. Notably, inhibition of EV generation with neural sphingomyelinase inhibitors, such as GW4869, were found to significantly halt tau propagation in PS19 tau mutant mouse model. Current studies also highlight that APOE is involved in EV synthesis through its primary role as a lipid carrier. However, the therapeutic effects of neural sphingomyelinase inhibitors on targeting EV generation in AD, especially the potential difference between APOE3 and APOE4 genotypes, remains elusive. In this project, cortical organoids will be generated using AD-patient derived isogenic APOE3 and APOE4 induced pluripotent stem cell (iPSC) lines. Abnormal tau proteins will be expressed in an individual organoid and then fuse that organoid with a normal one. Once the fusion is completed due to the migration of cells within organoids, the research team will examine whether abnormal tau is spread from a tau-expressed organoid to a normal organoid. The Principle Investigator (PI) will also test if the AD risk gene APOE4 contributes to the spread of tau proteins using this organoids model. Next, the neutral sphingomyelinase inhibitors will be employed into the tau-expressed organoids to explore the differential effects on halting tau propagation between APOE3 and APOE4 genotypes. This research will develop a novel human brain-like model in the laboratory to study tau spread and identify whether inhibition of EV biogenesis may serve as a therapeutic target for AD. Further, it will help to provide fundamental insights into the precise medicine on AD with different APOE genotypes.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A05 Developing A Digital Pathology and Machine Learning-Based Diagnostic tool for Alzheimer's Disease and Related Dementia

Principal Investigator: Shunsuke Koga, MD

Organization: Mayo Clinic Jacksonville

Abstract: In this proposed study, the research project staff will investigate the clinicopathological correlations of Alzheimer's disease (AD) using brain samples from Florida's AD Initiative. The research aims to develop machine learning-based object detection tools for each pathologic hallmark (i.e., tau, amyloid-β, α-synuclein, and TDP-43) and assess the clinicopathologic correlations. For context, disease-modifying therapy for AD has been extensively investigated over the last decades, but most clinical trials failed to show efficacy. Accumulating evidence suggests that AD is a heterogeneous disease regarding clinical, pathological, and genetic aspects. Each patient may have different risk factors for AD; therefore, the uniqueness of each patient may need to be considered for the treatment and prevention of the disease. This approach, known as precision medicine, has been used in the field of oncology, leveraged by the advent of machine learning. More recently, the application of precision medicine for AD has gained traction. The significance of this proposed study is to provide deep phenotyping of AD using machine learning (ML)-based quantitative neuropathologic measures. To date, the research team has developed an algorithm that can automatically mask the tissue and split it into small tiles. Using this method, whole slide images are automatically converted into a number of small tiles that machine learning algorithms can efficiently process. Next, the research team utilized the clustering-constrained-attention multiple-instance learning (CLAM) algorithm to make a differential diagnostic tool for AD and other tauopathies, such as progressive supranuclear palsy, corticobasal degeneration, and Pick's disease. Three sections of tau-immunostained slides were used to compare the diagnostic accuracy between the sections. The three sections are: the motor cortex; the superior frontal and cingulate gyri; and the caudate nucleus and putamen. The most accurate model was developed using Section two, which showed an accuracy of 86%. Next, the research team tried to make a diagnostic model using hematoxylin and eosin staining slides. In the previous period, the same approach was used (CLAM), but the diagnostic accuracy of this model (approximately 50%) was significantly lower than the model based on tau-immunostained slides. During this period, the team utilized different machine learning algorithms, so-called self-supervised learning. As a result, the diagnostic accuracy improved, but it was still modest accuracy for diagnostic performance. The next step involves preparing the dataset of amyloid-β immunohistochemistry slides, and so far, 120 immunostained slides have been scanned. Using the CLAM algorithm, the research team trained the algorithm using this dataset. Impact to Floridians: The proposed research will develop two machine learning-based models that provide a diagnosis of AD using digital slide images and deep phenotyping of comorbid neuropathology in AD. Using these models, AD will be divided into more detailed subtypes, which may have different therapeutic targets. The results of this study will identify new therapeutic targets for AD, which can help improve the health of people in Florida.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A06 Brain Cell-Specific Epigenomic and Transcriptomic Impact of AD Risk Variants

Principal Investigator: Mariet Allen, PhD

Organization: Mayo Clinic Jacksonville

Abstract: Microglia play important roles in the progression and propagation of Alzheimer's disease (AD). This notion has been supported by *in vitro* and *in vivo* studies. Also, several genome-wide association (GWAS) and sequencing studies discovered many AD risk variants in/near genes that are microglia-specific or enriched, that include ABI3 risk variant (rs616338-T) and PLCG2 protective variant (rs72824905-G). This proposal plans to dissect the precise roles of PLCG2 and ABI3 variants in microglia cells by leveraging the deeply phenotyped brain samples and single nuclei approaches. The research team will obtain microglial single nucleus transcriptomics and DNA accessibility profiles from frozen temporal cortex of AD patients (n=18) and a disease-control, progressive supranuclear palsy (PSP) (n=12) patients, which harbor PLCG2 protective allele or ABI3 risk allele or neither. Using fluorescence-activated nuclei sorting (FANs), the research team will enrich for microglial nuclei in which the team will perform single-nucleus gene expression (snRNAseq) and DNA accessibility measures (snATACseq) via 10X Genomics Chromium platform. The research team will integrate these two datasets via computational methods and reveal AD specific alterations in microglia subtype and activation states at further depth. The research team will also evaluate the activation states of microglia using the expression profile of hub genes and characterize the microglial clusters in AD and PSP patients who either carry or lack these mutations. The research team expects to detect differentially expressed genes (DEGs) and networks in mutation-carriers vs. non-carriers and test the association of risk factors (sex and age) and pathology scores. Hub genes of differentially expressed networks and DEGs will be validated by other experimental techniques. The research team expects that this proposal will yield critical information on the consequences of these variants in microglial subtypes, their gene expression and phenotypes. This knowledge may reveal microglia subtypes and activation states regulated by PLCG2 and ABI3 and provide novel insights into therapeutic potential. The primary beneficiaries of any treatment or cure for AD would be the elder population of the state of Florida due to age being a major risk factor for AD. To date, the microglial enrichment protocol has been successfully tested in the target brain region of temportal cortex, and selected samples have been selected and assessed for tissue quality using RNA integrity metrics. The next steps will be to collect and analyze single nucleus transcriptome and epigenome measures to accomplish the goals of the study.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A07 The Role of Ufmylation for Alzheimer's Disease

Principal Investigator: Fabienne Fiesel, PhD

Organization: Mayo Clinic Jacksonville

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting more than five million individuals in the US with close to 500,000 in Florida alone. Clinically, AD is characterized by severe cognitive, behavioral, and motor impairments resulting from progressive synaptic dysfunctions and neuronal loss. Neuropathologically, AD is defined by the formation of insoluble protein aggregates including extracellular amyloid beta (Aβ) plaques and intracellular tau tangles. However, the molecular mechanism of that cause neurotoxicity and the accompanying pathological aggregation are not understood. The goal of the research team is to understand the role of ufmylation for AD. Ubiquitin Fold Modifier 1 (UFM1) is a ubiquitin-like small molecule modifier that similar to ubiquitin is attached to lysine residues of substrate proteins as a post-translational modification (ufmylation). Mutations that are associated with strong reduction of function in the genes that mediate the activation, conjugation, and ligation of UFM1 are all linked to severe neurodevelopmental disorders. Knockout of these genes in mouse models is embryonic lethal underscoring the importance of ufmylation for neuronal survival. While there is a strong premise for a neuroprotective role of ufmylation during development, the role of ufmylation for age-related neurodegenerative disorders is not yet established. The biological significance of ufmylation has only recently emerged and new roles are still being identified. UFM1 is involved several different key cellular pathways, among them the DNA damage response, the unfolded protein stress response, and the degradation of the endoplasmic reticulum via autophagy (ERphagy). The preliminary analysis of human postmortem brain suggests that several key components of the ufmylation cascade are reduced in AD brain compared to controls. Further, the data hints at a crosstalk of mitochondrial autophagy with the ufmylation pathway. Here, the aim is to investigate the role of ufmylation for AD by expanding this analysis in human post-mortem brain. The research team will also analyze the functionality of several ufmylation pathways in neuronal cell culture models to dissect which of these are impaired. As a result, the study will help to define the mechanisms of neurodegeneration and provide novel therapeutic targets and strategies for AD.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A08 Apoe Genotype Effects on Cerebrovascular Integrity and Dementia-Related Pathology

Principal Investigator: Yasuteru Inoue, MD, PhD

Organization: Mayo Clinic Jacksonville

Abstract: This research projects aims to define the Apolipoprotein E (APOE) genotype-related effects on cerebrovasculatures using human brain samples. Researchers successfully isolated and separated the vascular fractions into large vessels (V1: pial arteries) and microvessels (V2: capillaries) from frozen human brain samples using 2 different mesh size filters (100 μm and 40 μm). During these three months, the method was further optimized which enabled the purification of the vascular component with high accuracy. The Brain Bank of Mayo Clinic was contacted and the research team requested middle temporal cortex with [ApoE2/3, 2/4], [ApoE3/3], [APoE3/4, 4/4], each group is 20 cases (age, sex matched). The next step will be using these brain tissues to purify vascular component and measure the levels of amyloid beta (Aβ)40, Aβ42, ApoE, vascular extracellular matrix (ECM) forming basement membrane (collagen Ⅳ, nidogen, perlecan, and fibronectin) using enzyme-linked immunosorbent assay (ELISA).

The research team also aims to determine the APOE genotype effects on cerebrovascular functions using human induced pluripotent stem cells (iPSCs) derived cerebrovascular model. Research staff aim to establish the iPSCs derived cerebrovascular model system to analyze apoE genotype specific effect on cerebrovascular integrity. Rresearchers have also successfully differentiated isogenic human iPSCs with different APOE genotypes (APOE2, APOE3, APOE4, and APOE-KO) generated by Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR)/Cas9-mediated genome editing into brain microvascular endothelial cells (BMECs) and vascular mural cells (pericytes and smooth muscle cells) (VMCs). During these three months, the integrities of endothelial cells were validated using trans-endothelial electrical resistance (TEER). They maintained higher TEER values over 1000 Ohm, indicating strong BBB barrier formation. As a next step, researchers will use different ApoE genotypes and compare TEER values. Researchers will measure the tight junction marker using whole blood (WB) or quantitative polymerase chain reaction (qPCR).

The research staff will also aim to determine APOE genotype effects on cerebrovascular permeability using iPSCs derived cerebrovascular model. Permeability assays have been conducted as a pilot study, using sodium fluorescein (NaF) using ApoE3 cell line. In the coming months, the primary investigator will compare permeability efficacy between different ApoE genotypes.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A09 Identification of Novel Genetic Risk Factors for Cerebral Amyloid Angiopathy and Characterization of The Implicated LINC-PINT Locus

Principal Investigator: Nilufer Ertekin-Taner MD, PhD

Organization: Mayo Clinic Jacksonville

Abstract: Cerebral amyloid angiopathy (CAA), a common neuropathological finding in the brains of Alezheimer's disease (AD) patients, is characterized by an accumulation of amyloid beta (Aβ) in the brain cerebrovasculature. This impacts blood vessel integrity leading to brain hemorrhages and accelerated cognitive decline. Established risk factors for CAA include AD neuropathology (Aβ plaques and tau neurofibrillary tangles), male sex and the Apolipoprotein E (APOE)e4 allele. More recently, a splice variant was identified within long intergenic non-protein coding RNA (LINC-PINT) that decreases risk for CAA in AD cases that do not carry APOEe4. The central hypothesis is that additional genetic risk factors for CAA remain to be identified and that some of these may have different effects in the context of the established risk factors. However, identifying these will require larger study cohorts. The state of Florida has an aged population; age is a major risk factor for AD, and is the fifth leading cause of death in individuals over 65 (Alzheimer's disease facts and figures 2020, *Alzheimer's Association*). CAA plays a key role in AD pathogenesis, leading to brain hemorrhages and accelerated cognitive decline. Distinguishing patients at risk of brain hemorrhages is especially important given that a significant side-effect of the now Food and Drug Administration (FDA)-approved anti-Aß therapy aducanumab is brain hemorrhages. However, there are currently no low-cost peripheral biomarkers for CAA. This proposal aims to address these knowledge gaps through a genome-wide association study (GWAS) in an expanded neuropathology cohort of patients with neuropathologic diagnosis of AD or non-AD, and with existing CAA, Aβ (Thal) and tau (Braak) measures. This expanded cohort includes AD patients from a prior study (Reddy. J et al, 2021), and >1,000 additional participants, more than doubling the sample size. The research team will assess the functional consequence of CAA genetic variants on blood transcriptome and brain microhemorrhages leveraging samples, clinical and neuroimaging data from the Mayo Clinic Florida ADRC. The team will also correlate blood gene expression levels with radiographic microhemorrhages to determine their future potential as biomarkers of this vascular outcome. The team expects to identify additional genetic risk factors for CAA, applicable to both AD patients, and the broader at risk population and determine if the implicated variants and genes translate to candidate peripheral biomarkers for CAA. To date, the team has isolated DNA from almost 400 donors with a pathological diagnosis of AD and available CAA scores. These have been randomized according to key demographics and sample quality metrics and are ready for collection of genome-wide genotypes using arrays. The research team also proposes to performing analysis of using existing genetic and neuropathological data available on both AD and non-AD donors. Three independent datasets with existing genome-wide genetic data and CAA scores have been identified. An optimized Quality Control Pipeline (QC) developed by bioinformatics scientists in the team will be used for ensuring quality control of the three datasets separately and together. Relatedness and sample duplication will be identified with the combined QC process. QC process has been initiated for these three datasets. In summary, excellent progress has been made towards the goals of this award.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A10 Florida Consortium to Reduce Misinformation and Exploitation In AD

Principal Investigator: Nichole Lighthall, PhD

Organization: University of Central Florida

Abstract: Losses due to elder fraud have reached epidemic proportions. One in five Americans over age 65 are victims of financial exploitation, costing billions each year, with devastating consequences for wellbeing. A 2020 Federal Trade Commission (FTC) report revealed that Florida continues to have the highest incidence of fraud and financial exploitation compared to any US state. Major contributors to this problem include the proliferation of misinformation campaigns and scams that target the rapidly expanding older population. Older individuals with Mild Cognitive Impairment (MCI) and Alzheimer's Disease (AD) are at heightened risk for exploitation due to disease-related neurocognitive changes, which is a particular concern in Florida—home to over nine percent of US Alzheimer cases. Further, older adults' vulnerability to misinformation and online scamming increased during the COVID-19 pandemic, as safety requirements increased their reliance on digital technology to stay connected. This "perfect storm" was magnified in Florida's growing number of minority elders with cognitive impairments—many of whom faced isolation, limited resources, and lack of English fluency that further increased their vulnerability to misinformation and scams. Using a team science approach, the proposed consortium will utilize joint efforts at the University of Central Florida (UCF), the University of Miami (UM), and the University of Florida (UF) to fight fraud and the "infodemic" in Florida targeting older adults with AD and their families. This team's unique expertise in cognitive neuroscience of aging, clinical neuropsychology, and cybersecurity provides the necessary foundation to identify and address the primary drivers of exploitation among elders at risk for, and diagnosed with, AD. The project builds on foundational research showing that decline in frontal-executive functions characteristic of MCI and AD are crucial for the detection of deception. Also, poor inhibition promotes impulsive execution of irreversible actions or difficulty with complex decisions. Leveraging this prior knowledge, the research team will collect longitudinal data to identify and probe psychological and neural mechanisms involved in deception detection. This work also has genuine application: the research team has developed behavioral, ecologically valid measures of fraud susceptibility and will use these novel methods with standardized measures to identify diverse cognitively vulnerable individuals and develop effective intervention. The team science approach offers an unprecedented opportunity to address susceptibility to misinformation and exploitation among the most vulnerable. Achieving these goals can help to reduce health disparities, lessen caregiver burden, improve quality of life, and help individuals with AD age in place. The proposed consortium leaders (Primary Ivestigator (PI): Lighthall, UCF; Co-PIs: Levin, UM; Ebner, UF) are experienced investigators who have previously worked together on conceptually-related projects. The consortium will prospectively address this growing health crisis by: leveraging behavioral assessments to pinpoint cognitive, affective, and social predictors of misinformation and exploitation susceptibility; identifying structural and functional neural circuitry involved in detection of misinformation and exploitation; developing strategic intervention for elders with cognitive impairment and their caregivers to mitigate deception risk; and partnering with legal entities specializing in elder fraud as well as media liaisons in elderly residential living to promote culturally-sensitive intervention.

Follow on Funding: UF Consortium on Trust in Media and Technology, $7,500

Collaborations: This is a consortium grant involving three Florida universities (University of Central Florida, University of Florida, and University of Miami). Implicit in this project is the

strong cross-institutional collaboration. The response of "none" indicates that the research team has not added any additional collaborating institutions (beyond the core three) at this time.
Journals: Pehlivanoglu D, Lin T, Lighthall NR, et al. Facial trustworthiness perception across the adult lifespan. *The Journal of Gerontology. Series B*. 2022;gbac166. Published Oct 15, 2022. doi:10.1093/geronb/gbac166.

Lin T, Pehlivanoglu D, Ziaei M, et al. Age-Related differences in amygdala activation associated with face trustworthiness but no evidence of oxytocin modulation. *Front Psychol*. 2022;13:838642. Published Jun 23, 2022. doi:10.3389/fpsyg.2022.838642.

Patents: None at the time of reporting.

Grant#: 22A11 Novel Training In Interventions for Treatment Adherence In Underserved Diverse Patients At Risk for Alzheimer's Disease

Principal Investigator: Jeremy Grant, PhD

Organization: University of Florida

Abstract: Metabolic syndrome (MeSy) refers to a cluster of chronic health conditions that increase the risk of stroke and heart disease, including high blood pressure, high cholesterol, diabetes, and obesity. In addition to its effects on the body, MeSy has also been widely associated with increased risk for cognitive decline and Alzheimer's Disease (AD). It is shown that 55% of older adults in the United States are estimated to have MeSy, and this estimate is expected to increase in line with the country's increasingly aging population. Medication and healthy lifestyle modifications—including physical activity and dietary changes—are the frontline treatments for MeSy. However, treatment adherence rates remain low, and disability rates continue to rise. Notably, African Americans and Hispanics show disproportionately higher rates of MeSy as well as lower treatment adherence rates relative to White Americans. Identifying factors that contribute to poor treatment adherence among older adults with MeSy, particularly in ethnically-minoritized populations, is essential. The research project will leverage an ongoing One Florida Alzheimer's Disease Research Center (1FL ARDC) funded study examining psychosocial and cognitive risk factors for treatment adherence in older individuals with MeSy, as well as previously collected neuropsychological and neuroimaging data of the 1Florida ADRC to directly address the training goals for this fellowship. These training goals include: Learning the socio-cultural factors that influence the evaluation of patients and inform diagnostic accuracy, including reviewing best-practices literature in this domain while engaging and recruiting ethnically diverse research participants, developing hypotheses and methods in order to implement tailored interventions that mitigate AD progression, developing protocols to receive Institutional Review Board (IRB) approval, and gaining expertise in advanced statistical analysis and public dissemination of research findings. Notably, the proposed training will also involve both empirical data collection and secondary data analysis. Under the supervision of his mentoring team, which has expertise in cross-cultural neuropsychology and interventions for cognitive decline, this fellowship will support Dr. Grant's developing expertise in Alzheimer's disease research and advancing his understanding of the social determinants of brain health. Furthermore, the existing infrastructure at the 1FL ARDC and facilities at the University of Florida will provide Dr. Grant with the tools to achieve his research aims, such as advanced neuroimaging techniques and access to experts in clinical care for older adults. This postdoctoral fellowship opportunity will culminate in a competitive submission of a K23

application to the National Institute on Aging (NIA) to design and implement novel behavioral interventions to prevent cognitive decline and Alzheimer's disease in older adults, improving and diversifying the number of highly trained patient-centered researchers.

Follow on Funding: None at the time of reporting.

Collaborations: The postsecondary institution involved in this research project is the University of Florida. The PI (Jeremy Grant, PhD) is co-mentored by Dr. Shellie-Anne Levy, PhD., and Glenn Smith, PhD, who are both faculty in the Department of Clinical and Health Psychology in the College of Public Health and Health Professions at the University of Florida. There are two graduate students in the Doctoral Program in Clinical Psychology at the University of Florida who are also receiving training under Dr. Levy in relation to the research program: Ambar Perez-Lao, MS, and Tamare Adrien, BS.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A12 Novel Behavioral and Neural Markers of Alzheimer'S Disease Progression: A Case for Visual Orienting

Principal Investigator: Adam Barnas, PhD

Organization: University of Florida

Abstract: Spatial navigation is an essential task, and the way in which humans perceive the world relies on both the information that is processed and upon which aspects of the information are focused on. Spatial navigation detriments are prevalent symptoms of Alzheimer's Disease (AD), resulting in limited mobility and decreased quality of life among patients and their caregivers. Typically, spatial navigation behavior is supported by visual navigation cues—signs, verbal instructions, and maps—that show the way to go. But before these cues can be useful, people need to find them, a task requiring two distinct aspects of attention: re-orienting toward a particular kind of object and re-orienting to a location in space. Whereas AD patients show general deficits in attentional re-orienting, meaning that AD patients have trouble attending to objects and locations in visual space, individuals with Mild Cognitive Impairment (MCI) show specific deficits in space-based attention. Research staff hypothesize that deficits in attentional function underlie specific spatial navigation detriments among AD patients which manifest in distinct patterns of neural activity. This hypothesis will be addressed in two research aims: Determine the extent to which individuals with MCI show a deficit in space- and/or object-based attentional re-orienting; and examine connectivity between attentional neural network nodes underlying space- and object-based attentional re-orienting deficits in MCI. These research aims will directly link to two training goals: Develop an empirical and theoretical background in AD pathology focusing on spatial navigation, visual attention, and general cognitive deficits and; learn advanced functional magnetic resonance imaging (fMRI) techniques, including functional and structural connectivity and network analysis. The proposed research and training as part of this fellowship will leverage the existing infrastructure from the ongoing grant FDOH 21A09 on ameliorating age-related decline of spatial navigation success. This clinical fellowship opportunity will allow Dr. Barnas to increase the doctor's publication record in clinical psychology, neuroimaging, and cognitive aging science and, crucially, the collection of

preliminary data for a competitive submission of the NIH K99/R00 in 2022 to design and implement a training intervention toward improving attentional processing subserving spatial navigation skills in MCI and AD. This project has tremendous potential for basic science, translational, and clinical training-related impact, providing significant advancements to understanding brain-mediated function and dysfunction in AD and toward the development of empirically grounded and theoretically motivated rehabilitation intervention that ameliorates attention deficits in persons with AD.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A13 Characterization of New CamK2-Tau Strains of Mice to Model Dementia

Principal Investigator: Jada Lewis, PhD

Organization: University of Florida

Abstract: The goal of this proposal is to perform behavioral characterization on tau transgenic mice that are designed to model dementia observed in diseases like Alzheimer's Disease. Transgenic mice are ones manipulated to contain a foreign gene (in this case one that is associated with dementia) is the genome (the genetic sequence that makes up an organism). Research staff will then examine the brains of the mice that are behaviorally characterized for abnormal features that align with Alzheimer's Disease and related dementias. This grant has been active since April 1, 2022 and during this time research staff have focused on recruiting and training personnel in the study and identifying the best lead line for the behavioral and pathological studies for this proposal. Research staff successfully recruited and trained a student seeking a Masters degree in Neuroscience to execute the neuropathological analysis that research staff will perform on the behaviorally characterized mice. Research staff have identified a lead transgenic line which is one that develops key brain abnormalites observed in dementias like Alzheimer's Disease and a form of frontotemporal dementia. Research staff are now in the process of building the mouse colony to provide a wide range of ages to use for behavioral analysis and the research staff will determine if the brain abnormalities correlate to the behavioral changes observed.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A14 The Beneficial Role of Exercise-Induced Neuronal DNA Damage in Aging and Alzheimer's Disease

Principal Investigator: Claes Wahlestedt, MD, PhD

Organization: University of Miami

Abstract: Each year, nearly one in three seniors (adults age 65+) in the U.S. dies with Alzheimer's disease (AD) or other related dementias. Florida has a disproportionally high number of seniors, 80% of which have at least one chronic condition impacting their quality of life. One of the many pathologies that links AD and other chronic diseases of aging is accumulation of DNA damage in tissues throughout the brain and body. Aerobic exercise is a safe, affordable, and accessible intervention that has consistently demonstrated its potential to decrease the risk of AD and co-morbidities associated with a sedentary lifestyle. Interestingly, it is known that aerobic exercise acutely induces DNA damage in peripheral tissues including muscle, liver, and blood cells. This paradox prompts the question: how does exercise prevent chronic disease, stave off cognitive decline, and extend healthspan? The hypothesis was that exercise-induced DNA damage is a necessary stressor that upregulates DNA damage repair processes in peripheral tissues and in the brain. Research in the field of hormesis generally supports this hypothesis, concluding that acute biochemical stress is necessary to improve cellular function (e.g., fasting). However, the mechanistic link between exercise-induced DNA damage in the brain, long-term neuroprotection, and DNA-damage related peripheral biomarkers of healthy aging is unexplored. The preliminary data shows that multiple DNA damage repair enzymes are upregulated in the brain of mice post-exercise. Until recently, sensitive methods to detect the most common type of DNA damage, single-stranded DNA breaks (SSBs), did not exist. Efforts from the research team and collaborators have led to the development of a next-generation sequencing method that allows for spatial detection of SSBs throughout the genome, referred to as SSiNGLe. This is important because DNA damage does not occur randomly. Indeed, depending upon the stimulus or the cell type, unique SSB signatures can be characterized. When SSiNGLe was applied to blood cells from human subjects of various ages, the research team was able to derive a "breakome age" based on the frequency of breaks at specific locations in the genome. Such a breakome profile can plausibly serve as an early biomarker to differentiate healthy aging from diseased aging. With all of this in mind, the research team will investigate the relationship between exercise-induced DNA damage and neuroprotection in aging wild-type and AD mice. Characterizing the post-exercise DNA damage response in the blood and brain at various ages will allow for the establishment of a blood-based biomarker that tracks how exercise influences cognition in both healthy and AD mice as they age. Innovation and Significance: While there is a clear benefit of aerobic exercise to AD and its co-morbidities, deep mechanistic insight of the paradoxical role of DNA damage in brain aging is missing. Emerging technologies like SSiNGLe combined with established, albeit less sensitive methods, will reveal new insight into the mechanisms of healthy and diseased aging in both the brain and blood. These studies will help establish a novel biomarker of exercise response and aging in the context of DNA damage, and provide a novel modality for the assessment of potential therapies that promote a healthy aging profile.

Follow on Funding: None at the time of reporting.

Collaborations: The Principal Investigators, three graduate students all at the University of Miami Miller School of Medicine, and two undergraduate students from the University of Miami Coral Gables campus have been involved with this research project.

Journals: None at the time of reporting.
Patents: None at the time of reporting.

Grant#: 22A15 Characterization of African American Alzheimer Disease GWAS Hit RBFOX1

Principal Investigator: Karen Nuytemans, PhD

Organization: University of Miami

Abstract: There was a significant delay in hiring personnel (research associate). Novel research associate was hired end of July 2022. The Principal Investigator (PI) completed some preliminary analyses for aim one, assessing efficiency of short hairpin (sh)RNAs to knockdown RNA Binding Fox-1 Homolog 1 (RBFOX1) expression. Vectors harboring published shRNAs (pLKO.1-shRBFOX1-3260 / pLKO.1-shRBFOX1-3261) as well as the corresponding control vector (scramble shRNA; pLKO.1-scramble) were used for packaging in lentivirus. Additionally, a vector using human complementary DNA (cDNA) RBFOX1 and a corresponding control (empty vector) were used for packaging in lentivirus. Neuronal progenitor cells of a healthy control were plated and were either untreated or transduced with any of the five above viruses. Twenty-four hours after transduction, the cells were put under selective medium (puromycin) to select for transduced cells. At differentiation day 35, RNA was selected from each treatment and converted to cDNA. Quantitative polymerase chain reaction (qPCR) analyses targeting a common exon for all RBFOX1 transcripts was used for assessment of RBFOX1 expression levels compared to housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). As expected, treatment with the overexpression vector significantly increased level of expression (>500 fold). However, in assessing the knockdown vectors, the research team also identified reduction of RBFOX1 expression for the control vectors (scramble and empty vector). Performing qPCR on an unrelated gene in the same RNA sample did not identify any changes in expression, potentially indicating the observed effect of reduced expression in the cells treated with control vectors is RBFOX1 specific. The research team procured three more control vectors for the shRNA vectors to pinpoint the potential issue; an empty backbone of the same vector (shc001), a vector with specific shRNA for Green Fluorescent Protein (GFP; shc005) and a vector with specific shRNA for luciferase (shc007). GFP and luciferase are not present in the human genome. Inclusion of these vectors will clarify whether the scramble sequence somehow still had capability to interact with the human cell's RNA and/or whether RNAinterference activity in general has an effect on RBFOX1. The research team created a large DNA stock of these plasmids, and generated lentiviral stocks of them for transduction of the neuroprogenitor cells (NPCs) used in this project. The research team identified a suitable control induced pluripotent stem cell (iPSC) cell line (African American healthy control) for use in this project. Available stocks of NPC for this line are insufficient for the proposed experiments. The research associate has been trained on the protocols and quality control steps of NPC creation from iPSC and neuronal differentiation from NPCs, including cell culture, immunocytochemistry, nucleic acid extraction, etc. Work to create a new stock of NPCs from the selected iPSC cell line is currently underway. All specific reagents for NPC creation and neuronal differentiation have been purchased.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A16 Postdoctoral Fellowship In Neuropsychology and Cognitive Health

Principal Investigator: Philip Harvey, PhD

Organization: University of Miami

Abstract: The postdoctoral research fellowship was granted to Dr. Diana Hincapie, a bilingual and bi-cultural post-doctoral fellow and neuropsychology trainee to continue specialty training in Alzheimer's disease (AD) and AD related disorders (ADRD). The research fellowship program is devoted to mentoring a qualified individual who desires to pursue a career as a clinician-scientist in the field of AD and ADRD. AD is a devastating condition, with immeasurable detrimental effects on the affected individuals and their families, as well as society as a whole, that is expected to lead to an economic and public health crisis of immense proportions in the decades ahead. This fellowship training year is focused on the development of advanced skills in clinical, cognitive, and functional assessment, research methodology, grant writing, psychometric test development, and cognitive remediation, in a diverse sample of older Floridians who are at-risk for developing neurodegenerative disorders. A unique training emphasis for Dr. Hincapie has included the delivery of empirically supported cognitive interventions with the aim of improving brain health. Dr.Hincapie has learned about various intervention strategies and is able to select interventions that are most appropriate to the older depending on their level of impairment. Dr. Hincapie has participated in the preparation of multiple manuscripts that are being reviewed for publications in peer-reviewed journals. The fellow is also working on an independent project that is being piloted in the One Florida Alzheimer's Disease Research Center (1FL ADRC) related to novel plasma biomarkers and relationship to cognitive performance, particularly among Black/African American and Hispanic/Latino older adults. Dr. Hincapie has become a valued and integrated member of the Center for Cognitive Neuroscience and Aging (CNSA) team and the 1Florida ADRC, and works productively with mentors, other trainees and staff. The University of Miami Brain Health Pavilion and the large National Institutes on Health (NIH)-funded clinical research program at the CNSA serve as the ongoing training environment for Dr. Hincapie, as planned. Dr. Hincapie has significantly advanced the fellow's own training and that of predoctoral students who are training in clinical research methods, and psychometry at the CNSA. Dr. Hincapie and mentors also designed the 2022-2023 Neuroscience Didactic Lecture Series, which is a weekly series that is aligned with Houston Conference Guidelines for the training of specialty neuropsychologists. As it relates to leadership, in addition to supervisory duties, Dr. Hincapie has also transitioned to lead the neuropsychological reporting during etiological consensus diagnostic conferences, and has taken on higher level junior faculty oversight of a longitudinal NIH grant under the guidance of Co-Mentor, Dr. Loewenstein. There is an urgent need to adequately train the next generation of clinician-scientists that will contribute greatly to the health of older adult Floridians and their families. Specialists in the field of AD need to develop advanced clinical research skills to expand upon ongoing efforts aimed at the development of accessible diagnostic methods that could potentially detect AD before irreversible brain degeneration occurs. The current training opportunity is of particularly high impact, in that the field of neuropsychology plays a critical role in the diagnosis and management of AD.

Follow on Funding: None at the time of reporting.
Collaborations: Dr. Hincapie is involved in the supervision of predoctoral practicum students from Nova Southeastern University and Albizu University using a developmental model of supervision.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 22A17 Role of Microbiome In Aging of Gut and Brain In Floridian Older Adults

Principal Investigator: Hariom Yadav, PhD

Organization: University of South Florida

Abstract: The research team has received Internal Review Board (IRB) approval for Miami Jewish Health Site and finalized the subcontract for Florida Atlantic University (FAU). Ongoing meetings occur to discuss progress and data and sample collection procedures are harmonized in all the sites. Research staff have completed around 130 recruitments. Additionally, the RedCap project has been developed for this study and harmonization for receiving data from other sites is under-way. Research staff have also analyzed data of around 90 samples from gut microbiome and around 60 samples for oral proteomics.

The research team has also applied for additional grant funding based on Florida Department of Health (FDOH) funded project. In collaboration with Dr. Shalini Jain (co-investigator (co-I) in FDOH funded Microbiome in aging of Gut and Brain (MiaGB) study) recently submitted an ancillary National Institutes of Health (NIH) R21 study titled "Saliva based protein markers for predicting the risk of cognitive decline and dementia in older adults" on July 8, 2022 with budget $412,344. In collaboration with Drs. Yadav Primary Investigator (PI) and Michal Masternak (UCF site PI) submitted another ancillary NIH R21 on July 15, 2022 investigating miRNAs using MiaGB samples.

Follow on Funding: None at the time of reporting.

Collaborations: Research staff has initiated collaboration with University of North Florida for further studies to involve their students and recruitments at their site. However, no FDOH related funding is extended to them.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix B
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2020-2021

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
21A01	Florida Atlantic University	Monia Rosselli, PhD	99,051.00	02/28/23	No	Yes	Yes
21A03	Florida Atlantic University	Howard Prentice, PhD	99,050.00	02/28/23	No	No	No
21A04	Florida Atlantic University	Qi Zhang, PhD	247,620.00	02/28/25	No	Yes	Yes
21A05	Florida State University	David Meckes, PhD	99,051.00	02/28/23	No	No	No
21A06	University of Central Florida	Suren Tatulian, PhD	247,620.00	02/28/25	No	No	Yes
21A07	University of Florida	Yong Ran, MD, PhD	247,620.00	02/28/25	No	No	No
21A08	University of Florida	Karen McFarland, PhD	99,051.00	02/28/23	No	No	No
21A09	University of Florida	Steven Weisberg, PhD	247,613.00	02/28/25	No	No	Yes
21A10	University of Florida	Stefan Prokop, MD	246,991.00	02/28/25	No	No	No
21A11	University of Florida	Barry Setlow, PhD	247,620.00	02/28/25	No	No	No
21A12	University of Florida	Karina Alvina, PhD	99,051.00	02/28/23	No	Yes	No
21A13	University of Miami	William (Dalton) Dietrich, PhD	247,620.00	02/28/25	Yes	Yes	Yes
21A14	University of Miami	Coleen Atkins, PhD	247,620.00	02/28/25	No	No	No
21A15	University of Miami	Claes Wahlestedt, MD, PhD	247,542.00	02/28/25	No	No	No
21A16	University of Miami	Bonnie Levin, PhD	99,051.00	02/28/23	No	No	No
21A17	University of Miami	Holly Cukier, PhD	247,620.00	02/28/25	No	No	No
21A18	University of Miami	Katrina Celis, MD	99,051.00	02/28/23	No	No	No
21A19	University of Miami	Rosie Curiel Cid, PhD	86,615.00	02/28/23	No	No	Yes
21A20	University of Miami	Tatjana Rundek, MD, PhD	247,620.00	02/28/25	No	No	Yes
21A21	University of Miami	Grace Zhai, PhD	247,620.00	02/28/25	No	No	Yes
21A23	University of South Florida	Mark Kindy, PhD, FAHA	247,620.00	03/31/23	No	Yes	No
21A24	University of South Florida	Laura Blair, PhD	247,620.00	04/30/25	No	No	Yes
21A25	University of South Florida	Nan Sook Park, PhD, MSW	80,000.00	02/28/23	No	No	No

Grant#: 21A01 Postdoctoral Research Fellowship in Neuropsychology and Brain Biomarkers of Abnormal Aging

Principal Investigator: Monica Rosselli, PhD

Organization: Florida Atlantic University

Abstract: The research team has been preparing papers for publication under the mentorship of Dr. Monica Rosselli from Florida Atlantic University (FAU). Preparation for publication tasks included: reviewing and analyzing literature, writing reviews, and methodological procedures; preparing data for analysis; collaborating on writing, editing, and preparing coauthored manuscripts and conference presentations. There has been continued training on analysis of brain and blood biomarkers and volumetric data, under the mentorship of Dr. Ranjan Duara from Mount Sinai Medical Center (MSMC) as well as continued training on ratings of amyloid

positivity/negativity on positron emission tomography (PET) scans and rating brain atrophy in structural magnetic resonance imaging (MRI). With this training the research team has been detecting abnormalities such as ventricular enlargement, white matter hyperintensities, infarcts, and hemorrhages and analyzing MRI sequences needed in each case. The research team has also been participating on the Alzheimer's Disease Resource Center (ADRC) data core, ensuring data reliability, and updating data. The team has received continued training on case revision and preparation of case reports for case reports aimed for publication andintersite diagnosis for standardization of diagnostic procedures and reliability.

The research staf has been responsible for preparing patient information, assigning the work to clinicians from the University of Miami, the University of Florida and MSMC, to obtain cross-rater reliability scores for testing diagnostic consistency across ADRC sites, and collaborating with the ADRC's Clinical and Data Cores in ensuring the clinicians across sites. The team has been responsible for coordinating the biweekly clinical consensus conferences and preparing cases for presentation, which includes: reviewing patient data and selecting cases based on pre-established criteria; ensuring the relevant data is uploaded and available; and reparing relevant information. The relevant information would include patient history, longitudinal neuropsychological test scores, biomarkers, MRI and PET scan images, quantitative amyloid data, brain volumes, Neurofilament Light (NfL) and genetic information, and incorporating biomarkers information: amyloid beta (Aβ)40, Aβ42, Glial fibrillary acidic protein (GFAP), Ptau181. Research staff have been reviewing literature about said biomarkers and collaborating on preparing a Clinicopathological case for presentation. The team have been attending regular meetings at the FAU Neuropsychology Lab meetings and ADRC meetings to discuss MSMC research, Clinical core, ORE core, Data core, and intersite diagnosis calibration.

Mentorship sessions with Dr. Monica Rosselli (FAU) and Dr. Ranjan Duara (MSMC) have been ongoing. The research team have been training and performing clinical interviews to ADRC participants and study partners, including Clinical Dementia Rating Scale (CDR), Neuropsychiatric Inventory (NPI), and additional clinical forms integrating clinical and neuropsychological findings. Research staff have also been training in the administration of cognitive assessments for clinical trials. Additionally, staff and mentors have collaborated on preparing and submitting National Institutes of Health (NIH) grant proposal, including IRB document preparation and submission, NIH Just in Time request for information, and response to Grant reviewer comments.

Follow on Funding: National Institute of Aging, $3,089,709

Collaborations: None at the time of reporting.

Journals: Rosselli M, Uribe IV, Ahne E, Shihadeh L. Culture, ethnicity, and level of education in Alzheimer's disease. *Neurotherapeutics*. 2022;19(1):26–54. doi:10.1007/s13311-022-01193-z.

Arruda F, Rosselli M, Kurasz AM, et al. Stability in cognitive classification as a function of severity of impairment and ethnicity. (Doctoral dissertation, Florida Atlantic University). https://www.proquest.com/openview/2e4805ce66edc7ae592561ce9e719c8a/1?pq-origsite=gscholar&cbl=18750&diss=y

Greig Custo MT, Lang MK, Barker WW, et al. The association of depression and apathy with Alzheimer's disease biomarkers in a cross-cultural sample. *Applied Neuropsychology*. 2022 doi:10.1080/23279095.2022.2079414.

Patents: None at the time of reporting.

Grant#: 21A03 Role of hypoxia in triggering Alzheimer's disease pathogenesis: sulindac as a potential therapeutical intervention.

Principal Investigator: Howard Prentice, PhD

Organization: Florida Atlantic University

Abstract: This research project aims to characterize and measure the effect of sulindac on amyloid beta (Aβ) aggregation and tau hyperphosphorylation in SHSY-5Y amyloid precursor protein (APP) overexpressing cells under hypoxic conditions. An *in vitro* model for examining Alzheimer's disease (AD) pathology was established using a human neuronal cell line under normoxic conditions and low oxygen (hypoxic) conditions. It was confirmed by protein analysis that the AD familial mutations (Swedish/Indiana mutations) could be introduced into this cell line. The duration of hypoxia (from six hours to two days) was optimized for assessing percent cell survival in these cells under hypoxic stress. For baseline studies, the effect of sulindac was analyzed at a range of doses using the control neuronal cell line with no AD mutations. Doses of sulindac were optimized in terms of levels of production of a pro-survival kinase (p-Akt). Using the optimized doses protection by sulindac was demonstrated in conjunction with high levels of expression of the p-Akt kinase and optimal doses were determined for effectively protecting this cell line from hypoxic stress.

The research team also aims to test the effect of chronic treatment with sulindac in Aβ aggregation in an AD mouse model when subjected to hypoxia. An important collaboration was initiated with Dr. Hung Wen (Kevin) Lin, Louisiana State University. Dr. Lin's relevant expertise includes research studies on Alzheimer's mouse lines as well as exposure of mice to hypoxic conditions. The experiments for collaboration with Dr. Lin have involved testing of 3xTg Alzheimer's mice (Jackson Labs.) subjected to hypoxia with and without administration of sulindac. In preliminary data using transgenic AD mice a number of key findings were obtained. Firstly, in aged AD female mice flux images (cerebral blood flow) of cortical vasculature were obtained by laser speckle contrast imaging. Aged female AD mice (nine to twelve months) showed impaired cerebral blood flow compared to control non-transgenic mice. In a second study on blood brain barrier (BBB) permeability, mice received retroorbital injections of Evan's blue stain and then perfused fixed tissue was assessed and quantified.

The data indicated that the BBB permeability was enhanced in aged AD mice. Gamma secretase is involved in generating A-beta peptides of which A-beta-42 is the major component of pathological amyloid plaques in AD. Presenilins (PSEN-1 and PSEN-2) are the catalytic subunits of gamma -secretase. In an analysis of the role of gamma- secretase in mouse brain it was found that cortex and hippocampus protein levels for PSEN-1 were higher in aged AD transgenic mice than in control. In young (four month) AD mice treated with oral sulindac for seven days it was found that alpha-secretase and beta-secretase were increased whereas gamma-secretase expression levels were reduced in cortex as reflected in decreased mRNA expression for PSEN-1 and PSEN-2. Ongoing experiments are assessing protein expression

levels of nicastrin, a component of the gamma secretase multiprotein complex, in hippocampus and cortex of aged AD (nine to twelve month old) mice. Ongoing behavioral measurements include assays of working /short term memory as well as novel object recognition for reference/long term memory.

Follow on Funding: None at the time of reporting.

Collaborations: A collaboration is ongoing on Alzheimer's disease mice with Dr. Kevin Lin Louisiana State University, Shreveport, LA. Dr. Lin's expertise is facilitating progress on the scientific aims. The experiments for the collaboration with Dr. Lin involve testing of 3xTg Alzheimer's mice (Jackson Labs.) subjected to hypoxia with and without administration of sulindac. Cerebral blood flow will be assessed using laser speckle contrast imaging and blood brain permeability changes will be determined by measurement of Evan's blue penetration into the brain. Behavioral measurements include assays of working /short term memory as well as novel object recognition for reference/long term memory.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A04 Rebalancing Brain Cholesterol – A Novel Therapeutic Strategy for Alzheimer's Disease

Principal Investigator: Qi Zhang, PhD

Organization: Florida Atlantic University

Abstract: This funded project is to better understand the role of cholesterol (Chol) in the origination of Alzheimer's disease (AD) and to test the effect of re-balance neuronal cholesterol in mitigating neurodegeneration and other AD pathologies. Research staff hypothesize that deficiency in presynaptic membrane Chol (mChol) homeostasis caused by factors including aging, Apolipoprotein E (APOE)4 or mutations in amyloid precursor protein (APP), collectively contributes to synaptic dysfunction and neurodegeneration in AD. This model is different from existing ones, which are mostly about Chol's influence on APP cleavage, amyloid formation, or the ability of the APP C-terminal fragment to regulate genes related to Chol metabolism. The research staff will focus on three specific aims: examining the role of mChol and APP in synaptic vesicle (SV) lipid and protein turnover and presynaptic sustainability; elucidating how APP cleavage by secretases affects mChol homeostasis, SV turnover and neurotransmitter release; and evaluating the pathological contribution of APP mutations and mChol dysregulation to neurodegeneration, and pharmacological tools to restore mChol homeostasis, rescue synaptic dysfunction and reduce neuropathology. During the period from October 1, 2021 to September 30, 2022, research team members have been focusing on aims one and two while starting aim three. In collaboration with Dr. Maciej Stawikowski at Florida Atlantic University (FAU) Chemistry Department, research staff invented a group of bright, membrane-sensitive, and pH-sensitive fluorescent lipid reporters. One of them, CND2 allows researchers to visualize and quantify Chol distribution and trafficking at nerve terminals. Using those dyes, researchers have found that APP modulates Chol retrieval and reuse at nerve terminals and certain AD-related APP mutations impair such modulation, which leads to nerve damage or neuronal loss over time. In pursuing aim three, the research team has discovered that the absence of APP

indeed causes dysregulation of brain Chol but only in aging mouse brain, which indicates a multi-hit model of AD originated from different AD risk factors including ApoE4 and aging. In addition to live-cell imaging and transgenic manipulation, research staff have introduced RNA-seq and volume-imaging to explore the landscape changes in transcriptome and proteome in the brains of APP-null mice. The preliminary results point to age-dependent alternations in synaptic proteins and Chol regulators, which supports the model developed by the research team. Therefore, the research team continues on three specific aims by using APP-null mice to conduct cellular and molecular investigation for the cause of AD and then use that information to find pharmacological tools to re-balance brain cholesterol.

Follow on Funding: National Institute of General Medicine, $299,337.00.

Collaborations: Postsecondary educational institutions involved: Harvard University. Description: Research team members have built collaboration with Dr. Lei Liu at Harvard University. Dr. Liu, an expert in Alzheimer's disease genetics and protein assays, has been working closely with the team to assess changes in APP, secretase, synaptic proteins, and all AD-related gene expression changes using both biochemical and genomic methods. Number of students receiving training or performing research under the research project.

Journals: Thomas D, Rubio V, Iragavarapu V, et al. Solvatochromic and pH-sensitive fluorescent membrane probes for imaging of live cells. *ACS Chem Neurosci*. 2021;12(4):719-734. doi:10.1021/acschemneuro.0c00732.

Alamgir S, Pelletier OB, Thomas D, Rubio V, Stawikowski MJ, Zhang Q. Measuring membrane lipid turnover with the pH-sensitive fluorescent lipid analog ND6. *J. Vis. Exp*. 2021;(173):e62717. doi:10.3791/62717.

Patents: None at the time of reporting.

Grant#: 21A05 Mesenchymal Stem Cell-Derived Extracellular Vesicles for the Treatment of Alzheimer's Disease

Principal Investigator: David Meckes, PhD

Organization: Florida State University

Abstract: The research team's recent findings (Yan Li et al) have indicated that microglial differentiation is affected by amyloid-beta42; more specifically, it is directed to the M1 phenotype (proinflammatory state). When exposed to low levels of extracellular vesicles from induced-neuronal precursor cells (iNPCo) the amyloid beta (Aβ)42-stimulated microglial showed slightly reduced mRNA expression of the M1 markers, and decreased M2 markers. Enzyme-linked immunosorbent assay (ELISA) results also showed reduction of IL-6, which was consistent with quantitative polymerase-chain-reaction (qPCR) results. These data support the hypothesis that extracellular vesicles (EVs) from iNPCo can serve to mitigate inflammation.

Follow on Funding: None at the time of reporting.

Collaborations: The research team has determined that after conventional electroporation and ultracentrifugation, less than 10% of input EV proteins are recovered, whereas with the

application of the previously published ExtraPEG method (PMID: 27068479) protein recovery levels were greatly enhanced nearly 10-fold to 50%. Significantly, the levels of miRNA-21 in recovered EVs (determined by qPCR) were also enriched by 1500-fold. Additional experiments on the effects of miRNA21-loaded EVs on *in vitro* microglial proliferation showed that the well-known inhibitory effects of hMSC-derived EVs on microglial cells could be completely rescued with miRNA-21-loaded EVs. Further work will include proteomic and miRNA profiling studies, and experiments on the neuroprotective properties of MSC-derived EVs on amyloid-beta-damaged neurons and glia.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A06 Amyloid beta oligomers' structure, membrane pore formation, and toxicity

Principal Investigator: Suren Tatulian, PhD

Organization: University of Central Florida

Abstract: Progress has been made in structural characterization of four amyloid beta (Aβ) peptides in aqueous buffer and reconstituted in lipid membranes. The peptides selected for these studies are the most abundant and toxic Aβ species, i.e. Aβ1-42, Aβ1-40, and the hypertoxic, pyroglutamylated AβpE3-42 and AβpE3-40. One of the mechanisms of Aβ toxicity is membrane binding, destabilization, and formation of ion-conducting pores. The purpose of these studies was to identify the structural mechanism of toxicity through membrane perforation, which may lead to novel therapeutic strategies for Alzheimer's disease (AD). Various biophysical methods have been employed, including attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, circular dichroism (CD), fluorescence spectroscopy, and atomic force microscopy (AFM).

Fluorescence studies identified that the tyrosine can be excited in the 210-240 nm range. Splitting of tyrosine emission band into two components (~307 nm and ~340 nm) has been detected and attributed to solvent-protected and exposed fractions, respectively; the 340 nm component results from strong H-bonding between the tyrosine hydroxyl and phosphate of the buffer. Moreover, the two populations can be selectively excited at ~220 and ~240 nm, allowing determination of the degree of solvent accessibility and hence the compactness of the tertiary fold of the peptides. Combined with CD, the data indicated that Aβ1-42 forms tightly packed, twisted β-sheet structure and in membranes acquires fraction of α-helix. Aβ1-40 forms less stable and more solvent-exposed β-sheet structure, and the vesicles induce partially unordered structure. AβpE3-42 forms relatively loose β-sheet; weaker overall intensity and red-shifted CD band indicate less twisted β-sheet. Membranes protect from solvent and induce partial α-helical structure. AβpE3-40 forms loose, solvent accessible β-sheet in buffer and solvent-protected α/β structure in membranes.

These data suggest that Aβ1-42 can form β-barrel-like ion channels. AβpE3-40 can do so less effectively. Aβ1-40 and AβpE3-42 may be less efficient in forming channels because of insufficient β-sheet fraction or narrow β-barrels. ATR-FTIR experiments showed that all four peptides exhibited α-helix + β-sheet secondary structure. The content of β-sheet was 44%, 37%, 17%, and 27% in Aβ1-42, AβpE3-42, Aβ1-40, and AβpE3-40 and the content of α-helix

was 20%, 33%, 15%, and 33 %, respectively. The largest β-sheet content of Aβ1-42 is consistent with its ability to form β-barrel-like ion-conducting pores in membranes, followed by AβpE3-42, AβpE3-40, and Aβ1-40. In collaboration with Dr. Themis Lazaridis of the Department of Chemistry, City College of New York, molecular dynamics structural model has been generated for the membrane pore formed by Aβ1-42. AFM studies on Aβ1-42 reconstituted in lipid vesicles have been carried out to visualize the peptide in lipid membranes and to identify the effect of the peptide on the integrity of vesicle membranes. Control measurements of vesicles without the peptide showed spherical particles. The presence of the peptide strongly affected the morphology of the vesicles, indicating partial loss of integrity of vesicle membranes. These data suggest that in addition to membrane pore or channel formation, Aβ1-42 is able to cause global membrane disruption, which may contribute to toxicity through a membrane damaging mechanism.

Follow on Funding: None at the time of reporting.

Collaborations: Dr. Themis Lazaridis, Department of Chemistry, City College of New York. Dr. Lazaridis generated molecular dynamics models for Aβ peptides. Rowan Hassan, undergraduate student, University of Central Florida. Rowan received training and conducted the above-described biophysical work. Dr. Laurene Tetard, Department of Physics, University of Central Florida. Dr. Tetard conducted the AFM work. Dr. Ratnesh Lal, Dept. of Bioengineering, Mechanical Engineering, Materials Science and Engineering, University of California San Diego. Abhijith Karkisaval Ganapati, PhD student in Prof. Lal's lab. Dr. Lal and his student Abhijith conducted ion channel studies on Aβ peptides.

Journals: Abedin F, Kandel N, Tatulian SA. Effects of Aβ-derived peptide fragments on fibrillogenesis of Aβ. *Scientific Reports*. 2021;11(1):19262. doi:10.1038/s41598-021-98644-y.

Tatulian SA. Challenges and hopes for Alzheimer's disease. *Drug Discovery Today*. 2022;27(4):1027-1043. doi:10.1016/j.drudis.2022.01.016. (Invited Keynote Review).

Patents: None at the time of reporting.

Grant#: 21A07 Function of Chimeric Phagocytic Receptor Targeting Aβ and Tau Protein

Principal Investigator: Yong Ran, MD, PhD

Organization: University of Florida

Abstract: Alzheimer's disease (AD) is the most common form of dementia among the elderly. Over five million Americans currently have AD, and the number of cases is expected to rise to >13 million by 2050. No effective AD therapies exist, and many aspects of AD pathogenesis remain enigmatic. AD is hypothesized to be proteinopathies. Despite the growing understanding of the pathogenic mechanisms underlying these disorders, progress with respect to development of new therapeutics has been lagging. Based on the premise that protein aggregation and accumulation in AD and many other neurodegenerative disorders is a key, causal, pathologic event, it is logical to pursue therapeutic strategies to prevent aggregate formation, enhance clearance of the aggregate, or neutralize "toxic" signaling by the aggregate. Many strategies targeting protein aggregates have been developed, but these strategies have proven difficult to translate, or in some cases, found to have dose limiting side-effects. Further,

even in the preclinical studies supporting clinical development, most of these therapeutic approaches have shown modest effects. Robust effects may have been reported from prophylactic or early interventions studies, but evidence for efficacy is much more limited, or absent, for treatment studies initiated when pathology is widespread.

Enhancing clearance of the target protein via phagocytic mechanisms is arguably the most direct way to test the hypothesis that enhancing clearance will be beneficial. Here, staff will directly evaluate this premise using novel chimeric phagocytic receptors (CPRs). These studies are analogous to chimeric antigen receptor T-cell (CAR-T) studies, but instead of engineering T-cells to target a specific protein, they will harness phagocytic cells to target either amyloid beta (Aβ) or tau. The research staff have already created and functionally validated multiple CPRs targeting Aβ and tau. These CPRs are fusion molecules composed of an extracellular single chain variable fragment (scFv) that binds the target protein with high affinity that is fused via a linker to the transmembrane and cytoplasmic domains of different phagocytic receptors (FCERG1, MRC1, MERTK, CLEC4L). Notably, for each target protein, research staff have identified two or more CPRs (based on the different phagocytic receptor fusion) that selectively bind to the target protein and mediate rapid internalization of the targeted protein. In the central nervous system (CNS) both microglial cells and astrocytes can be phagocytic. Researchers are able to selectively transduce astrocytes in neonatal and adult mice using appropriate rAAV vectors. Microglia cells remain difficult to transduce with high efficiency in vivo, but with novel rAAV vectors research staff can transduce them efficiently ex vivo. These engineered microglial can then be studied ex vivo in relevant models or transplanted into the brains of mice. Using select *in vivo* and ex vivo models of amyloid beta (Aβ) and tau pathology, including the research staff's novel brain slice culture models of tauopathy, researchers will evaluate the ability of CPRs to have disease modifying effects when delivered before pathology develops or when pathology is already robust. If successful, like CAR-Ts, it is likely that appropriately targeted CPRs may have applicability to AD and many other disorders.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A08 Epitranscriptomics in models of Alzheimer's disease

Principal Investigator: Karen McFarland, PhD

Organization: University of Florida

Abstract: The societal and economic impact from Alzheimer's disease is only expected to grow over time. Within the brain, hallmarks of Alzheimer's disease include pathological accumulation of beta-amyloid and Tau proteins in neurons and surrounding glial support cells. The accumulation of these proteins is toxic and leads to cell dysfunction and death of neurons. This grant proposes to examine whether changes in the epitranscriptome contribute to the accumulation of toxic proteins in the brains. The epitranscriptome describes chemical, reversible changes to individual nucleosides of Ribonucleic Acid (RNA) transcripts which are able to

influence RNA stability and downstream protein levels in a rapid manner. In this pilot grant proposal, the goal was to determine whether N6-methylation at adenosine residues (m6A), one of the most well-studied epitranscriptomic modifications, are present in RNA transcripts from beta-amyloid treated microglial cultures (aim one) and in the brains of an amyloid mouse model, Tg CRND8 (aim two).
The research team has used a long-read, nanopore sequencing technology to sequence samples from both of these groups (microglial cultures and mouse brains). Nanopore sequencing technology (Oxford Nanopore Technologies) allows for sequencing of native RNA species and can detect modified nucleoside on the RNA molecules. Sequencing experiments are completed for these two groups. Analysis of differential expression of RNA transcripts is also completed. These initial experiments to detect differential gene expression were important to benchmark the nanopore sequencing technology against results obtained previously used standard short-read technologies. Current efforts are concentrated on adapting software algorithms to detect modified RNA bases within the sequencing reads. Additional efforts will focus on determining whether there is differential usage in the RNA modifications that are identified between experimental groups (treated vs untreated microglial cultures and normal vs transgenic mouse brains) and then to correlate these changes to proteomic alterations in the mouse brains of the amyloid model. These experiments are aimed to further understand molecular changes that lead to the accumulation of amyloid and Tau proteins in the brains of patients with Alzheimer's disease with the ultimate goal to identify new targets for disease modifying therapies.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A09 Go your own way: Evaluating neurofeedback-induced plasticity to improve spatial navigation behavior in older adults at risk for Alzheimer's disease

Principal Investigator: Steven Weisberg, PhD

Organization: University of Florida

Abstract: Spatial navigation is an essential task, without which even finding the kitchen from the bedroom becomes a challenge. With decreased ability to navigate comes decreased safety and an overall decline of independence and quality of life. The goal is to assess and attenuate age-related decline of navigation success (whether a navigator reaches their goal) by dissociating success from navigation strategy (the cues and cognitive processes a navigator employs to encode the environment). This approach combines behavioral, neural, and genotypic assessment with a novel real-time neurofeedback intervention. Research staff propose three specific aims: determine the behavioral and neural correlates of spatial navigation strategies in healthy older adults and older adults who are at risk for developing Alzheimer's disease (AD), i.e., diagnosed with amnestic mild cognitive impairment (aMCI); to evaluate the efficacy of a real-time functional magnetic resonance imaging (rtfMRI) guided neurofeedback training to increase hippocampal or caudate activation, thereby improving navigation success; to

determine whether carriers of a genetic marker, apolipoprotein E4 (ApoE4), which predisposes carriers to develop AD, are more likely to show navigation deficits or may be more (or less) amenable to rtfMRI interventions. In particular, the team hypothesizes a shift from a hippocampal-based navigation strategy in younger adults to a caudate-based strategy in older adults, with no age-related change in navigation success. Further, research staff hypothesize that this neural shift and associated behavioral effects are more pronounced in individuals at elevated risk for developing AD (i.e., ApoE4 allele carriers) and in aMCI. These data have direct translational impact by developing an improved mechanistic understanding of the link between neural processes, genetics, and human behavior allows for more precise determination for who may need clinical support before they get lost or wander.

Since the award was activated on May 2, 2021, the research team has hired one post-doctoral fellow who received follow-on funding to research visual attention components to spatial navigation in aMCI (Dr. Adam Barnas). Research staff have made substantial progress in data collection for Aim one and three. Research members have now collected fMRI data from 30 younger adults and 23 healthy older adults, and one older adult with subjective cognitive complaints and a familial history of Alzheimer's disease—a non-clinical precursor to MCI. Researchers have collected behavioral data from an additional 20 participants. Preliminary data analyses reveal that when participants find the goal in a virtual environment navigation task, older adults are more likely to use a route-following strategy compared to a spatial strategy. The research team has presented these data at several conferences and are preparing a manuscript to report the results now. For Aim two, staff have hired a Master's student in data science who has developed the real-time fMRI paradigm and tasks. The research team is implementing those tasks now and plan to initiate data collection by the end of the calendar year.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Barnas AJ, Ebner NC, Weisberg SM. (Under Review). Spatial direction comprehension is guided by efficient allocation of space-based attention. Submitted on August 9, 2022 to *PsyArXiv*. doi:10.31234/osf.io/uwhq9.

Patents: None at the time of reporting.

Grant#: 21A10 Impact of severe systemic infections on brain pathology and local immune response

Principal Investigator: Stefan Prokop, MD

Organization: University of Florida

Abstract: Alzheimer's disease (AD) is the most common cause of dementia, currently affecting more than 580,000 Floridians. It is known, that severe systemic infections can trigger cognitive decline and the current COVID-19 pandemic has brought a surge of severe viral illness highlighting the importance of understanding the short- and long-term impact of acute infections on cognition and precipitation of neurodegenerative disease in survivors.

In this context, the overarching goal of this proposal is to recruit Floridians who survived COVID-19 infections into the brain donation program of the University of Florida Neuromedicine Human brain and tissue bank (UF HBTB) to allow for a detailed neuropathological workup, as well as an in depth analysis of local immune responses in the brains of these patients. During the first year and a half of funding, research staff have undertaken outreach efforts, including personal communications with interested participants, distribution of flyers, and launch of a webpage. These efforts have been extremely successful, allowing the research team to recruit a total of 33 COVID-19 survivors into the brain donation program. All of these participants subsequently died and research staff were able to procure their brains for the UF HBTB.

A neuropathological workup has been completed on the majority of these brains, revealing a variety of neuropathological changes, including but not limited to AD neuropathological changes, Lewy body pathology, as well as cerebrovascular disease. The research team have analyzed the local immune response in a subset of these brains and compared the findings with existing data sets of patients who suffered from neurodegenerative diseases, but did not have a COVID-19 infection. These preliminary analyses revealed elevated inflammation in the brains of COVID-survivors, indicating that the infection had a lasting effect on brain immunity. So far, no differences in the extent of neurodegenerative pathology in COVID-19 survivors compared to cases without a history of COVID-19 infection have been detected, but these studies are still ongoing as more and more patients are recruited into the brain donation program.

Recruitment efforts in the first year and a half of funding have been extremely successful and the research team is confident that recruitment efforts will continue on this trajectory to allow for a comprehensive assessment of the impact of COVID-19 infections on the burden of neurodegenerative disease in Florida. In addition to the immediate results of the autopsy studies, this project will also be able to create a registry of patients signing up for brain donation, to provide a longitudinal view of the emergence of neurodegenerative disease in the state of Florida in the upcoming years following the conclusion of the COVID-19 pandemic.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None

Grant#: 21A11 Effects of cannabis on Alzheimer's disease-related pathology and cognitive decline

Principal Investigator: Barry Setlow, PhD

Organization: University of Florida

Abstract: Adults over age 65 represent the fastest growing group of cannabis users, but they are also at the greatest risk for Alzheimer's disease (AD) and age-related cognitive decline. This project uses rodent models to determine how chronic exposure to cannabis and cannabinoids affects AD pathology (Aim one) and age-related cognitive impairments (Aim two). Under Aim one, a study in mice was completed that evaluated blood levels of delta-9-tetrahydrocannabinol

(THC, the primary psychoactive component of cannabis) following exposure to different amounts of cannabis smoke, to determine the optimal conditions for subsequent studies that will evaluate how chronic exposure to cannabis smoke affects AD pathology. Data analysis is in process from a second study in mice that is designed to determine how chronic exposure to cannabis smoke affects age-related dysregulation of inflammatory/immune system signaling, which is thought to contribute to AD pathology. Under Aim two, a study in rats was completed that evaluated the effects of chronic oral consumption of THC on two forms of cognition. In this study, young adult and aged rats of both sexes were first trained in a working memory task, in which they had to remember information over short delay periods (one to 24 seconds). In humans, this form of memory is impaired in older adults compared to younger adults. Once trained, rats of each sex and age group were split into two groups balanced for initial working memory performance, and underwent daily consumption of either gelatin containing a low dose of THC or plain (control) gelatin, while continuing testing in the working memory task for three weeks. The results showed that, as expected, aged rats had less accurate working memory compared to young. Among young rats, THC had no effect on working memory accuracy. In contrast, aged rats that consumed THC performed significantly more accurately than aged control rats, to the extent that aged-THC rats were comparable to young control rats. The same rats described above were then tested for their spatial memory ability (which is also impaired in aged compared to younger humans), while continuing daily consumption of THC or control gelatin. The data showed that, in contrast to the enhancing effects of chronic THC on working memory accuracy in aged rats, chronic THC consumption fails to enhance spatial memory (which, as expected, was impaired in aged rats compared to young). Together, these data show that the effects of daily oral THC consumption differ as a function of both age and form of cognition, in that daily THC can remediate age-related deficits in working memory, but has no effect on spatial memory. As these two forms of memory are mediated by distinct brain systems that can show different trajectories of decline in older adults, the results to date suggest that (at least under the conditions tested) chronic THC may be useful for some forms of age-related cognitive impairment.

Follow on Funding: None at the time of reporting.

Collaborations: All components of the project are conducted at University of Florida (UF), in the Colleges of Medicine and Pharmacy. Four graduate students have conducted research under the project: Sabrina Zequeira and Emely Gazarov (UF College of Medicine): Erin Berthold and Alexandria Senetra (UF College of Pharmacy). In addition, one undergraduate, Alara Guvenli (Setlow laboratory), assisted with the chronic oral cannabinoid project (aim two). Another undergraduate, Bailey McCracken (Setlow laboratory) assisted with the cannabis smoke exposure/THC pharmacokinetics study (aim one). These students will be authors on resulting presentations and publications.

Journals: None at the time of reporting.

Patents: None

Grant#: 21A12 Role of Irisin as mediator of exercise-related cognitive improvement in Alzheimer's Disease

Principal Investigator: Karina Alvina, PhD

Organization: University of Florida

Abstract: The original plan was to use postmortem samples from human patients from the University of Florida Brain Bank (sample database to be provided by Dr Stefan Prokop) and to use a commercially available antibody to stain for irisin. Research staff have continued to evaluate different antibodies using both mouse brain and muscle tissue as controls to find the most effective one and use to stain human brain samples as proposed. The research team has tried two different commercial sources for the antibody, and are troubleshooting to get the best staining results. So far, these antibodies have not produced the expected results and thus research staff have used western blots as an alternative to measure protein expression in mouse brain tissue. The research team has seen very interesting results comparing different brain regions and conditions (sedentary vs exercise). Results have shown that the expression of FNDC5 and irisin is different when comparing different brain areas such as olfactory bulb and hippocampus in conditions of exercise or sedentary lifestyle in young adult male mice. Research staff are following up on these results and including groups of sedentary and exercised mice. The research team is also expanding these experiments to use female mice. For the second aim of the project, the research team is using a mouse model of Alzheimer's disease (mouse strain called CRND8). Research members started a breeding colony and already used the first cohort produced from the first breeding pairs. An exercise protocol was successfully started (daily swimming for three weeks) and several behavioral tasks were performed to probe memory function, exploratory behaviors. The research team also collected a variety of tissues to later evaluate different patterns of protein or transcript expression. Research staff are currently in the process of analyzing the data collected. In addition, a new collaboration was established with Dr Kyle Allen, Associate professor in Biomedical Engineering, from the College of Engineering at the University of Florida. The research team finished the second cohort of AD mice placed in running cages for eight weeks and another group that ran for four weeks. These male mice ran on a voluntary basis using running cages designed by the Allen lab. The results showed different patterns of body weight changes in different conditions. Importantly, AD mice that exercise showed less anxiety like behaviors and changes in adrenal glands size. Currently the research team is analyzing expression of Irisin in circulation and also in other tissues.

Follow on Funding: The Joe and Dorothy Dorsett Brown Foundation Program in Neuroscience Grant ($15,000)

Collaborations: University of Florida, Department of Neuroscience. The number of students involved are one graduate student from the Master in Neuroscience program (Jonah Juergensmeyer) and four undergraduate students (Sam Vilarino, Sophia Moret, Allison Comite, Erin Kang). University of Florida, Department of Biomedical Engineering. The number of students involved are one undergraduate student (Eiko Alzamora). The College of Wooster, OhioH The number of students involved are one undergraduate student (Jill Murray).

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A13 The Importance of the Innate Immune Response as a Mechanistic Link between TBI and AD

Principal Investigator: William (Dalton) Dietrich, PhD

Organization: University of Miami

Abstract: Traumatic brain injury (TBI) is a significant risk factor for the development of Alzheimer 's disease (AD) and AD related dementias. Although this interrelationship represents an important heath problem to the citizens of Florida especially due to the high incidence of TBI and a growing aging population, the causative relationships between these two conditions is not known. In mouse models that incorporate genetic risk factors for AD, evidence for TBI accelerating the emergence and severity of AD-like pathologies has been proposed. The central hypothesis of the research team is that an increase in neuroinflammation in AD augments TBI-induced inflammation and contributes to memory impairments in models of AD. The first aim which has been completed during this past year investigated the temporal activation and cellular distribution of TBI-induced inflammasome activation on AD pathology. After injury in AD transgenic mice, protein analysis of cortical tissue showed significantly increased activate (cleaved) caspase-1, caspase-8, and IL-1β when compared to shams at one hour post injury and maintained at one day and at one week post injury. These findings provide evidence for brain injury resulting in increased neuroinflammatory activity which was maintained over the course of one week after injury. The team did not see this increase in hippocampal tissue which is a structure involved in learning and memory. To determine whether AD predisposition impacts the neuroinflammatory response to TBI, research members performed protein analysis in cortical tissue from wildtype (WT) and AD mice at one day post injury or sham surgery. The findings showed increased IL-1β after brain injury in AD mice when compared to brain injured WT mice. The team also registered significantly increased levels of activated caspase-1, caspase-8, and IL-1β in WT and AD mice after CCI compared to sham controls. To assess alterations in cognitive function after brain trauma in AD and WT mice, mice were tested in open field at three days post-surgery and novel object recognition at 14 days post-surgery. Although some increased anxiety behavior was observed in the AD mice after trauma, the research team did not observe any differences in cognitive function using the novel object recognition test. Overall, the findings indicate that TBI increased inflammasome activation in mice with a genetic predisposition towards AD. These findings are further evidence that a treatment targeting neuroinflammation has the potential to reduce the development of AD after TBI in the clinic.

Follow on Funding: NIH/HINDS, $2,894,996.00.

Collaborations: None at the time of reporting.

Journals: Johnson NH, Hadad R, Taylor RR, et al. Inflammatory biomarkers of traumatic brain injury. *Pharmaceuticals*. 2022;15(6):660. doi:10.3390/ph15060660.

Johnson NH, de Rivero Vaccari JP, Bramlett HM, Keane RW, Dietrich WD. Inflammasome activation in traumatic brain injury and Alzheimer's disease. *Trans Res*. 2022;S1931-5244. doi:10.1016/j.trsl.2022.08.014.

Patents: Inflammatory Biomarkers of Traumatic Brain Injury. de Rivero Vaccari JP, Keane RW, Dietrich WD, Jennifer Christine Muñoz-Pareja, Johnson NH, Jon Perez-Bárcena. (UMIP-754 & UMIP-770) US 63/334,218 (provisional) (04/25/2022)

Grant#: 21A14 Cyclic Nucleotide Regulation in Alzheimer's Disease and Brain Trauma

Principal Investigator: Coleen Atkins, PhD

Organization: University of Miami

Abstract: Traumatic brain injury (TBI) and Alzheimer's disease (AD) are significant public health problems in Florida. In 2020, there were 21,540 hospitalizations related to TBI in Florida and there are approximately 370,000 Floridians living with long-term disabilities from TBI. It is estimated that there were 580,000 people diagnosed with AD in Florida in 2020 and this number is projected to increase by 24.1% to reach 720,000 in 2025. TBI is a risk factor for AD, but how TBI is linked to the development of AD is unclear. Given the prevalence of AD in the aging Floridian population, understanding how a TBI that accelerates cognitive deficits related to AD is important. In preclinical studies, a single TBI in mouse models of AD increases beta-amyloid deposition and tau phosphorylation, key AD pathologies. Another commonality for TBI and AD is synaptic loss, which contributes to cognitive deficits and is caused by neuroinflammation. The goal of this research study is to determine if TBI accelerates cognitive dysfunction in mouse models of AD and evaluate an anti-inflammatory drug treatment to reduce cognitive dysfunction. Mice were studied that have gene mutations that result in early onset AD. These mice have mutations in amyloid precursor protein and presenilin 1(APP/PS1), which accelerates beta-amyloid deposition mice. Another set of mice studied have mutations in amyloid precursor protein and presenilin 1 as well as tau (3xTg-AD mice). AD mice and their wild type controls received sham surgery or mild controlled cortical impact at two months of age (presymptomatic for AD) or at twelve months of age (symptomatic stage). Mice were treated with an anti-inflammatory drug, T2409, which inhibits phosphodiesterase 4B. After treatment, mice were analyzed in learning and memory tasks to study cognitive decline. Research staff were blinded to the animal genotype, surgery allocation and treatment during behavioral testing and analyses. Behavioral deficits were not observed in two-month-old 3xTg-AD mice after TBI when tested for learning and memory using contextual fear conditioning. In contrast, two-month-old APP/PS1 mice with TBI had significant impairments in contextual fear conditioning which were rescued with T2409 treatment. Both 12-month-old APP/PS1 and 3xTg-AD mice were significantly impaired in contextual fear memory formation after TBI and these deficits were improved with T2409 treatment. These results indicate that the combination of TBI in the context of AD predisposition accelerates cognitive decline which can be mitigated by a phosphodiesterase 4B inhibitor. The interesting finding that APP/PS1 mice but not 3xTg-AD mice, developed early cognitive decline after TBI at two months of age suggests that levels of beta-amyloid in the hippocampus may be a determinant in the manifestation of cognitive decline. 3xTg-AD mice have extracellular beta-amyloid deposits in the hippocampus at 12 months of age, whereas APP/PS1 mice already have extracellular beta-amyloid deposits at six months of age. These results could support clinical development of T2409 in TBI patients before and during the development of AD. Findings from this study have the potential to impact the health of Floridians by improving their cognitive functioning and slowing underlying AD-associated pathology after a TBI.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A15 Contributions of Menopause-Induced Epigenetic Mechanisms on Alzheimer's Disease Pathogenesis

Principal Investigator: Claes Wahlestedt, MD, PhD

Organization: University of Miami

Abstract: The mechanisms that drive the sexual dimorphism observed in Alzheimer's disease (AD) are not well understood. This project aims to identify epigenetic mediators linking female sex and aging to AD. Based on the data collected by the research team, this study will highlight the need for drug trials to stratify patients based on sex, and perhaps the need to study efficacy in single sex cohorts. The long-term goal of this research is to better inform future AD therapies. Context and progress to date: Women represent two-thirds of AD cases, and experience more rapid cognitive decline and worse pathology than men, for reasons that remain unknown. One hypothesis for the higher prevalence of AD in women lies in the drastic changes in sex hormones women experience as they traverse menopause, resulting in a depletion of estrogen and progesterone in post-menopause. Menopause has been reported to cause changes in epigenetic modifications, including histone acetylation. The research team has previously shown that regulation of epigenetic modulators through histone deacetylase (HDAC) inhibition improves memory in murine models of AD, while normalizing AD-related genes. Human imaging studies have uncovered that increased levels of amyloid beta (Aβ) begin in peri-menopause, even in cognitively normal women, when compared to men of the same age or pre-menopausal women. A decline in glucose metabolism in the brains of women, a factor that has been implicated in AD, also commences in peri-menopause. Since October 2021, the research staff has established a mouse model to facilitate the investigation of the menopause transition in the context of AD. In that model, accelerated ovarian failure is induced, which recapitulates the human condition better than the commonly used ovariectomized models. The research staff has validated the model at the follicle stages, replicating the early stages of menopause. The research team has also demonstrated that at the post-menopause stage, triple transgenic AD mice present impaired glucose tolerance when compared to age-matched controls and peri-menopause AD mice. Studying epigenetic changes that are occurring in the brain during the neuroendocrine state of peri-menopause, using this model, might be key to understanding AD onset in women. To date, no rodent studies have studied epigenetic modifications in the brain during the peri-menopausal state. Therefore, these studies propose to uncover the acetylation and gene expression changes occurring in the brain in peri- and post-menopause-like states in this mouse model, with the long-term goal of being able to develop more personalized or sex-specific treatments to AD. Studies are underway to complete the aims of the grant. Impact to Floridians: Florida has the second highest population of individuals living with AD in the U.S. and is projected to increase nearly 30% over the next five years. Data suggest that early intervention may help delay AD symptoms, but early biomarkers are lacking. Through the work proposed here, the role of menopause-transition-mediated changes occurring in the brain could be key to understanding AD onset in women, and may improve early AD detection in a sex specific manner, in Florida and beyond.

Follow on Funding: None at the time of reporting.

Collaborations: The Principal Investigators, two scientists and two graduate students all at the University of Miami Miller School of Medicine, as well as two undergraduate students from the

University of Miami Coral Gables campus have been involved with this research project for the reporting period.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A16 Detection and reduction of scams susceptibility among Hispanic/Latinx and non-Hispanic/Latinx individuals with mild cognitive impairment and Alzheimer's Disease

Principal Investigator: Bonnie Levin, PhD

Organization: University of Miami

Abstract: During this grant year (October 2021-September 2022), the research team has accomplished several critical project milestones. Research staff have consistently met two to three times per week to discuss study design, physician and participant recruitment, and community outreach strategies. The research team has established partnerships with leaders in the Latinx community who have encouraged the team to raise awareness about scamming of vulnerable elders through informative presentations, both in person and on radio as well as two upcoming talks scheduled this fall. The research staff have also begun to incorporate a screen into the clinic that addresses susceptibility to scamming to help individuals with cognitive impairment become more aware of their own vulnerability to deception. Staff have finalized the innovative tasks after making several adjustments to enhance comprehension of test instructions and material, particularly among elders with cognitive limitations. All test materials have been translated into Spanish. The research team has also developed and finalized the pre- and post-intervention protocol, which now raises awareness about both financial scamming and false information pertaining to medical treatments, as the latter has increasingly led elders to spend money on non-evidenced based treatments with poor outcomes. Research team members have begun testing the training program and translated questionnaires on both healthy elders and elders with mild cognitive impairment, with particular emphasis on ensuring that all participants are able to understand the training. Several changes were made to the presentations to make them more engaging and animated—for instance, the slides are now brighter and more colorful with the aim of increasing the salience of the information. With regards to recruitment, research staff are working with the University of Miami's Consent to Contact program, a resource specifically designed to search for potential patients who wish to become involved as research participants at the University. Research team members have completed the necessary background checks and submitted the proposal to the Internal Review Board (IRB) to make the forms more readable, relevant, and to ensure that the content has a wider application for multiple types of scams. Finally, two new fellows will be joining the team: Dr. Ileana Pacheco-Colón and Dr. Emma Ducca. Drs. Pacheco-Colón and Ducca will be doing the training portion of this pilot study.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A17 The Role of ABCA7 in Alzheimer's Disease in African Americans

Principal Investigator: Holly Cukier, PhD

Organization: University of Miami

Abstract: For the research period of the past year, from October 1, 2021 to September 30, 2022, the team has made steady progress on using induced pluripotent stem cells (iPSCs) to model Alzheimer's disease (AD). Efforts to transition three iPSC lines that were generated internally—one African American case with an ABCA7 deletion and two African American control lines—from growing on mouse embryonic fibroblasts (MEFs) to grow on the feeder-free matrix vitronectin were not working and so the team switched efforts to reprogramming two of the lines directly onto a feeder free matrix (control AD 380188 and case AD38152). These lines were successfully reprogrammed, and multiple clones established on the feeder free matrix of vitronectin and growing in StemFlex Medium (STEMCELL Technologies). Each of the lines are in the process of were validated by immunocytochemistry (ICC) of pluripotency markers, karyotyping to ensure that there are not any gross chromosomal abnormalities, and testing negative for Sendai virus. Research team members are also in the process of writing a paper describing one of the ABCA7 lines from an Alzheimer's disease patient to be submitted for publication in the journal Stem Cell Research. For the microglia specific work, the research staff continued working with African American iPSC lines from Alzheimer's disease patients with the 44 base pair ABCA7 deletion (387780 and 392081) and African American control lines (380188, and iPS80, obtained from the University of California, Irvine). Hematopoietic progenitor cells (HPCs) were generated for all four cell lines, in case-control pairs to ensure that if there was variation due to the differentiation protocol itself, it wouldn't be attribute to the ABCA7 deletion. Next, the HPCs will be further differentiated into microglia cells using the STEMCELL Technologies reagents and evaluated for phenotypes in the cells that could be a result of having the ABCA7 deletion. There have also been minor delays in the research due to supply chain issues and the Primary Investigator (PI) of the project going on Family Medical Leave (FMLA) for three months during this time frame. Through the experiments outlined in this proposal, the team aims to reveal the role of ABCA7 in AD pathogenesis, a gene involved in AD across diverse populations.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A18 Functional analysis of UNC13B gene polymorphism in a Hispanic population with Alzheimer Disease.

Principal Investigator: Katrina Celis, MD

Organization: University of Miami

Abstract: Research staff continue to work on the functional characterization of the induced pluripotent stem cell (iPSC) derived neurons generated from five lines, three from individuals carrying the UNC13B Asp238Glu variant and two without the variant from the family studied. Previoulsy, researchers presented the bulk RNA sequencing data results looking at expression levels withing the linkage area in chromosome 9. This report is presenting the results of the Alzheimer Disease (AD) phenotype analysis performed using the Human amyloid beta (Aβ)40 and Aβ42 enzyme-linked immunoassay (ELISA) Kits (catalog number: KHB3481 and KHB3441) for detection of Amyloid Beta 40 (Aβ40) and 42 (Aβ42) in the five iPSC derived neurons previously generated. To accomplish this, the supernatant was collected from three technical replicates of each line at day 60 and 75 of maturation and measured the concentration of Aβ40 and Aβ42. For the analysis, research staff first generated standard curves over the range of 0 to 500 pg/mL for both Aβ40 and Aβ42 and then compared concentrations at different maturation days and Alzheimer Disease status. The results showed that Aβ40 and Aβ42 levels are age-dependent even in cognitive normal individuals, supporting previous evidence. The research also demonstrated an increased Aβ40/Aβ42 ratio at day 75 compared to day 60 of maturation, which has been reported to drive tau pathology in neural cell models of Alzheimer Disease. The research team is still in the process of increasing the sample size and to corroborate previous results using four additional iPSC lines from two individuals with the UNC13B Asp238Glu variant and two unrelated non-AD individuals. A setback was experienced while differentiating these lines into neuron progenitor cells (NPC) as the lines needed multiple rounds of cleaning and passaging to obtain the number of cells needed for functional analysis. In addition, as part of the transcriptomic analysis, the research team performed in situ Hi-C from one AD iPSC derived neuron line at day 60 of maturation. The in situ Hi-C library was prepared from ~ two to five million neurons using the protocol adapted from Rao et al. For robust enhancer-promoter interaction mapping, DeepLoop and HiCorr pipeline was used to correct Hi-C bias at sub-TAD level to identify Hi-C loops4,5. This data is currently being analyzed.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A19 Postdoctoral Fellowship in Cross-Cultural Neuropsychology and Cognitive Neuroscience

Principal Investigator: Rosie Curiel Cid, PhD

Organization: University of Miami

Abstract: During Dr. Hincapie's postdoctoral fellowship training year at the Center for Cognitive Neuroscience and Aging, Psychiatry Department, University of Miami Miller School of Medicine, Dr. Hincapie has continued to receive extensive training related to the administration, scoring, and interpretation of traditional neuropsychological assessments methods used for the detection and diagnosis of AD+ADRD. Dr. Hincapie is extensively involved in procedures related to clinical research protocols, and the operational aspects of active research studies including

recruitment efforts, data entry and management, protocol implementation, and providing institutional trainings to research staff and doctoral students. Dr. Hincapie has collaborated on three different scientific manuscripts and four poster presentations directly related to the clinical manifestations of AD and biological markers related to AD progression. Dr. Hincapie attended weekly consensus conferences with a multidisciplinary team and is managing neuroscience didactic series. During the past year, Dr. Hincapie has clinically evaluated dozens of individuals in order to foster proper evaluation methodology for different ethnic and cultural groups for early-stage mild-cognitive impairment.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Loewenstein DA, Curiel-Cid RE, Kitaigorodsky M, et al. Persistent failure to recover from proactive semantic interference on the cognitive stress test differentiates between amnestic mild cognitive impairment, pre-mild cognitive impairment and cognitively unimpaired older adults. *J Alzheimers Dis*. 2022;90(1):313-322. doi:10.3233/JAD-220348.

Curiel-Cid RE, Hincapie D, Kitaigorodsky M, et al.The computerized LASSI-BC test versus the standard LASSI-L paper-and-pencil version in a community-based study. *J Prev Alzheimers Dis.* 2021;8(2):135-141. doi:10.14283/jpad.2021.1.

Kitaigorodsky M, Curiel-Cid RE, Hincapie D, et al. A-144 A cross-validation study of the Hopkins verbal learning test (HVLT-R) with Hispanic Spanish speaking older adults. *Archives of Clinical Neuropsychology*. 2022;37(6):1298. doi:10.1093/arclin/acac060.144.

Patents: None at the time of reporting.

Grant#: 21A20 Carotid Ultrasound Imaging Markers of AGINg and Endothelial function in Risk of Alzheimer's Disease: The Florida IMAGINE Study of AD Risk

Principal Investigator: Tatjana Rundek, MD, PhD

Organization: University of Miami

Abstract: The purpose of this study is to determine the burden of carotid ultrasound Imaging Markers of AGINg and Endothelial function (IMAGINE) in participants at risk of Alzheimer's disease (AD), investigate the impact of the IMAGINE burden on magnetic resonance imaging (MRI) measures of cerebral small vessel disease (CSVD), on the severity of neurodegeneration on volumetric magenetic resonance imaging (MRI) and amyloid positron emission tomography (PET), and investigate the impact of IMAGINE burden on cognitive performance.

The first research hypothesis is that carotid IMAGINE measures of carotid artery structure and function are prevalent, with over 50% of participants having plaque or other ultrasonographic measures above the median (or BFV below the median) and that these measured more prevalent in Hispanic-Latino (HL) participants. The research team continues performing a high-resolution carotid ultrasound imaging in the participants enrolled to the One Florida Alzheimer's Disease Research Center (1FL ADRC). Research members are analyzing carotid IMAGINE measures of arterial wall structure including carotid intima-media thickness (cIMT), presence of

carotid plaque and characterization of carotid plaque area, the Gray Scale Median (echodensity index), geometry (common-to-internal carotid artery angle), and assessment of endothelial function (arterial stiffness, blood flow velocity-BFV).

The second research hypothesis is that carotid IMAGINE measures are associated with greater burden of CSVD (WMHV above the median or any presence of SBI, CMB, ePVS), total cortical volume in AD regions, and presence of amyloid. It is expected that these associations are likely more pronounced in HL participants and likely modified by the Apolipoprotein E (APOE)*ε4 status. The research team plans to correlate the IMAGINE measures (once they are collected and measurements fully completed) with MRI markers of CSVD (white matter hyperintensity volume-WMHV, silent brain infarcts-SBI, microbleeds-CMB, and enlarged perivascular spaces-ePVS), neurodegeneration (in AD prone regions), and amyloid load. The analytical plan for these analyses is prepared, the programing codes are completed and ready for implementation once the measurements of IMAGINE markers are completed.

The final research hypothesis is that greater burden of carotid IMAGINE measures are associated with cognitive dysfunction and that these associations are more pronounced in HL participants; mediated by the burden of CSVD, neurodegeneration and amyloid load; and likely modified by the APOE*ε4 status. Team members plan to analyze the effects of IMAGINE markers on both novel as well as standard neuropsychological measures tapping different cognitive domains (failure to recover from semantic interference, attention, global memory impairment, executive function, language, visuospatial skills/praxis). The analytical plan for these analyses is prepared, the programming codes are completed and ready for implementation once the measurements of IMAGINE markers are completed. Subject to periodic review due to statutory requirements Rev. 10.1.2022 v.2.

Additionally, the research team has continued to perform research and operational IMAGINE meetings were held bi-weekly. The meetings focused on: study progress, recruitment techniques, and strategies for subject transportation; reviewing protocol safety measures; maintaining quality of data collection; ensuring quality of post-processing and analyses of ultrasound and MRI scans; and planning the next steps with respect to quality control and analyses.

The Principal Investigator and study coordinator/lead technologist recently made significant progress after employing successful recruitment techniques. The recruitment total increased with 11 new study subjects enrolling in the study for a total of 19 participants enrolled. The study visits were conducted using good clinical practice and in accordance with the protocol. The team continues to receive positive feedback about learning their carotid ultrasound scan results. The research team still face the challenge of enrolling from the 1FL ADRC enrollment pool, which widely consists of subjects from lower socioeconomic income scales. Research team members addressed this challenge with partners at 1FL ADRC and the Center for Cognitive Neuroscience and Aging (CNSA). These successful recruitment techniques were focused on ways to help provide transportation and/or offset the travel cost for the study visits.

The study team PhD student at the University of Miami School of Biomedical Engineering (BME), Taylor Ariko (under mentorship of Dr. Rundek, the PI) continues to work on the MRI post-processing pipeline under the supervision of the study physicist, Dr. Mohammed Goryawala. Dr. Goryawala has already programed and coded the pipeline for WMHV and DTI image sequences and continues to process these images. There are no direct challenges with

this activity. However, many groups that process MRI images and other large sets of data (big data) experience challenges with storing large datasets and efficiently managing data. The research team is working with the University of Miami IT to resolve this issue and find the best solution to support research MRI data storage and data management

Follow on Funding: None at the time of reporting.

Collaborations: The IMAGINE core research team has been collaborating with the UM Center for Cognitive Neuroscience and Aging (CNSA, led by Dr. David Loewenstein-Director and Dr. Rosie Curiel-Co Director), research team and the One Florida Alzheimer's Disease Research Center (1FL ADRC). The IMAGINE core and CNSA research collaborators met frequently resulting in successful recruitment strategies that increased enrollment. The recruitment strategizing will continue. Several subjects are already scheduled for the next reporting period. Subject to periodic review due to statutory requirements Rev. 10.1.2022 v.2. Collaboration with the University of Miami Department of Radiology has been maintained via scheduled bi-weekly meetings assessing magnetic resonance imaging (MRI) imaging quality control (QC) with Dr. Mohammed Goryawala, the study physicist-investigator. The PhD Biomedical Engineering trainee in the IMAGINE study Taylor Ariko, continued to be mentored and trained on QC for the MRI pipeline process and analysis plan of the MRI and ultrasound scans.

Journals: Rundek T, Del Brutto VJ, Goryawala MZ, et al. Associations between vascular risk factor and perivascular spaces in adults with intact cognition, mild cognitive impairment, and dementia. *J Alzheimers Dis*. 2022;89:437-448. doi:10.3233/JAD-215585.

Patents: None at the time of reporting.

Grant#: 21A21 Regulation of proteostasis by sleep in Alzheimer's disease

Principal Investigator: Grace Zhai, PhD

Organization: University of Miami

Abstract: Sleep disturbance and aberrant sleep patterns are commonly observed in patients with Alzheimer's disease (AD). Growing evidence suggests that in addition to being a symptom, sleep disturbances can also drive the progression of neurodegeneration. Neurodegeneration in AD patients is marked by the formation of protein aggregates in the brain. The complex interplay between sleep and protein homeostasis remains largely uncharacterized in part due to the limitations of the animal models that could allow simultaneous sleep behavior monitoring/manipulation and cellular and biochemical analysis *in vivo*.

Research staff proposes an integrated approach to characterize the molecular interplay between sleep regulation and neurodegeneration *in vivo* in Drosophila. The main goal of this project is to investigate the cellular and molecular mechanisms by which sleep regulates protein misfolding, aggregation, and clearance in Drosophila models of AD. The proposed research is built on preliminary data showing the impact of sleep disruption on protein aggregation. This study will integrate high resolution immunofluorescent and electromicroscopic imaging, comprehensive biochemical assays, sleep monitoring and analysis, and neuronal functional recordings to answer the following key questions: how sleep disruption affects protein aggregation and clearance; whether a disrupted sleep pattern changes the amyloid-like

biochemical properties of protein aggregates; and how disrupted sleep homeostasis alter neuronal cellular changes, including mitochondrial dynamics and function, microtubule-based axonal trafficking, and synaptic integrity. This project will reveal the cellular mechanisms that connect sleep disruption to proteotoxicity and in so doing, will form the basis for innovative neuroprotective strategies that may halt or reverse AD progression.
This proposed collaborative project will address for the first time, how sleep regulates protein homeostasis in neurodegeneration. The proposed work will potentially lead to the design of novel disease-modifying therapies based on targeting sleep dysregulation, which would have far-reaching implications for alleviating neurodegenerative diseases and improving the quality of life of patients suffering from AD.

Progress report: In the first year of the grant, research staff has successfully gathered experimental tools and reagents. Specifically, the research team has: established the sleep monitoring system for AD model flies; set up the experimental protocols for sleep modulation paradigms, which include the sleep disruption and sleep induction; and set up genetic crosses to obtain AD model flies that would subject to sleep modulation. The work done by the research team has contributed to a publication in eLIFE where the team characterized the structural basis of Tau aggregation. The project is proceeding on schedule and on-time delivery of the results is expected.

Follow on Funding: None at the time of reporting.

Collaborations: This is a multi-disciplinary collaborative project between Dr. Grace Zhai, PhD in Department of Molecular and Cellular Pharmacology in Miller School of Medicine and Dr. Sheyum Syed, PhD in Department of Physics in University of Miami. Given that Dr. Zhai's research lab is located in the medical campus and Dr. Syed's lab is on the Coral Gables undergraduate campus, this project provides training opportunities to both undergraduate, graduate and medical students to collaborate on addressing the issue of Alzheimer's Disease.

Journals: Zhang S, Zhu Y, Lu J, et al. Specific binding of Hsp27 and phosphorylated Tau mitigates abnormal Tau aggregation-induced pathology. *eLife*. 2022,11:e79898. doi:10.7554/eLife.79898.

Patents: None at the time of reporting.

Grant#: 21A23 Effects of novel glucagon-like peptide-1 receptor (GLP-1R) agonists on comorbities in Alzheimer's disease with diabetes mellitus

Principal Investigator: Mark Kindy, PhD, FAHA

Organization: University of South Florida

Abstract: The research team has started work and has exciting data. The team has continued the generation of quantities of the P5 and Fc-P5 for the studies. The team is also screening more compounds that might have beneficial effects and have been testing the compounds *in vitro* (neuronal cell lines and primary neuronal cultures) to determine the impact of the agonists on neuronal survival and outcomes. The research team has shown that the glucagon-like peptide-1 receptor (GLP-1R) agonists can attenuate the expression of both amyloid beta (Aβ) generation and tau phosphorylation in a dose dependent fashion. The research team is

continuing testing the compounds in several different animals models of Alzheimer's disease (AD). In the three different amyloid precursor protein (APP) transgenic mice (APP/PS-1 (presenilin-1), 5XAPP, and tau mice), the P5 and P5-Fc both show a dose dependent reduction in Aβ peptide levels and attenuate the deposition of Aβ in the brains of the mice. In the tau transgenic mice, the P5 and P5-Fc both show a reduction in tau phosphorylation and accumulation of neurofibrillary tangles in the brain. Team members are continuing to carry out studies to determine the impact on behavior, inflammation, and other parameters. The data indicates that the compounds show improvement in the behavioral parameters, reduction in inflammation and oxidative stress. In the APP X db/db mice, the AD pathology is worse in the presence of diabetes. Using the P5 and P5-Fc both showed improved (reduced) diabetic parameters (glucose and A1c) and also significantly reduced the AD pathology in the mice. Research team members are beginning to write up the results for submission of publications.

Follow on Funding: Veterans Affairs, $650,000

Collaborations: This is a collaboration with Moffitt Cancer Center. The Co-Investigator is Dr. Patsy McDonald in the Cancer Physiology Program at the Moffitt Cancer Center. Both undergraduate and graduate students are working on the project for USF. Undergraduate students (two) from the College of Arts and Sciences (CAS) and graduate students (three) from the Taneja College of Pharmacy (TCOP) master's graduate program. There are also have five PharmD students that are working on the project. In addition, research staff are collaborating with Drs. Cheryl Kirstein and David Diamond in the Psychology Department at the University of South Florida (USF). Research members are also collaborating with Dr. Randy Seeley at the University of Michigan who is providing the team with the GLP-1r KO mice for subsequent studies.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 21A24 Exploiting molecular chaperones to understand the impact of tau aggregation on prion-like spreading in AD

Principal Investigator: Laura Blair, PhD

Organization: University of South Florida

Abstract: The main goals of this research project are to identify molecular chaperones that alter tau seeding and release, which is implicated in Alzheimer's disease (AD) pathogenesis and may identify novel therapeutic targets. During this reporting period, the research staff used a cell-based assay that was optimized in the prior funding period to identify molecular chaperone regulators of tau seeding. This is important, since tau accumulation and spreading are associated with Alzheimer's disease progression. Molecular chaperones were selected as the proteins to be screened, since they are enriched with members that control protein triage. This screen identified six specific molecular chaperones that reduce tau seeding and one that significantly increases tau seeding. Interestingly, these "hits" were concentrated in three molecular chaperone families, which supports additional focus on these families (Cyclophilins, DnaJs, and Hsp90) in particular for the regulation of tau seeding. Secondary and tertiary assays of tau accumulation in separate cell lines were used to confirm the results of these assays. The

follow up assays confirmed that most of these hits lower tau levels when they are overexpressed, but only two of the hits were found to increase tau accumulation when their levels were reduced. These data have prioritized two discrete molecular chaperones, DnaJB1 and DnaJB6, for their role in regulating tau accumulation. Research is ongoing to understand the direct and indirect effects of these proteins on tau and their ability to regulate tau in neurons isolated from animal models of tau accumulation. Upcoming studies will determine the effects of these molecular chaperones on regulating tau accumulation and functional outcomes in vivo. The models for this work were established in the lab this funding period and additional tools for these studies are currently in development. One paper was written, revised, and accepted during this funding period based on the data from the screen on the cyclophilin family, together with other data from the research staff. This project will identify novel targets to regulate tau pathogenesis in Alzheimer's disease.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Hill SE, Esquivel AR, Rodriguez Ospina S, Rahal LM, Dickey CA, Blair LJ. Chaperoning activity of the cyclophilin family prevents tau aggregation. *Protein Sci*. 2022;31(11):e4448. doi:10.1002/pro.4448.

Patents: None at the time of reporting.

Grant#: 21A25 Project Title: Building Dementia Care Network and Community Capacity for Older Asian Immigrants with Limited English Proficiency in Florida

Principal Investigator: Nan Sook Park, PhD, MSW

Organization: University of South Florida

Abstract: Based on the dementia care network (DCN) conceptualization, the project has three research objectives: 1) To conduct needs assessment for older Asian Americans (aged 50 and older) and assess the community resources and barriers to deliver ADRD services. In part, older Asian Americans will participate in a survey to assess their knowledge and awareness of both ADRD and access to ADRD-related services. 2) To build community capacity for dementia care by establishing ADRD care network with community leaders and older Asian Americans and their families. 3) To establish web-based resources for ADRD and relevant services and disseminate the resources. Taken together, the goal of the project is to build community capacity for early detection of Alzheimer's disease and related dementias (ADRD) by promoting the knowledge and service utilization of the three largest groups of older Asian Americans in West Florida (Chinese, Korean, and Vietnamese), their families, and ethnic community leaders on ADRD knowledge and related services.

Due to the ongoing threats of the pandemic, in-person surveys had to be delayed for several months but the following activities have been conducted: development of needs assessment and survey protocol and survey instruments among older Asian Americans. The research team submitted a proposal on the survey with older Asian Americans, received approval of the Institutional Review Board (IRB), and compiled the survey instruments based on the literature. Based on the comments from the investigators and consultants, the research team revised,

finalized, and translated the survey questionnaire into four different Asian language versions (Simplified Chinese, traditional Chinese, Korean, and Vietnamese). Research staff have been developing community resources for recruitments of survey participants and community leaders. The research staff were able to conduct the surveys with 330 participants and entered the survey data in Excel and REDCap. Research staff validated the accuracy of the data entry and followed up with participants where possible when there were missing responses.

The research team has begun establishing an ADRD care network with community leaders by conducting focus group interviews, building social connection with community leaders in the three Asian communities by engaging in community activities and serving on advisory boards. Research staff have begun building the list of community leaders of the Chinese, Korean, and Vietnamese communities. Community resources for the Korean community have been updated and recorded. The research team submitted proposal on the focus group interviews with the community leaders to the IRB. The research team has also been developing web-based resources for ADRD and ADRD-related services by building local resources and connections, which are integral components of the built-in dementia care network for Asian Americans.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix B
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2019-2020

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
20A05	University of Miami	Hong Jiang, MD, PhD	250,000.00	03/31/23	No	No	Yes
20A06	University of South Florida	Lianchun Wang, MD	250,000.00	09/30/22	No	No	No
20A08	University of Florida	Melissa Armstrong, MD, MSc, FAAN	374,660.00	04/30/23	No	Yes	Yes
20A09	Florida State University	Aaron Wilber, PhD	250,000.00	03/31/24	No	Yes	No
20A11	University of Miami	Elizabeth Crocco, MD	248,590.00	10/31/22	No	No	No
20A13	Carlos Albizu University	Miriam Rodriguez, PhD	250,000.00	09/30/22	No	Yes	No
20A14	University of Miami	Claes Wahlestedt, MD, PhD	249,959.00	05/31/23	No	No	No
20A16	University of Florida	Sara Burke, PhD	250,000.00	03/31/23	No	Yes	Yes
20A17	Florida Atlantic University	Qi Zhang, PhD	100,000.00	04/30/22	No	Yes	Yes
20A18	University of South Florida	Saeid Taheri, PhD	250,000.00	03/31/24	No	No	No
20A19	University of Miami	Noam Alperin, PhD	171,790.00	04/30/23	No	No	No
20A20	Mayo Clinic Jacksonville	Maisha Robinson, MD	171,790.00	09/30/22	No	No	No
20A21	Mount Sinai Medical Center of Florida	Ranjan Duara, MD	171,790.00	11/30/22	No	No	No

Grant#: 20A05 Retinal biomarkers for monitoring vascular contributions to Alzheimer's disease

Principal Investigator: Hong Jiang, MD, PhD

Organization: University of Miami

Abstract: The research team started to recruit study subjects in this period from the parental National Institutes of Health (NIH) R01 studies (PIs: Loewenstein R01 AG047649 and Curiel R01 AG05563801A1) for in-person study visits. 17 study subjects who met the inclusion/exclusion criteria were enrolled and imaged.

Meanwhile, research staff continued to recruit study subjects from the Department of Neurology and these study patients who met the inclusion/exclusion criteria were contacted. Total 46 study subjects finished in person visits. Therefore, total 63 study subjects finished study visits. Meanwhile, the team continued to perform the work, under the scope of work, supported by this grant, which focused on further analysis of previously acquired data and manuscript preparation.

To determine the effect of different software versions on the measurement of retinal vessel densities using optical coherence tomography angiography (OCTA) in normal subjects, thirty-two eyes of eighteen healthy subjects were imaged using two OCTA devices: the Optovue RTVue and the Zeiss Cirrus. Research team members found the differences of the vessel density measurements between software versions and between devices. This is the first study to determine that different software versions with various intraretinal layer segmentation methods affect the vessel density measurements. The manuscript has been published with Current Eye Research. 2021 Mar;46(3):341-349. PMID: 32767906. Research staff also did a

critical review of the literature regarding retinal vascular changes in Alzheimer's disease and its prodromal stages, focusing on functional and structural changes of large retinal vessels (vessels visible on fundus photos) and microvasculature (pre-capillary arterioles, capillary, and post-capillary venules) that are invisible on fundus photos. The manuscript has been published online by Journal of Neuro-ophthalmology. PMID: 33136677. Previously acquired data was analyzed to characterize the changes of retinal microvascular density and their relations to cognitive function in the healthy older people without known cognitive impairment after an eight-week high-speed circuit resistance training program (HSCT). This is the first study to reveal that the individual response of the singuar value decomposition (SVD) was related to the improvement in the cognition in cognitively normal older people after high-speed circuit resistance training program (HSCT). The manuscript has been published by Experimental Gerontology. 2020 Dec;142:111114, PMID: 33132156.

Research team members continue to analyze previously acquired data to examine the associations between retinal microvascular density, cognition, and physical fitness in healthy older adults with no reported cognitive decline. This is the first study to reveal the association between retinal vessel density and cognition as measured with Montreal Cognitive Assessment (MoCA) in healthy older adults with no reported cognitive decline. The manuscript has been published by Experimental Gerontology 2021 Jun 6;152:111433. PMID: 34091000 The research team developed a new method to analyze retinal capillary function after high-speed circuit resistance training in healthy older adults. The abstract tilted "Improvement of Retinal Capillary Function after High-Speed CircuitResistance Training in Healthy Older Adults" was accepted by the Subject to periodic review due to statutory requirements. The work will be presented as a featured poster in the annual meeting at the North American Neuro-Opthalmology Society (NANOS) 48th Annual Meeting taking place February 12-17, 2022 at the JW Marriott, Austin in Austin, Texas. The research team also analyzed repeatability of retinal vessel density acquired using optical coherence tomography angiography (OCTA) and the abstract has been accepted by the Association for Research in Vision and Opthamlmology (ARVO) annual conference being held in Denver, May 1-4, 2022.

Follow on Funding: None at the time of reporting.

Collaborations: Two medical students at the University of Miami are receiving training.

Journals: Wang H, Hu H, Gregori G, Zhang J, Jiang H, Wang J. The effect of software versions on the measurement of retinal vascular densities using optical coherence tomography angiography. *Curr Eye Res*. 2021;46(3):341-349. doi:10.1080/02713683.2020.1801756.

Jiang H, Wang J, Levin BE, et al. Retinal microvascular alterations as the biomarkers for Alzheimer disease: are we there yet? *J Neuroophthalmol*. 2021;41(2):251-260. doi:10.1097/WNO.0000000000001140.

Fang M, Strand K, Zhang J, et al. Characterization of retinal microvasculature and its relations to cognitive function in older people after circuit resistance training. *Exp Gerontol*. 2020;142:111114. doi:10.1016/j.exger.2020.111114. (Subject to periodic review due to statutory requirements).

Zhang J, Strand K, Totillo M, et al. Improvement of retinal tissue perfusion after circuit resistance training in healthy older adults. *Exp Gerontol*. 2021;146:111210. doi:10.1016/j.exger.2020.111210.

Fang M, Strand K, Zhang J, et al. Retinal vessel density correlates with cognitive function in older adults. *Exp Gerontol*. 2021;152:111433. doi:10.1016/j.exger.2021.111433

Patents: None at the time of reporting.

Grant#: 20A06 The Role of Extracellular Tau in Endothelial Cell Biology

Principal Investigator: Lianchun Wang, MD

Organization: University of South Florida

Abstract: The research team is reporting the following research progress on the Specific Aims.

Aim one. Delineate the proangiogenic function of tau *in vitro*, ex vivo and *in vivo*.

In preliminary studies reported in the proposal, the research team has shown that tau increased biliary epithelial cells (BEC) permeability and promoted BEC proliferation and migration *in vitro*. Tau also robustly induced neovascularization in a Matrigel plug angiogenesis mouse model *in vivo*. From April 4, 2020 – June 3,2020, the research team examined the effect of tau on endothelial cell morphogenesis on coated Matrigel. Tau potently promoted endothelial cell tube formation, similar to the positive control VEGF165. From January 1, 2021 – October 30, 2021, the research team optimized the experimental condition and determined that tau potently induced vascular permeability in mouse brain, comparable to positive control VEGF165. Research team members have worked to test if the cell surface receptor lipoproteins receptor-related protein 1 (LRP1) mediates the proangiogenic effect of tau in endothelial cells. From January 1, 2021 – October 30, 2021, research staff have generated endothelial cell-specific LRP1 knockout mice and observed that deficiency of endothelial LRP1 diminished tau-induced neovascularization *in vivo* in the angiogenesis bioreactor assay. From November 1, 2021-January 30, 2022, using the Crispy-Cas9 technique, research staff generated LRP1-deficient human brain endothelial cell line and are ready to test if tau binds to LRP1 to induce brain endothelial cell proliferation, migration and cord formation. The research team also generated inducible endothelial cell-specific Ext1 (heparan sulfate) deficient mice, and will test if tau-induced angiogenesis *in vivo* depends on endothelial heparan sulfate.

Aim two. Delineate the molecular mechanisms underlying the proangiogenic function of tau and its crosstalk with (Vascular Endothelial Growth Factor) VEGF signaling.

In *in vitro* studies, research staff have determined that tau and VEGF165 mutually inhibit their activity to induce brain endothelial cell migration as shown in Preliminary studies in application. From April 2, 2020 – June 3, 2020, the team worked to optimize cell surface tau binding assay with heparin as testing molecule. Heparin acts in a concentration-dependent manner to inhibit human brain cell surface tau binding. During From July 1, 2020 – September 30, 2020, the research team was able to extend this study to search for the heparan sulfate proteoglycan that mediates cell surface tau binding and cellular uptake. By proteomics analysis, staff observed that tau pulled down much less cell surface heparan sulfate proteoglycan CD44 from heparan

sulfate-deficient mouse lung endothelial cells (the Ext1 knockout cell line) compared to wildtype mouse lung endothelial cells. From October 1, 2020 – January 30, 2021, research staff determined both mouse and human brain endothelial cells do not express CD44. To search for the heparan sulfate proteoglycans that mediates proangiogenic tau protein, from January 1, 2021 – June 30, 2021, the research team worked to isolated primary mouse brain endothelial cells. The research team can get highly purified mouse brain endothelial cells, but the yield remains very low. Research staff need to further optimize the current procedures to get enough cells for proposed further experimentation. Researchers also carried out angiogenesis array and found that tau significantly downregulated the expression of C-X-C motif chemokine 10 (CXCL10), a chemokine that inhibits angiogenesis. From July 1, 2021 -October 30, 2021, research staff have determined that recombinant CXCL10 inhibits tau-induced angiogenesis in the matrigel plug mouse model. Now are moving to generating AAV-CXCL10 which will be used to test if CXCL10 could normalize dysregulated angiogenesis in tau-overexpressing mouse model. From November 1, 2021 – January 30, 2022, staff were still working to get the plasmids to prepare AAV90CXCL10. Through collaboration, the research team received the plasmids for AAV9-VEGFA preparation, will work to generate AAV9-VEGFA.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A08 Communication of Dementia Diagnoses: Investigating Patient, Family, and Physician Experiences and Developing Best Practices

Principal Investigator: Melissa Armstrong, MD, MSc, FAAN

Organization: University of Florida

Abstract: The University of Florida (UF) research team has published one article and has to additional manuscripts in development (one submitted and in revision, one in preparation) resulting from interviews with clinicians, patients, and caregivers about experiences giving and receiving dementia diagnoses. The published manuscript, titled "Clinician approaches to communicating a dementia diagnosis: An interview study" was published April 2022 in PLOS ONE. This manuscript reports the results of interviewing 15 Florida-based clinicians about their approaches to giving dementia diagnoses. Clinicians reported using patient- and family-centered communication practices including checking patient understanding, communicating empathically, and involving family members. Clinicians reported positively framing information, including instilling hope, focusing on healthy behaviors, and discussing symptom management. Finally, clinicians reported providing patient/family education and arranging follow up. In the second manuscript from the analysis of clinician interviews, clinicians identified three categories of barriers to giving a dementia diagnosis well - patient and caregiver-related barriers, clinician-related barriers, and barriers related to the triadic interaction. Patient and caregivers-related barriers included lack of social support, misunderstanding the diagnosis, and denial. Clinician barriers included difficulty giving bad news, difficulty communicating uncertainty, and lack of time. Triadic interaction barriers included challenges meeting multiple goals or needs and family

requests for non-disclosure. When asked about their recommendations for best practices relating to giving a dementia diagnosis, participating clinicians recommended fostering relationships, educating patients and families, and taking a family-centered approach. The manuscript reflecting the results of patient and caregiver interviews is currently being edited by team members. The third study aim is to develop consensus-based best practices for giving a dementia diagnosis. The initial multistakeholder meeting was conducted on 5/3/2022 via Zoom. Attendees included general neurologists, dementia specialists, communication researchers, dementia social workers, community education organizers, neuropsychologists, people living with dementia and carepartners for people with dementia who were spread across the state. Dr. Armstrong met with the patient and caregiver participants prior to the in person meeting to solicit their ideas in a one-on-one setting. At the working group meeting, the study team presented an overview of the study purpose, results of the systematic review and Aims one and two, and training on the modified Delphi process. Following training, the Co-Primary Investigator (PI) led a discussion regarding strategies for communicating a diagnosis of dementia and solicited input from all participants.

Follow on Funding: NIA, $3,154,767

Collaborations: University of Florida (Gainesville, FL) is the primary grant site responsible for grant management, regulatory activities (e.g. Internal Review Board (IRB) approval), study management and conduct, inter-site communications, analysis, and manuscript preparation. Dr. Melissa Armstrong (MD, MSc, Department of Neurology) and Dr. Carma Bylund (PhD, Department of Health Outcomes & Biomedical Informatics) co-lead the grant, share responsibilities, and oversee the research coordinator (Noheli Bedenfield) and post-doc (Easton Wollney, PhD). Dr. Mónica Roselli, PhD oversees study conduct at Florida Atlantic University (Boca Raton, FL; Department of Psychology), including regulatory issues, coordination with other sites, interview recruitment, and participation in the Aim three modified Delphi consensus process. Dr. Roselli brings the perspective of an experienced neuropsychologist and multicultural researcher. Dr. Roselli also oversees the site coordinator, Ximena Levy, MD, MPH, MBA. At the University of Miami (Center on Aging; Miami, FL), Rosie Curiel, PsyD oversees study conduct including regulatory issues, coordination with other sites, interview recruitment, and participation in the Aim three modified Delphi process. Dr. Curiel also brings unique experiences working with individuals from varied racial-ethnic backgrounds. Dr. Curiel oversees the site coordinator, Marcela Kitaigorodsky, PsyD, who helped with recruitment and is participating in the modified Delphi consensus process. The modified Delphi consensus process is also engaging other individuals/institutions in Florida, including a representative from Mayo Clinic Jacksonville and one from the University of South Florida (participants in consensus but not grant recipients). Two individuals representing Florida chapters of the Alzheimer's Association are also participating in the consensus process.

Journals: Armstrong MJ, Weisbrod NJ, Bylund CL. Strategies to improve clinician-patient communication experiences for patients with neurologic conditions. *Neurol. Clin. Pract*. 2021;11(6):e896-e900. doi:10.1212/CPJ.0000000000001091.

Wollney EN, Bylund CL, Bedenfield N, et al. Clinician approaches to communicating a dementia diagnosis: an interview study. *PLoS One*. 2022;17(4):e0267161. doi:10.1371/journal.pone.0267161.

Patents: None

Grant#: 20A09 Cortical-hippocampal interactions during sleep in Alzheimer's disease

Principal Investigator: Aaron Wilber, PhD

Organization: Florida State University

Abstract: Alzheimer's disease (AD) is devastating for individuals and society. Impaired learning and memory, particularly in the context of spatial navigation (e.g., driving to the new store across town), is one of its major symptoms. Similarly, rodent models of AD also exhibit impaired navigation. For this proposal, the research team developed and are using a spatial navigation task that mimics the impairments observed in humans (getting lost in new surroundings). There is a great deal of scientific evidence suggesting abnormal communication between two parts of the brain, the parietal cortex and hippocampus, in humans with AD. The team previously published a paper that achieved part of this proposal by showing that interactions between the parietal cortex and hippocampus during sleep are critical to form new memories in a triple transgenic mouse model of AD which present with Tau and amyloid beta (Aβ) pathology. Changes in these brain interactions during sleep could cause impaired learning in AD, especially in early (pre-symptomatic) disease progression as the research team is assessing here. Because activity within the parietal cortex remained intact, the paper also suggests that the mechanism of impaired memory in AD is a failure to bind the components of a memory while the parts of a memory may be intact (e.g., remember taste of breakfast food but not where eaten). The research team is also using a novel approach to understand the contributions of Tau and Aβ in the hippocampus-parietal network to impaired parietal-hippocampal interactions during sleep by reversing the pathology in these brain regions. This approach uses a non-invasive treatment that has undergone human clinical trials with several papers and conference reports showing it is effective. This work is furthering understanding of the fundamental mechanisms of this new treatment approach and is critical for further improving the effectiveness in humans. Also, in parallel the team is confirming these findings in a second mouse model, in which the Aβ sequence is replaced with a non-mutated human Aβ sequence, to more closely mimic sporadic AD. These data suggest that getting lost in new surroundings emerges much later in these mice than the triple transgenic mice (14 vs six months). The team's research is relevant to public health because it increases knowledge about the role of changes in cortical-hippocampal functional interactions and use current technologies to reverse impaired learning. The proposal will expand the knowledge base and establish a new research platform for understanding impaired memory in AD.

Follow on Funding: NIH/NIAAA, $442,433

Collaborations: Undergraduate students from FSU received training and performed research activities as part of this project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A11 Building an Advanced Cognitive and Biomarker Registry for African American Older Adults At-Risk for Alzheimer's disease

Principal Investigator: Elizabeth Crocco, MD

Organization: University of Miami

Abstract: All planned project start up procedures have been completed successfully. The research personnel has been hired,the study has obtained Internal Review Board (IRB) approval, and the infrastructure for neuroimaging and blood collection has been established. This includes all standard operating procedures that ensure mandatory compliance with restrictions imposed by the COVID-19 pandemic. Outreach and recruitment efforts have been made including contact with local community partners that will facilitate the recruitment of participants for this study. The study timeline was delayed from the initial start date of June 15th, 2020 due to the COVID-19 pandemic; however, as of October 2020, the research team received the institutional approval to resume all in-person outreach, imaging, blood work and clinical and neuropsychological evaluations. To improve recruitment/retention, participants were provided with the option of completing the cognitive evaluation in-person at the Center for Neuroscience and Aging or virtually from their home environment. At that time, the research team engaged with many community centers via teleconference to promote the study. However, many community centers that serve African American individuals in the surrounding area were closed and not offering opportunities that yielded recruitment. It was not until the Spring of 2021 that research staff were able to actively begin recruitment in the community. These circumstances significantly delayed the research team's recruitment targets. To date, 42 participants have been enrolled in the study; 12 during this reporting period. The study team continues to work diligently in recruitment efforts by identifying prospect study participants, reaching out to community partners to share the going research projects and offer community education, and organizing virtual webinars, education forums, and presentations targeted to older adults in conjunction with community partners (e.g. Alzheimer's Florida Association) to increase awareness about brain health and promote the activities performed at the Center. During this reporting period, an additional seven outreach presentations/events were held with a combined total of 33 outreach presentations/events with an attendance of over 3,087 individuals since project start up.

Follow on Funding: None at the time of reporting.

Collaborations: This work has led to stronger community partnerships and collaborations with the One Florida Alzheimer's Disease Research Center (1Florida ADRC) and the southeast Chapter of the Alzheimer's Association.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A13 Relationship between functional measures, cognitive performace, and AD biomarkers between hispanic and white non-hispanic

Principal Investigator: Miriam Rodriguez, PhD

Organization: Carlos Albizu University

Abstract: The manuscript to report the transcriptome analysis of tumor-associated high endothelial venules (HEVs) was recently published by Cancer Immunology Research, and this article was highlighted in, "In the Spotlight" of the same issue of the journal, recognizing the significance of the finding for the immuno-oncology field. This study was also featured online by the Johns Hopkins All Children's Hospital News Letter for general public. The molecular signature of HEVs identified in this study may be useful for guiding immunotherapies and provide a new direction for investigating tumor-associated HEVs and their clinical significance. Research team members are currently investigating the induction of HEVs and tertiary lymphoid structures (TLS) developed around the HEVs in mouse tumors. Staff have been successful to induce these lymphoid structures using a cocktail of cytokines that were selected based on the pathway analysis that was conducted from the differential transcriptome of the vasculature of TLS-rich tumors vs. TLS-free tumors. A therapeutic induction of mature TLS has never been achieved in mouse tumors, and this is a significant breakthrough as it provides mouse models of TLS and anti-tumor immunity development in various types of cancer. More importantly, this cytokine cocktail strategy is fully translatable to clinical use in the future to heighten immune response against cancer by promoting intratumoral TLS formation, which is known to be associated with responses to cancer therapies and patients' survival. Currently, research staff are characterizing the TLS formed in mouse mammary tumors and pancreatic tumors by immunostaining of several marker genes that represent the maturation of TLS, such as the markers for follicular dendritic cells and follicular helper T cells. Researchers are also conducting a characterization of these cells in the tumors by fluorescenceactivated cell sorting (FACS) in parallel. This TLS-inducing regimen appears to have a strong tumor suppressive effect based on the pilot studies, and staff have also observed much improved survival in a mouse model of tumor recurrence. In this model, mice were treated with the cytokine cocktail (neoadjuvant) and surgical tumor resection followed by re-inoculation of the same tumor cells. These results suggest a development of strong anti-tumor immunity by the experimental therapy.

Follow on Funding: NIH/NCI, $228,750

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A14 Contributions of Histone Deacetylase 8 (HDAC8) to Alzheimer's Disease Pathogenesis

Principal Investigator: Claes Wahlestedt, MD, PhD

Organization: University of Miami

Abstract: The purpose of this project is to validate whether histone deacetylase-8 (HDAC8) inhibition can exacerbate Alzheimer's disease (AD)-like pathogenesis, and whether increased HDAC8 activity can prevent and/or rescue AD-like pathogenesis in AD models. The research team is using both genetic (plasmid-mediated) and pharmacological interventions to increase or

inhibit HDAC8 activity. These studies will help confirm whether HDAC8 upregulation is a suitable target for AD and whether inhibition of this HDAC isoform should be avoided. Context and progress to date: This is a three-year grant that was fully executed in October 2021. The research staff has bought necessary materials, and is conducting experiments on brain cells and animals. Considering the recent shortcomings of AD clinical trials, the research project team and others have proposed targeting epigenetic enzymes as a potential therapeutic approach for AD. One such group of enzymes is Class I HDACs comprising of four members: HDACs 1, 2, 3 and 8. The research staff and others have shown that inhibition of HDACs 1, 2 and 3 can individually present beneficial effects for AD by either increasing neuroprotective genes such as brain-derived neurotrophic factor (BDNF), reducing AD-like pathogenesis and/or increasing learning and memory in animal models. Remarkably little has been shown about the effects of HDAC8 activity on AD-like pathogenesis, although inhibition of HDAC8 has been well-documented as a drug target for the treatment of various types of cancers. Using validated tools, the data suggested that interrupting HDAC8 signaling potentially aggravates AD-like pathogenesis. Since July 2021, research project staff has confirmed that increasing HDAC8 expression and activity in brain cells increases the expression of the neuroprotective molecule BDNF. Research staff has also treated transgenic AD mice with the HDAC targeting small molecule CTI-701 and observed significant increase in novel object object recognition in the CTI-701-treated group compared to vehicle-treated controls. This is meaningful because AD patients lose the ability to recognize objects and faces. The research project staff has also recently discovered that treatment with CTI-701 ameliorates glucose tolerance—a feature that is highly desirable because diabetes is a risk factor for Alzheimer's disease. These data support the hypothesis that HDAC8 activity is neuroprotective and potentially beneficial for Alzheimer's disease. The research team thus hypothesizes that either targeting HDAC class 1 without affecting HDAC 8 or having an HDAC8 activator will be protective against AD and result in increased cognitive performance in AD animal models. In the state of Florida, approximately half a million people currently suffer from AD. Data from the proposed work can validate whether increasing HDAC8 activity is protective and whether inhibiting HDAC8 exacerbates AD, defining a novel therapeutic strategy for AD. This project has the potential to yield a small epigenetic molecule suitable for AD treatment in Florida and beyond.

Follow on Funding: None at the time of reporting.

Collaborations: The Primary Investigators (PIs) (Department of Psychiatry and Center for Therapeutic Innovation), two graduate students (Department of Neuroscience and Programs in Biomedical Sciences (PIBS) and Department of Molecular and Cellular Pharmacology) all at the University of Miami Miller School of Medicine, and two undergraduate students (University of Miami Coral Gables Campus) have been involved with this research project. One Summer undergraduate research fellow (SURF) from Barry University chemistry program also worked on this project .

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A16 Cyclic Ketogenic Therapy as Treatment for Alzheimer's Disease-Related Metabolic Decline, Tau Pathology and Cognitive Impairments

Principal Investigator: Sara Burke, PhD

Organization: University of Florida

Abstract: A defining feature of Alzheimer's disease (AD) is a reduced ability of the brain to use glucose to meet its energetic needs. Within the brain, dysfunctional insulin signaling can promote the pathological aggregation of tau protein that ultimately leads to neurodegeneration, brain atrophy, and cognitive loss. The research team has shown that high fat/low carbohydrate ketogenic diets can improve brain metabolism and enhance cognitive function. Ketogenic diets increase circulating and brain levels of ketone bodies to substitute for glucose in the production of energy. While glucose utilization in the brain is impaired in AD, the utilization of ketone bodies remains intact. Thus, ketogenic diet therapy is likely to improve brain metabolism in individuals with AD thereby changing the disease course to improve cognitive function.

A major barrier to the implementation of nutritional ketogenic therapy, however, is low compliance associated with long-term carbohydrate restriction in AD patients that have increased cravings for sweet foods. The objective of this proposal, which has not changed since initial funding, is to restore brain metabolism in a rat model of age-related metabolic syndrome and AD-related tauopathy with a cyclic ketogenic diet. Cyclic ketosis allows for windows in which one can consume carbohydrates thereby showing greater translational potential than a chronic ketogenic diet. In this last reporting period, research staff have observed that rats with pathological tau protein in the brain had normal spatial memory, but were impaired at the Paired Associates Learning (PAL) task. A comparable task is also used clinically to to measure cognition in humans with Mild Cognitive impairment. Research staff have also observed cyclic ketogenic diet therapy benefits performance in this task. Research staff have recently published a paper documenting changes in the gut microbiome from a ketogenic diet or intermittent fasting therapy that were associated with better cognition.

Finally, the collaboration initiated during the last reporting period with Stephen Anton (Institute on Aging, University of Florida (UF)), and Glenn Smith (Clinical Health Psychology, UF) to initiate ketosis through intermittent fasting recently received a fundable score on an R21 exploratory grant. This will support establishing a human clinical trial to use diet-based therapy to improve cognitive function in older adults. The preliminary data funded by this award were instrumental the study design.

Follow on Funding: NIA/NIH, $419,375

Collaborations: None at the time of reporting.

Journals: Hernandez AR, Watson C, Federico QP, et al. 12 months of time-restricted feeding improves cognition and alters microbiome composition independent of macronutrient composition. *Nutrients*. 2022;14(19):3977. doi:10.3390/nu14193977.

Patents: None

Grant#: 20A17 Amyloid Precursor Protein And Cholesterol As A Novel Druggable Axis For Alzheimer's Disease

Principal Investigator: Qi Zhang, PhD

Abstract: Researchers are close to finishing Aim 1: Establish iN-based platform to test how familial Alzheimer's Disease (fAD) mutations affect neuronal mitochondrial cholesterol (mChol). Using immunocytochemistry, electrophysiology, live-cell imaging, researchers have confirmed that the neuron-like cells differentiated from the wildtype and the amyloid precursor proteins (APP)-knockout human induced pluripotent stem cells (iPSCs) possess all features of neurons being tested. The research team named those cells induced human neurons (hiNs). Using wildtype and APP-knockout hiNs, the research team first tested their membrane mChol content using two refined Filipin staining protocols targeting surface and total mChol separately. In both cases, researchers also co-applied a pan membrane dye (AM1-43) to measure surface or total membrane area, which is a normalization measure in order to control the variation of membrane areas. Researchers confirmed that there was significantly less surface mChol in APP null hiNs than wildtype control. Then, researchers used two new lipid reporters as well as two existing fluorescent dyes to examine mChol homeostasis especially in neuronal synapses, and researchers found that the lack of APP significantly delayed mChol retrieval from surface membrane, which induced a reduction of overall surface mChol likely as a compensatory measure by neurons. Notably, such changes are specific to Chol in the plasma membrane but not other lipids or in other parts of the cell membranes. Then, researchers tested five APP mutants bearing representative fAD mutations. The research team did find that point mutations affecting APP's affinity to mChol or its surface membrane localization have phenotypes resembling those in APP-null cells. Now, researchers are testing more fAD mutations and new mutations targeting on mChol-binding, APP trafficking and its C-terminal fragment (i.e., automatic implantable cardioverter defibrillators (AICD)).

Researchers are also working on Aim 2: Examine how direct manipulation of neuronal mChol affect APP and AD-like pathology. Using novel fluorescent mChol reporters, the researchers quantitatively assess the effects of drugs and protein factors that can alter Chol synthesis, uptake, transport, dispersion, and catabolism. Moreover, researchers are combining those reporters with other neuronal and synaptic markers to perform live-cell imaging of mChol trafficking and synaptic transmission in live hiNs. Researchers have found that exhaustive stimulations challenge neurons ability to maintain mChol homeostasis, especially at axon terminals. And such adversary effects are chronic and likely caused neuronal loss in the cultures. Similarly, reduction of Chol synthesis or uptake, disruption of intracellular mChol transport to surface membranes, or removal of surface mChol increased the burden of mChol homeostasis, and thus made neurons much more vulnerable to stress like prolonged stimulation. While wildtype hiNs can reasonably sustain those challenges to certain degree, APP-null or mutant-expressing ones are vulnerable and cannot sustain for long or when some of those challenges are combined. Moreover, researchers are employing immunostaining, electrophysiology and live-cell imaging to probe how the dysregulation of mChol homeostasis can chronically affecting neuronal functions, especially synaptic transmission and plasticity in the wildtype and APP-null or mutant hiNs.

Follow on Funding: NIMS, $435,581

Collaborations: Researchers have collaborated with Dr. Charles R. Sanders at Vanderbilt University to explore the point mutations of APP that may affect mChol regulation and that are related to the pathogenesis of the disease. (Subject to periodic review due to statutory requirements; Page 14 of 15 rev. 7.1.2021). The researchers have collaborated with Dr. Lei Liu at Harvard University to study the changes of proteins related to Alzheimer's diseases and cholesterol regulation in aging APP-knockout mice.

Journals: Thomas D, Rubio V, Iragavarapu V, et al. Solvatochromic and pH-Sensitive Fluorescent Membrane Probes for Imaging of Live Cells. *ACS Chemical Neuroscience*. 2021;12(4):719-734. doi:10.1021/acschemneuro.0c00732.

Alamgir S, Pelletier OB, Thomas D, Rubio V, Stawikowski MJ, Zhang Q. Measuring Membrane Lipid Turnover with the pH-sensitive Fluorescent Lipid Analog ND6. *Journal of Visualized Experiments*. 2021;(173). doi:10.3791/62717.

Patents: None at the time of reporting.

Grant#: 20A18 Impact of cerebrovascular pathology on Alzheimer's disease and other dementia

Principal Investigator: Saeid Taheri, PhD

Organization: University of South Florida

Abstract: In most of age-related cognitively declined multiple pathologies coexist that vary across the aging spectrum. The manifestation of these pathologies can be observed via various biomarkers, including behavioral and psychological symptoms, *in vivo* imaging, cerebrospinal fluids and blood markers.

However, behavioral and psychological symptoms, as leading markers, have different prevalence onset and course. Therefore, the course, onset and pattern of biomarkers on cerebrovascular pathology are valuable tools in understanding the disease. Research staff hypothesizes that longitudinal epidemiological data of cerebrovascular pathologies aid in understanding the role of vascular pathology in Alzheimer's disease (AD). To tests this hypothesis staff will recruit cognitively impaired patients with and without the symptoms of AD and investigate the current state of disease by using magentic resonant imaging (MRI) and biochemical data, along with epidemiological data. Knowledge about the impact of vascular disease on AD enables the research team to tailor treatments.

Progress:

1- Research staff are advertising for patient recruitment by posting the study flyer in Tampa General Hospital (TGH) and University of South Florida (USF) Morsani.

2- Staff continue phone interviewing more volunteers for recruitment.

3- Staff continue recruiting more patients into the study by taking informed consents.

4- Staff are looking at the TGH Radiology in the downtown for MRI of the study subjects.

5- Staff have submitted a proposal to Bright Focus for funding this research.

6- Staff are working to prepare an RO1 proposal for the February submission. Staff are in touch with the National Institutes on Health (NIH) and National Institute on Aging (NIA) program officer. The program officer is collaborating with the research team to make the proposal successful.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A19 Lifestyle Stressors of Hippocampus and AD Related Brain Regions: Potential for Intervention

Principal Investigator: Noam Alperin, PhD

Organization: University of Miami

Abstract: The research team has made modest progress with subject recruitment over the last period. The subjective improvement of sleep equality as measured by the insomnia severity index (ISI) is impressive. The results from the last four subjects who received cognitive behavioral therapy for insomnia (CBTI) from Dr. Rene Hernandez-Cardenache, Psy.D. were as follow. Prior to CBTI, ISI ranged from ISI of 26 (severe clinical insomnia) to 17 (moderate clinical insomnia). Post intervention, the ISI ranged from 11 (below clinical level of insomnia) to two (no clinical insomnia). Evaluation at six-month post intervention is pending. The true efficacy of CBTI will be determined once three follow up magnetic resonance imaging (MRI) scans to assess the change in the rate of tissue volume loss in the hippocampus compared to subject who did not receive CBTI have been completed. The work done by this research team will be one of the first to assess the efficacy of CBTI using objective imaging biomarkers of AD.

In parallel to the team's recruitment efforts, research staff have extended the analysis of the data collected during the previous FL DOH award. The data collected allowed the research team to determine an important and basic question related to the pathophysiology of AD, does reduction of cerebral blood flow (CBF) precedes or follows the tissue loss in AD-prone brain regions. Reduction in total cerebral blood flow (tCBF) and increased brain atrophy are linked to cognitive impairment and Alzheimer's disease (AD). In this cross-sectional study, researchers used an MRI technique that image flow, and MRI brain volumetry to investigate differences in tCBF, global cerebral perfusion (GCP), volumes of gray matter and white matter, inferior lateral ventricle, and AD prone regions between cognitively normal (CN) and early amnestic mild cognitive impairment (aMCI) individuals as well as the associations of these measures with global and several specific cognitive domains performance including memory, executive function, language and visuospatial ability across and within CN and aMCI. Both hemodynamic measures, tCBF and GCP, were significantly smaller in aMCI relative to CN while the differences in global and AD prone volumetric measurers were not statistically significant. Additionally, tCBF and GCP were associated with global cognition and memory, but the associations of volumes of the AD prone brain regions with global cognition and memory were insignificant.

These findings imply that reduced tCBF and GCP occur prior to morphologic changes in the brain during the early phase of the cognitive impairment. This is a very important finding as it implies that a measurement of tCBF is likely a more sensitive biomarkers of early AD than degree of tissue loss in the hippocampus. Researchers submitted a manuscript of this work

titled, "Early Amnestic Mild Cognitive Impairment is Associated with Lower Total Cerebral Blood Flow with no Brain Tissue Loss" to the journal of Alzheimer disease. It was very favorably reviewed and the editor requested the team to perform additional statistical analysis to further validate the findings presented in the article. Having done so, the research team have resubmitted the revised manuscript to the journal and expect acceptance of the manuscript for publication in the coming days.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A20 Between Here and There: Addressing End-of-Life Disparities Among African Americans with Mild Cognitive Impairment and Dementia Through Community-Based Training in Advance Care Planning

Principal Investigator: Maisha Robinson, MD

Organization: Mayo Clinic Jacksonville

Abstract: The three identified people from the Working Group who underwent training to be advance care planning facilitators participated in a debrief of their experiences and plans for incorporating the information learned during the training into the larger community group sessions. The research team has been strategizing about how to best hold the final sessions given continued concern about the risk of covid during the large group gatherings. The gatherings will map on to the identified gaps in readiness to complete an advance directive: while nearly 60% of participants indicated that they felt confident that they could ask someone to be their decision maker, in the following six months, only 30% of them were thinking about doing so. Of the participants 35% thought they would be ready to complete a health care surrogate form in the following six months and approximately 50% indicated that they would complete a living will outlining their wishes for the end of their life in the following six months. Barriers to completion of advance directives include a lack of knowledge of what needs to be discussed, poor understanding of who is an ideal health care surrogate, fear of facing one's mortality, lack of prioritization of advance care planning, and the complexity of end of life decision making.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A21 Utility of Blood Biomarkers for Amyloid, Tau and Neurodegeneration to assist in the Diagnosis of Alzheimer's disease and other Dementias - Relationship to Cognition, Brain Atrophy and Amyloid Load

Principal Investigator: Ranjan Duara, MD

Organization: Mount Sinai Medical Center of Florida

Abstract: During the January to March 2022, samples from thirty-seven ADRC participants were collected, processed, and stored in a -70°C freezer at Mount Sinai in the Research Department. These samples were sent in three large batches to the University of Florida on January 18, 2022, February 23, 2022, and April 4, 2022. As of March 31, 2022, 47 samples for this study had been collected.

Follow on Funding: None at the time of reporting.

Collaborations: Research staff are collaborating with the University of Florida since the university has purchased a Quanterix machine to be used on blood drawn from ADRC participants. Karen McFarland, a molecular biologist in the UF Department of Neurology, has employed Thomas Ladd, an experienced technologist, to assist with analysis of the samples. This is more economical than sending samples to Quanterix in Massachusetts and may enable researchers to study blood biomarkers in more subjects. The research team will collaborate with Zvinka Zlatar, PhD, University of California San Diego (UCSD), on the NIH R01 grant to study Subjective Cognitive Decline in Older Hispanics/Latinos. Research staff will enroll subjects from the One Florida Alzheimer's Disease Research Center (1Florida ADRC) into this study to leverage the clinical and imaging data from the ADRC, and the biomarker data collected from the Ed and Ethel Moore protocol. Dr. Zlatar will also send samples from participants at UCSD to UF.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix B
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2018-2019

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
9AZ02	Florida Atlantic University	Henriette van Praag, PhD, MA	250,000.00	02/28/23	No	No	No
9AZ31	University of South Florida	Joshua Gamsby, PhD	237,500.00	09/30/22	No	No	No

Grant#: 9AZ02 The Role of Exercise-Induced Systemic Factors in Alzheimer's Disease

Principal Investigator: Henriette van Praag, PhD, MA

Organization: Florida Atlantic University

Abstract: With the increase in human lifespan, more aging-related cognitive disorders, including Alzheimer's Disease (AD) are being diagnosed. In the absence of effective medications, physical activity is a simple, low-cost intervention that may prevent or delay the onset of memory loss. Physical exercise may slow disease progression and is a potentially modifiable risk-factor that may delay or prevent cognitive decline. Indeed, this research team was the first to show that running increases the production of new neurons in the hippocampus, a region important for learning and memory. Since this discovery, this research team along with others have demonstrated that running enhances synaptic plasticity, performance on learning tasks, growth factor levels and vasculature in the rodent brain. Moreover, in mouse models of AD there is accumulating evidence that running counteracts amyloid-beta (Aβ) production, reduces neuroinflammation, increases adult neurogenesis and benefits learning. In humans, there is complementary evidence that exercise improves cognitive function, hippocampal volume and cerebral blood flow, and may slow the progression of memory loss. The underlying mechanisms for these effects remain unclear. In particular, the systemic, metabolic and peripheral triggers that elicit these processes have only been recently begun to be explored. Such research suggests that blood-borne systemic factors can counteract age-related decline of adult neurogenesis and brain function. Upon activation by exercise, skeletal muscle releases factors (myokines) that circulate and communicate with the brain. These studies indicate that myokines, can increase neural stem cell differentiation, and may be important for improvements in memory function in mice and humans. The research team proposes to determine whether myokines support the effects of exercise and exercise-mimetics on brain function and behavior using a mouse model of AD. Specifically, research staff are studying the effects of voluntary wheel running, a compound that activates muscle energy metabolism, the adenosine 5'-monophosphate-activated protein kinase (AMPK) agonist 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), and the novel myokine Cathepsin B, on memory function in amyloid precurser protein (APP)swe/PS1Δ9 transgenic mice. Research staff are evaluating adult hippocampal neurogenesis and synaptic plasticity after these manipulations. The mouse behavioral experiments have been completed and are being analyzed. In particular, have evaluated spatial memory function. In addition, research team members have prepared viral vectors and performed stereotaxic surgeries to target newly born neurons in the hippocampus of AD mice housed under control and running conditions, and are in the process of analyzing the fine morphology of adult-born neurons. The research team will also assay Aβ and tau levels in

the hippocampi and cortices of these subjects. Research team members also aim to discover novel myokines that may aid brain function. Research staff will compare the effects of conditioned media secreted by skeletal muscle cells derived from rodent and human subjects on neural stem cell differentiation in vitro. Proteomic analysis of the conditioned media will be performed and potential candidate factors will be studied for their effects on neurogenesis and neurotrophin levels. These studies will add significantly to the general understanding of the role of molecules secreted by skeletal muscle cells that translate exercise to improved brain function, providing important preclinical evidence for novel therapeutic strategies based on myokines that could benefit AD patients.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 9AZ31 Investigation of Alzheimer's Disease-induced Circadian Dysfunction on Tau Production and Phosphorylation

Principal Investigator: Joshua Gamsby, PhD

Organization: University of South Florida

Abstract: In the last report, data was presented that showed Microtubule Associated Protein Tau (MAPT) is indeed a clock-controlled gene (ccg) in humans whereby a Brain and Muscle ARNT-Like 1 (BMAL1) binds to discrete e-box consensus cites in the MAPT promoter to drive transcription. Since then, research staff have been focused on reproducing chromatin immunoprecipitation (ChIP) results in the mouse brain, specifically in the hippocampus and cortex, to determine whether BMAL1 occupancy of the MAPT promoter is also evident. Research staff chose to do this as there is a putatively conserved e-box located at approximately the same position upstream of the total suspended solids (TSS) in the human genome. Confirmation will demonstrate that BMAL1 regulation of the MAPT promoter is phylogenetically conserved and provide information about clock regulation of MAPT in regions of the brain that are impacted by tauopoathy. Research staff have already collected brain tissue from adult C56BL6/J mice and are currently working on testing primers for the diagnostic polymerase chain reaction (PCR) endpoint assay. Team members will have data to report in the next update. Additionally, staff have obtained progeny with the desired genotype from the BMAL1 Knock Out x hTau cross. Research team members are currently aging these mice, along with controls, for the pathogenesis experiments. This will occur when the mice reach six months of age.

Follow on Funding: None at the time of reporting.

Collaborations: Research staff continue to collaborate with the Hurely lab from Rensselaer Polytechnic Institute (RPI), the Lee lab at the University of Kentucky, and the Gulick lab at the University of South Florida (USF). Staff have also established a collaboration with Dr. Arunava

Roy, who is faculty in the same department as the primary investigator, to help with the research team's ChIP assays.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix B
Fiscal Year 2021-2022 Active Grants
Funded Fiscal Year 2017-2018

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
8AZ12	University of Central Florida	Kenneth Teter, MD	200,000.00	08/31/22	No	No	No
8AZ16	University of Florida	Paramita Chakrabarty, PhD	221,000.00	08/31/22	No	No	No
8AZ24	University of Miami	Michal Toborek, MD, PhD	221,000.00	08/31/22	No	Yes	Yes

Grant#: 8AZ12 Protein Disulfide Isomerase Uses Conditional Disorder as a Disaggregase Mechanism to Detoxify Amyloid Beta Fibrils

Principal Investigator: Kenneth Teter, MD

Organization: University of Central Florida

Abstract: Protein disulfide isomerase (PDI) has an abb'xa' structural organization that consists of two thioredoxin-like catalytic domains (a & a') separated by two non-catalytic domains (b & b') and an x-linker. The research team proposes that PDI can act as a "disaggregase" to dissolve and detoxify aggregated fibrils of the amyloid beta (Aβ) peptide. It is predicted that the disaggregase activity of PDI is activated when substrate binding to the b domain transmits a signal through the b'x domains for unfolding of the a' domain. The expanded hydrodynamic size of the unfolded a' domain subsequently functions as a wedge to push against two or more peptides in the Aβ aggregate. This provides a mechanical force to break apart nascent aggregates of Aβ. Thus, recombinant PDI could be used as a novel therapeutic agent for the clearance of extracellular Aβ fibrils. Research team members will pursue this possibility by identifying the minimal PDI fragment with disaggregase activity and the molecular mechanism for its neuroprotective function. In the current (15th) reporting period, the repaired surface plasmon resonance (SPR) instrument was returned by the manufacturer. Research team members are now using the instrument to study the binding parameters between PDI and Aβ. The research results have established a protocol for monitoring protein-protein interactions with the instrument and have shown that PDI does not bind to monomeric Aβ (Fig. 1A). This confirmed earlier work with a now-defunct Reichert SPR instrument that found PDI will not bind to monomeric Aβ (Fig. 1B) but will bind to fibrils of aggregated Aβ (Fig. 1C). The OpenSPR instrument will now be used in conjunction with the panel of PDI deletion constructs to determine which domains of PDI are required for its binding to aggregated Aβ. Additional quantities of the PDI deletion constructs were purified for this purpose. Using a Thioflavin-T (Th-T) aggregation assay, it has previously been found that PDI effectively inhibits the fibrillization of Aβ when added at the onset of aggregation. Yet, the SPR data shows that PDI does not bind to the Aβ monomers present at the onset of aggregation. This strongly suggests that PDI interacts with the oligomeric forms of Aβ that are generated early in the aggregation process and either prevents further Aβ aggregation or returns Aβ to a monomeric state. These possibilities will be examined using the recently established native gradient PAGE system for visualizing Aβ monomers, oligomers, and aggregates. The overall order of priority for completing project experiments will be: (i) SPR binding assays; (ii) ThT aggregation assays to determine which domains of PDI are required to disrupt the fibrillization Aβ; (iii) tryptophan fluorescence structural

assays to monitor how binding to Aβ alters the folding state of PDI; and (iv) native gradient PAGE aggregation assays. The order of experiments is based upon the expected speed and ease of completion. Notably, assays (ii) and (iii) can be performed in parallel using a high-throughput format. When completing the native gradient polyacrylamide gel electrophoresis (PAGE) assays, researchers will also revisit the nascent enzyme linked immunosorbent assay (ELISA) protocol for detecting the oligomeric forms of Aβ.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 8AZ16 Towards understanding the biological role of newly discovered Alzheimer's disease susceptibility genes affecting immune function

Principal Investigator: Paramita Chakrabarty, PhD

Organization: University of Florida

Abstract: In this grant period, the research team is continuing to finalize the data on the effect of Plcg2 deletion on amyloid beta plaque deposition. Research team members are also conducting primary microglia phagocytosis assays using both Abi3 deletion and Plcg2 deletion mice. This is towards the fulfillment of Aim 2.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 8AZ24 Extracellular vesicles as novel therapeutic targets in Alzheimer's Disease

Principal Investigator: Michal Toborek, MD, PhD

Organization: University of Miami

Abstract: Virtually all cells of the human body shed vesicles into the extracellular space, which then travel via the blood stream and can reach distant organs. These vesicles, named "extracellular vesicles" (EVs), carry content characteristic to the cells they originate from, including a protein called amyloid beta (Aβ). Deposits of Aβ in the brain have been linked to the memory loss and cognitive decline in individuals suffering from Alzheimer's disease (AD). The research team proposes that ECV can bring Aβ from the periphery into the brain by crossing the bloodbrain barrier (BBB), a critical interface built by small brain vessels that normally protects the brain from the majority of blood-borne factors. Specifically, it is hypothesized that BBB-

derived ECV-Aβ may be transferred to neural progenitor cells (NPC) and affect their differentiation into neurons. Receptor for advanced glycation end products (RAGE) may be involved in these events. This may be relevant to the brain Aβ pathology in neurodegenerative diseases. The work on the project progressed as planned; however, research staff experienced unprecedented hardship related to the COVID-19 pandemic. As the result of extraordinary effort of the entire research team, staff managed to work on this grant without any major interruptions and are proud of the discoveries that were made. All goals of the projects have been accomplished.

Follow on Funding: NIH/NIHM, $422,125; NIH, $2,281,243; NIH, $140,256

Collaborations: This proposal resulted in collaboration with Dr. Marta Garcia Contreras from the Diabetes Research Institute at the University of Miami School of Medicine on analysis of extracellular vesicles, with Dr.Shanta Dhar from the Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Dr. Daniel Adesse from the Instituto Oswaldo Cruz, Fiocruz, Brazil, and Dr. Joice Stipursky from the Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Brazil.

Journals: Cho HJ, Velichkovska M, Schurhoff N, András IE, Toborek M. Extracellular vesicles regulate gap junctionmediated intercellular communication and HIV-1 infection of human neural progenitor cells. *Neurobiology of Disease*. 2021;155(July):105388. doi:10.1016/j.nbd.2021.105388.

Osborne O, Peyravian N, Nair M, Daunert S, Toborek M. The paradox of HIV blood-brain barrier penetrance and antiretroviral drug delivery deficiencies. *Trends in Neurosciences*. 2020;43(9):695-708. doi:0.1016/j.tins.2020.06.007.

András IA, Sewell BB, Toborek M. HIV-1 and amyloid beta remodel proteome of brain endothelial extracellular vesicles. *Int. J. Mol. Sci.* 2020;21(8):2741. doi:10.3390/ijms21082741.

András IE, Garcia-Contreras M, Yanick C, et al. Extracellular vesicle-mediated amyloid transfer to neural progenitor cells: implications for RAGE and HIV infection. Mol Brain 2020;13(21). doi:10.1186/s13041-020-0562-0.

Patents: None at the time of reporting.

Appendix C
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2020-2021

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
21A22	University of Miami	David Loewenstein, PhD, ABPP/CN	88,466.00	02/28/23	No	No	No

Grant#: 21A22 Postdoctoral Fellowship in Neuropsychology and Cognitive Neuroscience

Principal Investigator: David Loewenstein, PhD, ABPP/CN

Organization: University of Miami

Abstract: The research team successfully trained the post-doctoral fellow in working with magnetic resonance imaging (MRI) neuroimaging data and amyloid positron emission tomography (PET) data and relating these biomarkers to novel cognitive challenge tests employed in laboratories. The post-doctoral fellow was involved in National Institutes of Health (NIH) grant submissions, had posters accepted for national meetings and co-authored some papers currently under review in peer-reviewed journals. The post-doctoral fellow benefited from the post-doctoral lecture series and didactic graduate student seminars. The post-doctoral fellow was also active in diagnostic team conferences in the research team's One Florida Alzheimer's Disease Research Center (1Florida ADRC) and learned more about cutting edge and state-of-the science results. The fellow is so outstanding and will remain as a member of the research laboratory.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Appendix C
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2019-2020

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
20A02	University of Miami	David Loewenstein, PhD, ABPP/CN	87,959.00	03/31/22	No	No	Yes
20A04	University of Miami	Rosie Curiel Cid, PhD	86,211.00	03/31/22	No	No	Yes
20A10	Mayo Clinic Jacksonville	Pritam Das, PhD	250,000.00	04/30/22	No	No	No
20A12	Florida Atlantic University	Elan Barenholtz, PhD	99,863.00	04/30/22	No	Yes	No
20A15	University of Florida	Natalie C. Ebner, PhD	249,930.00	02/28/22	No	Yes	No
20A22	Mayo Clinic Jacksonville	Rickey E. Carter, PhD	171,790.00	03/31/22	No	No	Yes

Grant#: 20A02 Postdoctoral Fellowship in Neuropsychology and Neurosciences

Principal Investigator: David Loewenstein, PhD, ABPP/CN

Organization: University of Miami

Abstract: Christian Gonzalez-Jimenez, Ph.D. has completed his second year of postdoctoral fellowship and the Center for Cognitive Neuroscience and Aging (CNSA) at the University of Miami Miller School of Medicine. Dr. Gonzalez-Jiminez succeeded in his goal of gaining expertise in Neuropsychology and Cognitive Neuroscience. Dr. Gonzalez-Jimenez, has greatly contributed to the development of a successful virtual platform for the administration of neuropsychological evaluations through a secure telehealth protocol as outlined below. Kitiagordodsky M, Loewenstein D, Curiel Cid R, Crocco E, Gorman K, González-Jiménez C. A Teleneuropsychology Protocol for the Cognitive Assessment of Older Adults During COVID-19. Front Psychol. 2021 May 13;12:651136. doi:10.3389/fpsyg.2021.651136. PMID: 34054655; PMCID: PMC8155705.

Dr. Gonzalez-Jimenez played a critical role in the amyloid carotid Imaging Markers of AGINg and Endothelial function (IMAGINE) measures positron emission tomography (PET) scan and Tau acquisition for the team's National Institutes of Health (NIH) grants, has assisted in the process of developing training protocols, and detailed manuals following the recommendations provided by the National Academy of Neuropsychology and the National Alzheimer's Coordinating Center in order to transition the team's research visits to a telehealth platform. Dr. Gonzalez-Jimenez has become proficient in relating neuropsychological measures to amyloid PET and magnetic resonance imaging (MRI) biomarkers. Dr. Gonzalez-Jimenez contributed to several scientific manuscripts and a number of poster presentations and has been involved with the well phenotyped older adult population. Dr. Gonzalez-Jimenez has functioned with both autonomy and independence in terms of the supervision and management of a state funded project and is now a well-trained as a clinician-scientist in Alzheimer's Disease. Dr. Gonzalez-Jimenez has been very productive and a pleasure to train.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Kitaigorodsky M, Loewenstein D, Curiel Cid R, Crocco E, Gorman K, González Jiménez CA. A teleneuropsychology protocol for the cognitive assessment of older adults during COVID-19. *Front. Psychol*. 2021;12:651136. doi:10.3389/fpsyg.2021.651136.

Kitaigorodsky M, Curiel Cid RE, Crocco E, et al. Changes in LASSI-L performance over time among older adults with amnestic MCI and amyloid positivity: A preliminary study. *Journal of Psychiatric Research*. 2021;143(November):98-105. doi:10.1016/j.jpsychires.2021.08.033.

Patents: None at the time of reporting.

Grant#: 20A04 Postdoctoral Fellowship in Neuropsychology

Principal Investigator: Rosie Curiel Cid, PhD

Organization: University of Miami

Abstract: Katherine Gorman, Psy.D. completed two years of postdoctoral fellow in Neuropsychology with an emphasis on Clinical Research in Alzheimer's Disease (AD) and Alzheimer's disease related dementias (ADRD) at the Cognitive Neuroscience and Aging (CNSA), Department of Psychiatry at the University of Miami, Miller School of Medicine. During the doctor's tenure, Dr. Gorman became a licensed psychologist, played an important scientific and operational leadership role across multiple state and National Institutes of Health (NIH)-funded studies, and participated actively in disseminating scientific findings including poster presentations, platform presentations, and peer-reviewed publications. Dr. Gorman also helped co-develop and implement a unique telehealth platform in order to continue the clinical research operations during the COVID-19 pandemic. Dr. Gorman was instrumental in working with CNSA faculty in developing and updating training protocols (including training videos that could be delivered remotely) and detailed manuals following the recommendations provided by the National Academy of Neuropsychology and the National Alzheimer's Coordinating Center in order to continue research visits using a telehealth platform. Dr. Gorman led efforts in the training of research associates, pre-doctoral students, and research staff members on how to administer virtual assessments. Dr. Gorman has also been instrumental in ensuring the implementation of all novel procedures and protocols. The doctor has created well-organized and efficient systems for administering, scoring, and interpreting traditional and experimental cognitive outcome measures. Dr. Gorman has also contributed to scientific manuscripts and posters. Dr. Gorman was co-author on two manuscripts (see Q4 below); two that were accepted by the Journal of Alzheimer's Disease and another in Frontiers in Neurology. Dr. Gorman submitted a poster to this year's American Psychological Association (APA) Conference titled, "Deficits in Odor Identification and Susceptibility to Proactive Semantic Interference in Older Adults with Mild Cognitive Impairment," which was accepted. Dr. Gorman also gave a presentation on the same topic at the Mild Cognitive Impairment Symposium and has joined a collaborative effort working with investigators at Columbia University regarding this topic that is of great personal interest and relevant in the field. Dr. Gorman and the predoctoral students involved all experienced a significant advancement in training. Dr. Gorman completed the "Behavioral and Cognitive Neurology" course. Dr. Gorman participated in etiological consensus diagnostic conferences with the faculty and staff of the One Florida Alzheimer's Disease Research Center (1FL ADRC) and the CNSA.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Kitaigorodsky M, Curiel Cid RE, Crocco E, et al. Changes in LASSI-L performance over time among older adults with amnestic MCI and amyloid positivity: A preliminary study. *Journal of Psychiatric Research*. 2021;143(November):98-105. doi:10.1016/j.jpsychires.2021.08.033.

Loewenstein DA, Curiel Cid R, Kitaigorodsky M, Crocco E, Zheng D, Gorman K. Amnestic mild cognitive impairment is characterized by the inability to recover from proactive semantic interference across multiple learning trials. *Journal of Prevention of Alzheimer's Disease*. 2021;8:181-187. doi:10.14283/jpad.2021.3.

Kitaigorodsky M, Loewenstein D, Curiel Cid R, Crocco E, Gorman K, González Jiménez CA. A teleneuropsychology protocol for the cognitive assessment of older adults during COVID-19. *Front. Psychol*. 2021;12:651136. doi:10.3389/fpsyg.2021.651136.

Patents: None at the time of reporting.

Grant#: 20A10 Detection of Vascular and Inflammatory Plasma Biomarkers in Patients Diagnosed with Obstructive Sleep Apnea and Cerebral Small Vessel Disease

Principal Investigator: Pritam Das, PhD

Organization: Mayo Clinic Jacksonville

Abstract: Measure changes in vascular, inflammation biomarkers and neurofilament light chain in the plasma of patients diagnosed with obstructive sleep apnea (OSA) with varying degrees of cerebral small vessel disease (CSVD) pathology using the Mayo Clinic Familial Cerebrovascular Diseases Registry (Internal Review Board (IRB): 08-003878).

The research team has previously shown some gender specific changes with a subset of markers, including Angiopoetin-1 in the plasma of patients diagnosed with OSA with varying degrees of CSVD pathology. It was found that Angiopoetin-1, an important protein involved in vascular integrity, was decreased in male OSA patients that had a stroke, with lower plasma levels in cases with more severe white matter pathology. Previous studies have shown that lower levels of Tie-2, a specific receptor for Angiopoetin-1, increased risk for stroke. To determine whether lower expression levels of Angiopoetin-1 could also increase risk for stroke, research staff are currently analyzing plasma levels in a larger cohort of stroke samples using the Mayo Clinic Familial Cerebrovascular Diseases Registry (IRB: 08- 003878). Significantly, several samples have been identified, including plasma from unaffected family members, which will aide in this type of association studies. Research staff are still in the process of obtaining additional plasma samples for this analysis, and hope to update in the next reporting cycle.

Perform a pilot prospective longitudinal study to measure changes in plasma biomarkers, cognitive impairment and white matter pathologies in OSA patients enrolled in the Mayo Clinic sleep center. Name of Study: Detection of Vascular and Inflammatory Plasma Biomarkers in Patients Diagnosed with Obstructive Sleep Apnea and magnetic resonance imaging (MRI)-defined Cerebral Small Vessel Disease IRB # 20-000692 Phase # prospective, single-center, longitudinal cohort study.

The research team previously generated some preliminary data from plasma of seven OSA patients enrolled in this study using various times. The research team will continue to collect plasma and have now enrolled additional patients for the Aim. Research staff will update on plasma markers in the next cycle using these additional patient samples and more times points.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A12 Development of a Gaze and Speech-Behavior Based Cognitive Exam to Assist in the Detection of Early-Stage Alzheimer's Disease and Related Disorders

Principal Investigator: Elan Barenholtz, PhD

Organization: Florida Atlantic University

Abstract: Data collection was unable to be completed during the course of this grant, principally due to delays from the pandemic which impacted both recruitment as well as processes related to opening the new research lab at the University of Miami (construction, licensing, hiring staff), where this project takes place. Many participants who previously stated willingness to participate are snowbirds that have been out-of-state since data collection began in late February. However, data collection is still ongoing at no cost to the Florida Department of Health, supported by either compendium grants awarded to Dr. Galvin (site Primary Investigator (PI)) or research fellowships awarded to Dr. Kleiman.

Follow on Funding: Alzheimer's Association, $175,000 ; American Academy of Neurology, $150,000

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A15 Evaluating neurofeedback-induced plasticity to improve spatial navigation behavior in older adults at risk for Alzheimer's disease

Principal Investigator: Natalie C. Ebner, PhD

Organization: University of Florida

Abstract: This project is the first to demonstrate feasibility of the real-time functional magnetic resonance imaging (rtfMRI)-guided neurofeedback approach in aging and Alzheimer's disease (AD). The key findings of the project are demonstration of a potential for functional plasticity in frontal brain regions via contingent rtfMRI-based neurofeedback training in aging and in AD; and

demonstration of a behavioral benefit from contingent rtfMRI-based neurofeedback training on selective attention. Additional deliverables fo the project include implementation of crucial infrastructure for rtfMRI-guided neurofeedback at the magentic resonance imaging (MRI) scanning facility at the University of Florida (UF) as part of this grant, and development of standard operation procedures for its use in aging and AD. Preliminary data from this grant furthermore essentially contributed to the acquisition of extramural funding (through the Florida Department of Health (FDOH) and National Institutes of Health (NIH)) to extend this methodology into other research domains in aging and AD (trust related decision-making, spatial navigation, and immersive meditation). Also, various trainees (undergraduate, post-bac, graduate, postdoc) received and/or are actively receiving extensive training in the context of this project (e.g., one NIH T32 trainee received funding for a related project) and had opportunities to present preliminary data at scientific meetings.

Follow on Funding: NIH/NIA, $3,164,331 ; FDOH Ed and Ether Moore Alzheimer's Disease Research Program, $247,613 ; NIH/NIA, $3,575,174 ; UF Research Opportunity Seed Fund, $100,000

Collaborations: The University of Central Florida and the University of Arizona respectively in the context of a newly funded NIH R01 that was based on data from this project (see below under funding for details). The University of Florida in the context of a newly funded FDOH standard on spatial navigation in aging the University of Florida in the context of a pending NIH R01 on immersive mediation and health outcomes.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

Grant#: 20A22 Racial and Ethnic Differences in Gene Expression Data

Principal Investigator: Rickey E. Carter, PhD

Organization: Mayo Clinic Jacksonville

Abstract: During this period, the research team shipped the RNA samples extracted from frozen archival human hippocampi to the Mayo Clinic Genome Analysis CORE in Rochester, MN after electronically filling out the required sample submission form (REQ-000000075278). Research staff requested bulk RNA sequencing on 120 samples and eight replicates for a total of n=128 (15ng/ul with total of 50ul each). The CORE technicians process the samples in tubes and plate them using a robot to avoid human error. It was recommended that the team use RNA Access/Exome prep to allow for RNA integrity numbers (RIN)s as low as six to be used. Capture will be four samples pooled per capture and NovaSeq S4 PE100 for sequencing x one flow cell. The NovaSeq S4 using 32 samples/lane and Xp Loader has each lane of the flow cell loaded with an equal number of samples. The Genome Analysis CORE access and process the RNAs in the order they are listed on the Excel sample submission sheet, therefore the samples were randomized (the four groups include: ethnoracial AD cases, white AD cases, ethnoracial Normals and white Normals). The CORE has been in contact and they said it should take four to six weeks but may be longer due to the holidays and reagent availability/supply chain. The most time-consuming part is the library prep and capture (the sequencing can be completed in a few days once the libraries are in the sequencing queue).

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Farrell K, Kim S, Han N, et al. (2021) Genome-wide association study and functional validation implicates JADE1 in tauopathy. *Acta Neuropathologica*. 2021; 143:33-53. doi:10.1007/s00401-021-02379-z.

Patents: None at the time of reporting.

Appendix C
Fiscal Year 2021-2022 Closed Grants
Funded Fiscal Year 2017-2018

Grant #	Institution	PI	Award Amount	End Date	Patents	Follow on Funding	Publications
8AZ04	Florida International University	Madhavan Nair, PhD	224,643.00	08/31/22	No	No	No

Grant#: 8AZ04 Therapeutic role of Withaferin A and CRID3 in the prevention of AD. A Novel Nanotechnology approach

Principal Investigator: Madhavan Nair, PhD

Organization: Florida Internatonal University

Abstract: Liposomal formulations containing CRID3 bound multipole expansion for nanophotonics (MENP) with Withaferin A (WA) embedded in the lipid bilayer was prepared based on Specific Aim one. Before preparation of liposomal formulation, fine sized MENP was collected by removing aggregation using centrifuge. This process improved the polydispersity index of MENP. The binding of CRID-3, Potent NLRP3 inflammasome inhibitor on the fine sized MENP was confirmed after 14h of reaction. The encapsulation efficiency of CRID3 bound on fine-sized MENP was confirmed to be 95%. Loading capacity of MENP for CRID-3 was found to be 47.6%. The binding efficiency of CRID-3 with MENP was improved by longer incubation time. Liposomal formulations were prepared by adding WA to lipid solution before lipid film formation, followed by hydration process with the buffer containing MENP-CRID3. Drug bound MENP liposomes were extruded through 0.4µm membrane and the Lipo_MENP-CRID-3-WA formulation was finally reconstituted in polybutylene succinate (PBS) for further *in vivo* studies. The % encapsulation efficiency for WA was 79.15% in the complete Nanoformulations. One of the main goals of this study is to understand and safety and efficacy profiles of these complete liposomal formulations under *in vivo* settings. The data highlights the characterization of MENP and liposomal nanoformulations with respect to drug loading, bound percentage. These formulations were used for ongoing *in vivo* studies, which were presented below. For *in vivo* studies four female amyloid precursor protein/specific peptide-peptoid hybrid (APP)/(PPS1) mice were used and they are sepearated into four groups. In order to study the safety, efficacy and five neurobehavrioual analysis, research staff choose one mouse as control to inject with saline and three mice for complete nanoformulations at different concentration as indicated in by the data. Four female APP/PPS1 mice were used for the behavior T-maze studies to get baseline measurements. They were given a five minute acclimation period to explore the maze. For the behavioral trials, one arm of T-maze is lit up and baited with food, the mice are given minutes to find the food. The latency is recorded as well as the number of times the mouse enters the wrong arm. The mice are given ten trials a day, for three consequetive days, and the latency is recorded and the average is taken for each mouse each day. At this time of the study, all mice are naïve, and pretreatment behavior analysis is completed and baseline parameters were recoreded for each mice to compare with post treatment. They are given the treatment doses following this behavior study. The behavior is currently being repeated for post treatment study. Completion of *in vivo* studies will give a full idea on safety and effiacay of these formulations and hope to complete these ongoing studies before the end of the grant.

Follow on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Kolishetti N, Vashist, A, Arias AY, Atluri S, Dhar S, Nair M. Recent advances, status, and opportunities of magneto-electric nanocarriers for biomedical applications *Mol. Aspects Med.* 2022;83:101046. doi:10.1016/j.mam.2021.101046.

Patents: None at the time of reporting.

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