AGE RELATED DEMENTIAS

By

Shahab Soltani, M.D.

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in

Chemistry

University of Alaska Fairbanks

December 2021

APPROVED:

Lawrence K. Duffy, Committee Chair
Bradley K. Cruz, Committee Member
Kriya L. Dunlap, Committee Member
Thomas K. Green, Department Chair
Department of Chemistry & Biochemistry
Kinchel C. Doerner, Dean
College of Natural Science & Mathematics
Richard Collins,
Director of the Graduate School

Abstract

During the last few decades, the average life expectancy has dramatically increased in the U.S.A. and globally. As a result, the diseases associated with age, such as dementia, significantly impact families, society, and government. Dementia is a broad term describing memory loss, cognitive dysfunctions, and limited social skills caused by many factors such as aging, stroke, and genetic background. Although the primary etiology remains unclear but inflammatory reactions, neurodegeneration, vascular abnormalities play an important role in the progression of these disorders. Therefore, treatment is mainly supportive to relieve the symptoms of dementia and not to cure dementia or slow down its progression. This review discusses the most common disorders that cause dementia, including Alzheimer's disease, vascular dementia, and Parkinson's disease, as well as their symptoms, diagnosis, and treatments. Also covered are the studies that were performed on these disorders and animal models for each disease.

Table of Contents

List of Figures

Page

List of Tables

Page

Chapter one: Introduction

1.1, Dementia: is a collection of multiple disorders that could cause memory loss, communication, and sleeping difficulties, as well as limit social abilities. All these symptoms severely impact the person's life [1]. Globally, 44 million people suffer from dementia, making it a common disease [2]. It is estimated that about five million Americans older than 65 suffer from dementias. The current economic cost of dementia treatment inside the U.S. is approximately $159 billion to $215 billion per year, and it will rise to $511 billion by 2040 (inflation included). Home and long-term care are only included in this cost, not medical expenses or the economic impact of the disease [3]. Dementia is not a part of normal aging; Dementia is not a normal part of aging; 2 out of 3 people over 90 are usually completely free of dementia symptoms [4]. Dementia risk factors are divided into two categories, changeable factors (such as diabetes, depression, diet, smoking, cardiovascular disease, and alcohol abuse) and non-changeable factors (such as aging and family history, along with Down syndrome). Advanced age is the most recognized risk factor for dementia. Dementia cannot be prevented yet. However, some preventive measures may be helpful, such as quitting smoking, being physically active, and taking vitamin D [5]. Despite the fact that there are multiple forms of dementia, we will focus on age-related dementias, which are diseases associated with dementia and progressive dementias.

1.2, Disorders linked to dementia:

1.2.1, Huntington's disease: is a rare inherited autosomal dominant disease that mainly affects basal ganglia's caudate nucleus and putamen. As the definition of Huntington's disease implies, the prevalence of the disease is very low (0.38 per 100,000 per year in the U.S.A.) [6]. Diagnosis is based on clinical symptoms and imaging in the patients, confirming familial Huntington’s. Psychological symptoms include anxiety, depression, and loss of judgment, as well as memory loss, and language problems. It is characterized by chorea and spasms as its main neurological symptoms, and they constitute its primary phenotype. Diagnosis is based solely on clinical phenotypes. There are many diseases with similar psychological symptoms, including depression, anxiety, dementia, and Alzheimer's. Differential diagnosis is difficult because their symptoms overlap. The only

defined risk factor is the HTT gene. Family members with an H.D. patient should undergo early genetic testing, and then a group of experts should assess the patient's condition. Currently, there is no defined treatment for these patients. Multidisciplinary management of disease improves the quality of life [7].

Huntington's disease is progressive and generally manifests in middle age. Despite Huntington's disease being well studied genetically, there is little understanding of epigenetic and pathophysiological changes in the disease. At some point in the future, individuals who carry 36 or more CAG repeats of one allele of HTT will show symptoms of Huntington disease [8,9]. Researchers have traditionally studied this disease using mice, but recent studies have confirmed that some rodent findings do not apply to humans [10,11]. The new proposed models are minipigs, sheep, and nonhuman primates that are anatomically more relevant to human diseases. As humans live longer, researchers are able to study the long-term effects of treatments [12].

1.2.2, Parkinson's disease: Following Alzheimer's, Parkinson's is the second most prevalent neurodegenerative disease. Parkinson's disease is a progressive neurological disease characterized by tremors, rigid muscles, bradykinesia, and cognitive difficulties [13]. Parkinson's disease is diagnosed in one or two out of 1000 people, increasing to one out of 100 after age 60. This is equivalent to 60,000 new cases every year. The central pathology for Parkinson's disease is the loss of cells in the ventral component of the pars compacta of substantia nigra and the presence of Lewy bodies containing α-synuclein [14,15]. Among the risk factors are drugs, pesticides, toxins, brain trauma, and genetics [16]. Typically, a diagnosis is made on the basis of medical history, physical examination, and imaging. Specific single-photon emission computerized tomography (SPECT) scan, called a dopamine transporter scan (DaTscan), is used for diagnosis confirmation. C.T and MRI scans rule out other neurological disorders such as depression and Alzheimer's disease [17,18]. The purpose of most Parkinson's medications on the market is to raise dopamine levels in the Substantia Nigra Pars Compacta in order to reduce symptoms, yet there is no rehabilitation [19]. Multiple neurochemical and neuroimaging biomarkers are available. Neurochemical biomarkers include Hydroxy-2′-Deoxyguanosine, α-Synuclein, Apolipoprotein A1. Neuroimaging methods include Magnetic Resonance

Imaging (MRI), Single-Photon Emission Computed Tomography (SPECT) Scan with 23I-ioflupane 123I-FP-CIT, 123I-iometopane, 123I-β-CIT [20]. Recently, genetic studies on the confirmed Parkinson's patients identified several genetic mutations related to LRRK2, PINK1, and α-syn genes [21]. Mice and rats are the leading animal models for studies on genetic and environmental factors. These models are also used to study the effectiveness of vaccines and drugs [22].

1.3, Progressive dementia:

There are three types of dementia commonly associated with aging: 1- Vascular dementia, 2- Alzheimer's disease, 3- Lewy body dementia.

1.3.1, Vascular dementia: Memory, reasoning, planning, and judgment problems are associated with blood vessel abnormalities. Vascular dementia is the second most common form of dementia worldwide after Alzheimer's. About 1.2% to 4.2% of people over 65 have this type of dementia [23]. Vascular dementia is caused by cerebrovascular disorders, but the symptoms and severity of the condition vary depending on the region and the vessel affected [24]. The most typical causes of vascular dementia are subcortical vascular disease and large subcortical infarcts [25]. Vascular dementia is also associated with several risk factors such as age, gender, and stroke location [26]. Vascular dementia is a multi-stage disease. Cognitive function decline is the main symptom during the early stage, and memory loss is the main symptom in the late stage. Unlike Alzheimer's disease, the severity and symptoms of vascular dementia are more variable and depend on the vessel and location of the vascular pathology.

The diagnosis of the disease is made based upon the medical history (memory loss and cognitive dysfunction), neurological examination, neuropsychological examination, CT scan, and MRI [27]. CT scanning usually identifies vascular infarcts, but MRI is more accurate in showing the extent of stroke and its effect on white matter. The only approved treatment for vascular dementia is supportive management [28]. Supportive management involves treating comorbidities and assisting the patient with cognitive dysfunction issues [23]. Several Cerebral Amyloid Angiopathies caused by mutations were identified in

patients with vascular dementia [27], [29]. Rats are the primary animal model used to study Vascular Dementia. The study conducted by Ihara and colleagues on the effects of bilateral carotid artery occlusion or stenosis on vascular dementia emphasized the role of inflammation in producing the condition [30].

1.3.2, Lewy body dementia: Lewy bodies are proteins that develop inside the mammalian brain nerve cell that interrupt the normal function of the cells, causing symptoms such as memory loss, cognitive issues, depression, and movement impairment. Lewy body dementia is the most common neurodegenerative dementia in elderlies and accounts for 10 to 15% of all geriatric dementias [31]. Lewy body accumulation in the nerve cells causes acetylcholine deficiency and α-synuclein toxicity (same as Alzheimer's disease). The main affected areas in the brain include the dorsal raphe, locus ceruleus, substantia nigra. Age, sex, and family history of LBD or Parkinson's consider as risk factors. The main phenotypes of LBD are progressive dementia, fluctuating cognitive decline, non-visual hallucinations, and Rapid eye movement [32]. Diagnosis is based on medical history, neurological examination, and brain image. The main challenge to the diagnosis of LBD is the differential diagnosis. Patients affected by other types of dementia, such as Parkinson's disorder and Alzheimer's disease, could show the same phenotype. The imaging methods such as MRI, CT-scan could help to differentiate and confirm the disease [33]. The course of the disease is progressive, and the similarity in symptoms makes the differential diagnosis very critical. Like other types of progressive dementia, there is no treatment to modify the course of the disorder. The management of the disease is based on reducing the symptoms related to the disorder [32]. Several studies on α-synuclein toxicity in mice and different techniques targeting heat shock proteins tried to modulate the toxicity [34]–[36].

1.3.3, Alzheimer's disease: is the most common cause of dementia. Alzheimer's disease is defined as a progressive decline in thinking, behavior and social skills that interferes with a person's ability to function independently [37]. Alzheimer's disease is the primary cognitive disease affecting the elderly in the U.S. It affects 11.5% of people over the age

of 65 [38], and roughly 24 million people live with the disease worldwide. Scientists expect this number to be doubled in the next 20 years [39]. A major risk factor for this disease is age, along with genetics and family history [40]. There is some evidence that the neuropathological mechanisms underlying Alzheimer's disease start in humans decades before the disease reaches the mild stage [41]. Alzheimer's disease research before the 1990s was primarily focused on different metals, especially aluminum, and the effects they have on the brain [42]. Amyloid plaques became the main concern during the '90s, and beta-amyloid plaques were thought of as the primary cause of Alzheimer's disease until recently [43]. Several studies have shown that anti-inflammatory drugs can slow the progression of A.D, and different studies attempted to explain how this works by examining amyloid plaques [44]. Recently, some studies showed that beta-amyloid plaques result from the disease progression, not the cause of the disease advancement. However, it was found that inflammatory responses in the neural tissue were responsible for the progression of A.D [45], [46]. The symptoms of Alzheimer's disease progress gradually in three stages: mild, moderate, and severe. In the mild stage, common difficulties include misplacing or losing valuable objects, having difficulty performing tasks in social or work settings, and having trouble coming up with the right word or name. In the moderate stage, patients experience a variety of symptoms, such as feeling moody and withdrawn, confusion about the time or where they are located, and altered sleep patterns. In the severe stage, the main symptoms or manifestations are loss of awareness or memory of recent events, confusion about their environment, difficulty communicating, and vulnerability to infection [37].

The main characteristic feature of Alzheimer's disease is neurodegeneration, and the most readily observed manifestation of this process is cerebral atrophy. Hippocampus and amygdala are two of the main brain areas affected by Alzheimer's disease. T1 weighted MRI imaging is the best tool to detect morphologic changes and atrophy [47]. Studies have shown that vascular risk factors are associated with Alzheimer's progression [48]. In addition to cerebrovascular diseases, hypercholesterolemia, hypertension, and diabetes are among VRF. Li, J. et al. noted that subjects with higher VRF show a faster progression in cognitive decline and function loss than those with low VRF. Additionally, patients with MCI may benefit from VRF treatment by slowing the onset

of dementia [49]. MRI is one of the best non-invasive techniques to detect vascular disease in the brain and detect vascular defects such as vascular rupture (brain stroke). Hematoma with conventional MRI is feasible. At the same time, MRA (M.R. angiogram) could monitor blood flow in the vessels of the circle of Willis.

Currently, there is no treatment at the later stages to stop the progression or a vaccine to prevent this disease. The first-line therapy of Alzheimer's patients is cholinesterase inhibitors. These drugs are prescribed to reduce the symptoms and improve behavioral symptoms, but they do not affect the progression of the disease. Razadyne® (galantamine), Exelon® (rivastigmine), and Aricept® (donepezil) are the cholinesterase inhibitors. Some vaccines like ABVAC40 and AADvac1 are under investigation, but the results are not promising [50], [51]. Today, the only hope is to detect this disease earlier to diagnose it better and decelerate the progression. Some evidence suggests that DHA found in Salmon, Tuna, and sardines may help to prevent the progression of Alzheimer's disease in patients with mild and moderate symptoms.

Animal models are the primary tools for Alzheimer's disease studies, and different animals have been used as a model for this disease. Two examples are Hsiao et al., which used transgenic mice as a model to investigate the disorders [52], and Johnstone et al., which worked on polar bears and dogs [53]. The aged dog shows a similar cognitive decline, especially in memory and learning. Since the '90s, old dogs have been standard animal models to investigate beta-amyloid (Aβ) accumulation and plaques [54]–[57], inflammatory reactions in the hippocampus, and white matter [58] [59]. The compressed time scale of the lifespan of dogs means that the disease can be studied more expeditiously in dogs. Based on our hypothesis, degeneration of brain tissue caused by the inflammatory reaction will correlate with the loss of brain tissue and inflammatory biomarkers. We proposed MRI imaging is the best tool to monitor the dog's brain over time. MRI is a non-invasive and common technique to diagnose and monitor Alzheimer's disease [60], [61]. A new method that we will also use is M.R. spectroscopy, which helps detect inflammatory biomarkers and their relationship to degeneration progression [62].

Table 1.1, Dementia types, prevalence, and risk factors

Disorder	Type	Prevalence	Risk factors
Huntington's disease	Progressive	0.38 per 100,000 per year in the U.S.A.	CAG repeat length in the huntingtin gene
Parkinson's disease	Progressive	1-2 persons out of 1000 have Parkinson's disease, and this can increase to 1 person out of 100 after the 60s	drugs, Toxins and pesticides, brain vascular trauma, and some genetic factors
Vascular dementia	Progressive	1.2 to 4.2% of people over 65 years old	age, female sex, vascular risk factor, and stroke location
Lewy body dementia	Progressive	10 to 15% of all geriatric dementias	Age, sex, and family history of LBD or Parkinson's disease
Alzheimer's disease	Progressive	11.5% of people at age 65 or older	age, genetic, and family history

Table 1.2, Age, symptoms, and treatments

Disorder	Age	Symptoms	Treatment
Huntington's disease	Middle age, Elderly	Anxiety, depression, and loss of judgment, memory loss, reasoning dysfunction, and language perturbance, Chorea, and Spasm	No treatment, disease management
Parkinson's disease	Elderly	cause tremor, rigid muscles, bradykinesia, and cognitive dysfunction include memory loss	Levodopa, increase dopamine level, symptoms relief
Vascular dementia	Elderly	memory, reasoning, planning, and judgment impairment	Supportive treatment
Lewy body dementia	Elderly	memory loss, cognitive issue, depression, and movement impairment	No treatment
Alzheimer's disease	Elderly	Dementia, continuous and progressive decline in thinking, behavioral and social skills that disrupt a person's ability to function independently	Razadyne® (galantamine), Exelon® (rivastigmine), and Aricept® (donepezil) are the cholinesterase inhibitors (symptoms relief)

Chapter two: Material and Methods

2.1, Diagnostic imaging techniques:

2.1.1, Magnetic resonance imaging (MRI): produces a detailed picture using powerful magnets (ranging from 0.35 -3.0 Tesla), radio waves, and the magnetic dipole properties of the nuclei of the mobile hydrogen atoms in water, fat, and proteins. The abundance of hydrogen atoms inside the body makes it an ideal atom for imaging. The resonant frequency of hydrogen nuclei, a single proton, at 1.5 Tesla is 73MHz. The powerful magnetic field causes alignment and precession of the hydrogen protons, which can then be manipulated with radio magnetic signal inputs from the RF coil inside the machine. The protons echo back magnetic radio signals based on the chemistry in the tissues. MRI is a minimally invasive technique; no ionizing radiation, so we can study living subjects with no harm. The heat deposition from the RF coils is limited to avoid heating the tissues significantly. The ability to discern chemical differences from the behavior of the hydrogen protons in response to MRI pulse sequences makes MRI an ideal method for soft tissue scanning, especially in the brain. Different RF pulse patterns and subtle frequency shifts can be used to elucidate other chemical processes or tissues in an organ. Gadolinium-based contrast agents can be administered to examine blood flow and contrast uptake kinetics. Gadolinium affects the signal strength of the adjacent water hydrogens. For example, functional MRI (fMRI) scanning can assess cerebral cortical activity, which uses deoxy-hemoglobin concentration, measured indirectly by the effect on surrounding water hydrogens, as a proxy for electrical activity in the activated regions. Spatial localization of echo signals from within is achieved with minor manipulations in the magnetic field made by electromagnetic coils inside the bore of the major magnet. Two major properties determine the signal intensity emitted from the protons, relaxation time (T1) and echo coherence time (T2). MRI can also assess other parameters with specific pulse techniques, including blood flow, CSF flow, perfusion, and diffusion. MRI is widely used in the medical setting for brain scans. MRI has become more than a morphological imaging technique by these pulse sequences and now yields practical information. All

these advantages attracted researchers to use the MRI instruments for neurobiology research purposes as well [63,64,65].

2.1.2, MR-spectroscopy: a non-invasive method to detect biochemical changes in the brain. The feature of spatially localized signal input and echo collection allows interrogation of small blocks of tissue to get spectral signals that indicate concentrations of molecular species in a tissue sample volume. MR-spectroscopy can be performed on MRI machines equipped with Spectroscopy software. The operational simplicity and non-invasiveness of this Spectroscopy technique bundled with the valuable anatomic data from standard MRI technique in the same MRI machine at the same session are the main reasons for the interest by researchers. From a technical point of view, MR-spectroscopy uses the same rules as nuclear magnetic resonance (NMR) in chemistry; therefore, sometimes, researchers call it in-vivo NMR. The different molecules of interest in the tissues under study have differing, distinct spectral characteristics. The spectra represent the distribution of processional frequencies of hydrogen protons in each chemical species since 1985, which Bottomley P. A did the first in-vivo brain MR-spectroscopy [66]. This technique has identified many biomarkers, and several applications have been suggested, including in other body parts. Still, currently, the main application of this technique is for brain tissue and mainly for cancers and neurodegenerative disorders. Biomarkers include but are not limited to Myo-inositol (M); Choline (Cho); Creatine (Cr); N-acetyl aspartate (Naa); Lactate(L) [67].

2.1.3, Computed tomography (CT) scan: a widely available and quickly accomplished mainly morphologic imaging technique based on X-ray transmission or attenuation values of tissues, as shown by an X-ray tube and a detector array orbiting rapidly around the patient, generating a transversely oriented data set of X-ray attenuation numbers (Hounsfield Units or HU) that can be reformatted into other image planes and displayed as needed for the tissues of interest. The Hounsfield scale is defined at the low end at minus 2000 HU for air around the patient or gas within the patient, 0 HU for water, and up to +2000 HU for dense cortical bone. Specific tissues have characteristic attenuation values, such as white matter in the brain, typically about 25-30 HU, gray matter, typically about 35-40 HU, and CSF, about 0-12 HU. CT is singularly good for examining hard tissue

such as bone or tendons but is widely used for soft tissue imaging for a long list of conditions. The main drawback of CT is the radiation burden. Since the discovery of CT in the 1970s, continuous efforts to decrease radiation and thereby decrease the cancer risk caused by CT scans have resulted in almost order of magnitude reductions in absorbed X-ray dose for many CT scan protocols. Like MRI, CT scans can be made with or without contrast media, which means iodinated contrast injected intravenously or intraarterially or less commonly injected in the cerebrospinal fluid for neuroimaging. Iodinated contrast increases the density of tissues or structures depending on the pharmacokinetics or the route of administration. For example, under perfused or de-vascularized regions are made more conspicuous by enhancing the surrounding normally perfused tissues. CT is one of the main tools for clinicians and researchers to study brain tumors or degenerative disorders such as Parkinson's disease or Alzheimer's disease [49,68].

2.1.4, Nuclear Medicine and Positron emission tomography (PET): In both of these modalities, the signal that is detected emanates from within the patient from photons emitted by the radioactive isotope component of the tracer agents that were injected, and which distributed according to the pharmacokinetics of the tracer compound and the physiology or pathology of the patient. This is a functional imaging modality because the distribution and concentration of the tracer compounds can be quantitated by these computes that collect and reformat the photon events into images. The nature of the image receptors, the physics of the photons, and the low photon count statistics limit the spatial information from such studies. Modern equipment and software allow either simultaneous acquisition of high spatial information images from CT or MRI or fusion of the Nuclear Medicine images onto CT or MRI images to yield hybrid images with both quantitative functional information AND great spatial detail

The functional information can be tailored by choice of the molecule to which the radioactive isotope is coupled. Several compounds are in widespread use for many clinical conditions, including the evaluation of dementias. In addition, new agents are being developed to target metabolic processes and molecular entities involved in dementias.

PET is a particular subset of Nuclear Medicine imaging that involves different isotopes undergoing a special intranuclear reaction that produces a pair of high-energy particles called positrons, radiating out simultaneously in 180 degrees opposite directions. It requires a special machine and software, different from standard Nuclear Medicine cameras. Clinical use of PET so far has been predominantly based on 18-Fluoro-deoxyglucose (18FDG) molecule, uptake of which reflects the level of glycolysis in the tissues, the most common application currently being to detect and assess the state of neoplastic tissues [69] based on the abnormally increased glycolysis of most tumor tissues compared to most surrounding tissues. More recently, novel agents directed at specific biomarkers associated with neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease have been developed. However, the use of this technique is still limited because of cost and availability issues [70]; Thus, the use of mixed modalities such as PET/CT to study various types of dementia is more common now.

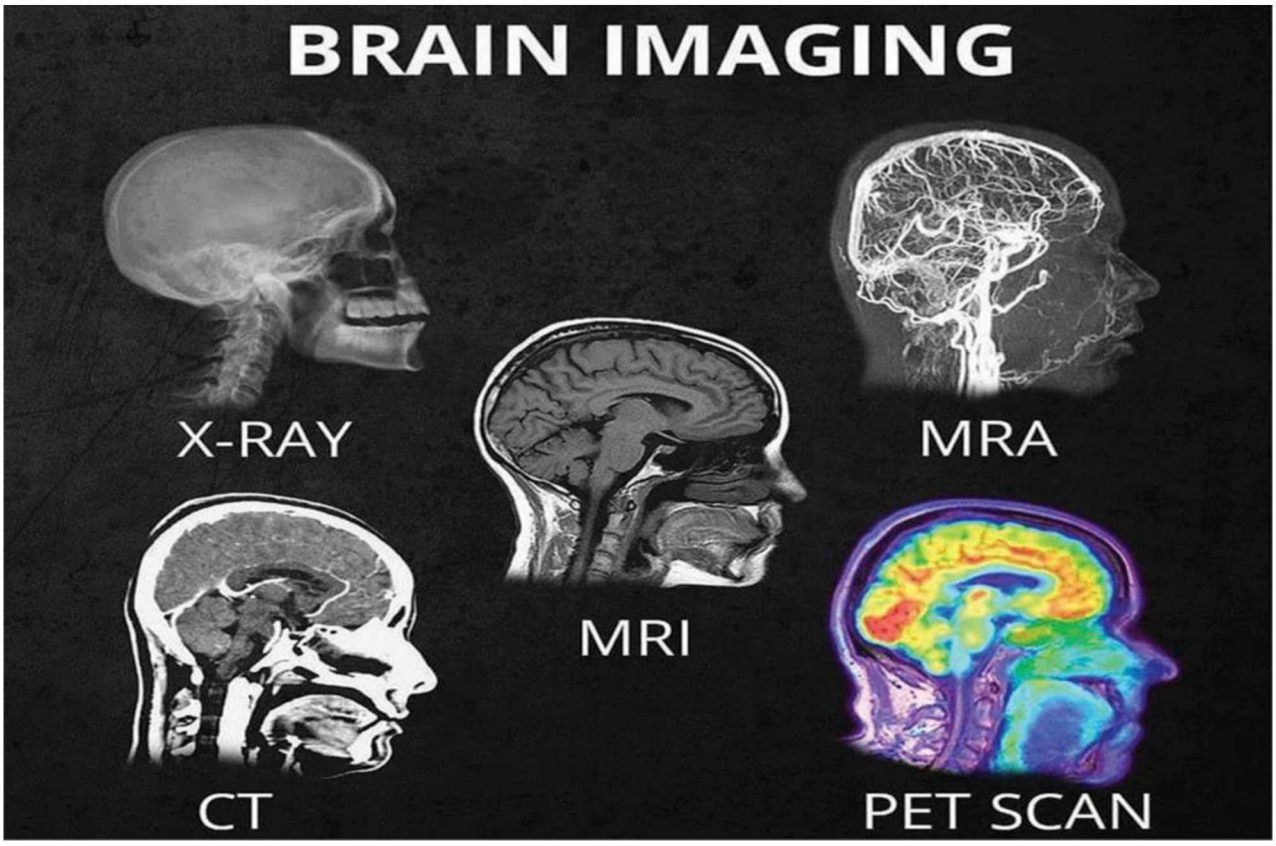

Figure 2.1, The difference between the images obtained from different imaging techniques (Tehrani, 2020) [71].

Table 2.1, Dementia diagnosis criteria

Disorder	Diagnosis
Huntington's disease	Medical history and medical examination, MRI, MR-spectroscopy, CT/PET
Parkinson's disease	Medical history and medical examination, MRI, I-123 ioflupane SPECT
Vascular dementia	Medical history and medical examination, MRI, CT, differential diagnosis from Alzheimer's disease is crucial
Lewy body dementia	Medical history and medical examination, MRI, SPECT/PET differential diagnosis from Parkinson's disease is crucial
Alzheimer's disease	Medical history and medical examination, MRI, CT, SPECT, FDG PET, Amyloid PET

2.2, Animal model: an animal model is a great tool to test new diagnostic techniques, drugs, or procedures before use in humans. Different animal models have been used (figure 2). The most common models are from rodent, canine, and primate families. Today, genetically modified mice are widely used for research and have several advantages and disadvantages. The main advantage is they can produce certain symptoms specific to humans, such as hallucination and sadness. This can happen through genetic modification. The issue is we can not be sure if the modification has the same effects on the animal. For example, for Alzheimer's disease, the same histopathological pattern was seen in dogs, but we can not be sure if they have the same symptoms as humans.

2.2.1, Rodent: The most widely used animal models for research are from this group. Rodent families include mice, rats, squirrels, guinea pigs, and rabbits. For different diseases and settings, various animals are used as the model. Here we mainly focused on the animal models used for neurodegenerative and neuropsychiatric disorders. Wild and transgenic mice models are widely used to study Parkinson's disease, Alzheimer's, and schizophrenia. Because of the short life span of rats and mice, these models mainly use genetic clones to evaluate amyloid toxicity, cytotoxicity of alpha-synuclein, or test newly synthesized drugs. Various genetically modified mouse and rat models have been designed over the last few years [71,72]. Usages of the rabbit and the guinea pig as a model for neural disorders are limited.

2.2.2, Primate: This model is the best model for human disease because of the similarities between primates and humans. Working on this model is very expensive, and the IACUC regulations are restricted, so the use of primates is limited. Monkey models for Parkinson's disease showed similar signs and symptoms and gave valuable insight into P.D. While symptoms could be induced in animals, evaluating the symptoms related to aging is difficult. Primates have a similar life span as humans. While the time course and symptoms can be related to humans, the cost of the study on the progression of Parkinson's disease or Alzheimer's disease is very high, and the study should be long-term [73]–[75].

2.2.3, Canine: The aged dogs showed similar cognitive dysfunction and personality change as seen in humans. The dog is used as a model for P.D. and A.D. disease. Unlike other models, no genetic modification or inducing is required to show the symptoms of aging. Only a few animals naturally show the aging effects similar to A.D. disease, from which dogs and the guinea pig are the most common models. Because of their shorter life span, availability, and similarities in aging-related behavioral changes, dogs are one the most favorite models for AD [76]—research being conducted at the University of Washington, Cornel University, among others.

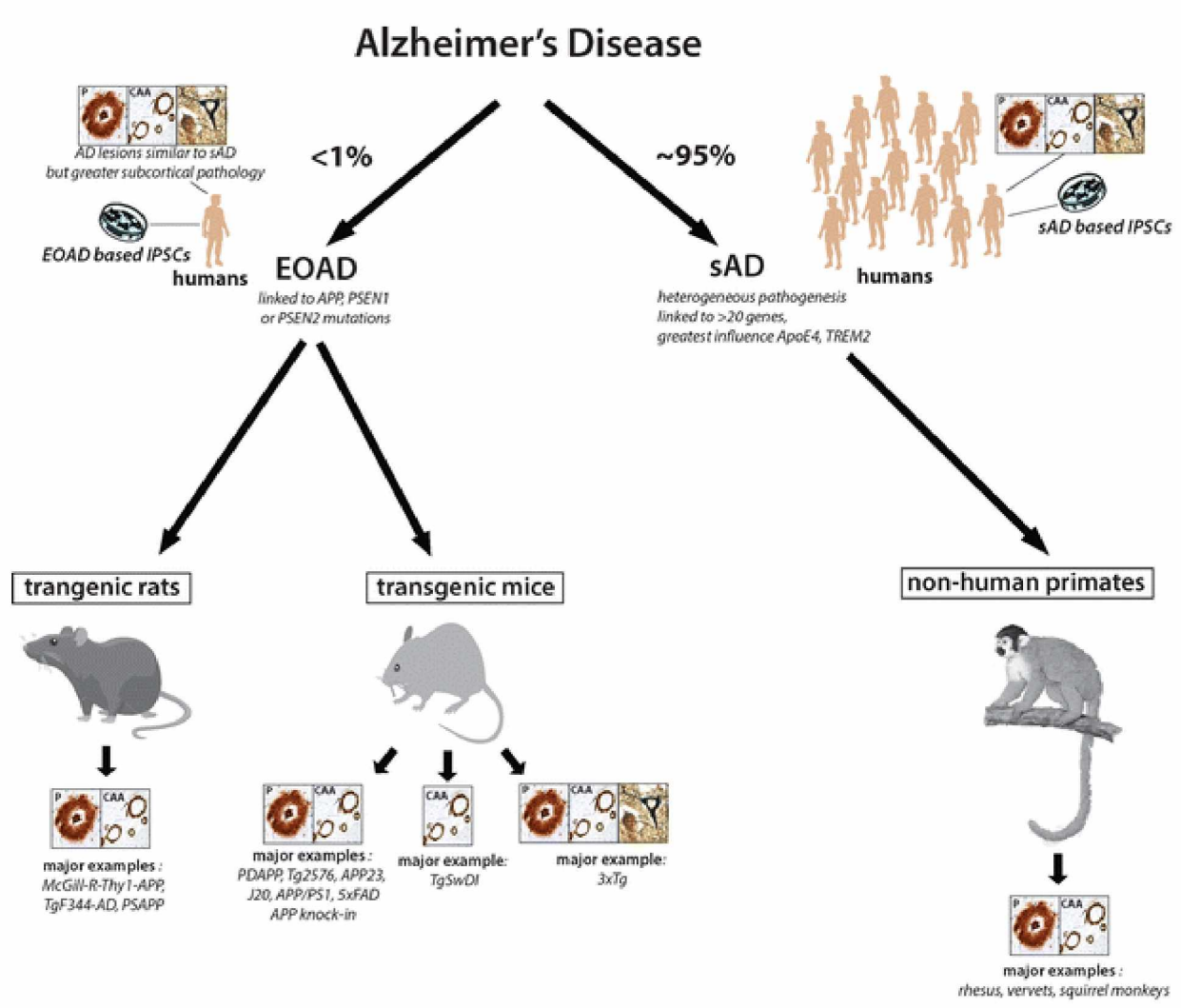

Figure 2.2, Main animal models for Alzheimer's disease. From Acta Neuropathologica (Drummond, Wisniewski, 2017, p 157-175) [77].

Chapter three: Alzheimer's disease, study design, and challenges

3.1, Definition: Alzheimer's disease is the primary cognitive elderly disease in the U.S.A., and many research groups studied different aspects. Alzheimer's disease is progressive, and the progression of this disease in humans mainly begins during the thirties or forties with very mild MCI. Unfortunately, no biomarkers have been found related to the progress of the early disease. Research on human subjects is costly and time-consuming. In order to analyze all stages of Alzheimer's disease, a person should be under investigation for more than thirty years. Because there are no early biomarkers, researchers could not be sure if the person is a suitable candidate for Alzheimer's disease treatments or not. Dogs are the best model for this disease for three reasons; first, their life compared to human life is compact, so investigating the subject in a shorter period is feasible. Second, research on dogs is much less expensive compared to research on a human model. Third, we suggest sled dogs are the best dog breed to study in Alaska because of Alaska's unique environment and foods.

3.2, Hypothesis: We hypothesize that degeneration of brain tissue caused by the inflammatory reaction over a lifetime will correlate with the loss of neuronal tissue and change in inflammatory biomarkers. This research will eventually lead to the development of a sled dog model for intervention studies. Based on our hypothesis, we design three specific aims.

3.3, Specific Aims:

3.3.1, Specific Aim 1: Monitor the brains of the subject animals for degenerative changes related to aging and hypertrophy of white matter. Rationale: The brain's gray matter volume decreases with age, and white matter (glial cells) replaces the gray matter. Hippocampus is the short-term memory center of the brain. Therefore, memory decline is the consequence of the degeneration of this structure. Hypothesis: the total volume of the brain and the volume of the hippocampus decrease, while the volume of ventricles and white matter increases with age. Approach: this aim will be accomplished by M.R. imaging the sled dog's brain to determine the Gary matter volume loss and hypertrophy of the white matter. Expected outcome: Finding the baseline and variation of the volume

alteration in the total dog brain and Hippocampus and correlating the finding to the aging process.

3.3.2, Specific Aim 2: Measure the inflammatory biomarkers in the hippocampus with M.R. spectroscopy. Rationale: Recently, the inflammatory reaction was suggested as the main reason for Alzheimer's disease progression [45,46]. Monitoring the inflammatory response with inflammatory biomarkers is now possible. In addition, a recent study has shown increased biomarkers (CSF t-tau) in humans with age [77]. Hypothesis: find a similar relationship between N-acetyl-aspartate (NAA), Choline (Cho), Creatine (Cr). Myoinositol and the NAA/Cr ratio with age. Approach: Monitoring the inflammatory biomarkers with M.R. spectroscopy technique by MRI. Expected outcome: Increase inflammatory biomarkers with age and find the correlation between inflammatory biomarkers and increased white matter volume.

3.3.3, Specific Aim 3: Standard blood tests and measurement of inflammatory biomarkers will be performed (hematologic and chemical profiles) before each session. Flow Cytometry will be used to determine the hematologic and chemical profiles of the blood and, the Elisa assay will use to measure the inflammatory biomarkers. Rationale: Inflammatory reactions increase with aging in the body and brain, and biomarkers can be monitored. Hypothesis: Increased inflammatory biomarkers in the blood of the older sled dogs. Approach: Use the ELISA test to find the inflammatory biomarkers in the blood and monitor them during this project. Expected outcome: We will define the relationship between inflammatory reactions in the body and effects on the neuronal tissue.

3.4, Approach

3.4.1, Specific Aim 1: Monitor the brain over time with MRI morphologic imaging to test the hypothesis that the volume of the brain and the hippocampus will decrease while the volume of ventricles and white matter increases with age. MRI imaging from brain vessels around the hippocampus, amygdala, and white matter will be analyzed. Measurement of the vessel's diameter will correlate with neurodegenerative disease progression. Monitoring and collecting data on a group of sled dogs will occur in year one and be repeated in year two. The MRI machine (TOSHIBA) provided by UAF and the director of the MRI Facility of UAF, Carl Murphy, will be supervised by a certified veterinarian.

Images are output in DICOM format. Conventional MRI sequences consist of T1 - weighted images, T2-weighted images, and Fluid Attenuated Inversion Recovery (Flair). In this study T1, T2 sequences are the main ones. The images can easily be differentiated as CSF is black in T1 images and white in T2 images.

The dogs for this study will provide by the owners of the sled dogs. Dogs will split into different groups to assess the effect of the Salmon (DHA fat) diet and darkness on the progression of the disease. Dogs will be divided into two groups; one group will feed strictly with the Salmon diet; another group will be on a commercial diet. In this way, assessment of the effect of DHA in the progression of Alzheimer's disease is possible. The same will happen for darkness, and in two groups, the impact of darkness will assess. MRI image acquisition is quite noisy, which may trigger the dogs to move. Therefore, we will use general anesthesia to tranquilize the dogs. The protocol is to have a veterinary technician sedate them with five mcg/kg dexmedetomidine + 0.2 mg/kg butorphanol intramuscularly. After 20 minutes, the technician will induce the dogs with 6 mg/kg propofol intravenously. Then they will intubate and be maintained on isoflurane (1-2% isoflurane usually keeps them). This procedure will be in the MRI unit in the Murie Building implemented by a vet technician.

Expected Results Interpretation: Shrinkage of the hippocampus and amygdala and hypertrophy of white matter expected in MRI images with age. Decreasing the size of the hippocampus and amygdala means degeneration, and if this size differential is less prominent, it means the progression of the disease is slow. If no change in size will detect during the experiment, we expect to see the degeneration accompanied by hypertrophy of white matter and the degenerated tissues substituted by white matter.

3.4.2, Specific Aim 2: Measure the inflammatory biomarkers in the hippocampus with M.R. spectroscopy. The level of these biomarkers is higher in human Alzheimer's patients, and we expect to measure more elevated levels of biomarkers in old dogs. First, measure N-acetyl-aspartate (NAA), a neurologic biomarker for neuronal damage in diffuse neurologic disorders. This biomarker helps us to determine the degree of neuronal damage in the hippocampus and amygdala. Second, choline (Cho): has a vital function in the production of acetylcholine, and the result of choline deficiency is memory loss and

mental dysfunction. Some studies suggested that a lack of acetylcholine significantly affects Alzheimer's progression [78]. This biomarker helps us determine the impact of acetylcholine deficiency in the disease course on sled dogs. Third, creatine (Cr): is a biochemical toxicity indicator and biochemical indicator of histopathological lesions in the brain. By measurement of this biomarker, monitoring the pathological progression of Alzheimer's disease in the hippocampus, amygdala, and other parts of the brain. Myoinositol (M) is a biomarker to demonstrate the pathological effect of amyloid (in this case, amyloid plaque) on brain tissue. By measuring these biomarkers, we can determine the impact of amyloid plaques on Alzheimer's disease progression. NAA/Cr ratio is a ratio of N-acetyl-aspartate to Creatine, a biomarker for neurological damage and cell proliferation.

Our goal is to find the relationship between the level of biomarkers and the progression of Alzheimer's disease. This finding could help to detect mild stage of dementia. In addition, this study will help us study the predictive validity of the biomarkers and promote their use in screening for disease, confirming the diagnosis non-invasively, or assessing the therapeutic or preventive value of various interventions.

Expected Results, Interpretation: We expect to measure a higher level of the inflammatory biomarkers with the progression of the disease. Measurement in each session will show us the level of biomarkers in comparison to the last session. Suppose we cannot detect any increase in inflammatory biomarkers, but we can see the changes in the size of the hippocampus and amygdala. In that case, it means the progression of the disease is not related to an inflammatory reaction, and this hypothesis of neurodegeneration needs to be reviewed.

3.4.3, Specific Aim 3: Standard blood tests (hematologic, chemical, and physical profiles) will be performed before each scanning session. We will expect a low red and white blood cell count and a high level of urea, creatinine, and ketones in aged dogs. Inflammatory processes alter blood profiles, and we may find a correlation between blood profile changes, MRI images, biomarkers, and disease progression. Inflammatory biomarkers (N-acetyl-aspartate (NAA), Choline (Cho), Creatine (Cr), Myoinositol, and the NAA/Cr ratio will be recorded. Other tests are the primary test to see inflammation in the body.

Routine blood and Elisa test will be performed before each session. Expected Results, Interpretation: Lower red and white blood cells and platelets level will be expected with aging. Inflammatory biomarkers will increase with age. Suppose the increased level of the inflammatory biomarkers in the blood is accompanied by an increased level of the biomarkers in the brain. In that case, there is a relationship between the two systems. Although Blood-Brain Barrier separates these two systems, the increment of biomarkers suggests a connection between them associated with aging.

3.5, Time Frame and Milestones: The first session held in Spring 2021. The blood test is performed by the UAF lab one week in advance for each session. After a thorough checkup and analyze the blood test results, MRI imaging was performed on dogs. The images and spectroscopy results were processed by DICOM software, and we have analyzed the MRI images and spectroscopy results. Based on IACUC approval, we will start the first session in Spring 2021. The project consists of 4 sessions, and hopefully, the last session will be in Spring 2023.

Chapter four: Conclusions

Dementia is a general term that we use for a broad spectrum of disorders compared to natural aging. A shared characteristic between all of them is the lack of treatment. Treatments for dementia are only supportive, and the goal of therapy is to relieve the symptoms associated with dementia. A.D. is the most common type of dementia in the U.S. and worldwide. Based on the prevalence of A.D., this draft mainly focused on A.D. Since discovering Alzheimer's disease in 1906, different strategies were followed to manage A.D. Eventually, they failed. The discovery of the relationship between Alzheimer's disease and inflammatory reactions in the CNS may shed light on the real cause for Alzheimer's disease and find a treatment.

Furthermore, finding the new inflammatory biomarkers make the diagnosis much more accessible, allowing early diagnosis and therapy. Finally, this study will demonstrate the sequence of neurodegenerative changes in the brain in sled dogs via MRI.

References:

[1] "What Is Dementia? | CDC." https://www.cdc.gov/aging/dementia/index.html (accessed Oct. 17, 2021).

[2] D. T and S. MB, "Dementia: definitions and types," Nursing standard (Royal College of Nursing (Great Britain) : 1987), vol. 29, no. 37, pp. 37–42, May 2015, doi: 10.7748/NS.29.37.37.E9405.

[3] "Planning for the Rising Costs of Dementia | RAND." https://www.rand.org/capabilities/solutions/planning-for-the-rising-costs-of-dementia.html (accessed Oct. 17, 2021).

[4] "The Demography of Dementia and Dementia Caregiving | PRB." https://www.prb.org/resources/the-demography-of-dementia-and-dementia-caregiving/ (accessed Oct. 17, 2021).

[5] "Dementia - Symptoms and causes - Mayo Clinic." https://www.mayoclinic.org/diseases-conditions/dementia/symptoms-causes/syc-20352013 (accessed Oct. 17, 2021).

[6] P. T, W. K, D. L, D. J, S. T, and J. N, "The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis," Movement disorders : official journal of the Movement Disorder Society, vol. 27, no. 9, pp. 1083–1091, Aug. 2012, doi: 10.1002/MDS.25075.

[7] R. A. Roos, "Huntington's disease: a clinical review," Orphanet Journal of Rare Diseases 2010 5:1, vol. 5, no. 1, pp. 1–8, Dec. 2010, doi: 10.1186/1750-1172-5-40.

[8] A. T. Lu et al., "DNA methylation study of Huntington's disease and motor progression in patients and in animal models," Nature Communications 2020 11:1, vol. 11, no. 1, pp. 1–15, Sep. 2020, doi: 10.1038/s41467-020-18255-5.

[9] D. Howland et al., "Large Animal Models of Huntington's Disease: What We Have Learned and Where We Need to Go Next," Journal of Huntington's Disease, vol. 9, no. 3, pp. 201–216, Jan. 2020, doi: 10.3233/JHD-200425.

[10] C. ZR and H. D, “Huntington’s disease: can mice lead the way to treatment?,” Neuron, vol. 69, no. 3, pp. 423–435, Feb. 2011, doi: 10.1016/J.NEURON.2010.12.035.

[11] B. V et al., “Phosphodiesterase 10A Inhibition Improves Cortico-Basal Ganglia Function in Huntington’s Disease Models,” Neuron, vol. 92, no. 6, pp. 1220–1237, Dec. 2016, doi: 10.1016/J.NEURON.2016.10.064.

[12] H. D et al., “Large Animal Models of Huntington’s Disease: What We Have Learned and Where We Need to Go Next,” Journal of Huntington’s disease, vol. 9, no. 3, pp. 201–216, 2020, doi: 10.3233/JHD-200425.

[13] “Parkinson’s disease - Symptoms and causes - Mayo Clinic.” https://www.mayoclinic.org/diseases-conditions/parkinsons-disease/symptoms-causes/syc-20376055 (accessed Oct. 17, 2021).

[14] O.-B. Tysnes and A. Storstein, “Epidemiology of Parkinson’s disease,” Journal of Neural Transmission 2017 124:8, vol. 124, no. 8, pp. 901–905, Feb. 2017, doi: 10.1007/S00702-017-1686-Y.

[15] S. Bhat, U. R. Acharya, Y. Hagiwara, N. Dadmehr, and H. Adeli, “Parkinson’s disease: Cause factors, measurable indicators, and early diagnosis,” Computers in Biology and Medicine, vol. 102, pp. 234–241, Nov. 2018, doi: 10.1016/J.COMPBIOMED.2018.09.008.

[16] F. N. Emamzadeh and A. Surguchov, “Parkinson’s Disease: Biomarkers, Treatment, and Risk Factors,” Frontiers in Neuroscience, vol. 12, no. AUG, Aug. 2018, doi: 10.3389/FNINS.2018.00612.

[17] “Parkinson’s disease - Symptoms and causes - Mayo Clinic.” https://www.mayoclinic.org/diseases-conditions/parkinsons-disease/symptoms-causes/syc-20376055 (accessed Oct. 17, 2021).

[18] J. C. Greenland and R. A. Barker, “The Differential Diagnosis of Parkinson’s Disease,” Neurology, vol. 43, no. 2, pp. S1-11, Dec. 2018, doi: 10.15586/CODONPUBLICATIONS.PARKINSONSDISEASE.2018.CH6.

[19] M. M. Goldenberg, “Medical Management of Parkinson’s Disease,” Pharmacy and Therapeutics, vol. 33, no. 10, p. 590, 2008, Accessed: Oct. 17, 2021. [Online]. Available: /pmc/articles/PMC2730785/

[20] F. N. Emamzadeh and A. Surguchov, “Parkinson’s Disease: Biomarkers, Treatment, and Risk Factors,” Frontiers in Neuroscience, vol. 0, no. AUG, p. 612, Aug. 2018, doi: 10.3389/FNINS.2018.00612.

[21] Z. XS, G. WS, J. JJ, C. L, and Z. PP, “Cellular and Molecular Basis of Neurodegeneration in Parkinson Disease,” Frontiers in aging neuroscience, vol. 10, no. APR, Apr. 2018, doi: 10.3389/FNAGI.2018.00109.

[22] E. A. Konnova and M. Swanberg, “Animal Models of Parkinson’s Disease,” Parkinson’s Disease: Pathogenesis and Clinical Aspects, pp. 83–106, Dec. 2018, doi: 10.15586/CODONPUBLICATIONS.PARKINSONSDISEASE.2018.CH5.

[23] O. JT and T. A, “Vascular dementia,” Lancet (London, England), vol. 386, no. 10004, pp. 1698–1706, Oct. 2015, doi: 10.1016/S0140-6736(15)00463-8.

[24] L. T. Grinberg and H. Heinsen, “Toward a Pathological Definition of Vascular Dementia,” Journal of the neurological sciences, vol. 299, no. 1–2, p. 136, Dec. 2010, doi: 10.1016/J.JNS.2010.08.055.

[25] P. ST and R. PM, “Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis,” The Lancet. Neurology, vol. 8, no. 11, pp. 1006–1018, Nov. 2009, doi: 10.1016/S1474-4422(09)70236-4.

[26] H. R and B. C, “Epidemiology of vascular dementia,” Neuroepidemiology, vol. 14, no. 5, pp. 240–257, 1995, doi: 10.1159/000109800.

[27] A. Khan, R. N. Kalaria, A. Corbett, and C. Ballard, “Update on Vascular Dementia:,” http://dx.doi.org/10.1177/0891988716654987, vol. 29, no. 5, pp. 281–301, Aug. 2016, doi: 10.1177/0891988716654987.

[28] R. GC, E. T, W. A, P. L, and C. HC, "Subcortical ischaemic vascular dementia," The Lancet. Neurology, vol. 1, no. 7, pp. 426–436, Jul. 2002, doi: 10.1016/S1474-4422(02)00190-4.

[29] H. J, R. RA, B. PE, H. MJ, L. W, and B. GT, "Hereditary cerebral hemorrhage with amyloidosis--Dutch type. Research-Group Hereditary Cerebral Amyloid-Angiopathy," Clinical neurology and neurosurgery, vol. 91, no. 4, pp. 285–290, 1989, doi: 10.1016/0303-8467(89)90003-6.

[30] M. Ihara and H. Tomimoto, "Lessons from a Mouse Model Characterizing Features of Vascular Cognitive Impairment with White Matter Changes," Journal of Aging Research, vol. 2011, p. 11, 2011, doi: 10.4061/2011/978761.

[31] I. McKeith, "Dementia with Lewy bodies," Dialogues in Clinical Neuroscience, vol. 6, no. 3, p. 333, Sep. 2004, doi: 10.31887/DCNS.2004.6.3/IMCKEITH.

[32] Z. Walker, K. L. Possin, B. F. Boeve, and D. Aarsland, "Non-Alzheimer's dementia 2: Lewy body dementias," Lancet (London, England), vol. 386, no. 10004, p. 1683, Oct. 2015, doi: 10.1016/S0140-6736(15)00462-6.

[33] A. Haider, B. C. Spurling, and J. C. Sánchez-Manso, "Lewy Body Dementia," StatPearls, Jul. 2021, Accessed: Oct. 17, 2021. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK482441/

[34] A.-M. KM, H. MY, A.-H. A, and E.-A. OM, "The role of α-synuclein in neurodegenerative diseases: from molecular pathways in disease to therapeutic approaches," Current Alzheimer research, vol. 10, no. 6, pp. 559–568, Jul. 2013, doi: 10.2174/1567205011310060002.

[35] J. DR, M. S, and M. P, "Targeting heat shock proteins to modulate α-synuclein toxicity," Therapeutic advances in neurological disorders, vol. 7, no. 1, pp. 33–51, 2014, doi: 10.1177/1756285613493469.

[36] W. J. Schulz-Schaeffer, “A transgenic mouse model of dementia with Lewy bodies suggests a link between α-synuclein expression, presynaptic vesicle pathology and cognitive deficit,” http://dx.doi.org/10.2217/fnl.11.57, vol. 7, no. 1, pp. 13–17, Dec. 2011, doi: 10.2217/FNL.11.57.

[37] “Alzheimer’s disease - Symptoms and causes - Mayo Clinic.” https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447 (accessed Oct. 17, 2021).

[38] “Alzheimer’s Facts and Figures Report | Alzheimer’s Association.” https://www.alz.org/alzheimers-dementia/facts-figures (accessed Oct. 17, 2021).

[39] R. Mayeux and Y. Stern, “Epidemiology of Alzheimer Disease,” Cold Spring Harbor Perspectives in Medicine, vol. 2, no. 8, 2012, doi: 10.1101/CSHPERSPECT.A006239.

[40] S. RA et al., “Toward defining the preclinical stages of Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease,” Alzheimer’s & dementia : the journal of the Alzheimer’s Association, vol. 7, no. 3, pp. 280–292, 2011, doi: 10.1016/J.JALZ.2011.03.003.

[41] “Alzheimer’s Stages - Early, Middle, Late Dementia Symptoms | alz.org.” https://www.alz.org/alzheimers-dementia/stages (accessed Oct. 17, 2021).

[42] W. HM, S. JA, and S. JW, “Aluminum chloride induced neurofibrillary changes in the developing rabbit a chronic animal model,” Annals of neurology, vol. 8, no. 5, pp. 479–490, 1980, doi: 10.1002/ANA.410080505.

[43] D. Morgan et al., “Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer’s disease,” Nature 2000 408:6815, vol. 408, no. 6815, pp. 982–985, Dec. 2000, doi: 10.1038/35050116.

[44] Y. Q et al., “Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer’s disease,” The Journal of neuroscience : the official journal of the Society for Neuroscience, vol. 23, no. 20, pp. 7504–7509, Aug. 2003, doi: 10.1523/JNEUROSCI.23-20-07504.2003.

[45] H. MT et al., “Neuroinflammation in Alzheimer’s disease,” The Lancet. Neurology, vol. 14, no. 4, pp. 388–405, Apr. 2015, doi: 10.1016/S1474-4422(15)70016-5.

[46] C. B and L. GE, “Inflammation, microglia, and Alzheimer’s disease,” Neurobiology of disease, vol. 37, no. 3, pp. 503–509, Mar. 2010, doi: 10.1016/J.NBD.2009.10.006.

[47] J. KA, F. NC, S. RA, and K. WE, “Brain imaging in Alzheimer disease,” Cold Spring Harbor perspectives in medicine, vol. 2, no. 4, 2012, doi: 10.1101/CSHPERSPECT.A006213.

[48] L. J et al., “Vascular risk factors promote conversion from mild cognitive impairment to Alzheimer disease,” Neurology, vol. 76, no. 17, pp. 1485–1491, Apr. 2011, doi: 10.1212/WNL.0B013E318217E7A4.

[49] J. Graff-Radford and K. Kantarci, “Magnetic resonance spectroscopy in Alzheimer’s disease,” Neuropsychiatric Disease and Treatment, vol. 9, p. 687, May 2013, doi: 10.2147/NDT.S35440.

[50] N. P et al., “AADvac1, an Active Immunotherapy for Alzheimer’s Disease and Non Alzheimer Tauopathies: An Overview of Preclinical and Clinical Development,” The journal of prevention of Alzheimer’s disease, vol. 6, no. 1, pp. 63–69, 2019, doi: 10.14283/JPAD.2018.45.

[51] A.-M. Lacosta et al., “Safety, tolerability and immunogenicity of an active anti-Aβ40 vaccine (ABvac40) in patients with Alzheimer’s disease: a randomised, double-blind, placebo-controlled, phase I trial,” Alzheimer’s Research & Therapy, vol. 10, no. 1, Jan. 2018, doi: 10.1186/S13195-018-0340-8.

[52] J. EM, C. MO, N. FH, P. R, and L. SP, "Conservation of the sequence of the Alzheimer's disease amyloid peptide in dog, polar bear and five other mammals by cross-species polymerase chain reaction analysis," Brain research. Molecular brain research, vol. 10, no. 4, pp. 299–305, 1991, doi: 10.1016/0169-328X(91)90088-F.

[53] C. BJ, S. JH, C. CW, W. R, and R. MJ, "Beta-amyloid accumulation in aged canine brain: a model of early plaque formation in Alzheimer's disease," Neurobiology of aging, vol. 14, no. 6, pp. 547–560, 1993, doi: 10.1016/0197-4580(93)90038-D.

[54] C. M et al., "Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production," Nature, vol. 360, no. 6405, pp. 672–674, 1992, doi: 10.1038/360672A0.

[55] G. G et al., "Cerebral preamyloid deposits and congophilic angiopathy in aged dogs," Neuroscience letters, vol. 114, no. 2, pp. 178–183, Jul. 1990, doi: 10.1016/0304-3940(90)90068-K.

[56] O. R, U. K, T. S, Y. R, N. H, and G. N, "The distribution of amyloid beta precursor protein in canine brain," Acta neuropathologica, vol. 87, no. 2, pp. 161–167, Feb. 1994, doi: 10.1007/BF00296186.

[57] O. WO et al., "Proteomic identification of brain proteins in the canine model of human aging following a long-term treatment with antioxidants and a program of behavioral enrichment: relevance to Alzheimer's disease," Neurobiology of aging, vol. 29, no. 1, pp. 51–70, Jan. 2008, doi: 10.1016/J.NEUROBIOLAGING.2006.09.012.

[58] H. E et al., "Oxidative damage increases with age in a canine model of human brain aging," Journal of neurochemistry, vol. 82, no. 2, pp. 375–381, 2002, doi: 10.1046/J.1471-4159.2002.00969.X.

[59] S. TM et al., “Individual classification of Alzheimer’s disease with diffusion magnetic resonance imaging,” NeuroImage, vol. 152, pp. 476–481, May 2017, doi: 10.1016/J.NEUROIMAGE.2017.03.025.

[60] B. AM et al., “An MRI measure of degenerative and cerebrovascular pathology in Alzheimer disease,” Neurology, vol. 91, no. 15, pp. E1402–E1412, Oct. 2018, doi: 10.1212/WNL.0000000000006310.

[61] W. H et al., “Magnetic Resonance Spectroscopy in Alzheimer’s Disease: Systematic Review and Meta-Analysis,” Journal of Alzheimer’s disease : JAD, vol. 46, no. 4, pp. 1049–1070, Jun. 2015, doi: 10.3233/JAD-143225.

[62] A. Berger, “How does it work?: Magnetic resonance imaging,” BMJ : British Medical Journal, vol. 324, no. 7328, p. 35, Jan. 2002, doi: 10.1136/BMJ.324.7328.35.

[63] S. T. Schwarz, T. Rittman, V. Gontu, P. S. Morgan, N. Bajaj, and D. P. Auer, “T1-Weighted MRI shows stage-dependent substantia nigra signal loss in Parkinson’s disease,” Movement Disorders, vol. 26, no. 9, pp. 1633–1638, Aug. 2011, doi: 10.1002/MDS.23722.

[64] B. PA, E. WA, F. TH, and A. WA, “In vivo solvent-suppressed localized hydrogen nuclear magnetic resonance spectroscopy: a window to metabolism?,” Proceedings of the National Academy of Sciences of the United States of America, vol. 82, no. 7, pp. 2148–2152, 1985, doi: 10.1073/PNAS.82.7.2148.

[65] “MR spectroscopy | Radiology Reference Article | Radiopaedia.org.” https://radiopaedia.org/articles/mr-spectroscopy-1?lang=us (accessed Oct. 17, 2021).

[66] R. de la Fuente-Fernández, “Role of DaTSCAN and clinical diagnosis in Parkinson disease,” Neurology, vol. 78, no. 10, pp. 696–701, Mar. 2012, doi: 10.1212/WNL.0B013E318248E520.

[67] C. J and P. ME, “Positron emission tomography scanning: current and future applications,” Annual review of medicine, vol. 53, pp. 89–112, 2002, doi: 10.1146/ANNUREV.MED.53.082901.104028.

[68] C. Marcus, E. Mena, and R. M. Subramaniam, “Brain PET in the Diagnosis of Alzheimer’s Disease,” Clinical nuclear medicine, vol. 39, no. 10, p. e413, Oct. 2014, doi: 10.1097/RLU.0000000000000547.

[69] H. Shinotoh and D. B. Calne, “The Use of Pet in Parkinsons-Disease,” Brain and Cognition, vol. 28, no. 3, pp. 297–310, Aug. 1995, doi: 10.1006/BRCG.1995.1259.

[70] V. Berti, A. Pupi, and L. Mosconi, “PET/CT in diagnosis of dementia,” Annals of the New York Academy of Sciences, vol. 1228, no. 1, p. 81, 2011, doi: 10.1111/J.1749-6632.2011.06015.X.

[71] “WHAT’S THE DIFFERENCE BETWEEN ALL THE DIFFERENT HEAD SCANS (X-RAY, CT, MRI, MRA, PET SCAN)? | SDBIF.” https://sdbif.org/index/whats-the-difference-between-all-the-different-head-scans ((accessed Dec. 14, 2021).

[72] T. M. Dawson, H. S. Ko, and V. L. Dawson, “Genetic Animal Models of Parkinson’s Disease,” Neuron, vol. 66, no. 5, pp. 646–661, Jun. 2010, doi: 10.1016/J.NEURON.2010.04.034.

[73] A. M. Hall and E. D. Roberson, “Mouse models of Alzheimer’s disease,” Brain Research Bulletin, vol. 88, no. 1, pp. 3–12, May 2012, doi: 10.1016/J.BRAINRESBULL.2011.11.017.

[74] M. E. Emborg, “Nonhuman Primate Models of Parkinson’s Disease,” ILAR Journal, vol. 48, no. 4, pp. 339–355, Jan. 2007, doi: 10.1093/ILAR.48.4.339.

[75] A. H. Nagahara et al., “Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer’s disease,” Nature Medicine 2009 15:3, vol. 15, no. 3, pp. 331–337, Feb. 2009, doi: 10.1038/nm.1912.

[76] E. A. C. Pereira and T. Z. Aziz, “Parkinson’s disease and primate research: past, present, and future,” Postgraduate Medical Journal, vol. 82, no. 967, p. 293, May 2006, doi: 10.1136/PGMJ.2005.041194.

[77] E. Drummond and T. Wisniewski, “Alzheimer’s Disease: Experimental Models and Reality,” Acta neuropathologica, vol. 133, no. 2, p. 155, Feb. 2017, doi: 10.1007/S00401-016-1662-X.

[78] L. Younes, M. Albert, A. Moghekar, A. Soldan, C. Pettigrew, and M. I. Miller, “Identifying Changepoints in Biomarkers During the Preclinical Phase of Alzheimer’s Disease,” Frontiers in Aging Neuroscience, vol. 0, no. APR, p. 74, 2019, doi: 10.3389/FNAGI.2019.00074.

[79] T. N, “Acetylcholinesterase inhibitors for Alzheimer’s disease: anti-inflammatories in acetylcholine clothing!,” Age and ageing, vol. 35, no. 4, pp. 336–338, Jul. 2006, doi: 10.1093/AGEING/AFL027.