2023

Reversal of Alzheimer’s disease with the Mediterranean diet through modulation of the gut microbiome

BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

REVERSAL OF ALZHEIMER'S DISEASE WITH THE MEDITERRANEAN DIET THROUGH MODULATION OF THE GUT MICROBIOME

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B.A. Williams College, 2017

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2023

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ACKNOWLEDGEMENTS

I would like to express my gratitude to my readers, Dr. Stephanie Oberhaus and Dr. John Weinstein, for their knowledge and guidance throughout this project, and ensuring a thorough final product. I would also like to thank the Boston University PA Program for the robust education and preparation, fulfilling my dreams to become a PA-C. I would also like to thank my family and boyfriend Jared, for their endless love and support on this journey.

REVERSAL OF ALZHEIMER'S DISEASE WITH THE MEDITERRANEAN DIET THROUGH MODULATION OF THE GUT MICROBIOME

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ABSTRACT

Alzheimer's Disease is a devastating disease and a major medical challenge of the 21st century without preventative treatment available and absence of a cure. Due to the profound clinical impact on patients with the disease, and emotional and financial impact on their loved ones, there has been extensive research into therapeutic agents. Unfortunately, many of the studies are abandoned or fail due to a lack of complete understanding of the complex pathophysiology of Alzheimer's Disease, and ineffective drug targets. While the etiology of disease remains controversial, the accumulation of both amyloid beta protein and neurofibrillary tangles has been the most supported theory for the past thirty years. Also in recent years, the gut microbiome has gained much attention for its unexplored potential and role in health and disease, and the Mediterranean Diet has been deemed the "healthiest" diet for the sixth consecutive year by health experts. This study reviews the current understood pathophysiology of AD and standard diagnostic criteria. This thesis aims to provide an overview of the neuroprotective effects of the Mediterranean Diet, the alterations of the gut microbiome that are associated with the Mediterranean Diet, and the alterations of the gut microbiome associated with Alzheimer's Disease. Based on the analysis of the large number of studies presented, this thesis concludes that the Mediterranean Diet optimally

modifies the ratio of Firmicutes:Bacteroidetes in the gut of people with Alzheimer's Disease and reduces pathological and symptomatic progression of disease.

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LIST OF ABBREVIATIONS

ABP	ALPHA-BETA PROTEIN
ACE	ABUNDANCE-BASED COVERAGE ESTIMATOR
AD	ALZHEIMER’S DISEASE
ADRC	ALZHEIMER’S DISEASE RESEARCH CENTER
APP	AMYLOID PRECURSOR PROTEIN
AQ	ALZHEIMER’S QUESTIONNAIRE
BMI	BODY MASS INDEX
CDR	CLINICAL DEMENTIA RATING
CNS	CENTRAL NERVOUS SYSTEM
CRP	C-REACTIVE PROTEIN
CSF	CEREBROSPINAL FLUID
CT	COMPUTERIZED TOMOGRAPHY
CVA	CEREBROVASCULAR ACCIDENT
DASH	DIETARY APPROACHES TO STOP HYPERTENSION
FFQ	FOOD FREQUENCY QUESTIONNAIRE
GBA	GUT-BRAIN AXIS
GDS	GERIATRIC DEPRESSION SCALE
GM	GUT MICROBIOME
HDG	HEALTHY DIETARY GUIDELINES
HOC	HIPPOCAMPAL OCCUPANCY
IBD	IRRITABLE BOWEL DISEASE

IBS	IRRITABLE BOWEL SYNDROME
LP	LUMBAR PUNCTURE
LPS	LIPOPOLYSACCHARIDE
LVV	LATERAL VENTRICLE VOLUME
MAP	MEMORY AND AGING PROJECT
MCI	MILD COGNITIVE IMPAIRMENT
MCT	MEAN CORTICAL THICKNESS
MD	MEDITERRANEAN DIET
MEGA	MEDITERRANEAN DIET, GUT MICROBIOME, ALZHEIMER’S DISEASE
MEND	METABOLIC ENHANCEMENT FOR NEURODEGENERATION
MIND	MD-DASH INTERVENTION FOR NEURODEGENERATIVE DELAY
MMSE	MINI MENTAL STATUS EXAM
MOCA	MONTREAL COGNITIVE ASSESSMENT
MRI	MAGNETIC RESONANCE IMAGING
NIA-AA	NATIONAL INSTITUTE ON AGING-ALZHEIMER’S ASSOCIATION
NMDA	N-METHYL-D-ASPARTATE
NPT	NEUROPSYCHOLOGICAL TEST
NTB	NEUROPSYCHOLOGICAL TEST BATTERY
PCR	POLYMERASE CHAIN REACTION
PET	POSITRON EMISSION TOMOGRAPHY
PUFA	POLYUNSATURATED FATTY ACIDS
RCT	RANDOMIZED CONTROL TRIAL

SAS	SHANGHAI AGING STUDY
SCFA	SHORT CHAIN FATTY ACIDS
SFFQ	SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE
SMS	SHANGHAI MEMORY STUDY
TBI	TRAUMATIC BRAIN INJURY
TBV	TOTAL BRAIN VOLUME
TGMV	TOTAL GRAY MATTER VOLUME
TWMV	TOTAL WHITE MATTER VOLUME
WHICAP	WASHINGTON HEIGHTS-INWOOD COLUMBIA AGING PROJECT
WRAP	WISCONSIN REGISTRY FOR ALZHEIMER'S PREVENTION

INTRODUCTION

Background

Alzheimer's Disease (AD) is considered a "disease of the elderly" with symptoms typically appearing after age 65. It is estimated that 11% of adults over the age of 65 and 50% of adults over the age of 85 in the United States are living with AD, affecting approximately 6 million adults.[2,3] Notably, two thirds of these individuals are women. African American geriatric patients and Hispanic geriatric patients are at increased risk -- twice as likely and 1.5 times as likely, respectively -- when compared to Caucasian counterparts.[4]

AD has a very high morbidity rate and is a major cause of disability across the country. Seniors living with AD or other forms of dementia have twice as many hospital stays per year relative to counterparts without the disease and are more likely to have other comorbidities, including heart disease, kidney disease, and diabetes.[4] According to the Alzheimer's Association, the lifetime cost for a person living with dementia is approximately $375,000. This value does not include the cost of unpaid caregiving: their caregivers spend on average 30 hours per week providing care, with the majority of families providing care for their loved one for greater than 4 years— an overall longer duration than the quantity of care necessitated for other conditions.[5] In 2019, greater than 16 million Americans provided roughly 18.5 billion hours of unpaid care. Consequently, 17% of AD caregivers had to quit their full-time job in order to take care of their loved one.[5] These caregivers have an increased risk of physical illness, depression, and social isolation. Seventy-four percent of dementia caregivers are worried about their own health maintenance due to missing personal medical appointments, trying to save money on their own medical prescriptions, food, and overall self-care and well-being.

Furthermore, in the last 5 years of the life of a person living with dementia, family out-of-pocket health care costs are, on average, greater than $83,000, partially secondary to the lack of Medicare coverage for a full-time caregiver, whether that be a home health aide, at a health center, or at a nursing home. A 2017 Cost of Care Survey, which was based on 15,000 surveys distributed throughout 440 regions nationwide, demonstrated the national median monthly cost of a home health aide for an individual with dementia to be $4,099, adult day health care to be $1,517, an assisted living facility to be $3,750, and a private room in a nursing home to be $8,121.[6] Due to the tremendous financial burden on families, 20% of families will delve into their retirement savings, 15% will borrow funds, and approximately 10% will reduce spendings on child education.

In terms of the high mortality, AD varies by report and is listed between the 3rd and 7th leading cause of death in the United States. Deaths from Alzheimer's Disease have more than doubled from 2000 to 2019. Seniors of age 70 living with AD have twice the mortality rate before the age of 80 compared to their counterparts without the disease.[4,7] As a result of the excessive morbidity and mortality, the annual cost worldwide has exceeded 1000 billion US dollars.[8] At the conclusion of 2022, AD and other types of dementia is projected to cost the United States $321 billion.[4] Based on healthcare costs, it is projected to cost $1 trillion in 2050.[4]

AD dementia is a paramount global health crisis of the 21st century. With the individual and familial burden and cost to society, along with the lack of a cure and effective treatment, the causes of AD and risk factors have gained much attention. The hallmark of disease pathology is the accumulation of amyloid protein forming amyloid plaques and tau protein forming neurofibrillary tangles (NFTs). Non-modifiable risk factors for AD include genetics, age, sex, and family history. The heritability of AD is 60-80% with the greatest genetic risk factor being the APOE4 gene.[9,10] The APOE gene encodes the protein, apolipoprotein E, which in combination with lipids, forms lipoproteins and has a strong role in amyloid beta metabolism. The APOE2 allele is protective against AD, while allele E3 is the most common, and allele E4 increases AD risk. The APOE4 allele is associated with decreased phagocytosis and reduced amyloid clearance. However, having one or two copies of this allele is not alone sufficient to cause disease.[11,12] It is estimated that 40-50% of adults who are homozygous for APOE4 will develop AD at the age of 70 or older.[13,14] One report suggests the etiology of late onset AD is 70% genetic and 30% environmental. Notably, modifiable risk factors include poor diet, obesity, diabetes, smoking, vitamin deficiencies, lack of exercise, inflammation, and medications, all of which can modify the gut microbiome, which directly communicates with the brain through the gut-brain axis.[15,16]

Statement of the Problem

Despite the severely detrimental physical, psychological, and financial impact on individuals living with AD and on their families and caregivers, along with the broader economic and societal burden, there remains no effective treatment for AD. There are few medications currently available for management of AD, including cholinesterase inhibitors and N-Methyl-D-aspartate (NMDA) receptor antagonists, however these treatments do not alter the pathology or molecular changes associated with the disease. More recently, the first disease modifying agent, Aducanumab, was approved by the FDA for mild AD. Aducanumab is a monoclonal antibody directed against beta amyloid plaques targeting them for destruction by the immune system. Sevigny et al. reported a substantial reduction in plaque burden in AD patients given the drug.[17] Unfortunately, the usage of this medication is hindered by the high price point.[18] The question remains and requires increased exploration– what are affordable ways to modify disease pathology in patients who are already symptomatic with AD?

The modification of diet and dietary timing has recently garnered much attention for its potential to reduce the risk and progression of AD. While it is well known that nutrition plays an important role in the pathogenesis of AD, more recent research has suggested these findings are particularly due to the influence of diet on the composition of the gut microbiome and subsequent gut-brain interactions.[19] Rapidly growing evidence analyzing the influence of environmental factors on the onset and progression of AD has demonstrated a substantial role of the gut microbiome on pathology and suggests that alterations to the gut microbiome can modulate the Central Nervous System (CNS) through a gut-brain axis.[20]

The Mediterranean Diet (MD) has been extensively studied and has been ranked for the sixth consecutive year on U.S. News & World Reports as the "healthiest diet" by health experts.[21] Sicily, the largest Mediterranean island, has 4.32-times more centenarians than the Italian national average. Nutritional assessments of those residing in Sicily demonstrated explicitly high adherence to the Mediterranean Diet.[22] The MD is associated with a variety of health benefits, including eubiosis, and is packed with high fiber foods, the main energy source for gut microorganisms, and polyphenols. Polyphenols are naturally found in plants and have numerous health benefits, including prevention of cardiovascular and inflammatory disease through regulation and reduction of detrimental reactive oxygen species (ROS) and modulation of the immune system. This mechanism of action also extends to the brain, offering neuroprotection and prevention of neurodegenerative disease by removing harmful species and decreasing neuroinflammation. The diet features minimal consumption of animal fat, cholesterol, and processed foods- all of which increase production of free radicals and increase aging. On the other hand, the MD is loaded with neuroprotective nutrients, including antioxidants and vitamins. Vitamin A, which is found in high quantities in leafy vegetables, has been shown to inhibit amyloid aggregation. Vitamin B, found in fish, leafy vegetables, and fruits, has been shown to suppress oxidative damage, reduce homocysteine (which is associated with formation of tau protein), and ameliorate cognitive decline. Vitamin D, found in fish, oil, and fortified cereals has been shown to mitigate amyloid plaque deposition and reduce neuroinflammation. Vitamin D deficiency has been linked to increased risk of cognitive decline and is associated with other neurodegenerative conditions (i.e., multiple sclerosis). Supplementation with Vitamin E, which is concentrated in oils, nuts, seeds, avocado, and unsaturated fatty acids, has been shown to delay onset of AD. Overall, recent research has demonstrated the protective effect of the MD against the accumulation of amyloid and tau protein in mouse studies, and the neuroprotective effects of the MD in human studies; higher adherence to a MD is associated with decreased risk of AD.[23,24] However, prospective randomized control trials analyzing the effects of the MD on the gut microbiome in the AD population are lacking, preventing establishment of causality of diet and dysbiosis in pathogenesis and severity of AD. This study will explore the ability of the MD to alter the gut microbiome and reduce progression and reverse signs/symptoms of AD in patients who have already been diagnosed.

Hypothesis

Adherence to the Mediterranean diet will result in improved cognitive function and a reduction and/or reversal of pathological markers of AD in patients with AD in association with its effects on modulating the composition of the gut microbiome.

Specific Aim 1: Measure changes in cognitive function in AD patients adhering to the Mediterranean Diet using neuropsychological testing via battery (NTB), MOCA score, Alzheimer's Questionnaire, and family reports.

Specific Aim 2: Determine whether changes in grey matter volume, hippocampal shrinkage, cortical thinning, amyloid and tau tangles, and/or glucose metabolism associated with improved cognitive function occur in AD patients adhering to the Mediterranean Diet, using specific imaging and CSF analytic techniques.

Specific Aim 3: Analyze changes in the gut microbiome postulated to be associated with optimal and/or improved cognitive function in AD patients adhering to the Mediterranean Diet.

REVIEW OF THE LITERATURE

Overview

While it is well documented that Alzheimer's Disease (AD) has a genetic component, research suggests that the disease is largely multifactorial with modifiable risk factors including diet, exercise, sleep, cardiovascular disease, smoking history, and alcohol use, among others.[9,10] Current literature demonstrates the positive impact of the Mediterranean Diet (MD) on the gut microbiome and the corresponding relationship with Alzheimer's disease, decreasing AD risk. However, the potential to reverse signs and symptoms of AD through use of the MD has not been examined.

This study will test the hypothesis that adherence to the Mediterranean diet will result in improved cognitive function and a reduction and/or reversal of pathological markers of AD in patients with AD in association with its effects on modulating the composition of the gut microbiome. This proposed study aims to explore the correlation between the MD, associated changes in the composition of the gut microbiome, and the potential to reverse AD signs and symptoms.

PATHOPHYSIOLOGY

Alzheimer's disease is a neurodegenerative disorder that causes approximately 60%-80% of the 50 million cases of dementia worldwide.[25] The etiology of the disease remains controversial, however the dominant theory for the past thirty years has been the amyloid-cascade hypothesis.[26–28] This hypothesis suggests the causative agent of the pathology of AD is the amyloid beta protein (ABP) coalescing into plaques and "neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a result of

[ABP] deposition”.[28] Therefore, the signature pathology of AD has become the accumulation of extracellular amyloid beta plaques and intracellular misfolded neurofibrillary tau (NFT) tangles (see Figure 1).

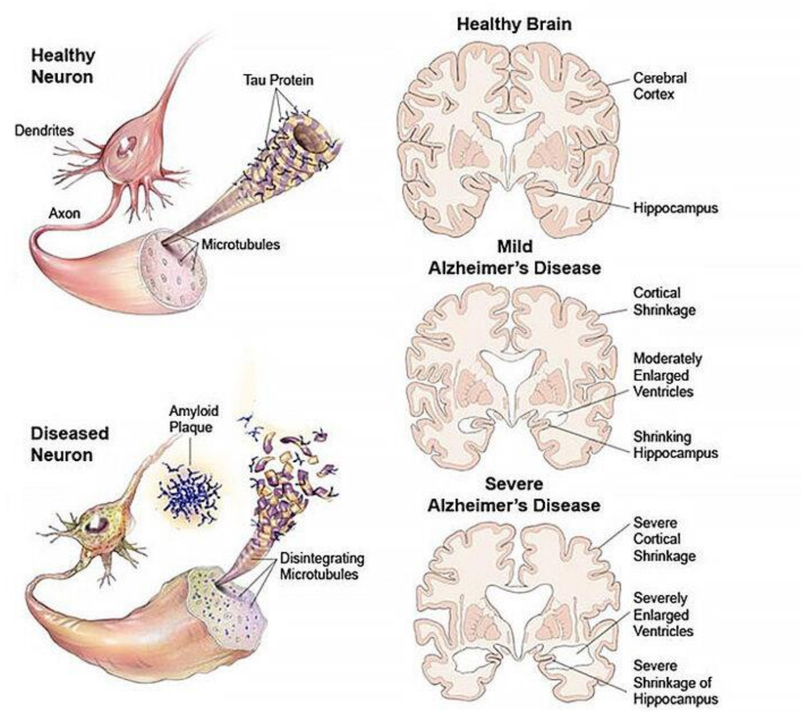


Figure 1. Neurologic changes as AD progresses[29]

Amyloid precursor protein (APP) is highly expressed in the brain and spinal cord.[30] Cleavage of APP can occur by the secretory pathway, which is nonpathogenic and occurs in healthy individuals, in which α-secretase cleaves APP first, then followed by γ-secretase (See Figure 2). Initial cleavage by α-secretase prevents the formation of Aβ peptides. In AD, APP is instead cleaved by β-secretase followed by γ-secretase, generating Aβ peptides, with the most common being $A\beta_{40}$ and $A\beta_{42}$ (the numbers indicate the length of the amino acids), that are released into the CNS.[30] Aβ proteins are insoluble and “sticky”, allowing them to accumulate together to form Aβ plaques, and reducing clearance. The higher burden of ABP leads to inflammation, decreased glucose metabolism, and disrupts synaptic communication between nerve cells. However, some studies of “healthy” brains of patients with no signs or symptoms of AD have demonstrated large deposits of Aβ plaques, suggesting a multifactorial cause. On the other hand, the absence of amyloid beta plaques is associated with a minimal risk of AD, however most elderly individuals (>65 years) have some degree of non-pathologic soluble amyloid protein (not amyloid beta protein) burden outside of neurons. This higher burden of amyloid protein, detected by PET scan or CSF analysis, in general has been shown to be associated with higher risk for AD, likely due to increased likelihood of cleavage errors and contributing to increased “aggregate stress” on surrounding CNS structures.[31,32]

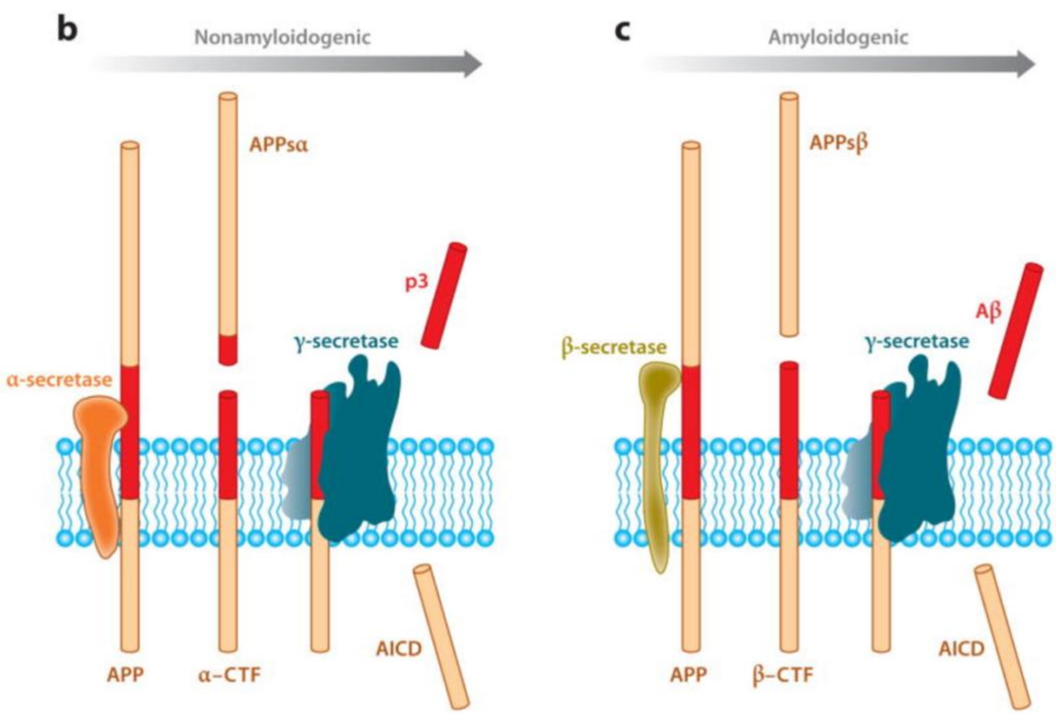


Figure 2. Pathophysiology of Aβ peptide generation[30]. Cleavage of amyloid precursor protein (APP) can occur by the secretory pathway/ “nonamyloidgenic” pathway, which occurs in individuals without Alzheimer’s Disease, during which α-secretase cleaves APP first, generating APPsα (secreted amyloid precursor protein alpha) and α-CTF (alpha C-terminal fragment) then followed by γ-secretase, generating a harmless p3 fragment and AICD (amyloid precursor protein intracellular domain). Therefore, initial cleavage by α-secretase prevents the formation of Aβ peptides. In the “amyloidgenic” pathway, cleavage of APP initially occurs by β-secretase, generating APPsβ (secreted amyloid precursor protein beta) and β-CTF (beta C-terminal fragment), followed by cleavage by γ-secretase, generating Aβ peptides that are released into the CNS, and AICD.

Unlike Aβ peptides, NFTs are found in all brains over the age of 55, mainly around the hippocampus, and occur inside of neurons. In healthy individuals, tau protein stabilizes microtubules facilitating communication between neurons. In AD, alterations in the chemical structure of tau and hyperphosphorylation causes the protein to detach from microtubules and instead bind to other tau protein. This process of tangle formation mediated by increased levels of phosphorylated-tau inhibits the neuronal transport system and ultimately leads to death of the neuron.[33] Hyperphosphorylation of tau in AD is defined as three times the amount of phosphorylated-tau that is found in healthy controls, therefore researchers compare phosphorylated-tau levels in healthy and AD brains.[34]

DIAGNOSIS

The National Institute on Aging and the Alzheimer's Association (NIA-AA) recently addended their research diagnostic criteria of Alzheimer’s Disease to include detection of both Aβ plaques and NFTs while individuals with an abnormal accumulation of Aβ with a normal level of phosphorylated tau protein would meet criteria for “Alzheimer’s pathologic change” or the early stage of the Alzheimer’s continuum.[35]

While amyloid (indicated by amyloid-PET or CSF $A\beta_{42}$) and tau (indicated by CSF phosphorylated tau, or tau-PET) are required for AD diagnosis, staging is based on cognitive symptoms (MOCA, NTB) and markers of neurodegeneration and neuronal injury (FDG-PET, structural MRI, CSF total tau).[36] More abnormal findings on FDG-PET, structural MRI, and CSF total tau are strongly associated with worse cognition throughout the Alzheimer's disease continuum.

History:

In 2010, the Patient Protection and Affordable Care Act required annual assessment of cognitive impairment in Medicare wellness visits in all patients over the age of 65. Routine diagnosis of AD typically begins by retrieving a detailed medical/surgical history, family history, and social history from patients and their loved ones. During the clinical visit, it is important to assess the impact of the patient's cognitive ability on their daily life and function, along with changes over time, and gain perspective from their caregiver. Healthcare providers should ensure the patient's medications are updated and the medication count is correct as polypharmacy (five or more medications daily) can play a major role in a geriatrics patient's behavior and induce dementia-like symptoms, including confusion, memory loss, and impaired executive function.

Physical Exam + Lab:

A complete physical exam and thorough neurological exam, including a mental status exam, should be performed in all patients over the age of 65 and in patients with symptoms concerning for AD. Healthcare providers often refer patients for a complete panel of bloodwork at this time. It is important to exclude other potential treatable causes of cognitive dysfunction, including anemia, hypothyroidism, vitamin B12/folate deficiency, hypoglycemia, serum electrolyte alterations, and hepatic/renal dysfunction. Though not standard practice, some providers will request APOE genotyping to be performed on a saliva or serum sample.

Cognitive Testing:

To further assess cognitive impairment and memory deficits, the Montreal Cognitive Assessment (MOCA) may be used.[28,37–40] The MOCA is used worldwide and is available in 65 different languages. It has proven to be a powerful cognitive screening tool, including tasks that evaluate attention, concentration, visuospatial skills, language, calculation, abstraction, and orientation, and has been validated for people between the ages of 55 and 85 years old. The assessment takes about 10 minutes to complete. A score on the MOCA between 26-30 is normal, 18-25 indicates mild cognitive impairment (MCI), 10-17 indicates moderate cognitive impairment, and <10 indicates severe cognitive impairment. The assessment is 90% sensitive for detecting MCI, however, is not diagnostic alone. The MOCA will often be administered before or after the Geriatric Depression Scale (GDS) in order to rule out depression due to the known negative impact of depression on a patient's MOCA score, leading to a misdiagnosis or overestimation of cognitive deficits.[41] It is important to diagnose and address depression since it is a reversible cause of cognitive impairment. The GDS consists of 15 questions that assess mood and has been reliable for detecting depression in geriatric patients. The questionnaire takes 5-10 minutes to complete.

Overall, the MOCA facilitates the ability of healthcare providers to detect cognitive impairment earlier, which allows early treatment and intervention, ultimately improving the quality of life of these patients. Upon completing the MOCA and establishing a baseline, patients who require more comprehensive evaluation may then be referred for neuropsychological testing (NPT). NPT is important because it is diagnostic for AD and provides multidimensional information, including prognosis of the patient's disease. It consists of a standardized administration of a group of cognitive tests, assessing memory, attention, processing speed, language, visual-spatial skills, executive functioning, and motor skills, by a trained neuropsychologist who will then interpret the results in an individualized manner based on patterns throughout the exam. The overall evaluation with the patient takes roughly 2-3 hours, making some individuals more reluctant to undergo testing and/or with decreased tolerance during the evaluation. The use of MOCA without complimentary NPT may miss a substantial proportion of cognitively impaired individuals. One study found that 59% of individuals who had scores in the normal range on the MOCA were found to have cognitive impairment when completing subsequent NPT.[42] Therefore, referral for NPT may be necessary in patients with risk factors for cognitive decline who have cognitive complaints despite having a normal MOCA score, especially if assessment of functional ability is needed for medical decision making and a treatment plan.

A neuropsychological test battery (NTB) was created in 2007 to assess changes in cognition in AD patients during research trials.[43] It takes about 40 minutes to complete and includes 9 validated questionnaires to assess memory and executive function (Table 1).

Questionnaire	**Score Range**
Wechsler Memory Scale visual immediate	0 - 18

Wechsler Memory Scale verbal immediate	0 - 24
Rey Auditory Verbal Learning Test (RAVLT) immediate	0 – 105
Wechsler Memory Digit Span	0 - 24
Controlled Word Association Test (COWAT)	N / a
Category Fluency Test (CFT)	N / a
Wechsler Memory Scale visual delayed	0 - 6
Wechsler Memory Scale verbal delayed	0 - 8
RAVLT delayed	0 – 30

Table 1. Questionnaires included in the NTB.

The compilation of these assessments provides an index of global cognitive function and is useful in staging AD. The COWAT and CFT assess language function, the Wechsler Memory Digit assesses attention and working memory, The Wechsler Memory Scale visual assesses visual perception, the Wechsler Memory Scale verbal and RAVLT assess verbal memory, and RAVLT assesses list learning.

Initial imaging:

MRI

MRI is initially used to exclude other potential treatable causes of cognitive dysfunction. Researchers and clinicians use MRI imaging to look at AD-vulnerable regions of the brain and to stage AD. Reduced gray matter volume and enlarged ventricles can be identified and are suggestive of AD (Figure 3) however the earliest changes are found in the medial temporal lobe structures, the entorhinal and perirhinal cortex and the hippocampus. Decreased hippocampal volume on MRI is a widely accepted assessment of AD pathology, often being reported as the key biomarker for identifying AD.[44]

Another signature sign of structural disease that can be visualized via MRI is cortical thinning. Im et al. studied regional changes in cortical thickness and found that the temporal, orbitofrontal and parietal regions are most impacted by AD. Researchers also linked disease severity (on a clinical dementia rating scale which scores six domains of cognitive function) to increased levels of cortical atrophy.[45] Other studies have demonstrated that cortical thinning is strongly correlated with cognitive impairment on the AD continuum.[46]

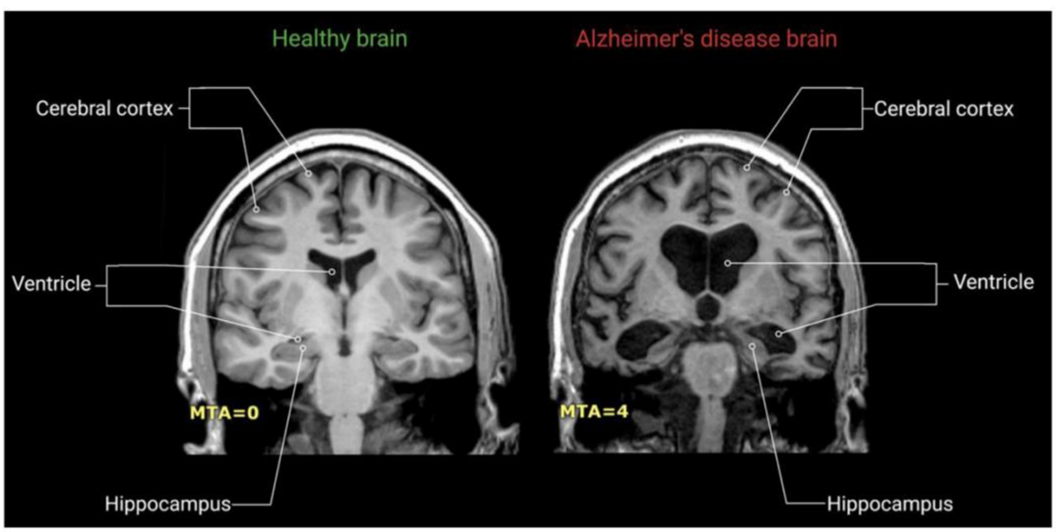


Figure 3. MRI changes in AD patient compared to patient without AD, showing cortical thinning in cerebral cortex, enlarged ventricles and decreased hippocampal volume.[47] MTA = medial temporal lobe atrophy; MTA = 0: no atrophy in medial temporal lobe; MTA = 4: severe volume loss of hippocampus.

FDG-PET

Fluorodeoxyglucose (FDG) is a radiolabeled glucose tracer used in PET scans to measure glucose uptake and serve as a marker of neuronal activity and cerebral metabolism. It is 94% sensitive in identifying AD patients. Reduced cerebral metabolism is normal in the aging process, however impairment of brain energy is a major feature of AD. In age-matched studies, AD patients had a 20-50% reduction in glucose uptake relative to those without CNS disease.[48] Reduced glucose metabolism is first observed in the temporoparietal areas of the brain, including the precuneus and posterior cingulate cortex. These pathologic changes are detectable earlier with FDG-PET than with MRI. As disease progresses through the AD continuum, glucose hypometabolism heightens.

Specialized Markers

Researchers can use PET scan analysis with injection of ^{18}F-Florbetapir[49] (radioactive tracer) for in-vivo identification of AD biomarkers in the brain, i.e. increased amyloid deposition and tau protein. Amyloid- and tau-PET scans (Figure 4) can detect signs of disease earlier than imaging using CT or MRI and have enabled researchers to study the accumulation of amyloid plaques and tau tangles without the need for biopsy.

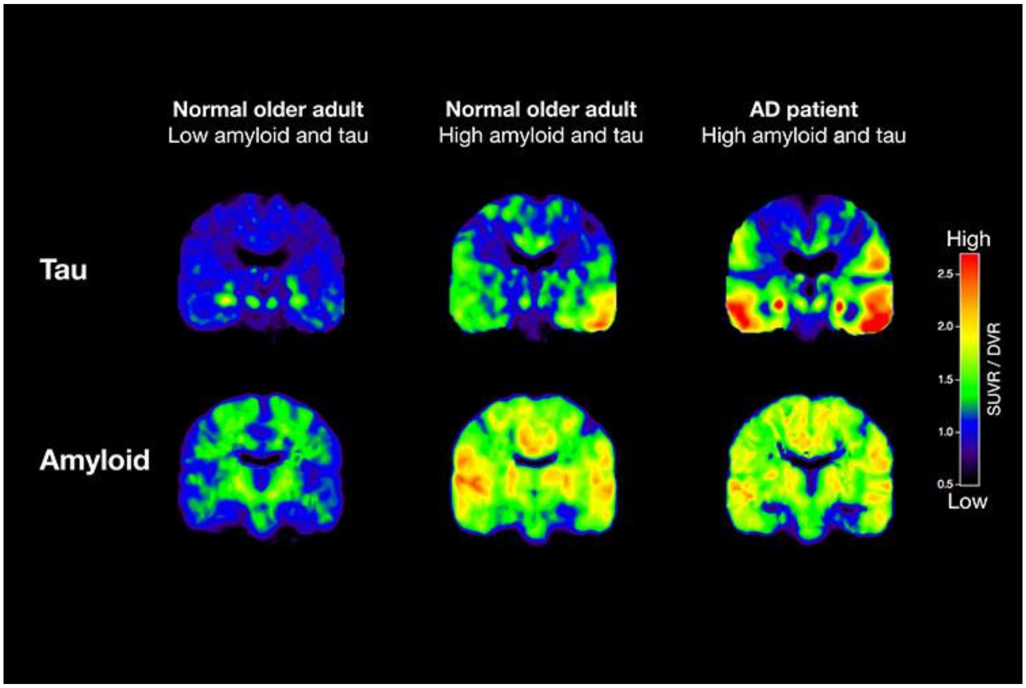


Figure 4. Tau and Amyloid PET scan changes[50] in two adults (>65 years) without AD compared to one adult with AD. There is minimal tau in the medial temporal lobe and minimal brain amyloid deposition in the participant without AD on the left, as indicated by high amounts of blue (SUVR:DVR ratio of 1). In the adult without AD in the middle, there is amyloid deposition throughout the brain, and tau has spread into the temporal cortex as indicated by increasing amount of green, yellow, and orange (SUVR:DVR > 1.5), and decreasing presence of blue. In the AD patient on the right, the amyloid deposition throughout the brain appears like that of the middle patient, however tau has spread more, indicated by more red areas, consistent with higher uptake and distribution. SUVR = standardized uptake value ratio, DVR = distribution volume ratio.

Researchers also analyzed levels of Aβ40 and Aβ42 in the blood and Aβ40, Aβ42, tau, and phosphorylated-tau181 in cerebrospinal fluid (CSF), along with respective ratios ($A\beta_{42}/A\beta_{40}$ ratio, p-tau/$A\beta_{42}$ ratio).[51,52] A CSF Aβ42 <600 ng/ml is suggestive of AD, however it is important to perform imaging along with CSF levels since low CSF $A\beta42$ does not always signify accumulation of amyloid plaque in the brain. CSF biomarkers can be low in other neuroinflammatory and neurodegenerative disease states, including cerebrovascular disease, meningitis, and multiple sclerosis.[53] Therefore, the combination of neuroimaging and CSF levels are routinely used in clinical practice for diagnosis of AD.[54] On the other hand, plasma levels have been ideal for large scale screening.[55] In compilation, a thorough history, physical exam, cognitive testing, labs and imaging are used to diagnose AD (Figure 5).

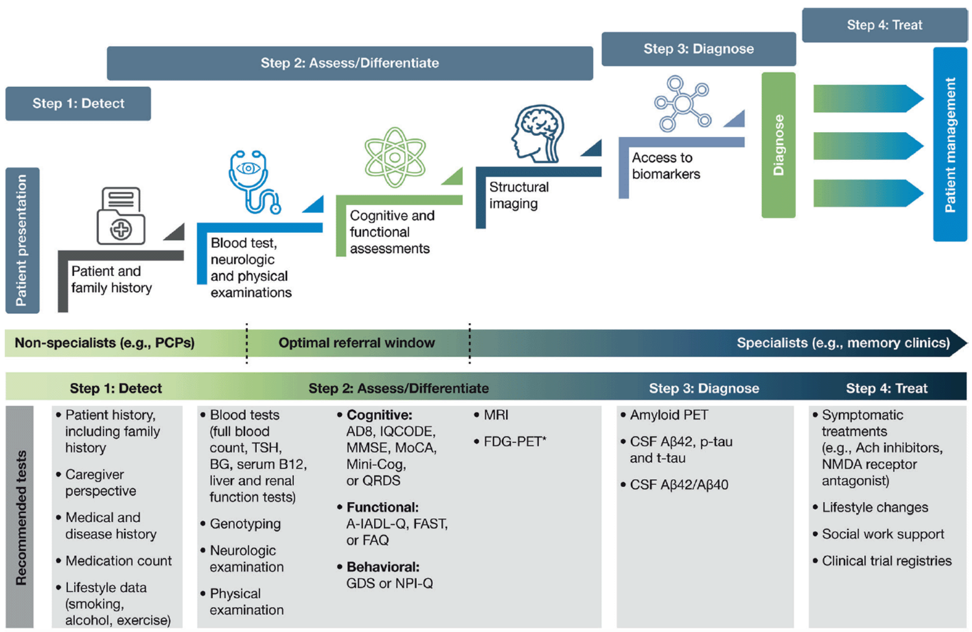


Figure 5. Diagnostic steps. Recommended steps and valuable tests to diagnose Alzheimer's Disease.[56] Steps include the following: detect, assess/differentiate, diagnose, and treat. Abbreviations: A-IADL-Q, Amsterdam Instrumental Activities of Daily Living Questionnaire. Aβ, amyloid beta. Ach, acetylcholine. BG, blood glucose. CSF, cerebrospinal fluid. FAQ, Functional Activities Questionnaire. FAST, Functional Analysis Screening Tool. FDG-PET, fluorodeoxyglucose-PET. GDS, Geriatric Depression Scale. IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly. Mini-Cog, Mini Cognitive Assessment Instrument. MMSE, Mini-Mental State Examination. MoCA, Montreal Cognitive Assessment. MRI, magnetic resonance imaging. NMDA, N-Methyl-D-aspartic acid. NPI-Q, Neuropsychiatric Inventory Questionnaire. PCP, primary care physician. PET, positive emission tomography. p-tau, phosphorylated tau. QDRS, Quick Dementia Rating System. TSH, thyroid-stimulating hormone. t-tau, total tau.

COURSE OF DISEASE and STAGING

The trajectory of AD is insidious and progressive, associated with various cognitive, functional, and behavioral changes on a lengthy continuum of defined stages. Early-stage AD is typically not diagnosed due to the insidious onset, poor diagnostics of Alzheimer's pathophysiology, and symptoms often being attributed to "the normal aging process". In stages 1 and 2 of AD (Figure 6), patients are asymptomatic, however AD pathology has begun; beta amyloid deposition can occur up to 20 years before any symptoms of disease arise, while NFTs from phosphorylated-tau can develop 5-10 years before symptoms. Therefore, this preclinical stage can last 10-20 years. As mentioned previously, both beta amyloid plaques and abnormal phosphorylated-tau tangles are required for AD diagnosis. Over time, these abnormal alterations in the brain result in neuronal dysfunction and cell death. These pathologic neural changes result in symptomatic disease, entering stage 3. During this stage, when an individual develops mild cognitive impairment (MCI), initial symptoms include missing appointments/events, forgetting conversations, and losing items (Figure 7). While the length of each stage varies by individual factors, including age, sex, and APOE status, 32.7-70.0% of individuals with MCI due to AD progress to stage 4 (AD with mild dementia) within roughly 3.4 years of follow-up.[57,58] As patients enter AD dementia (stages 4-6), there are greater functional impairments (Figure 6). Individuals become lost in familiar areas, develop personality changes, have difficulty communicating, and require perpetual care. Pathologically, there is reduced gray matter volume, enlarged ventricles, and hippocampal shrinkage (Figure 3) and glucose hypometabolism on FDG-PET. Based on the continuum of pathologic changes analyzed through neurological testing as well as the symptomatic changes, there are critical years after the start of AD pathology when early intervention can optimally occur prior to development of dementia.

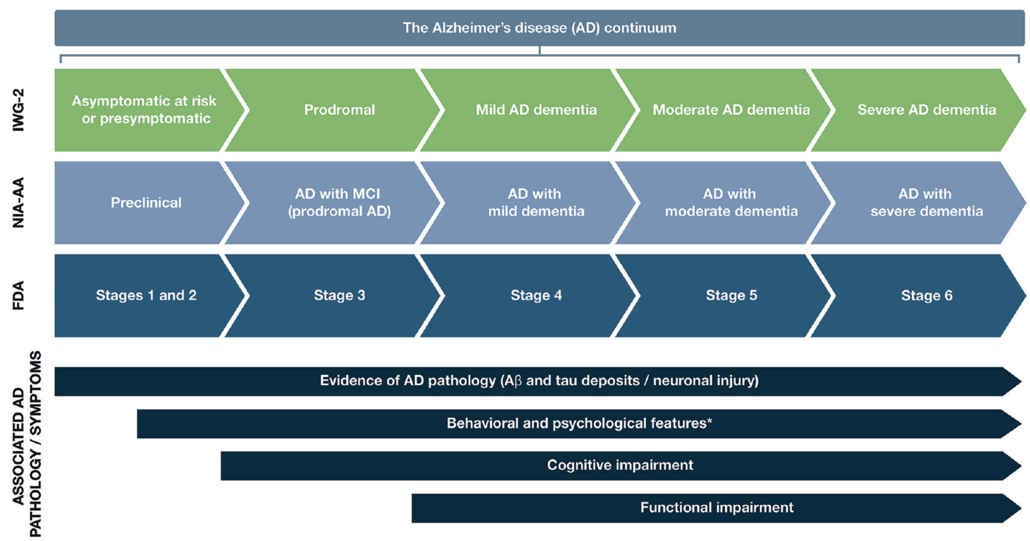


Figure 6. Stages of AD.[56] The nomenclature of various stages along the AD continuum differs based on specific clinical and research criteria. As seen in the figure, Stages 1 and 2, as defined by the FDA, align with the NIA-AA's Preclinical stage, which is most consistent with the "asymptomatic at risk or presymptomatic" phase defined by the IWG-2. Despite the differing naming systems, during this initial stage, there is evidence of AD pathology, and behavioral/psychological features start to appear. Cognitive impairment starts in stage 3 (also known as AD with MCI, or the "prodromal" phase), while functional impairments typically begin in stage 4 (AD with mild dementia). Abbreviations: Aβ, amyloid beta. AD, Alzheimer's disease. FDA, Food and Drug Administration. IWG, International Working Group. MCI, mild cognitive impairment. NIA-AA, National Institute on Aging—Alzheimer's Association.

TABLE: COMMON SYMPTOMS OF ALZHEIMER'S, BY STAGE		
Mild Alzheimer's (Early)	**Moderate Alzheimer's (Middle)**	**Severe Alzheimer's (Late)**
Problems retrieving words or names	Cannot recall details from personal history	Unaware of surroundings
Trouble retaining new information	Disoriented in terms of place and time	Major personality changes
Forgetting where objects have been placed	Difficulty choosing proper clothing for the season	Full-time care is required
Changes in mood	May wander or become lost	Loss of ability to walk, sit, and eventually swallow
Mathematical challenges	May experience behavioral and personality changes	Increasing difficulty communicating
Withdrawal from social situations		Vulnerable to infections

Figure 7. Symptomatology by stage along the AD continuum.[59] Initial symptoms of AD can include problems recalling names and learning new information while major personality changes and difficulty communicating is characteristic of severe disease.

THE GUT MICROBIOME

The gut microbiome (GM) is defined as the 100 trillion microorganisms (mainly bacteria, but also viruses, fungi, protozoa) inhabiting the gastrointestinal tract.[60,61] It also encodes over three million genes, many of which regulate metabolism, thus largely contributing to health vs. disease states.[62–64] Analysis has revealed the dominant microbial phyla found in the human digestive tract to be Firmicutes (79.4%), Bacteroidetes (16.9%), Actinobacteria (2.5%) and Proteobacteria (1%) (Figure 8).[65–67]

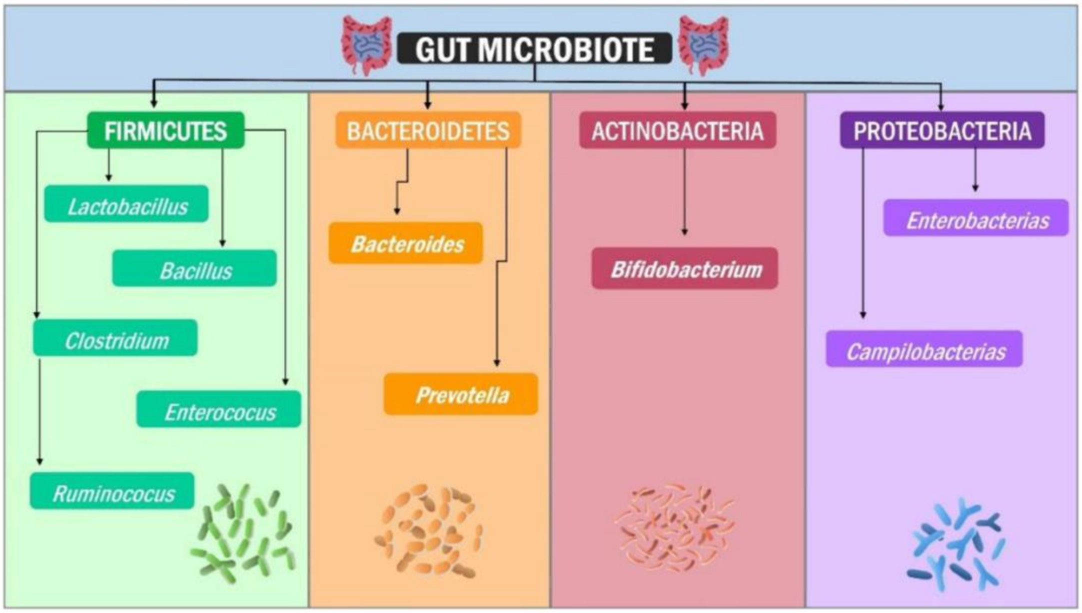


Figure 8. The dominant phyla of the human gut microbiome. Firmicutes (79.4%), Bacteroidetes (16.9%), Actinobacteria (2.5%) and Proteobacteria (1%) further divided into some notable genera.

The ratio of Firmicutes to Bacteriodetes, rather than absolute numbers, has been shown to play an important role in health, and has been observed to change from infanthood to adulthood to elderly stages.[68–70] Mariat et al. demonstrated Firmicutes:Bacteroidetes in healthy infants, adults, and elders to be 0.4, 10.9, and 0.6, respectively.[71] Every individual has a unique microbiome, so an exact "healthy GM" is undefinable, but the relative abundance and ratios of these bacterial taxa is similar among healthy individuals of similar geographic regions. This ratio tends to be higher in obese individuals and the ratio decreases with weight loss. A balanced healthy GM is crucial in maintaining intestinal barrier integrity, and preventing leaky gut and metabolic endotoxemia.[72] Short chain fatty acids (SCFAs), such as butyrate, propionate, and acetate, are critical in maintaining gut homeostasis and are produced by bacteria (largely species in the Firmicutes phylum).[73] They are anti-inflammatory and provide the major energy source for intestinal epithelial cells. Higher numbers of SCFA-producing strains of bacteria have been found in centenarians when compared to younger, healthy individuals, suggesting their potentially crucial role in healthy aging.[74] Centenarians were also found to have increased microbial diversity relative to younger healthy adults, further suggesting the importance of microbial diversity in healthy aging that may not be required at a younger age. Lower microbial diversity is associated with increased frailty, poor health markers (elevated fasting insulin levels, inflammatory markers (CRP), liver function tests, and cholesterol, among others), increased levels of inflammation, and chronic health conditions, including diabetes, obesity, IBD, and Parkinson's Disease.[75–78]

This study will focus on the Firmicutes and Bacteroidetes phyla due to their presence making up 75% of the GM and selected pro-inflammatory taxa: *Escherichia*/*Shigella* (Proteobacteria) and *Streptococcus* (Firmicutes). Firmicutes and Bacteroidetes are both widely diverse groups, however it is notable that Firmicutes include both Gram-negative and Gram-positive bacteria, while Bacteroidetes include only Gram-negative bacteria. This difference is important as the outer membrane of Gram-negative bacteria contains lipopolysaccharide (LPS), which is a potent inducer of the innate immune response and can lead to systemic inflammation. Notable genera and species of Firmicutes and Bacteroidetes can be found in Table 2 for reference throughout the paper.

Firmicutes	**Bacteroidetes**
Ruminococcus	*Bacteroides*
Enterococcus	*Alistipes*
Streptococcus	*Prevotella*
Clostridium	*Porphyromonas*
Lactobacillus	
Eubacterium	
Faecalibacterium	
Bacillus	
Staphylococcus	
Roseburia	
Erysipelatoclostridiaceae	
Erysipelotrichales	

Table 2. Notable genera and species of the Firmicutes and Bacteroidetes phyla.

STUDYING THE GM

Recent technological advancements have enabled researchers to study the gut microbiome through fecal DNA and RNA analysis with use of 16S ribosomal RNA sequencing, polymerase chain reaction (PCR) and shotgun metagenomics.[60,79,80] DNA is extracted from a sample and then sequenced by 16S PCR sequencing or metagenomic sequencing. 16S rRNA sequencing is the most widely used method. This type of sequencing uses PCR to target a portion of a gene encoding for the 16S ribosomal RNA, which is present and highly conserved in all bacteria, but not in eukaryotic cells. The 16S gene also contains 9 hypervariable regions (V1-V9) whose sequences are unique to different species and therefore useful in identifying members of specific taxa.[81] The V3 and V4 hypervariable regions are most frequently used. By looking at these variable regions, the types of bacteria present can be identified and bioinformatics can be used to create a taxonomic profile. 16S sequencing is limited to bacteria only, it does not provide cross-domain coverage (does not cover the Eukarya or Archaea domain), and resolution is limited to the species level, meaning there are different strains within a species that may not be distinguished, however the type of species, genus, family, order, etc. can be distinguished.

Shotgun metagenomics (i.e., whole genome sequencing) sequences all genetic information from a sample. Due to the extensive coverage of DNA, the results can be used for additional profiling including metabolic function analysis. In studies where researchers are interested in studying multiple domains (Eukarya, Bacteria, Archaea), metagenomics would be a better option than 16S rRNA sequencing since metagenomics has cross-domain coverage.[82] Shotgun metagenomics also has higher resolution with the ability to identify strains within a species. Therefore, it is more ideal when researchers need to identify specific pathogens or are interested in metabolic functions of genes encoded by the microorganisms.

THE GUT-BRAIN AXIS

Over 30 years ago, interest in the link between the gut and brain rose when patients with hepatic encephalopathy drastically improved following treatment with oral antibiotics.[83] Since then, studies have demonstrated that direct outputs from the gut microbiome contribute to autism, anxiety, and depression.[84,85] Furthermore, inputs to the gut from the central nervous system (CNS) and GM alterations have been shown to directly play a role in IBS.[86]

There has been increasing evidence suggesting numerous mechanisms by which bidirectional communication between the GM and the brain may occur via the gut-brain axis (GBA),[87–90] including through neural, immune, and endocrine networks (Figure 9). These networks are mediated by the CNS, autonomic nervous system (ANS), enteric nervous system (ENS), and the Hypothalamic-Pituitary-Adrenal (HPA) axis. In this study, the focus is on the role of the enteric microbiota in modulation of the brain.

Functions of the microbes in the GM include extraction and absorption of nutrients and production of metabolites, including SCFAs, neurotransmitters (acetylcholine, serotonin), amino acids, phenols, lactate, and vitamins B and K.[91,92] These health-promoting molecules interact locally with intestinal epithelia and the ENS, but are also released into systemic circulation and can cross the blood brain barrier, leading to direct communication between the GM and the CNS (Figure 9). When there is gut dysbiosis, there is an increase in pro-inflammatory bacteria and production of LPS which becomes neurotoxic.[93–95] The consequential increase in cytokines and inflammatory markers negatively impacts immune function, leads to systemic inflammation, compromises the blood brain barrier, and thus has been shown to contribute to CNS disorders, namely AD.[89,96–98]

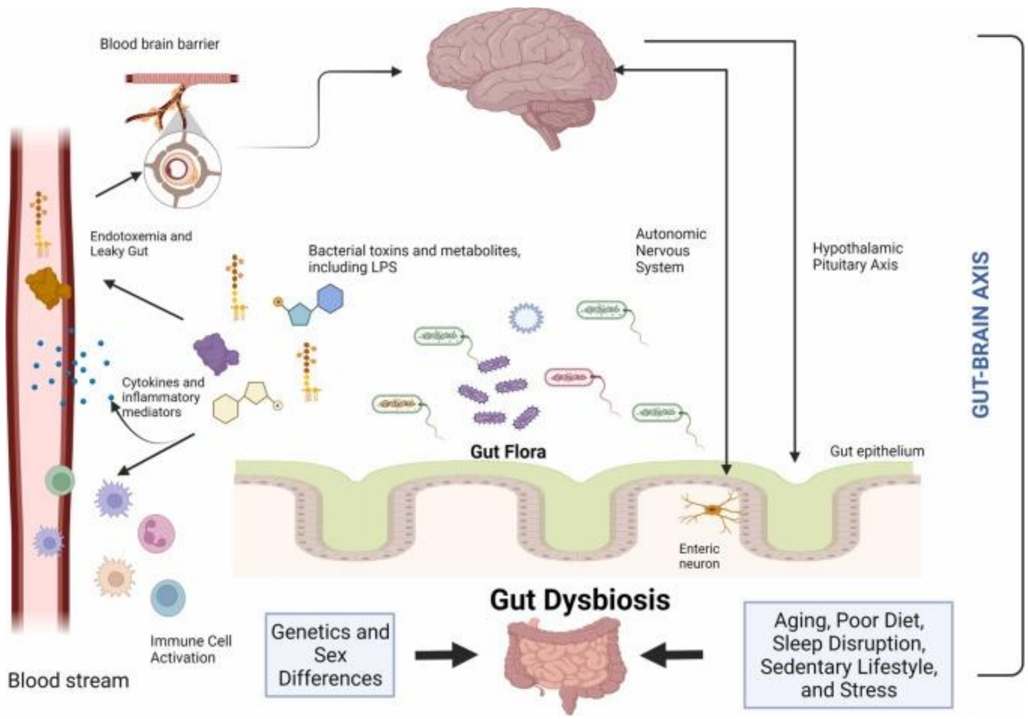


Figure 9. The Gut-Brain Axis.[20] Diagram demonstrates influences (poor diet, sleep disruption, etc.) on the gut that can lead to gut dysbiosis, which alters gut secretion of toxins and metabolites, and leads to increased cytokines and inflammatory mediators which results in a leaky gut and toxins throughout the blood, some with the capability of crossing the blood brain barrier. During this process, the gut also modulates the immune system. The brain modulates the gut via the hypothalamic pituitary axis and autonomic nervous system.

As previously mentioned, the pathogenesis of AD largely revolves around amyloid, tau, and neuroinflammation. Intestinal inflammation can trigger the misfolding of tau protein which can be delivered to the brain via the Vagus Nerve and has the capability to cross the BBB. Therefore, pathology does not have to originate within the CNS, but can spread from other organs through these bidirectional communication pathways.

THE GUT MICROBIOME AND ALZHEIMER'S DISEASE

The profile of the GM varies with race, ethnicity, gender, sex, and geographical location.[67,99,100] It is important to note that findings from various studies are inconsistent regarding how the composition of the microbiome of AD subjects can be affected by these factors, e.g. increase vs. decrease in ratios of each phyla being reported.[51,101,102] For example, Vogt et al. studied the GM in AD patients in the United States and demonstrated increased Bacteriodetes and decreased Firmicutes, while Zhuang et al. studied Chinese patients with AD and found a decrease in both Bacteriodetes and Firmicutes, compared to age- and gender-matched individuals without cognitive impairment.[51,101] Therefore, these differences may likely be attributed to the country in which the study is done and the population resides. Other characteristics that may play a role in the inconsistent findings include lifestyle, dietary habits, RNA sequencing area, and comorbidity burden.

Delving more into the details of these papers, Vogt et al. aimed to determine the gut microbial communities associated with AD due to recent evidence in mouse studies suggesting changes in the GM may increase amyloid deposition, a hallmark of AD pathology.[51] Twenty-five AD participants were recruited from Wisconsin Alzheimer's Disease Research Center (ADRC), and 25 age- and sex-matched controls were recruited from ARDC and Wisconsin Registry for Alzheimer's Prevention (WRAP) study in the

United States. Exclusion criteria included "other neurodegenerative disease, history of alcohol/substance dependence, major psychiatric/depressive disorders, the use of systemic antibiotics in the previous 6 months prior to providing the fecal sample; corticosteroid use, major dietary change during previous month (defined as eliminating or significantly increasing a major food group); major GI tract surgery in past 5 years (with the exception of cholecystectomy and appendectomy); major bowel resection at any time; active uncontrolled GI disorders or chronic constipation." The groups were comparable in age, sex, ethnicity, BMI, percentage of diabetic participants, and diet. Specifically, there was no difference in prevalence of subjects on the MIND diet (MD-Dash Intervention for Neurodegenerative Delay), as assessed through FFQs. It is notable that all except one AD participant was taking an acetylcholinesterase inhibitor and/or memantine, which may have influenced the GM. Researchers collected fecal samples, to be representations of the GM, in the morning and stored at $-80^{\circ}C$. Samples were DNA was extracted using NucleoSpin Gel and PCR Clean-up kit, and 16S rRNA sequencing using Illumina was used for genetic analysis. To evaluate AD biomarkers (amyloid and tau protein), lumbar punctures were performed in the morning after 12 hours of fasting. CSF measures included $A\beta_{42}/A\beta_{40}$ ratio, phosphorylated tau (p-tau), and the p-tau/$A\beta_{42}$ ratio. Researchers used the Abundance-based coverage estimator (ACE) and Chao1 to quantify microbial richness, which is defined as the number of unique operational taxonomic units (OTUs), including unique species, genus, phyla, etc., present in each subject. ACE and Chao1 are indices used to estimate the total microbial richness of a sample and to calculate expected OTUs based on observed OTUs.

In terms of associations between the GM and AD pathology, a predominantly negative correlation between specific bacterial abundance—increased *Blautia*, *Phascolarctobacterium*, and Gemella (Firmicutes), *Bacteroides* and *Alistipes* (Bacteroidetes), and *Bilophila* (Proteobacteria)—with decreased CSF $A\beta_{42}/A\beta_{40}$ suggested that increased abundance of those bacteria is associated with greater amyloid burden in the brain (lower levels of $A\beta_{42}/A\beta_{40}$). Additionally, a predominantly positive correlation between the same bacteria with increased CSF p-tau and p-tau/$A\beta_{42}$ was found, also supporting greater amyloid burden/AD pathology.

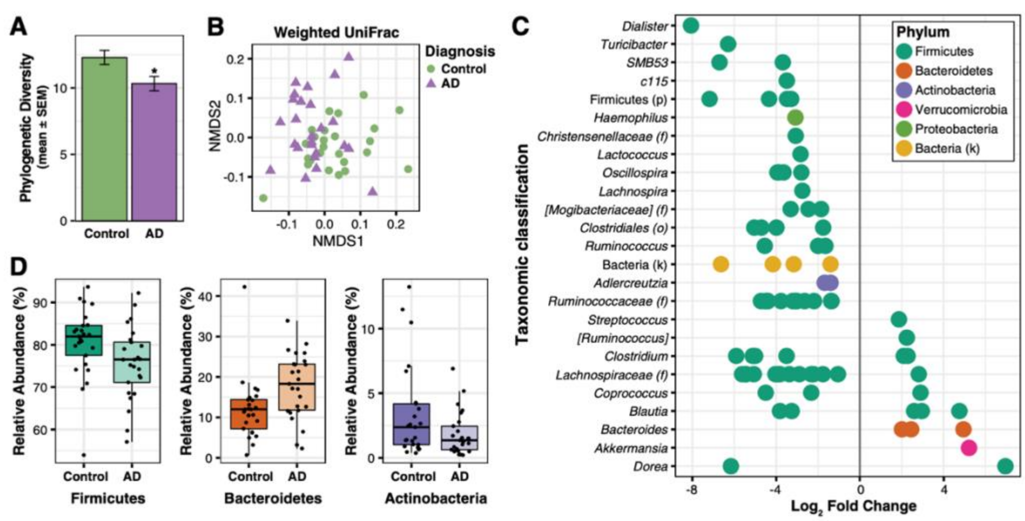


Figure 10. AD associated alterations in GM composition. (**A**) When comparing AD patients to control patients, the phylogenetic diversity is decreased in the microbiome of AD participants. (**B**) Non-metric multidimensional scaling (NMDS) plot of weighted UniFrac analysis of relative sample operational taxonomic units (OTU). The composition of the gut microbiome was characterized using measures of richness (the number of unique OTUs present in a participant) and alpha diversity (the richness and abundance of OTUs within each participant). Each dot represents a scaled measure of the composition and demonstrates the different gut microbial profile in AD patients vs. healthy controls. (**C**) Differential abundance analysis of taxa identified 14 OTUs that were increased and 68 OTUs that were decreased in AD relative to healthy controls ($p < 0.05$). Each dot

represents an OTU, plotted as $\log_2$ fold change. OTUs to the right of the zero line are more abundant in the AD group compared to healthy controls and OTUs to the left of the zero line are less abundant in AD compared to controls. (**D**) When OTUs were grouped at the phylum level, analysis demonstrated a decreased abundance of Firmicutes and Actinobacteria in AD patients, and an increased abundance of Bacteroidetes compared to healthy controls ($p < 0.05$).

Vogt et al. demonstrated reduced microbial richness in the AD group when compared to the control group in both ACE and Chao1 (*t*-test; ACE: DF = 48, $t = 3.05$, $p = 0.004$; Chao1: DF = 48, $t = 2.98$, $p = 0.004$, Figure 10a), with a significantly decreased presence of Firmicutes and Actinobacteria, and an increased prevalence of Bacteroidetes in AD patients compared to control participants ($p < 0.05$, Figure 10d). Researchers also identified the altered abundance of 13 different genera in AD that were correlated with CSF biomarkers of AD, indicating their association with greater AD pathology and increased severity of disease. This relationship was especially strong in *Bacteroides* (Bacteroidetes) and *Blautia* (Firmicutes). These findings suggest that the GM may be a target for therapeutic intervention in AD, however the study is limited by the small population size.

Zhuang et al. recruited 43 AD patients and 53 age- and gender-matched controls who had normal cognitive function, from three hospitals in China.[101] Exclusion criteria included a family history of dementia, any form of neurodegenerative disease aside from AD, mental illness, gastrointestinal diseases, or other severe chronic diseases. Researchers used a neuropsychological battery (as previously described) for neuropsychological testing. A diagnosis of AD was made according to the criteria of the National Institute of Neurological and Communicative Diseases and Stroke/AD and

Related Disorders Association. To analyze the GM, fecal samples were collected in the morning and stored at –80◦C prior to analyses. DNA was extracted using the standard Power Soil Kit and 16S rRNA gene sequencing using Illumina was performed. Data were analyzed by Partial Least Squares Discriminant Analysis. Groups were comparable in education attained and comorbidities, including hypertension, dyslipidemia, diabetes mellitus, and coronary heart disease, which otherwise could influence the GM, and this is a strength of this study. Researchers found a unique and altered microbial diversity in AD patients, notably decreased Firmicutes and Bacteroidetes relative to the control group. The main limitation of the study is the cross-sectional design, preventing causality from being established. Ultimately, the results further bolster the notion that the GM is altered in AD patients and may be involved in the pathogenesis of the disease.

Cattaneo et al. studied the association of amyloid deposition in the brain with a pro-inflammatory gut GM in elderly patients with cognitive impairment, defined as the presence of cognitive complaints reported by doctor and/or patient, abnormal scoring on ≥2 cognitive tests, history of progression/worsening symptoms, and absence of a metabolic or psychiatric cause of cognitive impairment.[103] Researchers analyzed anti-inflammatory taxa in the Firmicutes (*Eubacteria*, *Faecalibacteria*) and Bacteroidetes (Bacteroides) phyla, along with pro-inflammatory taxa (*Escherichia*/*Shigella* and *Pseudomonas* (Proteobacteria)) and pro-inflammatory cytokines through analyses of blood and stool samples. Two-hundred forty-one cognitively impaired participants were recruited from a previous study involving 18 memory clinics in Italy. Cognitively impaired, PET-amyloid positive (amy+; N=40) and cognitively impaired PET-amyloid negative (amy-) patients (N=33) were included, as well as individuals with normal cognitive function (N=10). There were no significant differences in demographic information between groups. NTB was used to assess global cognition. A Microbial DNA qPCR Assay Kit was used to analyze the abundance of taxa in stool, which was then calculated according to the comparative C_T method, a widely used method to quantify relative gene expression rather than absolute gene expression. C_T method uses the endpoint of PCR, during the exponential phase of amplification. Disadvantages include the assumptions regarding PCR efficiency, including that it is close to 1 and that the target gene is similar to the internal control gene. Researchers determined amy+ subjects had higher levels of pro-inflammatory cytokines, along with decreased *Eubacteria* (Firmicutes) and increased inflammatory *Escherichia/Shigella* (Proteobacteria) compared to the amy- ($p < 0.001$, $p = 0.003$) and control group ($p = < 0.001$, $p < 0.001$), respectively, suggesting a link between the GM and brain amyloidosis. It is important to note that researchers did not collect information regarding the participants' diets which may have influenced the GM data.

Other studies have also demonstrated this link between the GM and amyloidosis because certain species (*Bacillus subtilis* (Firmicutes), *Escherichia coli* (Proteobacteria), *Mycobacterium tuberculosis* (Actinobacteria), *Salmonella enterica* (Proteobacteria), *Salmonella typhimurium* (Proteobacteria), *Staphylococcus aureus* (Firmicutes)) were found to be capable of amyloid production.[104] These bacterial species grow as biofilms, or structured communities of microbes that are attached to various surfaces. Namely, *E. coli* was first discovered as capable of functional bacterial amyloid production with production of the amyloid curli, the main constituent of the biofilm's extracellular matrix, playing a major role in cell-cell adhesion.[105] Human beta amyloid and curli share an extremely similar quaternary structure and both are rich in beta sheets. This extreme resemblance results in the human immune system recognizing curli with the same receptors used to detect human beta amyloid, and consequently curli has a similar inflammatory effect in the human body to that of beta amyloid. Another example is the functional bacterial amyloid produced by *Pseudomonas*, known as Fap, which has been less studied compared to curli.[106] Sánchez-Tapia et al. conducted a cross-sectional study on volunteers from Mexico who were divided into the following groups: healthy subjects without comorbidities and not overweight (N=35), adults >60 years old with MCI (N=32), adults >60 years old with moderate cognitive impairment (N=42), and adults >60 years old with dementia (N=13). Blood samples were collected to evaluate lipid profile, LPS, tau protein tangles, beta amyloid plaque, APOE status. Stool samples were collected to analyze curli protein abundance. MMSE was used to determine cognition status. Researchers demonstrated that the abundance of curli was significantly associated with the abundance of *E. coli* ($p < 000.1$) and as the abundance of curli increased, MMSE decreased ($p < 000.1$). All subjects with dementia were found to be positive for curli, along with all subjects who were APOE4+.[107] Since these amyloid fibers can then exit the intestine and cross the BBB, more RCTs are needed to determine if bacterial amyloids produced by enteric bacteria can directly or indirectly increase beta amyloid plaque burden in the brain, contributing to AD pathology.

Liu et al. included 97 subjects (33 AD, 32 MCI, and 32 healthy controls) between the ages of 50 to 85 years and with at least six years of education to identify differences in the GM between groups.[108] The patients with AD and MCI were recruited from the Memory Clinic, Department of Neurology at School of Medicine, Zhejiang University. Cognitive function was assessed via the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA) and/or Clinical Dementia Rating (CDR) score. In patients who had completed 8th grade, a CDR score of 1+ or MMSE score ≤ 24 was indicative of AD, which was equivalent to a score ≤ 20 for those who only completed 4th grade, and ≤ 17 for those who did not have any education. MCI was defined as a MMSE score between 24 and 30 or a CDR score of 0.5. Individuals in the healthy control group were age- and sex-matched and the majority volunteered because they were the spouse of patients with AD or MCI who were also participating in the study. As a result, these subjects had lived in the same household for 20+ years and consumed similar diets. The control group had MMSE scores between 24 and 30, CDR scores of 0 (no dementia), and no significant memory complaints. Study exclusion criteria included IBS/IBD, severe chronic disease, brain tumor, traumatic brain injury, cerebrovascular accidents, psychiatric/mental illness, and history of alcohol/substance dependence. The composition of gut bacterial communities was determined by 16S rRNA sequencing. Researchers demonstrated unique GMs among all three groups. In AD participants, the prevalence of the Firmicutes phylum was significantly reduced ($P = 0.008$) relative to healthy controls, while the Proteobacteria phylum ($P = 0.024$) was significantly increased compared to healthy controls. A major novel finding of this study was the significant link between

Firmicutes predominance and AD progression rather than AD diagnosis alone. In addition, Bacteroidetes was significantly more abundant in healthy control individuals as compared with MCI patients. Additionally, there was a significant correlation between Bacteroidetes and MMSE scores in all 3 groups of participants. The increased Bacteroidetes in patients with cognitive impairment is consistent with the findings of Vogt et al.[51] Interestingly, the findings of this study were not consistent with Zhuang et al. who found decreased Bacteroidetes in Chinese AD patients, despite both studies being carried out in China with subjects who were Chinese AD patients.[101] This suggests that factors other than country of origin and residence affect GM composition in AD patients.

Interestingly, Liu et al. established a significant correlation between severity of AD and level of dysbiosis, adding functional differences of various taxa and species in AD populations to the literature.[108] Functional pathway analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) in AD patients revealed an increased prevalence of genes encoding for LPS synthesis compared to healthy controls. Pro-inflammatory *Escherichia-Shigella* (Proteobacteria) and *Streptococcus* (Firmicutes) were more abundant in AD patients while SCFA producers of Firmicutes were significantly decreased vs. the healthy control group. These alterations were positively correlated with the poorer cognitive function as reflected through lower MMSE and MOCA scores.[108] The reduction in SCFA producers impairs intestinal barrier function, alters acidity levels, and decreases protection against the growth of pathogenic bacteria, likely contributing to the increased abundance of inflammatory bacteria.[73,74] The microbial diversity in AD patients is significantly reduced relative to patients with MCI and to HC,[109] however dysbiosis may begin at the level of cognitive impairment since those with cognitive impairment prior to AD diagnosis also exhibited significant gut microbial alterations.[108,110,111] Importantly, Liu et al. also demonstrated the ability to analyze predominant phyla in an individual and based on those findings, determine whether the individual had MCI, AD, or were a HC.

Li et al. aimed to describe the role of gut microbiota in the pathogenesis of AD, elucidate GM alterations in MCI, and identify early biomarkers of neurodegeneration.[112] In light of the findings of Liu et al., these researchers hypothesized that gut dysbiosis begins in the MCI stage rather than during AD. Chinese patients with similar dietary habits were recruited. The diagnosis of AD and MCI was based on the criteria of the NIA-AA, including symptoms and neuroimaging. Three groups were analyzed: normal cognitive function (n = 30), MCI (n = 30), and AD (n = 30). There were no significant differences in age, gender, education, BMI, diabetes, or constipation. Patients with AD were taking AD medication, including acetylcholinesterase inhibitors and/or memantine. Patients with MCI were not on medication. Fecal samples were collected, DNA extracted by QIAamp DNA Stool Kit Method, and bacterial composition analyzed with 16S rRNA sequencing. Li et al. demonstrated differences in 11 genera from the fecal samples between the AD and control group, notably increased *Lactobacillus* (Firmicutes), and decreased Bacteriodetes. The decreased Bacteroidetes contrasts with findings from Liu et al.'s Chinese population, however, it is consistent with Zhuang et al. Also, in contrast to Liu et al., patients with AD and MCI both had decreased microbial diversity, and there were no differences detected in GM genera between AD and MCI. A weakness of this study was the lack of use of AD biomarkers to confirm the diagnosis of AD in the patients who were considered to have AD, along with the small sample size.

In a case-control study with participants recruited from China, Hou et al. used 16S rRNA sequencing of fecal samples to demonstrate that 18 taxa were significantly altered in AD patients when compared to non-AD subjects. AD patients (N=30) and sex- and age-matched (range 60-80 years) controls (N=47) were recruited from Xinjin, Chengdu, China. DNA was extracted from stool via the E.Z.N.A method and microbial composition was analyzed from 16S rRNA gene sequencing using Illumina. Researchers demonstrated increased prevalence of *Escherichia-Shigella* (Proteobacteria) and decreased SCFA production in AD subjects vs. healthy controls, consistent with Liu et al. Researchers also reported a strong association between the phylum Proteobacteria and Enterococcaceae (Firmicutes) with AD and, interestingly, their abundance was significantly elevated in patients with an APOE4 genotype, a major genetic risk factor for AD.[113] Despite the small sample size, a strength of the study is that the researchers factored in the effect of diet on the results by including questionnaires regarding dietary habits, nutritional supplements, probiotic usage, and any recent changes in diet in the past month. These results support the idea that targeting the GM microbiota may be an effective therapeutic strategy for AD, especially for those with the APOE4 allele who are at higher risk of developing the disease.

Haran et al. prospectively enrolled 51 healthy controls (HC) and 24 AD patients in the U.S., matched for age and sex, and collected stool samples once a month for up to five months.[102] Compared to the previously described studies, these researchers used a different method to study the GM- a DNA extraction method using PowerMag soil DNA isolation kit, or “shotgun metagenomics” and NextSeq 500 sequencing (rather than 16S rRNA). These researchers also used intestinal epithelial cell functional assays for P-glycoprotein (P-gp) expression *in vitro* to examine whether the GM of AD subjects (>65 years old) can induce an inflammatory state. This experimental approach allows researchers to develop models of GI tract disease with stem cells. P-gp, a transmembrane protein expressed on intestinal epithelial cells, is critical in intestinal homeostasis and health, and dysregulation leads directly to GI inflammatory disorders.[114] Since the function of P-gp is to facilitate removal of toxins and pathogens from the intestinal mucosa, dysregulation of the protein compromises intestinal integrity. The researchers also combined the metagenomic data with clinical data to identify the diverse species of the GM in AD compared to HC. Researchers found the GM of AD patients had fewer butyrate-producing species (a SCFA) and a higher abundance of pro-inflammatory taxa vs. HC. The GM alterations were accurate predictors of dysregulation of the P-gp pathway; stool samples from the AD group induced lower P-gp expression levels *in vitro* than stool samples provided by HC (p = 0.017). Additionally, shotgun metagenomic analysis revealed that there were more butyrate-coding genes present in all bacteria in HC relative to AD subjects. This research progresses the body of literature demonstrating associations between the GM and AD by using metagenomics to investigate intestinal microbial composition more robustly.

Zhu et al. investigated alterations of the GM in patients with MCI and AD by analyzing fecal samples from Chinese patients. However to advance the literature of the role of the GM in AD, the researchers also correlated specific taxa with APOE4 status, memory, and Clinical Dementia Rating (CDR) score.[115] HC (N=94) were recruited from the Shanghai Aging Study (SAS), a community-based study in Shanghai, China, while 125 patients with MCI and 83 patients with AD were recruited from the Shanghai Memory Study (SMS) from a memory clinic in Huashan Hospital.[115] Participants were all over 50 years of age. MCI or AD diagnosis was based on the 2011 NIA-AA criteria. HC had normal cognitive function, as indicated by NPT. Exclusion criteria included cognitive impairment caused by any neurodegenerative disorder aside from AD, tumors, traumatic brain injury, use of antibiotics in the past three months, history of major gastrointestinal surgery in past 5 years, history of irritable bowel disease/syndrome. Fecal samples were stored at −80°C until analysis. Microbial genomic DNA was extracted from feces using the E.Z.N.A. soil DNA Kit and 16S rRNA gene sequencing with Illumina was used. Groups were comparable in gender, history of diabetes, CVA, and alcohol intake (all $p > 0.05$). Significant differences among the groups included that the AD patients were younger than the MCI and HC groups (mean age, 71.8, 75.4, 74.3, respectively, $p = 0.004$), the HC group had more years of education relative to MCI and AD groups (12.4, 11.3, and 9.9, respectively, $p < 0.001$), the AD group or MCI group were both more likely to be APOE 4 carriers (HC: MCI: AD = 8%: 33%: 52%, $p < 0.001$) than the HC group, and hypertension was more frequent in the healthy controls (HC: MCI: AD = 55%: 54%: 33%, $p = 0.027$). Patients with MCI and AD had significantly different taxa than the control group, notably there were higher levels of Firmicutes (Erysipelatoclostridiaceae and Erysipelotrichales). The abundance of these taxa was also progressively more prevalent as cognitive decline heightened, as demonstrated from comparing taxa prevalence in HC to MCI to AD (all $p<0.001$). Furthermore, these microbiota changes were positively correlated with APOE4 genotype and negatively associated with memory, indicating a potential role in AD pathogenesis. Despite the relatively large sample size compared with previous research studies on the subject, the statistical power may not be sufficient to detect mild GM alterations. The study was also limited by the inability to control for confounding variables that may influence the GM. For example, the healthy control group had notably higher rates of hypertension, which may modulate microbial composition. Additionally, dietary habits of the participants were not accounted for, and biomarkers/pathology of AD were not used to confirm the AD diagnosis.

In summary, there have been inconsistent findings across studies regarding specific alterations at the phyla and genus level in AD patients. Animal and human studies have suggested a variety of mechanisms by which gut dysbiosis contributes to AD pathology and progression (Figure 11).

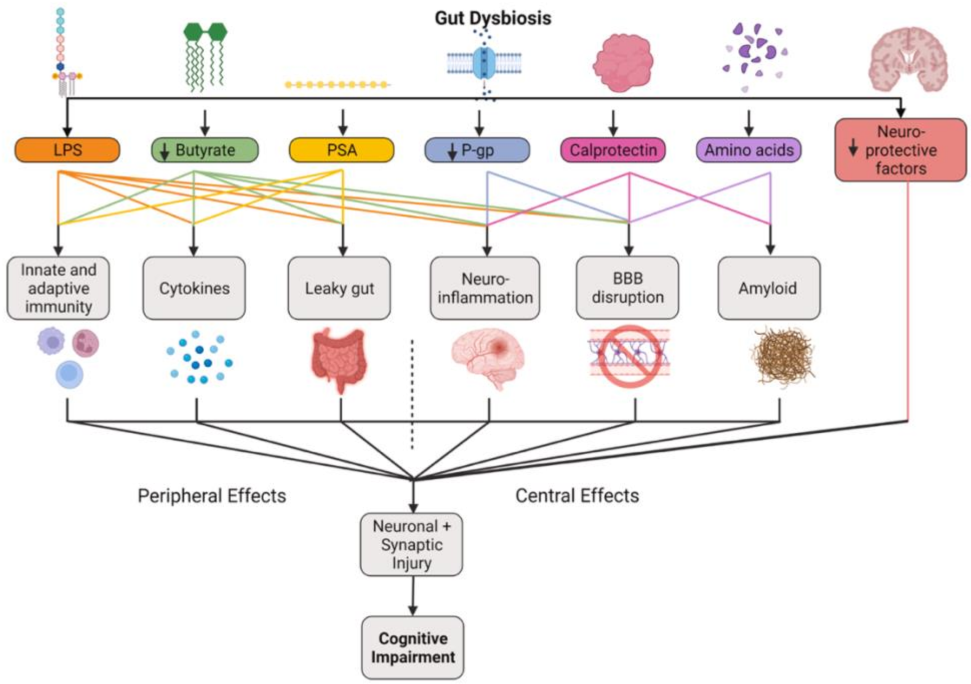


Figure 11. Potential mechanisms by which gut dysbiosis impacts AD pathology and progression, including influencing innate and adaptive immunity, altering the release of inflammatory mediators, namely cytokines, increasing neuroinflammation, altering the permeability of the blood brain barrier, increasing permeability of the intestinal epithelium, and increasing amyloid deposition, all of which directly impact neuronal and synaptic damage.[20] Abbreviations: LPS, lipopolysaccharide; PSA, polysaccharide A; p-gp, p-glycoprotein.

Studies suggest a link between dysbiosis in the gut microbiome and AD, including changes in GM composition during different stages of cognitive impairment in AD patients.[116] Modifying the GM is a promising target for therapeutic intervention.

MODIFICATION OF DIET AS A MEANS TO MODIFY THE GUT MICROBIOME

An individual's gut microbiome is altered by age, environmental factors, antibiotic use, stress, and infection, but one of the most powerful influences is an individual's diet.[117,118] Nutrition has continuously been demonstrated to be one of the greatest influences on the composition of the gut microbiome throughout all life stages.[119,120] Therefore, promoting a healthy gut microbiome via dietary alteration has been shown to be a promising strategy.

THE WESTERN DIET AND THE GUT MICROBIOME

The traditional Western diet is low in fiber, fruits, and vegetables and high in sugar, sodium, and fat. Calorie consumption from fats in the U.S. has recently approached about 45%.[121] A high fat diet is associated with insulin resistance, dyslipidemia, hyperinsulinemia, oxidative stress, sympathetic nervous system overstimulation, and hypothalamic-pituitary-adrenal axis dysregulation. As a result, obesity, chronic stress, anxiety, depression, diabetes, and many other poor health outcomes have risen. Researchers have identified the ramifications of a fat-enriched diet on the GM, quantifying an increase in inflammatory species of Firmicutes, LPS, and pro-inflammatory cytokines with a decrease in beneficial Bacteroidetes, SCFA, and butyrate, all modulations that trigger systemic inflammation and metabolic disease.[122–125] Evidence from numerous studies have supported that an increased Firmicutes:Bacteroidetes ratio is caused by a high fat animal-based diet and associated with disease.[126–130] The dysbiosis caused by excessive fat consumption increases intestinal permeability, reducing gut barrier function. In turn, there is increased leakage of toxins into systemic circulation. This accumulation of toxins contributes to neuroinflammation and blood brain barrier impairment, leading to neurodegeneration.

THE MEDITERRANEAN DIET AND THE GUT MICROBIOME

The Mediterranean Diet (MD) has been widely studied and is associated with a variety of health benefits, including eubiosis. The MD is packed with nutrient dense foods and is high in fiber. Fiber is a major energy source for gut microorganisms. It involves consumption of mainly plant-based foods and low consumption of red meat or processed foods. Protein sources include legumes, fish, nuts and seeds, beans, and small amounts of eggs and dairy. Carbohydrate sources are high quality, including local vegetables and fruit, potatoes, rice, breads, and unrefined grains. Olive oil is the primary source of fat, followed by a high intake of nuts. While the total fat content of the diet is moderate, ranging from 30-40%, there is a health promoting relationship between the balance of saturated fatty acids and unsaturated fatty acids. The MD also includes moderate consumption of wine. The accentuation on high oil content, nuts, and moderate alcohol (mainly red wine) make the MD unique relative to other dietary regimens.[131] The abundance of polyphenols in the MD likely contributes to the health benefits of the diet, due to their anti-inflammatory and antioxidant nature.[132] Polyphenols have been associated with protection from cellular aging and endothelial dysfunction in heart studies and have the crucial ability to cross the blood-brain barrier.[133] They have been found to reduce neuroinflammation caused by LPS injection in mouse studies and promote cell growth and hippocampal neurogenesis in chronically stressed adult mice.[134,135] Overall, a plant-based diet is associated with a decrease in opportunistic pathogens, LPS, and inflammatory cytokines, and an increase in beneficial bacteria and SCFA.[136]

Ultimately, the MD is rich in neuroprotective nutrients, including antioxidants, B vitamins, and polyunsaturated fatty acids (PUFA).

Numerous studies have examined MD-associated gut microbial changes. Due to increasing evidence of the role of the GM in metabolic disease, Cancello et al. aimed to demonstrate ways to modulate the GM and restore homeostasis. To do so, researchers evaluated the baseline genera of the GM before treatment with 15 days of a hypocaloric MD in 20 elderly, obese women from Italy via 16S rRNA sequencing of fecal samples. There were elevated pro-inflammatory *Streptococcus* (Firmicutes) levels in obese patients relative to non-obese controls and these findings were correlated with the Western diet.[70,137,138] Additionally, there were reduced numbers of beneficial SCFA producers.[139,140] Participants were then started on a 15-day hypocaloric MD, followed by an additional 15 days of a hypocaloric MD with probiotic supplementation. At the end of the dietary intervention, metabolic profiles improved with increases in bacterial species that are known to be associated with leanness (*Methanobrevibacter* (Euryarchaeota), *Akkermansia* (Verrucomicrobia), *Parabacteroides* (Bacteroidetes).[140,141] *Methanobrevibacter* has been shown to be associated with increased fiber intake.[142] Additionally, *Parabacteroides* has anti-obesogenic effects, is anti-inflammatory, and reduces insulin resistance while *Akkermansia* improves metabolism and insulin sensitivity.[143,144] Researchers concluded that transitioning to a healthy diet changes the GM in ways that benefit the individual's metabolism and can help restore homeostasis. Another study evaluated the GM composition in 140 young, healthy university students (males and females) in Italy, adjusted for anthropometric and demographic characteristics, who consumed the MD, with the aim to explore the composition of the GM in association with the MD.[145] Metagenomic sequencing of fecal samples revealed more abundant *Lactobacillus and Lactococcus* (Firmicutes), with MD adherence. These are health-promoting lactic acid bacteria found in high concentrations in extra virgin olive oil that reduce oxidative stress and inflammation.[146] In another study comparing the impact of two 5-year dietary interventions (MD or low-fat/high carbohydrate (LFHC)) on the GM of obese men with coronary heart disease, the MD decreased *Prevotella* (Bacteroidetes) and increased *Roseburia* (Firmicutes) genera, whereas the LFHC diet did the opposite.[147] The results of a 2-year follow-up study suggested that the extended consumption of either the MD or LFHC diet can partially restore eubiosis in patients with metabolic dysfunction.[148] Overall, it is well supported that the gut microbiome of obese individuals is largely characterized by increased Firmicutes and decreased Bacteroidetes when compared to leaner individuals. Obesity and insulin resistance are major risk factors for Alzheimer's Disease. The havoc wreaked on the gut microbiome by high fat diets and the coincident changes in the GM seen in obese patients then may be playing a role in the pathogenesis of Alzheimer's Disease.

THE MEDITERRANEAN DIET AND ALZHEIMER'S DISEASE

As noted, nutrition and altering dietary habits have been proposed as possible approaches to improve overall health, reduce inflammation, and prevent AD. In a study on the MD, incident-AD (new change in state of health to AD diagnosis), and inflammatory and metabolic markers, Gu et al. 2010 analyzed whether increased adherence to the MD could improve the metabolic profile of patients and reduce risk of AD.[149] Participants (N=2778) from The Washington/Hamilton Heights-Inwood Columbia Aging Project II (WHICAP II) Study were recruited.[150] This prospective study included Manhattan residents that were at least 65 years old. Medical histories were collected and a NTB was employed to assess cognitive function. 1559 participants were excluded from the study for one of the following reasons: lack of a sufficient blood sample, lack of follow-up, a prevalent AD diagnosis, or lack of complete dietary information. 1219 were included in the study (66.7% were female with an average of 76.7 years ± 6.4. Assessments recurred every 18 months. They assessed average diet over a one-year period through validated Food Frequency Questionnaires (FFQ), scoring participant's adherence to a MD on a scale of 0-9, with low adherence being categorized as 0-4, and high adherence 5-9. A point was given if the patient's average consumption was greater than that of the sex-specific population median for each of the following components: vegetables, legumes, cereals, fish, fruits/nuts, and monounsaturated fats: saturated fats. A point was also given if the subject's median consumption of meat and dairy products were lower than median of the sex-specific population and a point for mild-moderate alcohol consumption (quantified as 0-30 g/day). Researchers used high sensitivity C-reactive protein (CRP) as a measure of inflammation, and fasting insulin and adiponectin as measures of metabolic profile. After 4 years of follow-up assessments with FFQs completed every 18 months, 1,337 participants completed all the required dietary information and blood samples. There were 118 cases of incident-AD as indicated by NTB interpreted by trained physicians. Gu et al. 2010 demonstrated that higher adherence to the MD was associated with reduced CRP (p = 0.003), but there was no significant difference noted in metabolic profile markers. Additionally, researchers found that high adherence to the MD (score 5-9 on FFQ) was associated with a 34% reduced risk of AD compared to those with less adherence (p = 0.04). They demonstrated that poorer cognitive performance, graded by neuropsychological tests evaluating memory, language, processing speed, and visual-spatial ability, was associated with lower adherence to MD after adjustment for age, race, gender, and education (p = 0.5). The study findings are limited due to the inherent bias of self-reported data; participants may have overestimated or underestimated their average consumption on a typical day over the year of study participation. Furthermore, the 0-9 scale may have weaknesses in interpretation, with 4 being "low adherence" but one additional point classifying subject's habits as "high adherence" and lack of an in-between.

To further strengthen the literature on adherence to MD and brain health, the same researchers sought to determine whether increased adherence to the MD was associated with larger brain volume and cortical thickness, which are well accepted features of a healthy brain, in a cross-sectional study of 674 multiethnic Americans (males and females) residing in Manhattan without MCI.[151] Participants were again recruited from WHICAP.[150] To ensure subjects were dementia and MCI-free, participants had comprehensive cognitive assessments with neuropsychological testing and physician evaluations every 18 months. FFQs were used to assess average diet over the previous year, and a participant's adherence to the MD was scored as noted above on a 0-9 scale.[149] Gu et al. 2015 analyzed neurologic changes through MRI imaging, collecting information on total brain volume (TBV), total gray matter volume (TGMV), total white matter volume (TWMV), and mean cortical thickness (mCT). Regression models were adjusted for age, sex, ethnicity, education, and BMI to determine the association between adherence score (0-9) and specific food groups with brain volume. The FFQ data was collected 2.6 weeks prior to MRI imaging. They found that higher fish consumption (3-5 oz weekly) was associated with a larger mCT (0.019 mm, p = 0.03) and lower meat consumption (<100 g/day) was associated with larger TBV (p = 0.02). However higher adherence to the all-encompassing MD had the greatest benefit, suggesting the combination of foods rather than single components of the diet are responsible for the neuroprotective effect. Specifically, higher adherence was associated with a 13.11 mm larger TBV (p = 0.007), 5.00 mm larger TGMV (p = 0.05) and a 6.41 mm larger TWMV (p = 0.05). Interestingly, researchers reported that a weekly fish consumption of 3-5 oz or minimal meat consumption (<100 g/day) is protective from neurodegeneration, preventing brain atrophy equivalent to a 3-4 years of aging.[151] Researchers concluded that among adults over the age of 65, the MD is associated with less neurodegeneration and atrophy, with key elements of the MD likely being decreased meat consumption and increased fish consumption. While the study provides evidence on the impact of nutrition on brain atrophy in a group of individuals with typical cognitive function, it cannot be generalized due to the lack of demographic diversity.

Similarly, Mosconi et al. studied 52 individuals (age range 42-66 years, 70% women) with normal cognition in a cross-sectional study to assess the association between the MD and brain atrophy. Researchers used the same method of MRIs, FFQs, and MD adherence value calculation as Gu et al. 2010. The higher MD adherence group (n = 20) and lower MD adherence (n=32) group were similar in clinical and NPT measures, presence of a family history of AD, and APOE status. With and without controlling for age and total intracranial volume, higher MD adherence subjects showed greater thickness of AD-vulnerable brain regions as compared to the lower adherence group (p=0.026). Notably, the orbitofrontal cortex (p=0.001), entorhinal cortex (p=0.03) and posterior cingulate cortex (p=0.04) had less tissue loss in the higher MD adherent group, suggesting that the MD may be protective against AD neurodegeneration.[24]

In a study exploring incident-AD diagnosis and different diets, subjects were selected based on their participation in the Rush Memory and Aging Project (MAP), all of whom were volunteers residing in retirement communities in the Chicago area and over the age of 58. Volunteers in the MAP study (N=1545) were invited to participate in this dietary AD study which included validated semi-quantitative FFQs; 239 passed away or declined and 238 failed to complete two neuropsychological evaluations in order to confirm dementia-free at baseline, resulting in 923 eligible. Participants were followed for approximately 4.5 years in order to assess incidence of Alzheimer's disease and participants' food habits. Through the FFQ, subjects reported their typical frequency of consumption of 144 foods over the previous year and received scores on how much their diet resembled the DASH diet, the MD, and the MIND diet (Table 3).

DASH[a]		MedDiet[b]		MIND	
DASH components	**Max Score**	**Mediterranean Diet components**	**Max Score**	**MIND components**	**Max Score**
Total Grains ≥7/d	1	Nonrefined Grains >4/d	5	Whole Grains ≥3/d	1
Vegetables ≥4/d	1	Vegetables >4/d	5	Green Leafy ≥6/wk	1
		Potatoes >2/d	5	Other Vegetables ≥1/d	1
Fruits ≥4/d	1	Fruits >3/d	5	Berries ≥2/wk	1
Dairy ≥2/d	1	Full-fat Dairy ≤10/wk	5		
Meat, poultry & fish ≤ 2/d	1	Red meat ≤ 1/wk	5	Red Meats and products <4/wk	1
		Fish >6/wk	5	Fish ≥1/wk	1
		Poultry ≤3/wk	5	Poultry ≥2/wk	1
	1		5	Beans >3/wk	1

DASH[a]		MedDiet[b]		MIND	
DASH components	**Max Score**	**Mediterranean Diet components**	**Max Score**	**MIND components**	**Max Score**
Nuts, seeds & legumes ≥4/wk		Legumes, nuts & beans >6/wk		Nuts ≥5/wk	1
				Fast/fried food <1/wk	1
Total Fat ≤ 27% of kcal	1				
Saturated Fat ≤ 6% of kcal	1				
		Olive oil ≥1/d	5	Olive Oil primary oil	1
				Butter, margarine <1T/d	1
				Cheese<1/wk	1
Sweets ≤ 5/wk	1			Pastries, sweets <5/wk	1

DASH[a]		MedDiet[b]		MIND	
DASH components	**Max Score**	**Mediterranean Diet components**	**Max Score**	**MIND components**	**Max Score**
Sodium ≤ 2400mg/d	1				
		Alcohol < 300mL/d but >0	5	Alcohol/wine 1/d	1
TOTAL DASH Score	10	**TOTAL MedDiet Score**	55	**Total MIND Score**	15

Table 3. MD, DASH, and MIND scores
Table from Epstein et al. 2012. to highlight the key features and differences between the MD, MIND diet, and DASH diet.[152]

The DASH (Dietary Approaches to Stop Hypertension) diet emphasizes low sodium intake, plenty of vegetables, fruits and low-fat dairy products, and limited portions of red meats and processed food. The MIND (Mediterranean-Dash Intervention for Neurodegenerative Delay) diet is a hybrid of the DASH diet and MD diet with the following notable differences: the MIND diet specifies more frequent weekly consumption of green leafy vegetables and other vegetables; and the MIND diet does not specify daily fruit servings but emphasizes berry consumption.

Based on their scores on each diet, participants were classified into tertiles reflecting their adherence (high, medium, low). For instance, those who scored a 7-8 on the MIND diet, a 4.0-4.5 on the DASH diet, or a 30-34 on the MD had medium adherence for that specific diet and were deemed middle tertile. Of the 923 participants, 144 developed AD by the end of 4.5 years as determined through annual neuropsychological testing by medical professionals. When comparing the incident AD group compared to the healthy group, researchers considered education level, BMI, physical activity, depressive symptoms, and comorbid conditions. The MIND diet was associated with a significantly reduced risk of AD. Notably, when adjusted for age, sex, APOE4 status, physical activity, and education, individuals with the highest adherence to the MIND diet (score 8.5 – 12.5) had a 53% reduction in risk of AD compared to the low adherence group (score 2.5 – 6.5) (HR=0.47; 95% Confidence Interval: 0.29, 0.76). Even the middle adherence group to the MIND diet had a statistically significant 35% reduction in AD risk compared to the lowest adherence group. On the other hand, the highest tertile/adherence to the MD had a 54% reduction in AD (HR=0.46; 95% Confidence Interval: 0.29, 0.74; p = 0.001) while the highest tertile/adherence to the DASH diet had a 39% reduction (HR=0.61; 95% Confidence Interval: 0.38, 0.97, p = 0.07).[1] This study suggests that greater adherence to diets with emphasis on leafy vegetables, fruits, fish, legumes, nuts, olive oil, and mild/moderate alcohol intake with limited meat and dairy consumption can be protective against development of AD. Strengths of this study include the interpretation of NPT by clinicians, the use of FFQs that were validated in this elderly Chicago-based population, and the minimization of selection bias. The major limitation of this study is the observational design preventing the establishment of causality.

In a randomized clinical trial of 334 healthy participants from Spain, researchers aimed to investigate whether a MD supplemented with antioxidant-rich foods boosts cognitive function compared to a control diet.[153] Participants were randomly divided into one of three groups: MD + 1 liter/week of extra virgin olive oil, MD + 30 g mixed nuts per day, or the control group. Cognitive function was studied through interpretation of NPT. Participants were scored in memory, frontal (attention and executive function), and global function at baseline and after 4 years of dietary intervention. Mean z-scores of changes from baseline were used. Multivariate analyses were adjusted for confounders and there were no between-group differences observed for initial cognitive tests. For the MD + olive oil group, memory improved +0.04 mean z-score (-0.10 to 0.17, 95% CI), frontal cognition composite increased +0.23 (0.02 to 0.43; $P = .004$ vs controls), and global cognition improved +0.04 (−0.12 to 0.20; $P = .008$ vs controls). For the MD + mixed nuts group, memory improved +0.10 (−0.04 to 0.24; $P = .04$ vs controls), frontal function improved +0.03 (−0.26 to 0.32), and global cognition decreased −0.04 (−0.27 to 0.19). For the control group, memory diminished −0.16 (−0.32 to −0.01), frontal function diminished −0.33 (−0.57 to −0.09), and global cognition decreased −0.37 (−0.56 to −0.17) for the control diet. All cognitive composites significantly ($P < .05$) decreased from baseline in controls. Valls-Pedret et al. demonstrated that the MD supplemented with extra virgin olive oil or mixed nuts, both of which are high in antioxidants, mitigated age-related cognitive decline independent of sex, age, energy intake, education level, APOE4 genotype, and vascular risk factors.[153] The quality of evidence from this study is strengthened by the RCT design.

In an 18-month clinical trial, researchers aimed to explore the effect of diet on age-related brain atrophy at a research center in Israel. Among the 378 volunteers, 294 met inclusion criteria, >30 years old with abdominal obesity (men waist >102 cm, women >88 cm) or dyslipidemia. Participants were randomly divided into three dietary groups: a healthy diet guidelines (HDG) group, a MD group, and a Green MD group. All groups were provided a free gym membership and had in-person nutritional and physical activity sessions guided by dietitians, physicians, and fitness instructors. These interventions were weekly for the first month and monthly for the remainder of the study. The HDG group received typical nutrition consultations, the MD group were to follow a calorie-restricted, nutrient-dense diet focused on vegetables, fish, nuts, and legumes that was low in simple carbohydrates and red meat. The Green-MD group was instructed to follow a similar diet to the MD group, however they were required to completely avoid processed food and red meat, and drink 3-4 cups of green tea daily to increase polyphenol consumption. Adherence to respective diets was assessed through FFQs. Within 6 months of the study, 10 participants dropped out due to lack of motivation. 224 participants (79%) remained compliant for the full 1.5-year duration, resulting in 224 eligible MRI data. Notable characteristics of the overall group include: 88% men, average age 51 years, 15.7% having the APOe4+ genotype, and average BMI 31.2 kg/m^2. These were not statistically different between intervention groups and the APOe4+ genotype was equally distributed (n = 43). MRIs were analyzed for hippocampal occupancy score (HOC) and lateral ventricle volume (LVV) expansion, two signs of neurodegeneration. Most (79%) participants exhibited brain atrophy with aging. In subjects over the age of 50 on the MD diet or Green-MD diet, there was reduced hippocampal atrophy and reduced lateral ventricle expansion relative to the HDG group at the conclusion of the trial. The Green-MD diet had the most substantial difference when compared to the HDG group (HOC: −0.8% ± 1.6% compared with −1.3% ± 1.4%; 95% CI: −1.5%, −0.02%; P = 0.042; LVV: 2.3% ± 4.7% compared with 4.3% ± 4.5%; 95% CI: 0.3%, 5.2%; P = 0.021).[154] While there were significant differences in the MRI findings, it is important to note that there was no control group without intervention for comparison. The addition of physical activity in each group may have played an additive role in brain health that is not being accounted for. Furthermore, with 88% of the sample size being male, the results cannot be generalized to women. Finally, the education status of participants, baseline cognitive status (normal, MCI, dementia, etc.) and mental health conditions, including depression, are not known, all of which can play a role in neurological changes. Strengths of the study include the relatively large sample size for a clinical trial that was studied over a substantial period. Additional strengths were the setting of a research center that enabled close monitoring of participants, strict dietary guidelines and monitoring, group meetings with medical and health professionals, and access to the required dietary regimen free of expense.

Lee et al. explored the effect of grapes, which are loaded with the anti-inflammatory phenol, resveratrol, on cognitive function.[155] Resveratrol is rather predominant in the MD, notably from the increased grape, wine, berry, and nut consumption. In mouse studies, it has been demonstrated to protect against the natural aging process, and protect against beta-amyloid deposition, a hallmark of AD.[156] In a double-blind RCT, 10 subjects with MCI (age range 66 – 82, average age 72.2 years, 50% female) were randomized into a grape formulation group ("freeze-dried grape powder made of commercially grown fresh red, green and blue-black California grapes (Vitis vinifera) 36 g p.o. bid reconstituted in 8 oz. water") or a control group which received a placebo formula. The placebo formulation matched the color, texture, and aroma of the grape formulation but was absent of polyphenols. A monthly supply of respective formula packets was provided to participants, and they were instructed to take two daily for six months. Researchers ensured compliance by distributing calendars to subjects that were to be checked off daily and detailed with the timing of each dose. There were no differences in education level attained or age between the two groups. NTB and FDG-PET scans were performed at baseline and after 6 months of intervention. Exclusion criteria included under the age of 65, previous AD or dementia diagnosis, and thyroid disease. The placebo group exhibited significant decline in metabolism in the AD-vulnerable regions of the brain, including the posterior cingulate cortex ($p = 0.01$) and superoposterior temporal cortex ($p = 0.04$). However, there was not a significant decline observed in the grape formulation group, suggesting the antioxidant and anti-inflammatory effects of the grape may protect from metabolic decline. The two groups were comparable in performance in NPT. However, the grape formulation group had increased metabolism in areas of the brain (right superior parietal cortex and left inferior anterior temporal cortex) associated with stronger working memory and attention ($p = 0.04$) relative to controls. Additionally, it is important to note that despite the small sample size, brain regions were identified that exhibited statistically significant different metabolic function. Therefore, this study is limited by the extremely small sample size and is not generalizable. There is also inherent self-report bias of personally recorded consumption of resveratrol which is less accurate than if biomarkers were used to measure formulation consumption. Further analyzing the power of resveratrol, Turner et al. completed a double-blind RCT of resveratrol in individuals with mild-moderate AD dementia. Participants were recruited from 26 academic clinics in the United States affiliated with the Alzheimer's Disease Cooperative Study (ADCS).[52] One group (n = 56) was given encapsulated resveratrol powder (500 mg orally once daily (with a dose escalation by 500-mg increments every 13 weeks, ending with 1000 mg twice daily) while the control group (n = 48) received an identical placebo for 52 weeks. The general characteristics of the two groups were comparable at baseline. Researchers studied the effects of resveratrol administration on AD biomarkers (plasma Aβ40 and Aβ42, CSF Aβ40, Aβ42, tau, and phospho-tau181) and obtained MRI imaging. The resveratrol administered in the experimental group stabilized plasma and CSF markers of AD (plasma Aβ40 and CSF Aβ40) compared to the control group. Additionally, participants with biomarker-confirmed AD (baseline Aβ42 <600 ng/ml), resveratrol stabilized CSF Aβ42. From these studies, it is clear that components of the MD can have potent effects on brain health.

Moussa et al. performed a retrospective analysis of a subset of AD participants from Turner et al. who had CSF Aβ42 <600 ng/ml, a biomarker level confirming AD.[157] Subjects (N=19) were treated with resveratrol and 19 were treated with placebo. The plasma and CSF that were stored from Turner et al. were analyzed. In this subset of subjects, treatment with resveratrol diminished cognitive decline as assessed by MMSE scores and improved CSF Aβ42 levels during 52-week intervention. Tau levels did not significantly differ between groups.

Finally, Bredesen et al. studied the potential of metabolic enhancement for neurodegeneration (MEND) protocol to reverse cognitive decline in AD or MCI. Researchers followed ten patients, 6 who had MCI diagnosed and 4 who had AD diagnosed, all of whom were started on the MEND protocol for varying periods of time, from 5-24 months. Nine of the subjects were APOe4+ (5 homozygous, 4 heterozygous). Their prior diagnosis of MCI or AD was based on clinical presentation, subjective reports of changes in cognitive/functional status, MOCA scores, MMSE scores, family history of AD, APOe status, MRI scans, FDG-PET scans, amyloid-PET scans, and neuropsychological testing. Subjects were between the ages of 49-74 and 5 were male. The MEND protocol consists of greater than 24 interventions, including changes in diet (low glycemic Mediterranean Diet, fast 3 hours before bed and for 12 hours between dinner and breakfast), sleep, exercise, mindfulness, regular brain stimulation therapy, and a supplement routine (addition of fish oil, vitamin d, curcumin). All participants had subjective improvement post-MEND protocol as examined by neuropsychological testing, MMSE scores, MOCA scores, and/or hippocampal volume increase. Two patients returned to normal range for cognitive testing and no longer met criteria for MCI or AD, 9 patients had improvements in neuropsychological testing, and all patients reported subjective improvement.[158] Despite the unprecedented improvement in symptoms and cognition, limitations of the study need to be noted. First, there is a small sample size, and it is not evident how long the effects of the protocol will last. Additionally, participants were well-educated and a large percentage of them were ApoE4+. Therefore, the sample is not generalizable. The case series study design combined with the complex MEND intervention is likely further impacted by the placebo effect and selection bias. Unfortunately, researchers did not include a methods section, so it is not clear which elements of the protocol were followed. Due to the lack of specifics regarding the method, there is no information on the dose of supplements or duration of other MEND parameters (sleep, exercise, mindfulness, etc.). As a result, the study is not replicable. Lastly, the statistical significance of the changes is never noted throughout the paper, it is not clear if all participants had follow-up imaging, and subjects may have had a learning curve of the specific NPT repetitively used.

THE MD, GM, and AD

In a double-blind U.S. pilot study, researchers analyzed whether a modified Mediterranean-ketogenic diet modulated the GM in 17 subjects (6 HC and 11 MCI) and signature AD biomarkers in cerebrospinal fluid, including beta amyloid and tau protein.[159] While no significant differences were found in microbial composition between HC and MCI individuals, Proteobacteria trended positively with AD biomarkers, while fecal butyrate correlated negatively.

Zhang et al. studied the relationship between diet, the GM, serum miRNA, and risk of MCI in Chinese participants who were recruited from the Effects and Mechanism Investigation of Cholesterol and Oxysterol on Alzheimer’s disease (EMCOA) study.[110]

MiRNAs are important for posttranscriptional gene silencing by binding to messenger RNA and preventing translation of the gene. Therefore, miRNAs play a major role in gene expression. EMCOA is a multicenter prospective study of volunteers between 50 and 70 years old. Surveys were performed at baseline and every two years. Surveys consisted of interviews on demographic information (age, sex, and education years), lifestyle (smoking, drinking, and exercise), and medical history (hypertension, diabetes, hyperlipidemia, and heart disease). A battery of cognitive tests (MMSE, MOCA) and dietary surveys (CDGI-2018, E-DII) were also completed. Participants were excluded for any severe chronic disease, psychiatric/mental illness, brain injury or tumor, CVA, and/or history of substance/alcohol dependence. Participants (N=2239) completed the survey at baseline and at least one consecutive follow-up visit. Researchers measured the incidence of MCI at follow-up visits. From this subgroup, Zhang et al. evaluated the GM, diet, and serum miRNA of 75 newly diagnosed MCI patients and 52 controls. Blood and stool samples were collected. DNA was extracted by Power Fecal DNA Isolation Kit instructions and microbial diversity was characterized through 16S rRNA gene sequencing using Illumina platform. In terms of the dietary questionnaires used, the CDGI-2018 (from the Chinese Center for Disease Control and Prevention) assessed diet quality by scoring 13 components, tallying to a total from 0 to 110 points, with a higher score indicating a higher quality diet.[160] The other questionnaire, E-DII, assessed the inflammatory potential of diet, specifically evaluating proinflammatory parameters, including carbohydrate, protein, total fat, cholesterol, saturated fatty acids and total caloric intake, and anti-inflammatory PUFAs, fiber, and vitamins.[161] The two groups were comparable in age, sex, education, BMI, smoking, drinking, and exercise. MMSE and MoCA scores were lower in the MCI group compared to the control group ($P < 0.001$), along with lower scores on the CDGI-2018 ($P = 0.006$). Those with the highest diet quality had a 25% to 46% reduced risk of MCI while those with more inflammatory diets (higher E-DII scores) exhibited a 46% increased risk of MCI. Researchers also demonstrated significantly decreased alpha and beta diversity of GM bacteria among MCI subjects relative to the control group, and altered abundance of several taxa, notably reduced prevalence of various classes of Firmicutes (Ruminococcaceae, Faecalibacterium). Researchers established a predictive model that combined diet quality scores and the GM with serum miRNAs. The model that combined all these factors together accurately distinguished individuals with MCI from healthy individuals with improved performance (AUC = 0.91) than the model using miRNA data alone, suggesting that diet, the GM, and miRNAs may serve as a combinatorial biomarker for MCI.

In summation, the MD has substantial research supporting it as a promising intervention to attenuate cognitive decline in AD. However, more dietary intervention RCTs with the MD are needed to confirm the findings mentioned above and examine whether the MD may prevent and/or decrease progression of AD through gut microbiota modulation.

METHODS

Study design

To achieve study aims, a 3-year randomized control trial comparing a standard diet to the Mediterranean Diet for patients with Alzheimer's Disease will be conducted. Effects of diet on the gut microbiome and the relationship with clinical symptoms of AD, quantitative markers of AD, imaging and neuropsychological testing will be analyzed. Target enrollment is 1000 participants, randomized to MD or control.

Study population and sampling

Residents of the U.S. with Alzheimer's Disease that are over the age of 65 will be recruited from Alzheimer's Disease Research Centers (ARDC) and other AD centers and Neurology clinics across the country. Inclusion and exclusion criteria are noted below (Table 4). In general, participants 65 years and older with an AD classification of stage 4 (AD with mild dementia) by a physician without major chronic illnesses who have a family member/friend/caregiver willing to help answer questions regarding the patient's symptoms and overall function will be included. This close contact will also need to confirm adherence to dietary intervention for those in the treatment group and help with the FFQ for those not in the treatment group.

	Inclusion Criteria
1	Stage 4 AD diagnosis according to NIA-AA criteria by physician for at least 6 months

2	appropriate neurological consultation and CT/MRI obtained
3	capacity to provide informed consent and/or by family member/caregiver
4	65 years or older
5	residence in U.S for >10 years
7	must have a close family member, friend, or caregiver willing to participate
	Exclusion Criteria
1	non-stage 4 AD
2	history of thyroid disease
3	depression/psychiatric illness
4	history of CVA, neurological tumor, TBI
5	history of substance or alcohol dependence
6	IBD/IBS or GI inflammatory disorders
7	clinically significant electrolyte abnormalities and/or abnormalities in B12
8	the use of systemic antibiotics in the previous 6 months prior to providing the fecal sample
9	on a specific dietary plan
10	corticosteroid use
11	family member/friend/caregiver unable to participate in study interviews
12	Any contraindications to MRI or PET imaging

Table 4. Inclusion and Exclusion Criteria for study participation

Substance/alcohol dependence, psychiatric/depressive disorder, prior CVA/brain tumor/TBI, and other neurodegenerative diseases are all relevant exclusion criteria due to their impact on global cognition and function. The use of systemic antibiotics in the past 6 months, corticosteroid use, and adherence to a diet plan will influence the GM and could impact the dietary intervention, as could the participant’s willingness to stop or start a new diet. Overall, excluding patients with these histories will help eliminate confounding variables. Patients who have been excluded will be notified and thanked for their interest.

Participants who meet inclusion criteria will be randomly assigned to the control (no dietary intervention) or experimental group (MD intervention). The alpha level that will be used is 0.05 and beta level is 0.20. The plan will be to estimate the difference in means for continuous variables (NTB global cognition, MOCA scores, amyloid and tau burden on imaging and CSF, hippocampal volume, hypometabolism) between two independent populations (MD vs. control) and compare the baseline taxonomic profiles of the two groups to the taxonomic profiles post intervention. We used data from FINGER study which was a 2-year RCT with 1260 participants between the ages of 60-77 years old. The study assessed changes in cognitive impairment based on NTB results. The SD was 0.36, effect size 0.13. The following formula was used to calculate sample size for our study needed based on an effect size of 0.13 with a 95% confidence interval and 80% power:

$$N = 2\left((1.96 + 0.84)/(0.13)\right)^2 = 927.79$$

Based on these calculations, a sample size of 928 is needed. For enrollment, we will plan for a minimum of 1000 participants.

Intervention

Subjects will be able to live at home or their current residence for the duration of the study. The treatment group will receive weekly deliveries of quality premade meals by a meal delivery service. Weekly packages will consist of 7 breakfasts, 7 lunches, 7 dinners, a bottle of red wine, and extra fruits and nuts for snacks. The diet will consist of 35% fat (22% monounsaturated; 6% polyunsaturated and 7% saturated). The weekly meal plan will follow the characteristics of the MD highlighted (Table 5) below, ensuring a nutrient dense diet that is high in fiber and polyphenols. Participants, with their close contact, will be required to check off meals consumed on virtual REDCap linked survey and record average weekly minutes of exercise (see Appendix B).

Mediterranean Diet Components	
DAILY	**WEEKLY**
Olive oil ≥1 serving/d	Legumes, nuts & beans >6 servings/wk
Nonrefined Grains >4 servings/d	Full-fat Dairy ≤10 servings/wk
Vegetables >4 servings/d	Red meat ≤ 1 serving/wk
Potatoes >2 servings/d	Poultry ≤ 3 servings/wk

Mediterranean Diet Components	
DAILY	**WEEKLY**
Olive oil ≥1 serving/d	Legumes, nuts & beans >6 servings/wk
Fruits >3 servings/d	Fish >6 servings/wk
Alcohol < 300mL/d but >0	

Table 5. MD Diet Adherence from Morris et al.[1]

Recruitment

Method 1: Patients will be pre-screened using EMR and flagged for eligibility. Clinic patients presenting to an ADRC who have been diagnosed with AD and are at least 65 years old will be approached by research staff. Research coordinators on unit at ADRC will assess interest and obtain consent from participants (and caregiver/family member for those unable to provide consent). After giving consent, participants will be screened for inclusion/exclusion criteria (Table 4). Upon meeting inclusion criteria, participants will be provided with an iPad open to REDCap. Patients will be required to provide information, including date of birth, contact information, demographic information, medical history, family history, surgical history, medication use, lifestyle habits (diet, exercise, smoking). At this time, participants will also submit their baseline MOCA, GDS, AQ and saliva sample. Height and weight will be recorded to calculate BMI.

Method 2: Patients will be recruited through flyer distribution to Neurology clinics and AD centers across the country. Research staff will be contacted by volunteers via phone and/or email. Research staff will further discuss study details/requirements with participants over the phone, screen participants for inclusion/exclusion (Table 4) and obtain verbal consent. The initial REDCap survey link will be sent to participants email for completion and research staff will walk through the required information with the participant over the phone. As mentioned above, participants and their close contact will then submit a variety of information (contact information, demographics, medical history, etc.)

NOTE: Family member/caregiver/friend will need to consent to participating in study by completing the AQ at respective times and monitoring dietary adherence and average exercise minutes weekly.

Study variables, measures, and data collection

Specific Aim 1: Measure changes in cognitive function in AD patients adhering to the Mediterranean Diet using neuropsychological testing via battery (NTB), MOCA score, Alzheimer's Questionnaire, and family reports.

Specific Aim 2: Determine whether changes in grey matter volume, hippocampal shrinkage, cortical thinning, amyloid and tau tangles, and/or glucose metabolism associated with improved cognitive function occur in AD patients adhering to the Mediterranean Diet, using specific imaging and CSF analytic techniques.

Specific Aim 3: Analyze changes in the gut microbiome postulated to be associated with optimal and/or improved cognitive function in AD patients adhering to the Mediterranean Diet.

Collection Method	**Measures**
Semi-quantitative FFQ	Average annual food consumption
Weekly REDCap Survey (Appendix B)	Meal adherence and average exercise weekly
AQ (Appendix E)	Symptom report per caregiver/loved one
GDS (Appendix D)	Depressive symptoms (0-15)
MOCA (Appendix C)	Cognition (0-30)
NTB (Appendix F)	Global Cognition
Saliva Sample	Baseline APOE Status
MRI	Hippocampal shrinkage, mean cortical thickness, entorhinal cortex, perirhinal cortex, total gray matter volume
FDG-PET	Metabolic abnormalities are first observed in the temporoparietal areas of the brain, including the precuneus and posterior cingulate cortex
Amyloid-PET	Plaque burden
Tau-PET	Tangle burden
Lumbar Puncture – CSF	Aβ40, Aβ42, total tau, and phosphorylated-tau181
Blood Draw	CBC, B12/folate, CMP, blood glucose, TSH, plasma levels of Aβ40 and Aβ4

Fecal Sample – DNA extraction and 16S rRNA sequencing	Taxonomic profile

Table 6. Data Collection Measures

On the day of consent, data collection will begin. All volunteers will initially be screened by research staff with Inclusion/Exclusion Screening Sheet (Appendix A). Those who are excluded will be notified and thanked for their interest. Physical copies of screening sheets and the associated signed consent form will be kept in a research binder that will be stored in a secure room and locked in a research cabinet that only study staff will have access to. Subjects who meet inclusion criteria will be taken through a self-guided survey via REDCap with study staff on hand to answer any questions. Each participant's respective REDCap survey which will be associated with a unique identifier (e.g., 00001). For data organization, an encrypted worksheet will include participants' name, contact information, and unique REDCap identified.

The initial survey will obtain background information on the participant, which will be important in establishing general characteristics and comparing the two groups (intervention vs. control) during data analysis in terms of gender, BMI, ethnicity, etc. The baseline survey will also include the validated questionnaires:

The GDS, MOCA, semi-quantitative FFQ (sFFQ) and AQ. This survey will take 40 minutes to complete.

- THE GDS consists of 15 questions (emphasis on changes in sleep, energy, eating habits, and self-esteem) that have been validated in the geriatric population to determine which elderly individuals struggle with depression.

- The MOCA consists of 8 sections that test visuospatial skills, naming, memory, attention, language, abstraction, delayed recall, and orientation. It has been validated in the 55 - 85-year-old population.
- The sFFQ is a modified version of the Harvard FFQ, in which participants report their usual frequency of consumption of 144 food items over the past year.
- The AQ consists of 21 questions that are completed by a close contact (of patient with AD) and reflect patient's cognition and overall function.

Upon completion of the questionnaires, anthropometric measurements (height, weight) will be taken by the patient experience representatives (PERs) at the respective clinic for each participant's visit. These values will then be obtained by study personnel through review of the participant's medical record. A saliva sample will be taken by a research coordinator to establish APOE status. The patient's first clinical assessment visit then will be scheduled for within 2 weeks.

During the first clinical assessment, patients will have:

1. Blood drawn to check the following: CBC, B12/folate, CMP, blood glucose, TSH, plasma levels of Aβ40 and Aβ42.
    a. CBC, B12/folate, CMP, blood glucose, TSH will be checked at baseline to ensure there is not another contributor to dementia-like symptoms that can be treated.

   b. Serum levels of Aβ40 and Aβ4 will be checked at baseline as a measure of amyloid burden.

2. MRI scan at baseline will evaluate hippocampal shrinkage, mean cortical thickness, entorhinal cortex, perirhinal cortex, and total gray matter volume – all areas extensively studied and known to be impacted by AD.

3. PET scan

   a. FDG-PET

   b. Amyloid-PET

   c. Tau-PET

4. Lumbar puncture to assess baseline Aβ40, Aβ42, total tau and phospho-tau 181 in CSF.

   a. Protocol for lumbar puncture will follow Turner et al. (2015). CSF and blood samples will undergo an immunoassay with flow cytometry to analyze the presence and concentration of various molecules in the sample. To identify Aβ40 and Aβ42 in plasma and CSF, and tau and phospho-tau 181 in CSF, The Biomarker Core will be used. Meso Scale Discovery will be utilized to identify Aβ40 and Aβ42 and total tau. Innotest pTau181 will be used to identify phospho-tau 181.

5. Fecal samples will be collected based on protocol from Zhuang et al. (2018). to establish a baseline taxonomic profile for the control group and the intervention group.

   a. *Sample collection*

Fecal samples will be collected from all study participants in the morning and stored at –80˚C prior to analyses.

b. *DNA isolation*

DNA will be isolated using the standard Power Soil Kit according to the product instructions. Purification and quality of the DNA will be demonstrated by spectrophotometer and gel electrophoresis.

c. *16S rRNA sequencing*

PCR with primers will be used to amplify the V3-V4 region of the bacteria's 16S rRNA gene. Products from PCR will be extracted and purified using DNA Gel Extraction Kit. The purified products will be sequenced in Illumina MiSeq platform. The final sequences will be contributed to libraries of rRNA sequencing data, analyzed, and assigned to operational taxonomic units (OTUs) according to reference databases. Distributions of OTUs will be to calculate the abundance of specific microbiota.

6. NTB

After completion of the first clinical assessment, dietary intervention will begin. Participants will be sent home with their first week's package of meals if they are assigned to the MD group. During the time between the first clinical assessment and the next, patients and their caregivers will be responsible for tracking adherence to the MD by submitting weekly REDCap reports of meals consumed along with an estimate of average exercise minutes per week (Appendix B). This weekly submission will occur throughout the duration of the study and be linked to the patient's unique identifier number to keep the totality of data per participant together. The blood draw and fecal sample will be repeated at the first follow-up in 12 months to determine any initial changes in amyloid burden and taxonomic profile that may be occurring since baseline in that first year. During the third clinical assessment visit which will occur 24 months from the initial clinical visit, participants will have:

a. MOCA
b. GDS
c. AQ completed by family member/caregiver/friend
d. Blood draw
e. Fecal sample
f. Neuropsychological test battery

Upon completion of 3 years of dietary intervention or participation as a control, the following will be administered:

a. MOCA
b. GDS
c. AQ completed by family member/caregiver/friend
d. Blood draw (CBC, B12/folate, CMP, blood glucose, TSH, plasma levels of Aβ40 and Aβ4)
e. MRI scan
f. PET scan (FDG-PET, amyloid-PET, tau-PET)
g. Lumbar puncture
h. Fecal sample
i. Neuropsychological test battery

j. Control group: sFFQ

Data analysis

Average baseline characteristics will be calculated for each group, including proportion female, Caucasian, APOE4+ carriers, and average age, education length, AD duration from year of symptom onset, BMI, MOCA, brain volume (mL), ventricular volume (mL), CSF amyloid beta 40 (ng/mL), plasma amyloid beta 40 (ng/mL), along with standard deviations for each. A descriptive statistical analysis will be performed using the JMP statistical package (version 11.0 Pro, SAS Institute Inc.). Two-tailed, independent *t*-tests will be used to determine significant mean differences between groups for continuous variables. Pearson Chi-square tests or Fisher's exact tests will be used to determine significant associations between groups for categorical variables. Logistic regression will be used to report the odds ratio for the primary, secondary, tertiary, and quaternary outcomes. Additionally, descriptive statistics will be reported for these outcomes. Statistical significance will be set at 0.05. Information will be entered into a JMP and excel spreadsheet. Each row will represent a different patient and will correspond to a 5-digit study ID number. The identifying list of participant IDs will be saved in a different file on one encrypted computer. Only approved personnel will be able to access these files. Survey data will be managed on the REDCap database. Data will be double-checked by two independent reviewers

Timeline and resources

On the day of consent, data collection will begin. The initial survey will obtain background information on the patient, which will be important in establishing general characteristics and comparing the two groups (intervention vs. control) during data analysis in terms of gender, BMI, ethnicity, etc. The baseline survey will also include the validated questionnaires: The GDS, MOCA, semi-quantitative FFQ (sFFQ) and AQ. This survey will take 40 minutes to complete. Anthropometric measurements will be recorded, and a saliva sample will be taken by a research coordinator to establish APOE status. Two weeks later the patient will have the first clinical visit during which they will have blood drawn, a lumbar puncture, a brain MRI, a PET scan, and a fecal sample collected. Neuropsychological testing via battery will also be performed during this visit. Upon completion of the first clinical visit, dietary intervention will begin in the treatment group while the control group will continue with their typical diet. Participants in the Mediterranean Diet group will receive meals via a meal delivery system. Patients will submit weekly reports (Appendix B) checking off the meals they consumed to demonstrate adherence to the MD for the duration of the study. The blood draw and fecal sample will be repeated at the first follow-up in 12 months. During the third clinical assessment visit which will occur 24 months from the initial clinical visit, participants will have the MOCA, GDS, and AQ completed by family member/caregiver/friend, along with their blood drawn, a fecal sample collected and NTB performed. After 3 years of dietary intervention, the final clinical visit will consist of LP, blood draw, fecal sample, imaging, and all questionnaires (Table 7).

Total Study Timeline: 3 years		
VISIT	**TIMELINE**	**COLLECTED INFORMATION**
Background Information	Preclinical Visit	1. Consent form and consent of family member/friend/caregiver to help participant with answering the following survey questions and at future visits 2. Date of stage 4 AD diagnosis 3. Email address & phone number 4. Demographics (age, gender identity, racial identity, occupation) 5. Medical history 6. Surgical history 7. Medications 8. Family History of AD dementia 9. Exercise 10. Smoking/alcohol/substance use 11. sFFQ 12. GDS 13. MOCA 14. Height & Weight 15. Alzheimer's Questionnaire (AQ)[162] completed by family member/caregiver/friend 16. Saliva sample (APOE status)
Baseline Clinical Visit	0 months	1. Blood draw (CBC, B12/folate, CMP, blood glucose, TSH, plasma levels of Aβ40 and Aβ4) 2. MRI scan 3. PET scan (FDG-PET, amyloid-PET, tau-PET) 4. Lumbar puncture 5. Fecal sample 6. Neuropsychological test battery

Follow up 1	12 months	1. Blood draw (plasma levels of Aβ40 and Aβ4) 2. Fecal sample
Follow up 2	24 months	1. MOCA 2. GDS 3. AQ completed by family member/caregiver/friend 4. Blood draw (plasma levels of Aβ40 and Aβ4) 5. Fecal sample 6. Neuropsychological test battery
Follow up 3	36 months	1. MOCA 2. GDS 3. AQ completed by family member/caregiver/friend 4. Blood draw (CBC, B12/folate, CMP, blood glucose, TSH, plasma levels of Aβ40 and Aβ4) 5. MRI scan 6. PET scan (FDG-PET, amyloid-PET, tau-PET) 7. Lumbar puncture 8. Fecal sample 9. Neuropsychological test battery 10. Control group: sFFQ

Table 7. Study Timeline

Virtual Timeline	
GROUP	**PARAMETERS**
Intervention	weekly submissions of REDCap survey including adherence to diet and average exercise minutes
Control	weekly submissions of REDCap survey including FFQ with confirmation of lack of adherence to specific dietary plan and submission of average exercise minutes

Table 8. Study Timeline for Virtual Parameters

Resources Needed

People	**1.** Research coordinators **2.** Statistician **3.** Physician **4.** PhD in metagenomics **5.** Radiologist **6.** Phlebotomist
Materials	**7.** rRNA sequencing assays **8.** Gel electrophoresis **9.** Spectrophotometer **10.** MRI **11.** PET scan **12.** Lumbar Puncture kit **13.** iPads
Other	**14.** Meal delivery service **15.** Lab

Table 9. Resources Needed for Study

Institutional Review Board

This research proposal will be submitted for expedited review by the Boston University Medical Center Institutional Review Board. Despite the study being low risk to the participants, there are always risks associated with procedures including lumbar puncture and phlebotomy, namely infection, bleeding, and pain. There will also be exposure to radiation, however the exposure will be minimal. Lastly, information will be stored on a secure server and all work will be done on encrypted computers. No identifiable information will be stored on laptop.

CONCLUSION

Discussion

This study would be the first of its kind studying adherence to the Mediterranean diet and improvements in cognitive function, as well as improvements in pathological markers of Alzheimer's Disease in patients with Alzheimer's Disease in association with the diet on modulating the composition of the gut microbiome. Previous studies have included observational studies and risk mitigation for development of the disease rather than reversal of signs and symptoms in patients with the disease. Furthermore, few studies have analyzed the relationship between all three factors: the Mediterranean diet, the gut microbiome, and Alzheimer's Disease—and those that have did so in an observational manner. Strengths of this study include using multiple methods to assess cognitive function (NTB, MOCA), since there is lack of consensus among experts regarding the most accurate method. Additionally, the findings of this study will be strengthened by analysis of both cognitive symptoms and pathological markers of AD. Finally, by measuring CBC, B12/folate, CMP, blood glucose, and TSH from blood draws, along with depression via the GDS, potential confounders contributing to reversible cognitive symptoms are acknowledged and removed.

This study is limited by the lack of incentive to complete a lengthy, complex regimen -- all of which will increase the difficulty to maintain participation and comply with a dietary regimen. As a result, many volunteers may be lost to follow-up. Another limitation includes the self-report nature of measuring dietary adherence. Furthermore, there is inherent bias since the patients and caregivers will be aware which treatment arm the patient is in, based on the types of meals being delivered. This may lead to expectations of improvement and skew submitted subjective improvements of their loved one's symptoms. Finally, participants will be recruited from centers across the nation to meet a large and diverse sample size. This widespread recruitment will decrease feasibility and increase associated study costs- more research coordinators, physicians, sites, and materials will be needed.

The results of this study will provide information regarding the efficacy of a non-pharmacologic alternative intervention to improve cognitive deficits and pathological markers in patients with AD. While current drug regimens have helped patients manage their symptoms, they do not reverse pathological progression of disease. Many available treatments (acetylcholinesterase inhibitors, NMDA antagonists) function by targeting neurotransmitter imbalances of AD that lead to observable symptoms. However, these treatments are not addressing the major pathologic cause leading to this neurotransmitter imbalance—the amyloid beta plaques and NFTs. While extensive research has enabled advancements in therapeutic agents to now target the underlying pathology (disease-modifying treatments), these treatments are expensive and lacking data. As a result, there have been no disease-modifying treatments approved by the FDA and not a single new agent has been approved since 2003.[163] Given the lack of effective treatments in reducing progression of disease and the absence of a cure, the findings of this study could be invaluable. It is notable that in the last ten years, over 200 research projects involving potential therapies have failed due to starting therapies in late-stage disease, ineffective doses of medication, inadequate alteration of the pathologic mechanisms behind disease, and complications associated with including various stages of AD, along with prodromal and preclinical stages, in a single study. Experimental investigation of AD holds several challenges, as evident by the frequency of failed trials, namely the questions regarding the pathophysiology of disease and the substantial variability in diagnostic markers, along with the wide range of symptom severity and highly individualized experiences of various stages of disease. The combination of these challenges leads to many individuals being misdiagnosed or misclassified in certain stages. The NIA-AA proposed a clinical research framework to mitigate misclassification by requiring identification of ABP and NFT. This dietary study will attempt to avoid these issues by starting intervention in stage 4 of disease (AD with mild dementia), only including stage 4 disease (refer to Table 7), monitoring dietary regimen rather than dose of medication, and continuously monitoring ABP and NFT throughout the study period. This study will help provide information regarding alternative affordable methods to reduce progression of disease by altering the gut microbiome.

Summary

In summation, the MD has substantial research supporting it as a promising intervention to attenuate cognitive decline in AD. The research suggests that the health benefits of the MD are at least partly mediated by influencing the GM and, therefore, Alzheimer’s patients may benefit from treatment with a Mediterranean Diet as a means of altering the GM and attenuating AD pathology through the gut-brain axis. Overall, these studies have consistently suggested that alterations in the ratios and prevalence of bacterial species in the gut microbiome are associated with the decline of cognitive function and that certain dietary components can influence the prevalence of specific taxa. However, there have been inconsistent findings regarding specific alterations at the phyla and genus level in AD patients, along with changes in the microbiome associated with the MD. Therefore, more dietary intervention RCTs with the MD in AD patients are needed to elucidate the effects of the MD on the GM and whether the MD may prevent and/or decrease progression of AD through gut microbiota modulation. Furthermore, no RCTs have examined the relationship between adherence to the MD, the GM and AD progression.

While AD has been deemed irreversible, there has been substantial research on risk reduction of disease through lifestyle changes, including alterations in dietary habits, exercise, and mental exercises/stimulation. Though some research supports the beneficial results in prevention of AD, other studies have demonstrated minimal reduction in risk. However, these studies have not examined the reduction in pathological markers and symptoms in patients with a set stage of AD through the means of alterations in the GM. This research project addresses decreased symptoms and pathological changes associated with changes in the GM secondary to the MD. This study suggests that AD patients with high adherence to a MD will have a significantly improved beneficial balanced ratio of Firmicutes:Bacteroidetes and increased diversity compared to controls and will ultimately have decreased pathological markers and symptoms after three years.

Clinical and/or public health significance

Alzheimer's Disease is a devastating disease and a major medical challenge of the 21st century. With the complicated and not completely understood pathophysiology of AD, drugs have failed at controlling the crisis of this disease. Since drugs often have a particular target, they may not be a sufficient route to addressing disease pathology in entirety. Instead, this study may suggest the critical role the GM plays in pathogenesis of AD and will define an optimal "anti-AD" microbial profile. By defining these alterations of the GM associated with the MD, other methods (exercise, probiotic use, etc.) of promoting gut health and the consequential changes in specific taxa in patients with AD should be studied in RCTs. Furthermore, the MD is not associated with adverse effects, as drugs are, and do not compromise the health of other organ systems. If the Mediterranean Diet is found to boost gut health and improve cognitive symptoms and clinical markers of AD in AD patients, this would be ground-breaking as a therapeutic option and will function as a baseline to guide future research studies to target the GM. Furthermore, there will need to be exploration of fecal transplants as a potential cure.

APPENDIX

Appendix A. Inclusion/Exclusion Screening

NAME:	**DATE:**	
	Yes	No
The following answers below should be "YES" to meet inclusion criteria:		
Have you been diagnosed with stage 4 AD by a physician?		
Have you had appropriate neurological consultation and CT or MRI obtained?		
Are you at least 65 years old?		
Have you learned in the U.S. for >10 years?		
Do you have a close family member/friend/caregiver that is willing to participate in interviews, questionnaires, and record weekly habits?		
The following answers below should be "NO" to meet inclusion criteria:		
Do you have any other form of dementia or neurodegenerative disease?		
Do you have any history of thyroid disease?		
Are you currently depressed?		
Do you have any known/diagnosed psychiatric illness?		

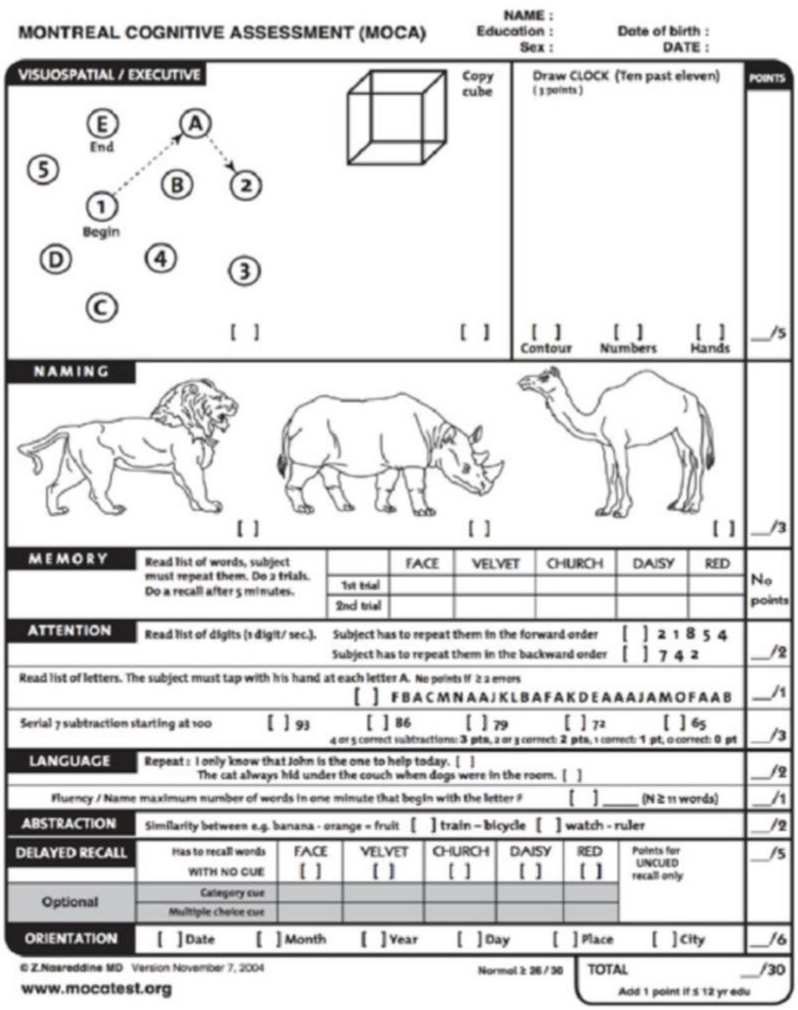
Do you have any history of CVA?		
Do you have any history of brain/spinal tumor?		
Do you have any history of TBI?		
Do you have any history of SUD/AUD?		
Do you have any history of IBD/IBS or GI inflammatory disorder?		
Do you have any known clinically significant electrolyte abnormalities and/or abnormalities in B12?		
Do you follow any specific dietary plan?		
Have you used systemic antibiotics in the past 6 months?		
Have you used corticosteroids in the past 6 months?		
Does the patient meet inclusion criteria?		

Appendix B. Weekly REDCap Survey

Day	Breakfast	Lunch	Dinner	Wine	Fruit/Nut Snack	Minutes of exercise
1	X					
2						
3						
4						

5						
6						
7						

Appendix C. Montreal Cognitive Assessment (MOCA)


MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education :
Sex :
Date of birth :
DATE :

			POINTS
VISUOSPATIAL / EXECUTIVE			
E End, A, 5, B, 2, 1 Begin, D, 4, 3, C [ ]	Copy cube [ ]	Draw CLOCK (Ten past eleven) (3 points) [ ] Contour [ ] Numbers [ ] Hands	__/5
NAMING [ ] [ ] [ ]			__/3

MEMORY	Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.		FACE	VELVET	CHURCH	DAISY	RED	No points
		1st trial						
		2nd trial						

ATTENTION	Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4 Subject has to repeat them in the backward order [ ] 7 4 2	__/2
	Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors [ ] FBACMNAAJKLBAFAKDEAAAJAMOFAAB	__/1
	Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65 4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt	__/3
LANGUAGE	Repeat : I only know that John is the one to help today. [ ] The cat always hid under the couch when dogs were in the room. [ ]	__/2
	Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ___ (N ≥ 11 words)	__/1
ABSTRACTION	Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler	__/2

DELAYED RECALL	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only	__/5
Optional	Category cue							
	Multiple choice cue							

ORIENTATION	[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City	__/6

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Normal ≥ 26 / 30
TOTAL __/30
Add 1 point if ≤ 12 yr edu

Score interpretation:

A score of 26 or more is considered normal
A score between 18 and 25 points suggests mild cognitive impairment
A score between 10 and 17 points suggests 15 or more points suggests moderate cognitive impairment

A score below 10 suggests severe cognitive impairment

Appendix D. Geriatric Depression Scale (GDS)

Choose the best answer for how you have felt over the past week:

1. Are you basically satisfied with your life? YES / **NO**

2. Have you dropped many of your activities and interests? **YES** / NO

3. Do you feel that your life is empty? **YES** / NO

4. Do you often get bored? **YES** / NO

5. Are you in good spirits most of the time? YES / **NO**

6. Are you afraid that something bad is going to happen to you? **YES** / NO

7. Do you feel happy most of the time? YES / **NO**

8. Do you often feel helpless? **YES** / NO

9. Do you prefer to stay at home, rather than going out and doing new things? **YES** / NO

10. Do you feel you have more problems with memory than most? **YES** / NO

11. Do you think it is wonderful to be alive now? YES / **NO**

12. Do you feel pretty worthless the way you are now? **YES** / NO

13. Do you feel full of energy? YES / **NO**

14. Do you feel that your situation is hopeless? **YES** / NO

15. Do you think that most people are better off than you are? **YES** / NO

Answers in **bold** indicate depression. Score 1 point for each bolded answer.

A score > 5 points is suggestive of depression.
A score ≥ 10 points is almost always indicative of depression.
A score > 5 points should warrant a follow-up comprehensive assessment.

Appendix E. Alzheimer's Disease Questionnaire (AQ)

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The Alzheimer's Questionnaire (AQ©)

How to score: Pick one answer to each of the 21 questions (yes or no). Then add up all the points to arrive at a final score. Note to score a "YES" response only to a decline in cognition and functional level compared to previous level.

	MEMORY	Yes	No
1	Does your loved one have memory loss?	1	0
2	If so, is their memory worse than a few years ago?	1	0
3	Does the patient repeat questions OR statements OR stories in the same day?	2	0
4	Have you had to take over tracking events OR appointments? OR Does the patient forget appointments?	1	0
5	Does the patient misplace items more than once a month? OR Does the patient misplace objects so that he or she cannot find them?	1	0
6	Does the patient suspect others are moving, hiding or stealing items when they cannot find them?	1	0
ORIENTATION			
7	Does the patient frequently have trouble knowing the day, date, month, year, time? OR Does the patient have to use cues like the newspaper or the calendar to know the day and date more than once a day?	2	0
8	Does the patient become disoriented in unfamiliar places?	1	0
9	Does the patient become more confused outside the home or when traveling?	1	0
FUNCTIONAL ABILITY			
10	Excluding physical limitations (e.g., tremor, hemiparesis, etc) does the patient have trouble handling money (tips, calculating change?)	1	0
11	Excluding physical limitations (e.g., tremor, hemiparesis, etc), does the patient have trouble paying bills or doing finances OR Are family members taking over finances because of concerns about ability?	2	0
12	Does the patient have trouble remembering to take medications or tracking medications taken?	1	0
13	Is the patient having difficulty driving? OR Are you concerned about the patient's driving? OR Has the patient stopped driving for reasons other than physical limitations?	1	0
14	Is the patient having trouble using appliances (e.g. microwave, oven, stove, remote control, telephone, alarm clock)?	1	0
15	Excluding physical limitations, is the patient having difficulty in completing home repair or other home related tasks (housekeeping)	1	0
16	Excluding physical limitations, has the patient given up or significantly reduced activities such as golfing, dancing, exercising, or crafts?	1	0
VISUOSPATIAL			
17	Is the patient getting lost in familiar surroundings (own neighborhood)?	2	0
18	Does the patient have a decreased sense of direction?	1	0
LANGUAGE			
19	Does the patient have trouble finding words other than names?	1	0
20	Does the patient confuse names of family members or friends?	2	0
21	Does the patient have difficulty recognizing people familiar to him/her?	2	0

Final Score: __________

Score interpretation:

A score of four points or less is regarded as normal
A score between five and 14 points inclusive suggests mild cognitive impairment
A score of 15 or more points suggests dementia
TOTAL = 27 POINTS

Appendix F. Neuropsychological Test Battery

Questionnaire	**Score Range**
Wechsler Memory Scale visual immediate	0 - 18
Wechsler Memory Scale verbal immediate	0 - 24
Rey Auditory Verbal Learning Test (RAVLT) immediate	0 – 105
Wechsler Memory Digit Span	0 - 24
Controlled Word Association Test (COWAT)	N / a
Category Fluency Test (CFT)	N / a
Wechsler Memory Scale visual delayed	0 - 6
Wechsler Memory Scale verbal delayed	0 - 8
RAVLT delayed	0 – 30

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