UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

PROGRAMA DE MAESTRÍA Y DOCTORADO EN CIENCIAS MÉDICAS, ODONTOLÓGICAS Y DE LA SALUD

ALTERACIONES VISUALES EN LA ENFERMEDAD DE ALZHEIMER

TESIS

QUE PARA OPTAR POR EL GRADO DE

MAESTRA EN CIENCIAS

PRESENTA:

PAOLA ITZEL JIMÉNEZ ESPÍNDOLA

DIRECTORES DE TESIS:

SERGE PICAUD: Instituto de la Visión, París, Francia
LUIS FERNANDO HERNÁNDEZ ZIMBRÓN: Asociación para Evitar la Ceguera en México I.A.P. CDMX, México

Ciudad de México
Junio, 2020

VISUAL DISTURBANCES IN ALZHEIMER'S DISEASE

By: Paola Itzel Jiménez Espíndola

REVIEW ARTICLE

INSTITUTE DE LA VISION
UMR S 968 INSERM, UMR 7210 CNRS, UPMC

Master Recherche de Sciences et Technologie
Mention Biologie Intégrative
Spécialité: Neurosciences
2020

Team Manager: Serge Picaud
Supervisor: Louiza Arouche-Delaperche

CONDITIONS FOR THE REALIZATION OF THE PROJECT

During my stay in the laboratory I learned different techniques such as Western blot, Immunohistochemistry and ELISA, which I was going to use to develop my research protocol, however due to the COVID-19 contingency I could not obtain conclusive results, so I made a bibliographic review.

During all the confinement I was in contact with my tutors by videoconference or by other electronic means, which allowed me to carry out this work.

Acknowledgements

To the universities; UNAM and Sorbonne Université for having accepted being part of them and opening the doors to create new experiences, new knowledge and a different vision of life.

To my teachers for their teachings and to be a guide during this process.

To my tutors, both in France and in Mexico for having allowed me to be part of their research team, for their dedication, understanding and advice that they have given me during the master's degree and that have allowed me to carry out this project.

To my family for trusting me, for guiding and accompanying me at all times.

To my friends and my boyfriend who were part of this adventure and to be a constant motivation for the culmination of this work.

A special thanks

To my mom *who has always been supporting every decision and project, for the teachings she has given me throughout my life and for always pushing me to keep going.*

AGRADECIMIENTOS

A las universidades; UNAM y Sorbonne Université por haber aceptado que formara parte de ellas y abierto las puertas para poder crear nuevas experiencias, nuevos conocimientos y una visión diferente de la vida.

A mis maestros por sus enseñanzas y ser una guía durante este proceso.

A mis tutores, tanto en Francia como en México por haberme permitido formar parte de su equipo de investigación, por su dedicación, comprensión y consejos que me han brindado durante la maestría y que han permitido realizar este proyecto.

A mi familia por confiar en mí, por guiarme y acompañarme en todo momento

A mis amigos y mi novio que formaron parte de esta aventura y ser una constante motivación para la culminación de este trabajo.

Pero, sobre todo

A mi mamá *que siempre ha estado apoyando en cada decisión y proyecto, por las enseñanzas que me ha dado a lo largo de mi vida y por siempre impulsarme a seguir adelante*

TABLE OF CONTENTS

VISUAL DISTURBANCES IN ALZHEIMER'S DISEASE

1. ABSTRACT

Alzheimer's disease AD is a neurodegenerative process that mainly affects people over 65 years of age and is the main cause of dementia, making it a major public health problem.

This disease is characterized by cortical atrophy that affects the visual area and, at the microscopic level, neuronal loss, neurofibrillary tangles containing hyperphosphorylated Tau protein and extracellular senile plaques formed by Aβ.

There are various hypotheses that try to explain the origin of this disease, having models allows generating of new information to understand the pathogenesis of Aβ and the hyperphosphorylated Tau protein during AD.

AD patients manifest ocular dysfunctions such as loss of visual acuity, changes in contrast sensitivity, alterations in complex visual function such as reading, visuospatial function, naming and identifying objects, among others. Changes in the visual morphology also occur, such as a reduction in the number of fibers, a decrease in the diameter and length of the nerve fibers. Microscopically, a reduction in the number of RGC, deposits of Aβ 1-40 and Aβ 1-42 has been reported in the retinas of patients with postmortem AD. Aβ deposits in the retina can be found earlier than in the brain. Animal models that emulate the clinical characteristics of the disease, such as mouse APP / PS1dE9 and rats TgF344-AD, allows the study of morphological changes in the retina, such as the presence and accumulation of different APP fragments and cellular activity. Knowing that the definitive diagnosis of AD is postmortem, this encourage us to search for new diagnosis tools that help to detect AD at presymptomatic stages using retina.

2. INTRODUCTION

2.1. Alzheimer's disease

Dementia is a set of signs and symptoms that lead a person to chronic failure in brain function (1). In 2015 worldwide, it was estimate that 47 millions of people were affected by dementia and it is expected that they will increase to 75 million people by 2030 and 131 million people by 2050 (2). Among senile dementia, Alzheimer Disease (AD) is representing the greatest proportion of case with 60 to 70%. Worldwide, there are 5.8 million people diagnosed with AD and it is inferred that this number will increase to 14 million by 2050. However, these numbers are clearly underestimated due to misdiagnosis and lack of accurate early diagnosis (3).

The main risk factors for AD is advance age and the increase in life expectancy is increasing the prevalence. More than 80% of AD patients are over 75 years of age. The World Health Organization (WHO) has recognized AD as a public health priority.

AD is defined in 3 characteristic phases (Supplementary Figure 1); the preclinical phase, mild cognitive impairment and dementia. Here is a gradual and progressive decrease in cognitive functions due to damage that has occurred at the neuronal level. Clinically, it manifests with agnosia, amnesia, followed by apraxia and aphasia, as well as loss of spatial memory and visual impairment (4) until the person becomes totally dependent.

In the preclinical phase, changes in mood, sleep, anxiety and apathy can be found (5). Amnesia can go unnoticed or is attributed to occasional forgetfulness, then the person or their family members can become aware of these oversights. Little by little these “forgetfulness” is becoming more frequent until they interfere with daily life and people cannot carry out their daily routine, forget how to drive a vehicle, or follow instructions. In the advanced stages, if the person leaves her daily environment, she may feel disoriented. In frank dementia, the person is completely disoriented and requires daily supervision (10% of subjects with AD develop Capgras Syndrome). However, activities such as socialization, routine behavior and superficial conversation remain intact. In the final stages of the disease the patient is stiff, mute, with incontinence, sometimes there is hyperreflexia and myoclonic spasms. The survival of the patients ranges from 10 to 20 years once they are diagnosed, the main cause of death of these patients is due to malnutrition, secondary infections, pulmonary embolism, bronchoaspiration or some heart disease. Changes in brain architecture can begin up to 20 years before clinical manifestations occur (Figure 1) (3,6,7).

2.1.1. Alzheimer's Disease Classification

The most frequent AD presentation is the sporadic form, which affects people older than 65 years. The percentage of people with AD increases according to age; There are 3% of people with AD between 65 to 74 years, 17% between 75 to 84 years and 32% of people with 85 years or older (3). A familiar or early form occurs at a younger age (40 years) with a Mendelian inheritance pattern representing 4 to 8% of cases (8,9).

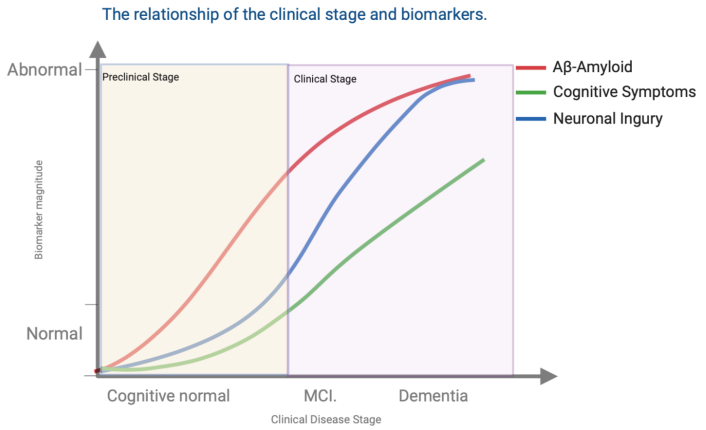


Figure 1. Alzheimer´s disease and biomarkers. It shows the relationship between the different stages of the disease and the biomarkers. Note that neuronal damage begins before clinical manifestations. Modified Hane F et. al. 2016.

This inheritance indicates the role of genetic factors associated with AD. Causing genes were identified for less than 1% of all cases.

Apolipoprotein E (APOE) is the major genetic risk factor for AD, the single amino acid differences among the APOE isoforms modulate APOE structure to profoundly affect its functions (10). Other causes can be caused AD is an extra copy of chromosome 21, mutations in the Amyloid Precursor Protein (APP) gene, or Presenilin 1 (PS1) and Presenilin 2 (PS2) genes. APP generates the β-amyloid peptides found in diffuse plaque characteristic of AD brains (see below). The normal hydrolysis of APP preventing the formation of β-amyloid peptides is performed by the γ-secretase activated by the presenilin proteins (8).

People who have mutations in PS2 are 95% more likely to develop AD than the general population (11). PS1 is found on chromosome 14 and encodes a protein called Presenilin 1 or S182. Mutations in this gene cause early-onset AD, with an early age for the presentation of symptoms (45 years), generating a brief but more marked evolution; with an autosomal dominant inheritance pattern (Ad). Presenilin 2 or STM2 is located on chromosome 1. These proteins provide the aspartate residues at the active site necessary for catalytic activation of γ-secretase which is an enzyme complex. They are found in the endoplasmic reticulum and Golgi apparatus, but can also be found in the nucleus, endosomal system, and plasma membrane (8).

2.1.2. Risk factors

Besides age and the causing gene reported above (Supplementary Figure 2), AD family history also represents a risk factor. People who have parents or siblings with AD are more likely to develop this disease and this risk increases if people have more than one family member. The APOE 4 allele was for instance recognized as a genetic risk factor for the disease. ApoE is a 299 amino acid lipoprotein encoded by the *APOE* gene present on chromosome 19 that is expressed in both the liver and brain. ApoE is involved in cholesterol transport and ß-amyloid clearance (12).

In the brain, the protein is produced by astrocytes and microglia, and then lipidated by ABCA1 to form lipoproteins. In the extracellular space, lipidized ApoE binds to Aβ leading to parenchymal amyloid plaque formation characteristic of AD. ApoE is endocited by cells with LDL receptors within the brain and facilitates the absorption of Aβ (8).

ApoE has three alleles ε2 (Cys112, Cys158), ε3 (Cys112, Arg158) and ε4 (Arg112, Arg158), therefore during conception, children can inherit any of the 6 possible combinations: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4. The penetrance of these pairs is different among ethnic groups, the ε3 allele being the most represented in 50-90% of the population, while ε4 and ε2 are only found in 5-35% and 1-5% respectively (8,13). The ε4 allele confers a higher risk of presenting AD in the general population (8), approximately 2% of AD patients are homozygous ε4/ε4 despite their very low representation. However, many AD patients do not carry the ε 4 allele and some carriers of this allele may never manifest the disease, so other environmental factors are required for the development of the disease (3).

Different environmental factors were also identified in AD etiology such as low educational level, smoking, obesity, diabetes mellitus and hypertension. In the case of the In the Mexican population, AD has a higher prevalence in females because women achieve a lower educational level than men, they receive a lower salary which affects their diet, they have less access to health systems and they are more long-lived (14). Modifying some risk factors can help reduce the risk of developing AD, however having late obesity and hypertension after the age of 80 have been associated with a lower risk of developing AD (3). People who have had a Traumatic Brain Injury (TBI) also have a higher risk of presenting AD, compared to the general population (3).

2.1.3. Neuropathology of Alzheimer's Disease

AD is characterized by brain changes visible in histopathology. At a macroscopic level (Supplementary Figure 3), there is a progressive, bilateral and diffuse cortical atrophy that

begins in the region of the entorhinal cortex, spreads to the hippocampus, then to the parietal and temporal neocortex prior to affecting the visual areas and showing a symmetric dilation of the lateral ventricles (hydrocephalus ex vacuo) (15). These changes are caused by the pathognomonic lesions of the disease (6). At the microscopic level, neuritic plaques formed from β-amyloid peptides and neurofibrillary tangles (NFT) are identified on brain slices of AD patients. NFT are composed with filaments of hyperphosphorylated Tau protein (16).

β-amyloid plaques can also be found on the walls of blood vessels in the cerebral cortex and in the leptomeninges (dura, pia, and arachnoid). This cerebral amyloid angiopathy is associated with neuropil threads, dystrophic neural processes, astrogliosis and activation of microglia (16).

2.1.3.1. Pathogenesis

In 1974, Drachman *et al.* studied the role of cholinergic neurotransmitter system in human cognitive functioning (17) and in the late 1960s it was shown that enzyme deficits led to decreased acetylcholine in AD (18). In 1976 Davies *et al.* studied the activity of enzymes associated with neurotransmitters such as acetylcholine, finding that Colin Acetyltransferase activity in AD brains was greatly decreased in the amygdala, hippocampus, and cortex, and ACh was dramatically decreased in the cerebral cortex, suggesting that could be a cause of this disease (19).

The cholinergic hypothesis was found by biochemical analyses a decrease in proteins at the cortical level, as well as neurotransmitters such as Acetylcholine (ACh), the enzyme acetylcholinesterase and nicotinic cholinergic receptors. The disease was attributed to the decrease in choline acetyltransferase and acetylcholinesterase activities in the cerebral cortex both enzymes being involved in cognitive function (8). Based on this theory, different treatment developed with various drugs: tacrine, rivastigmine, galantamine, donepezil (which are inhibitors of acetylcholinesterase). Neither of these drugs helps reverse the progression of the disease (20).

A major theory, the Tau hypothesis, incriminate the neurofibrillary lesions formed by the Tau protein in AD brains. The Tau protein is found under 6 isoforms that derive from an alternative splicing of a single gene located on the long arm of chromosome 17 (MAPT) (8,21). Their function is to bind to microtubules to stabilize them allowing thereby the transport of organelles, glycoproteins, neurotransmitters and other important elements for the neuron. Microtubule binding is achieved through a domain of 3 to 4 highly conserved repeats of 18 amino acids located in the carboxyl terminal (c-terminal). The protein is involved in the

polymerization and stability of the microtubules. Tau N-terminal portion contains acidic amino acids followed by a proline-rich basic region (projection domain) that can interact with the cytoskeleton and the plasma membrane (17). In AD brain, the hyperphosphorylated Tau protein polymerizes into Paired Helical Filaments (PHF) and Straight Filaments (SF) forming NFT suppressing thereby the protein function. NFTs are composed of neuronal cytoplasmic Tau protein fibers (10 nm in diameter) with abnormal phosphorylation that are pairs of helical filaments with a periodicity of 80 nm (15). There are other protein components associated with NTFs such as ubiquitin, cholinesterase and Aβ (Supplementary Figure 4) (15).

Dysregulation of the Tau protein affects the regulatory functions of the cytoskeleton such as maintenance of morphology, axonal transport, synaptic dysfunction and neurodegeneration (22). NFT´s formation begins in the allocortex of the medial temporal lobe (entorhinal cortex neurons of layer II and hippocampus CA1 and subicular region) before spreading to the association cortex (6) and neocortex in layers III, V and the superficial part of the VI layer (15).

An alternative hypotheses concentrates on the amyloidogenic pathway, which was described by Hardy and Higgins in 1992, arguing that the primary AD trigger is to be found in Aβ deposits caused by an imbalance between its production and elimination (8,23). NFT, neuronal loss, vascular damage and dementia are a consequence of these deposits (23) as indicated by genetic, biochemical and pathological evidence (8,24). The β-amyloid peptide is the primary component in diffuse plaque formation. They weight 4KD and contains 39 to 42 amino acids. There are obtained through the abnormal proteolysis of the type I transmembrane glycoprotein, APP, which is fragmented by α- and γ-secretases.

In the normal conditions, APP is cleaved near the membrane by the extracellular protease, α-secretase, releasing a soluble extracellular fragment (sAPPα). This fragment has several protective functions (8). A second cut into the membrane is made by another protein complex, the γ-secretase, who has one of its catalytic subunits encoded by the PRES1 or PRES2 gene. The second APP cut releases an intracellular peptide called Amyloid Intracellular Domain (AICD) and another small peptide residue between the cut of α-secretase and γ-secretase (25).

In contrast, in the pathologic amyloidogenic pathway, the first cut is made at a longer distance from the membrane by an aspartyl protease known as β-secretase, obtaining two fragments, one long that is called sAPPβ and the second that is anchored to the membrane and contains 99 amino acids, CTFβ, (26). This second fragment is immediately cut by the γ-secretase (multimeric enzyme composed of the APH1, PEN2, nicastrin and PS1 or PS2

subunit), thus generating β-amyloid peptides. This cut is imprecise causing heterogeneity at the C-terminus, with peptides of either 40 or 42 amino acids. Aβ42 is the most abundant in plaques due to its insolubility and higher fibrillation rate (Figure 2) (6,26). Polymerization of β-amyloid peptides can occur in the form of fibrils, protofibrils, and polymorphic oligomers. Aβ deposition and diffuse plaque formation favor local microglial activation, cytokine activation, reactive astrocytosis, and an inflammatory response, as well as a structural and biochemical change around axons, dendrites, and cell bodies leading to neuronal death, synapse loss and macroscopic atrophy (8). Senile or neuritic plates are a compact and spherical structural complex formed by a central accumulation of a 4Kda with a β-folded sheet configuration called βA4 or Aβ that is surrounded by "rosettes" that contain neurites (formed by neuronal processes, axons or dendrites), Tau immunoreactive neurons, dystrophic and activated microglia (15).

Dense plaques containing Aβ have also been found but do not show an accompaniment by dystrophic neurites called “burned-out plaques” and “end-stage” plaques that are considered remnants of neuritic plaques (15). Aβ deposition generally begins in the cortex and ultimately affects subcortical structures. Vascular amyloid deposits Aβ also tend to occur on the vascular walls of small arteries and arterioles of the leptomeninges that initially do not affect the normal function of the blood vessel. However, there is a greater accumulation of microhemorrhages in the thalamus and lenticular nucleus resulting from uncontrolled blood pressure. These last two theories are strongly intermingled in the formation of the pathognomonic lesions of the disease, suggesting a multi-causality in this disease.

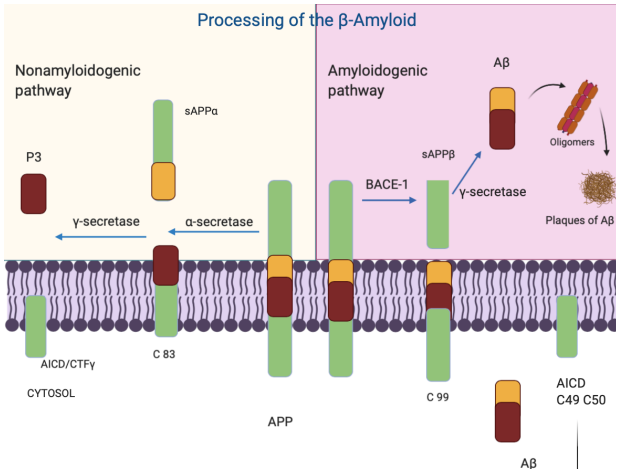


FIGURE 2. Schematic representation of AB processing. Representation of AB formation by the amyloidogenic pathway to the right of the image and non-amyloidogenic pathway to the left. Modified Barage S. 2015

2.1.4. Vision and Alzheimer Disease

2.1.4.1. Retina an extension of the brain

The retina as the Central Nervous System (CNS) derives from the neuroectoderm (24). The retina is a photosensor organ that receives light rays then converts it into signals that reach the brain where they are processed. Retina and brain shares similarities such: presence of

neurons, glial cells, it has a hemato barrier, it has strict control of the proliferation of endothelial cells and the optic nerve axons connect the retina directly to the brain.

Macroscopically the human retina is a transparent layer that is in contact with the choroid and the vitreous humor where 3 structures can be differentiated: the papilla or optic disc, which is the part where the optic nerve enters the eyeball, therefore it is a point blind. Then there is the fovea, which is an area devoid of rods, and finally, the ora serrata, which is the anterior boundary of the retina.

Microscopically it has 10 layers (27); three layers of neural bodies separated by two layers containing the synapses made by the axons and dendrites of the neurons. In the most posterior row of the retina are the photoreceptors, cones and rods (Figure 3).

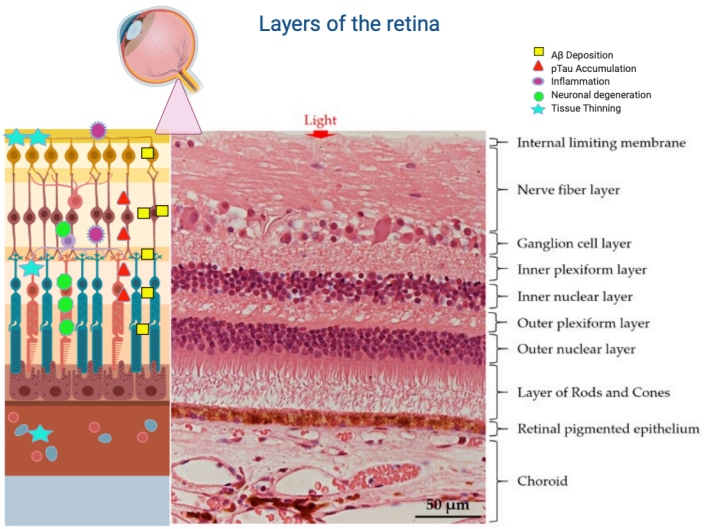


Figure 3. Layers of the retina. In the right part we can see the basic retinal structure. Histological appearance of choroid and retinal layers. The retinal layer harbors five retinal neuronal cells, primarily, the rod- and cone-photoreceptors, the Müller glia, the horizontal cell, the bipolar cell, the amacrine cell, and the Retinal Ganglion Cell, by Ding, S.L.S 2017. The left part shows the manifestation of AD in the human retina Modified Hart N. 2016.

2.1.4.2. Clinical alterations in vision during Alzheimer´s Disease

At early stages of the disease visual disturbances such; loss of visual acuity (VA) which is the main clinical manifestation, problems related to color vision and visual field, changes in pupillary response to mydriatics, fixation problems, saccadic eye movements and abnormal follow-up; changes in contrast sensitivity and visual evoked potentials and alterations of some complex visual functions such as reading, visuospatial function, naming and identifying objects were observed (5,28). Hallucinations have also been described mainly in patients with a decrease in visual and cognitive acuity.

Salobrar García *et al.* (2019) using the Snellen eye chart, the 28-tone Roth Color Test and the Digital Perception Test (PDT) found a decrease in VA in patients with mild cognitive impairment in the mild and moderate stages of AD, which correlates with the score obtained by the Mini-Mental State Examination (MMSE), therefore a decrease in VA is correlated with a decrease in cognitive ability. They also found a decrease in contrast sensitivity and color

perception. Patients with mild AD had fewer total errors compared to patients with moderate AD, who had 61.71% total errors (29).

2.1.4.3. Morphological changes in vision during Alzheimer´s Disease

Studying the retina morphology using Optical Coherence Tomography (OCT) showed a decrease of layer thickness in circular and concentric sectors of the macular region and the fovea of mild AD patients (Supplementary Figure 5) (30). However, when comparing these results with patients with moderate AD, it showed a significant increase in the thickness of the fovea as the thickness of the inner macular ring which could be associated with inflammatory processes and cellular movements (29). Also, patients with AD present a reduction in the number of retinal ganglion cells (RGC) that is associated with intracellular lesions such as the shrunken soma with the vacuolated cytoplasm and with lower density, swelling of the mitochondria and the endoplasmic reticulum (31), which together could explain the disc abnormalities and are mainly found in the upper and lower quadrants of the retina followed by the nasal quadrant(29,32), temporal (31) and the thickness of the nerve cell layer (24,31,33).

Using OCT, Aβ deposits was detected in perimacular areas, perivascular areas in the external plexiform layer, the ganglion cell layer and the nerve fiber layer (30).

The visual pathway is also affected, it presents a thinning in its fibers (33) decrease in the diameter and length of nerve fibers, therefore, axonal degeneration is reflected in a decrease in the number of RGC with its consequent visual cortex condition (Supplementary Figure 6) (32,34).

2.1.4.4. Histological alterations in vision during Alzheimer´s Disease

Koronyo-Hamaoui *et al.* identified Aβ in the retina of post-mortem AD patients (35). Retinal deposits are of the type: diffuse, immature, mature plaques, with Aβ 1-40 and Aβ 1-42, associated with lipid deposits (35). Aβ aggregates with diverse morphology accumulate around a subpopulation of RGC, which are highly photosensitive due to the expression of melanopsin, the melanopsin-expressing retinal ganglion cells mRGCs were observed in AD patients (32).
The RGC are the nervous retinal elements which connect the visual receptors to the brain forming the nervous visual system, the RGC send information to the thalamus, hypothalamus and midbrain. They receive information from the photoreceptors through the intermediate bipolar, amacrine and horizontal neurons. There are at least 22 types of RGCs that are specialized to encode aspects such as color, contrast movement, direction of movement, etc (36). One of the most important in neurodegenerative diseases are mRGCs. These cells constitute a system in the mammalian retina used for irradiance detection, regulating non-image

forming functions, such as photoentrainment of circadian rhythms, control of the pupillary light reflex, masking response, light-regulated melatonin secretion, and modulation of the sleep/wake cycle. There are five subtypes of mRGCs differentiated by morphology and function (37).

Degeneration of mRGCs and neurites suggests that Aβ is toxic to retinal cells and aggregation of Aβ with RGC degeneration in the upper quadrant and the Retinal Nerve Fiber Layer (NFL) and Ganglion Cell Layer (GCL) layers can distinguish ocular AD (24).

The suprachiasmatic nucleus is also affected in some patients with AD, this structure receives inputs from the retina and is in charge of regulating the sleep-wake cycle, so this damage could explain the sleep disorders presented by patients with AD, along with the degeneration of the mRGCs (32).

Other studies revealed also that amyloid deposits are associated with blood vessels in the upper quadrant (Figure 4) (35). Frost *et al*. (2013), using retinal photography, demonstrated that there was a correlation between the diameter of the blood vessel and the ramifications and the Aβ deposit in the asymptomatic phase of AD (38).

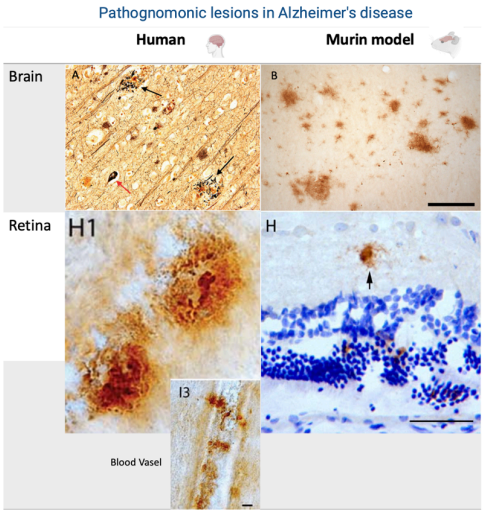


Figure 4. Pathognomonic lesions in AD. A. represent a microphotograph of the temporal cortex of an AD patient showing senile plaques (black arrows) and neurofibrillary tangles (red arrow), Perl D, 2010. B. represent a microphotograph showing deposition of Aβ in the mouse brain. 3xTg-AD, LaFerla F, 2005.
H1. shows classic mature plaques with a dense central nucleus Aβ and radiating fibrillar arms in a patient with AD. I3. shows deposition of Aβ along a retinal blood vessel in a patient with AD, La Morgia C, 2016.
H. shows Aβ plaques in OPL in the rat model TgF344-AD, Tsai Y, 2013.

Senile plaques and NFT have been found in the primary visual cortex specifically in the areas of visual association (V2, V3) than in V1 (Figure 5) (39). In addition, signs of inflammation or collapse of blood vessels, reduction of blood flow, tortuosity (24), decrease in the complexity of the branching pattern and cellular degeneration can be found, being consistent with the Changes in blood flow are also part of the pathogenesis of AD.

2.1.4.5. Physiological alterations in vision during AD

The pattern electroretinogram (PERG) showed delay abnormalities and a reduction in the amplitude N35, N50 and N95 in patients with AD, which could be observed from the early stages of the disease (40). The reduction in wave "b" could be attributed to the reduced number of retinal ganglion cells in patients with A, these results were consistent with what was shown in the ERG of patients with AD in advanced stages, where they also showed an increase in the latency of the response (40).

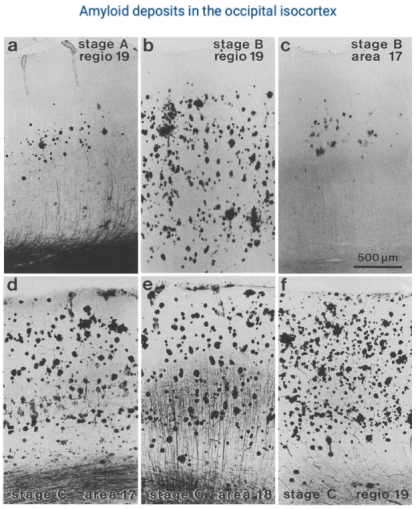


Figure 5. Amyloid deposits in the occipital isocortex (layers I to VI) of AD patients post mortem. a. Stage A displays a few patches of amyloid at mid-cortical level (basal occipital association cortex. b, c Stage B exhibits many amyloid deposits in virtually all association areas of the isocortex and occasionally a few dots in primary areas [b peristriate association cortex, c primary visual field (= area 17). d-f Stage C shows an abundance of amyloid in not only the association cortex but also in belt areas and core fields [d striate area (core, area 17), e parastriate area (belt, area 18), f peristriate region (association cortex, region 19). By Braak H 1994.

In advanced stages of AD, the ERG has indicated communication problems between groups of retinal neurons, which may be the result of neurotransmission damage, causing an excitation/inhibition imbalance towards the neuron (41). The ERG also showed neuronal hyperexcitability that can be found from the early stages of the disease (41).

Perez *et al.* (2009) also evaluated the integrity of the retina in the APPswe / PS1ΔE9 Tg mice using ERG, finding results very similar to those observed in humans, showing significant reduction in the amplitudes of a and b waves. These alterations may be the result of Aβ deposition in the retina that affects neuronal transmission (28).

2.1.4.6. Alzheimer's disease in animal models

Both, Tau protein and Aβ deposit are critical elements for the pathogenesis of AD; however, the linkage of these mechanisms is still not well understood. It is important to understand the link between these proteins and the evolution of the disease. To understand the disease different models that mimics AD pathology were developed and here were summarized few ones that are the most used and also where proofs of the vision dysfunctions were described.

2.1.4.6.1. Amyloid models

Amyloid models are the most used models due to the importance of amyloidogenic hypotheses. APP-KO animal models show reduced body size and microcephaly, hypersensitivity to seizures, learning disabilities and LTP, but Aβ is the main protein component of plaques and it is also known that the presence of Aβ can promote appearance of tau pathology (12,42,43). Recent studies in a mouse hippocampal primary neuronal cultures show that Aβ toxicity is Tau dependent (44), these studies will be discussed later.

The Tg2576 model is one of the most well characterized mouse models of AD. It overexpresses a mutant form of APP (isoform 695) with the Swedish mutation (KM670/671NL), resulting in elevated levels of Aβ and ultimately amyloid plaques. This model also presents cognitive deficits associated with age (45).

Hsiao *et al*. studies on swAPP (Tg2576) AD transgenic mouse model demonstrated that Aβ deposition in the hippocampus, cerebellum, and cortex is age-dependent and plays a fundamental role in cognitive processes, and in the retina, they found that Aβ deposition occurred mainly in GCL (45).

The abnormal folding of Aβ and its aggregates generates alterations in receptor-ligand interaction that modulate the activity of microglia and astroglia, which release cytokines, nitric oxide, and other cytotoxic agents in response to interaction with Aβ (40).

Also, Aβ produces an upregulation of NFκB gene in astrocytes, causing C3 a compound of complement complex release, generating neuronal dysfunction through C3aR receptor and intraneuronal calcium signaling, as it produces synaptic excitation and alteration of dendritic morphology (46). Lian H *et al.* demonstrated that the increase in NFκB and C3 activation exhales amyloidogenic pathology and promotes glial inflammation in addition to that C3 also interacts with microglial C3aR receptor causing alterations in cognitive function and deterioration of Aβ phagocytosis (47).

If astroglia comes into contact with Aβ, it generates astrogliosis that favors molecular and functional changes in astrocytes, elimination of Aβ (which produces the release of extracellular proteases such as neprilysin, angiotensin-converting enzyme-1 (ECA-1) and converting enzyme Endothelin-2 (48).

On the other hand, the Aβ oligomers and fibers can bind microglia surface receptors, such as (CD36), toll-like receptor (TLR)-4 and TLR-6, causing their activation and release of proinflammatory cytokines and chemokines, as well as the tumor necrosis factor alpha (TNFα), interleukin (IL) -1α and IL-1β (40).

Studies in the swAPP model (Tg2576) showed that early microglial decline is related to Aβ deposition and early mortality (40). Aβ can activate microglia to produce cytokines and neurotoxins that promote neurodegeneration, in contrast, microglia express receptors that promote Aβ clearance and phagocytosis.

Hickman *et al.* (2008) and; Krantic *et al.* (2016) Studied the inflammatory response in a young (1 month old) animal model, where they showed that increased levels of TNFα are found in the hippocampus in the transgenic model before Aβ can be detected, but the beta-C fragment of the Terminal fragment (Beta-CTF) already shows an increase (35,40).

Furthermore, retinal glial cells are responsible for the maintenance of the retina microenvironment, trophic and structural support, homeostasis, and immune response. In situations of stress, it is activated, producing changes in pro and anti-inflammatory markers, as well as in phagocytic activity and cell morphology (49).

Another study showed that retinal injection of Aβ induces degeneration of photoreceptor cells in a WT model and that exposure of EPR cells to Aβ *in vivo* induces oxidative stress (50).

The model APPswe/PS1ΔE9 was created by co-injection of two vectors encoding mutant APP and mutant PSEN1, respectively. These mice begin to develop Aβ deposits at six months of age, with abundant plaques in the hippocampus and cortex at nine months, culminating at 12 months. Astrictosis develops along with the Aβ plaques and generates severe gliosis that becomes evident at six months, in addition a modest neuronal loss can be seen, however the NFT are not typical in these animals (51).

In 2009 Pérez *et al*. labelled Aβ plaques with thioflavin-s in APPswe/PS1ΔE9 transgenic mice, this allowed them to observed Aβ aggregates in the retina at the age of 12 months using immunohistochemistry (28). The aggregates showed radial ramifications with a small central nucleus and their size ranged from 5 to 20μm. The largest aggregates were observed at the age of 15 to 16 months of age and the total number of aggregates was 20 times more than those observed at the ages of 12 to 14 months of age (28). The aggregates are located mainly in the inner (34.7%) and outer (41%) plexiform layer of the retina and showed that the deposition of Aβ aggregates begins earlier in females than in males.

Subsequently Koronyo-Hamaoui *et al*. used the same transgenic mice and evidenced the presence of Aβ in the retina of living mice, marked with curcumin that has a high affinity for Aβ *in vivo* in NFL, RGC, IPL, OPL, INL and some aggregates in the sclera (35). In their study they obtained images of the retina, cerebral cortex and hippocampus at ages 2.5, 5, 9 and 17 months of age, showing that the deposition of Aβ aggregates is dependent on age, however at

the age of 2.5 months a significant number of aggregates were detected in the retina but not in the brain, suggesting that the deposition of Aβ aggregates in the retina precedes the brain, where the first aggregate could be visualized from 5 months of age (35).

2.1.4.6.2. Tau Models

As previously mentioned, the hyperphosphorylated Tau protein generates one of the pathognomonic lesions of the disease that, together with Aβ, favors the appearance of AD. Therefore, this protein must be studied and various models have been created to help understand its impact on the pathology and also the connection between Ab pathways.

The hyperphosphorylated Tau protein is affected by genetic factors, aberrant kinase activation, or chronic stress that leads to excessive aggregation and NFT formation. The NFT cause neuronal synapse failure, danger in axonal transport, cytoskeleton dysfunction, among others (52).

In addition to the formation of Aβ in murine models, hyperphosphorylation of the Tau protein increases, which is why researchers have intracranially injected synthetic Aβ and have seen an increase in the Tau protein (52).

rTg4510 mice ("r" for regulatable) are produced by crossing the 4510-responder line, carrying a human MAPTP301L cDNA downstream of a tetracycline operon–responsive element (TRE), to an activator line expressing a tetracycline-controlled transactivator (tTA) under control of the CaMKIIα promoter. This model express high levels of mutant tau, and they develop progressive age-related neurofibrillary tangles, neuronal loss, and behavioral impairments (53).

In rTg4510 mice they demonstrated that the endogenous concentration of Aβ was higher both in the cortex and in the hippocampus, suggesting that the presence of pathogenic Tau may favor the accumulation of Aβ (54).

The Tau P301S (Line PS19) mice develop neuronal loss and brain atrophy by eight months, principally in the hippocampus but spreading to other brain regions, including the neocortex and entorhinal cortex. They develop widespread neurofibrillary tangle-like inclusions in the neocortex, amygdala, hippocampus, brain stem, and spinal cord. Tangle pathology is accompanied by microgliosis and astrocytosis, but not amyloid plaques (53)

Gasparini et al. using transgenic P301S tau homozygous mice showed the expression of the hyperphosphorylated Tau protein in the retina, specifically in NFL and RGC, axons and dystrophic dendrites from one month of age in the homozygous transgenic mouse and from 6 months in the heterozygous model (55)

2.1.4.6.3. Amyloid and Tau models

Mouse and rat transgenic models expression either amyloid plaques and tau tangles were developed.

TgF344-AD rats was generated on a Fischer 344 background by co-injecting rat pronuclei with two human genes driven by the mouse prion promoter: “Swedish” mutant human APP (APPsw) and Δ exon 9 mutant human presenilin-1 (PS1ΔE9) (56,57). This rat express high levels of human APP and show age-dependent increases in levels of detergent-soluble and detergent-insoluble Aβ40 and Aβ42 between 6 and 26 months in different parts of the brain (53).

TgF344-AD rat model, using immunofluorescence, showed a presence of Aβ plaques of variable size in the hippocampus and cerebral cortex, as well as in the internal and external plexiform layer of the 19-month-old, only a few plaques were observed at 14 months of age (57).

A thinning of the choroid was also demonstrated, which can be visualized at 14 months of age but becomes evident at 19 months. Hypertrophy was evident in the RPE, and cells binucleates were more evident in TgF344-AD rats of 14 and 19 months of age (57).

This team also showed the presence of inflammation by increased Iba-1 in the choroid, activation of microglia and presence of complement (C3) along the Bruch membrane (57).

The 3xTg-AD mice contain three mutations associated with familial Alzheimer's disease (APP Swedish, MAPT P301L, and PSEN1 M146V). This model is capable of forming plaque and tangle pathology. Aβ deposition is progressive, with intracellular immunoreactivity detected in some brain regions as early as three to four months of age. Extracellular Aβ deposits appear by six months in the frontal cortex and become more extensive by twelve months and presents some changes in the eyes (53).

The 3xTg-AD mice, extracellular Aβ deposits can be seen from 6 months of age and conformational changes of the Tau protein are evident until 10 and 12 months of age, reinforcing the Aβ hypothesis. Furthermore, this same team showed the conformational changes that the Tau protein has from the first stages of the disease to the advanced phase (Supplementary Figure 7) (21).

Using the same model, other group showed the presence of Aβ and NFT plaques, degeneration of ganglion neurons, astrogliosis and activation of microglia in presymptomatic stages of the disease (49)

The TgTauEC bigenic mice are made by crossing an activator line, neuropsin-tTA, with a responder line, Tg(tauP301L)4510. The neuropsin promoter drives the tetracycline transactivator (tTA) transgene preferentially in a subset of neurons in the entorhinal cortex, these mice develop a stereotyped progression of tau pathology as they age (53)

In rTgTauEC mice the presence of Aβ was found to exacerbate the propagation of transneuronal Tau from the entorhinal cortex to the brain regions interconnected by synapses between these two areas and accelerate the degeneration of human neurons expressing Tau (54).

2.1.4.7. *In vitro* models

Another way to study AD is through cell cultures, which allow the pathophysiological study of the disease as well as the gene expression of the target cells.

For this disease, two types of soft matrix scaffolding have been used, 2D and 3D. Neurons derived from iPSCs have an age of fetal development. While 3D organoids allow the generation of self-organized heterogeneous neuronal tissue, they have the advantage of being a complex tissue but have the disadvantage of the complexity of culture technic (58).

Induced pluripotent stem cells (iPSCs) can differentiate into glutamatergic and pyramidal neurons found in the neocortex and hippocampus, since according to the cholinergic hypothesis, the innervation of pyramidal cells by cholinergic neurons is lost early in AD (59).

Vazin and colleagues investigated Aβ-produced neurotoxicity in glutamatergic cortical and GABAergic neurons. They showed that the intermediate form of Aβ is toxic to culture-dependent glutamatergic neurons and that stable oligomeric Aβ has selective neurotoxicity to glutamatergic neurons (60). In 2016, using the single cell technique, sAPPα and Aβ were studied in depth in individual iPSC neurons and iPSC astrocytes. This team suggests that APP expression and/or processing increases as cells move from an immature mitotic fate to a postmitotic and distinct neuronal state and that astrocytes are capable of secreting large amounts of amyloid peptide and that excitatory glutamatergic neurons they secrete high levels of products from the cleavage of APP therefore they could favor the development of AD (61). These studies show cellular toxicity due to pathological dispersion in AD, loss of synapses, and ion imbalance in response to AD aggregates.

Familial AD has also been studied, using neuronal reprogramming with a genetic predisposition to AD. iPSC neurons derived from patients with familial AD have showed an increase in the Aβ42:40 ratio, as well as hyperphosphorylated Tau (58).

These cultures have also helped to clarify the risk factors for AD, such as Ad mutations in PSEN1, PSEN2 and APP, and genetic variants that favor late-onset AD have been identified,

in addition to Single Nucleotide Polymorphisms (SNP), finding that APOE stimulates the production of APP and Aβ through MAPK signaling and that SLOR1 is a necessary intermediate between signaling of the brain-derived neurotrophic factor (BDNF) and a decreased expression of Aβ (58).

The Tau protein has also been studied in iPSC-derived neurons from fAD cell lines that overexpress or have mutations in APP, finding a link between Tau phosphorylation and Aβ production, but this phosphorylation was not affected in patients with PSEN1 mutated, so it could be suggested that Tau may have an independent role for the Aβ peptide (62).

These types of culture not only represent an advantage for the understanding of the pathology, but for the development and evaluation of new drugs that can help slow down or reverse the pathology.

2.1.5. Diagnosis

The first pillar for proper care of the disease is an accurate and timely diagnosis (5), however, the definitive diagnosis of this pathology is made postmortem (7,63). In the preclinical phase it is very difficult to make the diagnosis. As AD progresses, certain changes can be evidenced such as an increase in CSF Tau or hypermetabolism in the posterior cingulate, however, it is very difficult to make a diagnosis at this stage since the patient has not yet manifested clinical symptoms (64).

For the diagnosis of AD, there are paraclinics such as: biomarkers for Aβ and Tau in CSF, however it is a highly invasive technique (64), whereas structural imaging techniques are fundamental and non-invasive techniques that corroborate the diagnosis and assess the progression of the disease (6). In Magnetic Resonance Imaging (MRI), cortical and hippocampal atrophy can be observed in the temporal and parietal lobes compatible with AD (5,41) or in Positron Emission Tomography (PET) show hypometabolism in the posterior cingulate and the parietal region and in stages delays in the prefrontal and occipital cortex (7), however the American Federation of Drugs (FDA) emphasizes that a positive PET does not confirm the diagnosis, it is only a diagnostic tool (2), in addition to having a high cost, has a limited resolution and cannot be used as a screening test (24).

Single-photon emission computed tomography (SPECT) shows hypoperfusion in the parietal lobes and the posterior part of the temporal lobes, generally the hypoperfusion can be symmetrical but not with the same magnitude and severity (65). However, these abnormalities are also similar in other pathologies.

Therefore, new non-invasive diagnostic tools must be sought that allow an accurate diagnosis to be made in preclinical or early stages of the disease in order to offer efficient treatment or to prevent the presentation of symptoms in this disease that is still irreversible.

2.1.6. Conclusion

The retina is an ideal site for the study of the brain and neurodegenerative diseases such as Alzheimer's disease. Both organs have the same embryological origin and the retina is an accessible organ allowing studies to be non-invasive and inexpensive to understand the brain and its pathologies, allowing the detection of the disease in preclinical stages.

They could also allow an accurate diagnosis of this disease and thereby monitor both the progression of the disease and the utility of the drugs. Furthermore, there is a growing need to identify new therapeutic targets and retinal biomarkers.

The retinal examination together with other ocular tests may be a specific biomarker for this disease, since the first manifestations that patients present are ocular. Therefore, having animal models that emulate Alzheimer's disease facilitates the study of this disease, as is the Tg344 rat model.

Recent advances show that the pathognomonic lesions of the disease in the retina precede brain lesions and can be assessed in vivo, therefore, it is proposed to characterize retinal dysfunctions in two models of AD; mouse APP/PS1dE9 and the rats TgF344-AD by identifying the presence and accumulation of different APP fragments in different compartments of the eye, measuring the morphological changes that the retina presented and measuring the cellular activity in the retina.

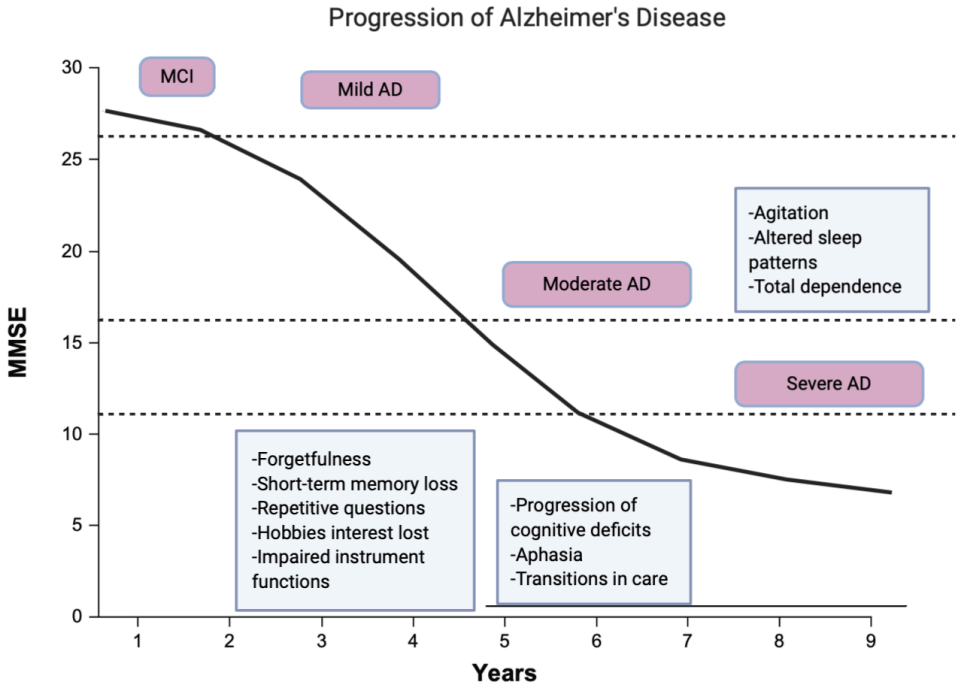
2.2. References

1. Donoso S A. La enfermedad de Alzheimer y otras demencias. 3rd edition. Santiago de Chile: Universitaria, Colección Manuales y monografías; 1998. 19–25 p.
2. Winblad B, Amouyel P, Andrieu S, Ballard C, Brayne C, Brodaty H, et al. Defeating Alzheimer's disease and other dementias: a priority for European science and society. Lancet Neurol. 2016 Apr;15(5):455–532.
3. Alzheimer´s Association. Alzheimer's Disease Fact and figures. Alzheimer Dement; 2019.
4. Iseri PK, Altina?? ??zg??l, Tokay T, Y??ksel N. Relationship between Cognitive Impairment and Retinal Morphological and Visual Functional Abnormalities in Alzheimer Disease: J Neuroophthalmol. 2006 Mar;26(1):18–24.
5. Atri A. The Alzheimer's Disease Clinical Spectrum. Med Clin North Am. 2019 Mar;103(2):263–93.
6. Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2018 Jan;25(1):59–70.
7. Pietrzak K, Czarnecka K, Mikiciuk-Olasik E, Szymanski P. New Perspectives of Alzheimer Disease Diagnosis – the Most Popular and Future Methods. Med Chem [Internet]. 2018 Jan 11 [cited 2020 Jun 4];14(1). Available from: http://www.eurekaselect.com/156039/article
8. Barage SH, Sonawane KD. Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. Neuropeptides. 2015 Aug;52:1–18.
9. Inserm - La science pour la santé. Maladie d'Alzheimer [Internet]. 2019 [cited 2019 Oct 24]. Available from: https://www.inserm.fr/information-en-sante/dossiers-information/alzheimer-maladie
10. Yu J-T, Tan L, Hardy J. Apolipoprotein E in Alzheimer's Disease: An Update. Annu Rev Neurosci. 2014 Jul 8;37(1):79–100.
11. Cita-alzheimer.org. El Alzheimer en números [Internet]. Fundación CITA Alzheimer. 2019 [cited 2019 Oct 23]. Available from: http://www.cita-alzheimer.org/la-enfermedad/el-alzheimer-en-numeros
12. Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2018 Jan;25(1):59–70.
13. Mahley R. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science. 1988 Apr 29;240(4852):622–30.
14. Álvarez-Cisneros T, Torres-Castro S, Mena-Montes B, Torres-Carrillo NM. Género y Salud en cifras. Secretaría de salud. 2017;15(3):4–29.
15. Perl DP. Neuropathology of Alzheimer's Disease. Mt Sinai J Med J Transl Pers Med. 2010 Jan;77(1):32–42.
16. Sociedad Española de neurología. Criterios CIE-10 para el diagnóstico de la demencia [Internet]. Neurología de la Conducta y Demencias. 2019 [cited 2019 Oct 25]. Available from: http://demencias.sen.es/articulos/criterios-para-el-diagnostico-de-la-enfermedad-de-alzheimer-u-otras-demencias/criterios-cie-10-para-el-diagnostico-de-la-demencia/
17. Drachman DA. Human Memory and the Cholinergic System: A Relationship to Aging? Arch Neurol. 1974 Feb 1;30(2):113.
18. Salazar M, Peralta C, Pastor J. Tratado de psicofarmacología bases y aplicación clínica. 2nd ed. Madrid: Editorial Médica Panamericana; 2009. 260–261 p.
19. Davies P. SELECTIVE LOSS OF CENTRAL CHOLINERGIC NEURONS IN ALZHEIMER'S DISEASE. The Lancet. 1976 Dec;308(8000):1403.
20. Mahajan D, Votruba M. Can the retina be used to diagnose and plot the progression of Alzheimer's disease? Acta Ophthalmol (Copenh). 2017 Dec;95(8):768–77.
21. LaFerla FM, Oddo S. Alzheimer's disease: Aβ, tau and synaptic dysfunction. Trends Mol Med. 2005 Apr;11(4):170–6.
22. Roy S, Zhang B, Lee VM-Y, Trojanowski JQ. Axonal transport defects: a common theme in neurodegenerative diseases. Acta Neuropathol (Berl). 2005 Jan;109(1):5–13.
23. Hardy J, Higgins G. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992 Apr 10;256(5054):184–5.
24. Hart NJ, Koronyo Y, Black KL, Koronyo-Hamaoui M. Ocular indicators of Alzheimer's: exploring disease in the retina. Acta Neuropathol (Berl). 2016 Dec;132(6):767–87.
25. Herrup K. The case for rejecting the amyloid cascade hypothesis. Nat Neurosci. 2015

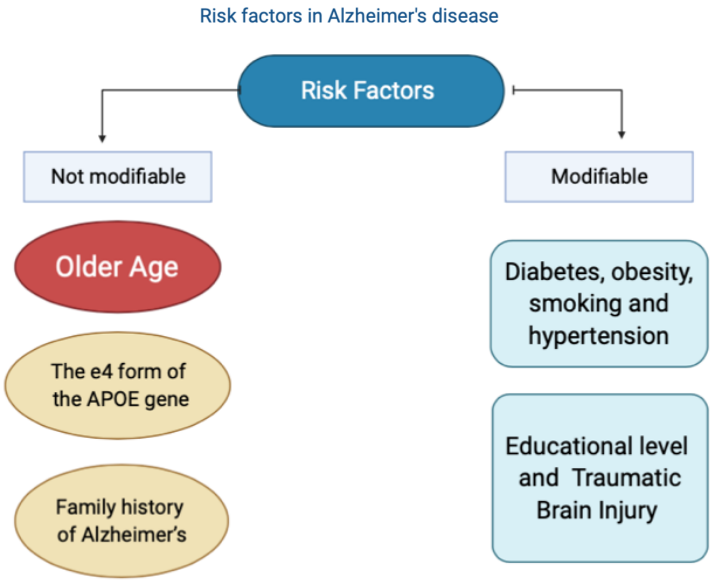
Jun;18(6):794–9.
26. Walsh DM, Selkoe DJ. AB Oligomers-alpha a decade of discovery. J Neurochem. 2007 Jun;101(5):1172–84.
27. Wojciech P. Ross Histología: Texto y atlas. 7th ed. Wolters Kluwer; 2015.
28. Perez SE, Lumayag S, Kovacs B, Mufson EJ, Xu S. β-Amyloid Deposition and Functional Impairment in the Retina of the APPswe/PS1ΔE9 Transgenic Mouse Model of Alzheimer's Disease. Investig Opthalmology Vis Sci. 2009 Feb 1;50(2):793.
29. Salobrar-García E, de Hoz R, Ramírez AI, López-Cuenca I, Rojas P, Vazirani R, et al. Changes in visual function and retinal structure in the progression of Alzheimer's disease. Barnes S, editor. PLOS ONE. 2019 Aug 15;14(8):e0220535.
30. Dehabadi MH, Davis BM, Wong TK, Cordeiro MF. Retinal manifestations of Alzheimer's disease. Neurodegener Dis Manag. 2014 Jun;4(3):241–52.
31. Blanks JC, Schmidt SY, Torigoe Y, Porrello KV, Hinton DR, Blanks RHI. Retinal pathology in Alzheimer's disease. II. Regional neuron loss and glial changes in GCL. Neurobiol Aging. 1996 May;17(3):385–95.
32. La Morgia C, Ross-Cisneros FN, Koronyo Y, Hannibal J, Gallassi R, Cantalupo G, et al. Melanopsin retinal ganglion cell loss in Alzheimer disease: mRGC Loss in AD. Ann Neurol. 2016 Jan;79(1):90–109.
33. Iseri PK, Altina?? ??zg??l, Tokay T, Y??ksel N. Relationship between Cognitive Impairment and Retinal Morphological and Visual Functional Abnormalities in Alzheimer Disease: J Neuroophthalmol. 2006 Mar;26(1):18–24.
34. Armstrong RA. Alzheimer's Disease and the Eye☆. J Optom. 2009;2(3):103–11.
35. Koronyo-Hamaoui M, Koronyo Y, Ljubimov AV, Miller CA, Ko MK, Black KL, et al. Identification of amyloid plaques in retinas from Alzheimer's patients and noninvasive in vivo optical imaging of retinal plaques in a mouse model. NeuroImage. 2011 Jan;54:S204–17.
36. García J, Pablo L. Manual de oftalmología. 1st ed. España: Elselvier; 2012.
37. Esquiva G, Hannibal J. Melanopsin-expressing retinal ganglion cells in aging and disease. Histol Histopathol. 2019 Dec;34(12):1299–311.
38. Frost S, Sohrabi H, Vignarajan J, Bourgeat P, Salvado O, Villemagne V, et al. Retinal vascular biomarkers for early detection and monitoring of Alzheimer's disease. Transl Psychiatry. 2013 Feb;3(2):e233–e233.
39. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol (Berl). 1991 Sep;82(4):239–59.
40. Masuzzo A, Dinet V, Cavanagh C, Mascarelli F, Krantic S. Amyloidosis in Retinal Neurodegenerative Diseases. Front Neurol [Internet]. 2016 Aug 8 [cited 2020 Jun 5];7. Available from: http://journal.frontiersin.org/Article/10.3389/fneur.2016.00127/abstract
41. Krantic S. From Current Diagnostic Tools and Therapeutics for Alzheimer's Disease Towards Earlier Diagnostic Markers and Treatment Targets). Curr Alzheimer Res. 2016 Nov 30;14(1):2–5.
42. Hardy J. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. Science. 2002 Jul 19;297(5580):353–6.
43. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, et al. Triple-Transgenic Model of Alzheimer's Disease with Plaques and Tangles. Neuron. 2003 Jul;39(3):409–21.
44. Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to -amyloid-induced neurotoxicity. Proc Natl Acad Sci. 2002 Apr 30;99(9):6364–9.
45. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, et al. Correlative Memory Deficits, A Elevation, and Amyloid Plaques in Transgenic Mice. Science. 1996 Oct 4;274(5284):99–103.
46. Lian H, Yang L, Cole A, Sun L, Chiang AC-A, Fowler SW, et al. NFκB-Activated Astroglial Release of Complement C3 Compromises Neuronal Morphology and Function Associated with Alzheimer's Disease. Neuron. 2015 Jan;85(1):101–15.
47. Lian H, Litvinchuk A, Chiang AC-A, Aithmitti N, Jankowsky JL, Zheng H. Astrocyte-Microglia Cross Talk through Complement Activation Modulates Amyloid Pathology in Mouse Models of Alzheimer's Disease. J Neurosci. 2016 Jan 13;36(2):577–89.
48. Pihlaja R, Koistinaho J, Kauppinen R, Sandholm J, Tanila H, Koistinaho M. Multiple cellular

and molecular mechanisms Are involved in human Aβ clearance by transplanted adult astrocytes. Glia. 2011 Nov;59(11):1643–57.
49. Grimaldi A, Brighi C, Peruzzi G, Ragozzino D, Bonanni V, Limatola C, et al. Inflammation, neurodegeneration and protein aggregation in the retina as ocular biomarkers for Alzheimer's disease in the 3xTg-AD mouse model. Cell Death Dis. 2018 Jun;9(6):685.
50. Bruban J, Glotin A-L, Dinet V, Chalour N, Sennlaub F, Jonet L, et al. Amyloid-β(1-42) alters structure and function of retinal pigmented epithelial cells. Aging Cell. 2009 Apr;8(2):162–77.
51. Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, et al. Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: evidence for augmentation of a 42-specific γ secretase. Hum Mol Genet. 2004 Jan 15;13(2):159–70.
52. Gao Y, Tan L, Yu J-T, Tan L. Tau in Alzheimer's Disease: Mechanisms and Therapeutic Strategies. Curr Alzheimer Res [Internet]. 2018 Jan 23 [cited 2020 Jun 8];15(3). Available from: http://www.eurekaselect.com/151650/article
53. Alzforum. Research models [Internet]. Alzforum, networking for a cure. 2020. Available from: https://www.alzforum.org
54. Pooler AM, Polydoro M, Maury EA, Nicholls SB, Reddy SM, Wegmann S, et al. Amyloid accelerates tau propagation and toxicity in a model of early Alzheimer's disease. Acta Neuropathol Commun. 2015 Dec;3(1):14.
55. Gasparini L, Anthony Crowther R, Martin KR, Berg N, Coleman M, Goedert M, et al. Tau inclusions in retinal ganglion cells of human P301S tau transgenic mice: Effects on axonal viability. Neurobiol Aging. 2011 Mar;32(3):419–33.
56. Cohen RM, Rezai-Zadeh K, Weitz TM, Rentsendorj A, Gate D, Spivak I, et al. A Transgenic Alzheimer Rat with Plaques, Tau Pathology, Behavioral Impairment, Oligomeric A , and Frank Neuronal Loss. J Neurosci. 2013 Apr 10;33(15):6245–56.
57. Tsai Y, Lu B, Ljubimov AV, Girman S, Ross-Cisneros FN, Sadun AA, et al. Ocular Changes in TgF344-AD Rat Model of Alzheimer's Disease. Investig Opthalmology Vis Sci. 2014 Jan 29;55(1):523.
58. Arber C, Lovejoy C, Wray S. Stem cell models of Alzheimer's disease: progress and challenges. Alzheimers Res Ther. 2017 Dec;9(1):42.
59. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry. 1999 Feb 1;66(2):137–47.
60. Vazin T, Ball KA, Lu H, Park H, Ataeijannati Y, Head-Gordon T, et al. Efficient derivation of cortical glutamatergic neurons from human pluripotent stem cells: A model system to study neurotoxicity in Alzheimer's disease. Neurobiol Dis. 2014 Feb;62:62–72.
61. Liao M-C, Muratore CR, Gierahn TM, Sullivan SE, Srikanth P, De Jager PL, et al. Single-Cell Detection of Secreted Aβ and sAPPα from Human IPSC-Derived Neurons and Astrocytes. J Neurosci. 2016 Feb 3;36(5):1730–46.
62. Rowland HA, Hooper NM, Kellett KAB. Modelling Sporadic Alzheimer's Disease Using Induced Pluripotent Stem Cells. Neurochem Res. 2018 Dec;43(12):2179–98.
63. Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic-Nerve Degeneration in Alzheimer's Disease. N Engl J Med. 1986 Aug 21;315(8):485–7.
64. Hane FT, Robinson M, Lee BY, Bai O, Leonenko Z, Albert MS. Recent Progress in Alzheimer's Disease Research, Part 3: Diagnosis and Treatment. J Alzheimers Dis. 2017 Apr 10;57(3):645–65.
65. Camargo EE. Brain SPECT in Neurology and Psychiatry. J Nucl Med. 2001 Apr 1;42(4):611–23.

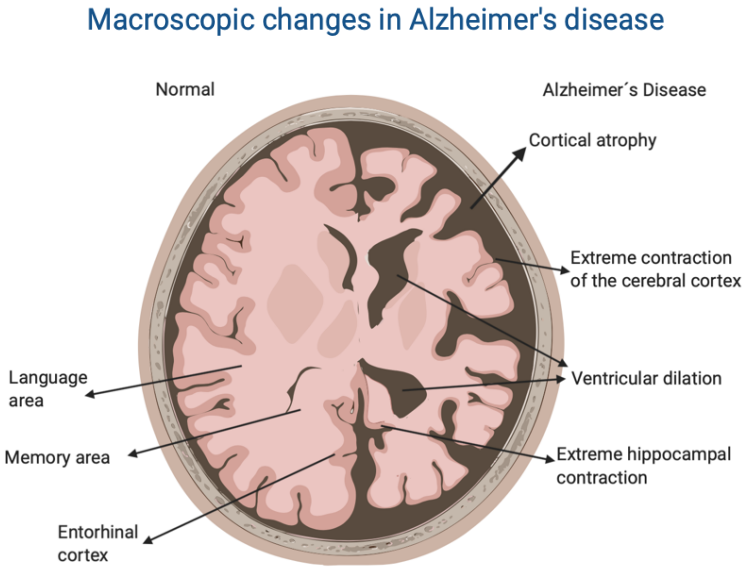
Supplementary Figures



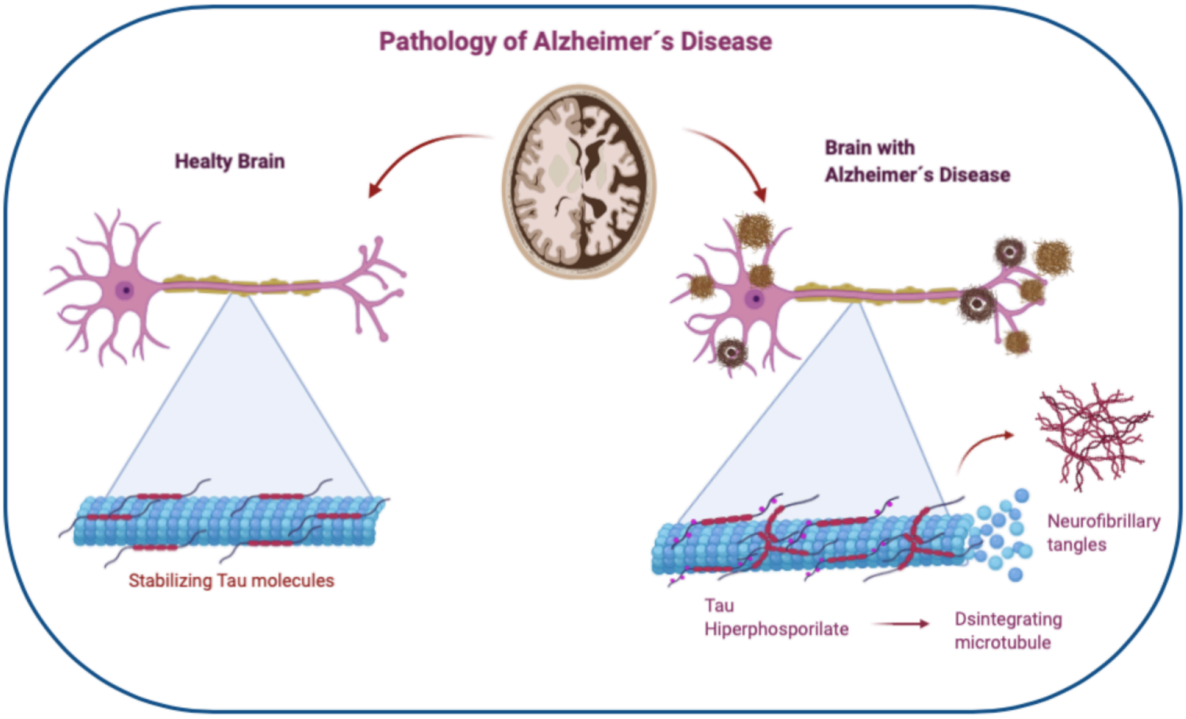
Supplementary Figure 1. Progression of Alzheimer´s disease. It shows the different stages of Alzheimer's disease and its clinical manifestations as well as the correlation with the MMSE. Modified Ferris F 2013



Supplementary Figure 2. Risk factors in AD. Shows the main risk factors associated with Alzheimer´s Disease. By Jimenez P. 2020

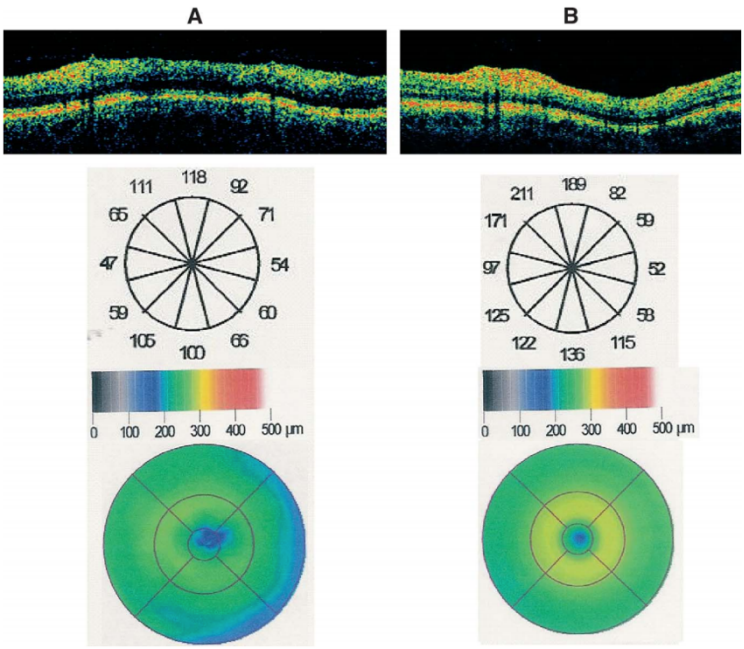


Supplementary Figure 3. Macroscopic changes in AD. Shows the main macroscopic alterations in the brain with AD, which are cortical atrophy, hippocampal and dilatation of lateral ventricles. By. Jimenez P, 2020.



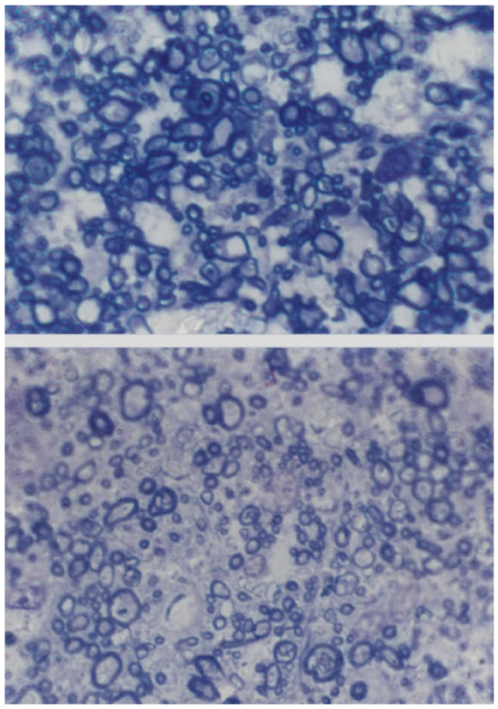
Supplementary Figure 4. Schematic carton of pathology of AD. In the left we can see a macroscopic view of a healthy brain and on the right the brain of a patient with Alzheimer´s Disease is shown schematically, where neuronal tissue loss is observed and at the microscopic level, extracellular neuritic plaques formed by ß-amyloid and intracellular neurofibrillary tangles are observed formed by filaments of the hyperphosphorylated Tau protein. By Jimenez P 2020.

OCT and Alzheimer Disease



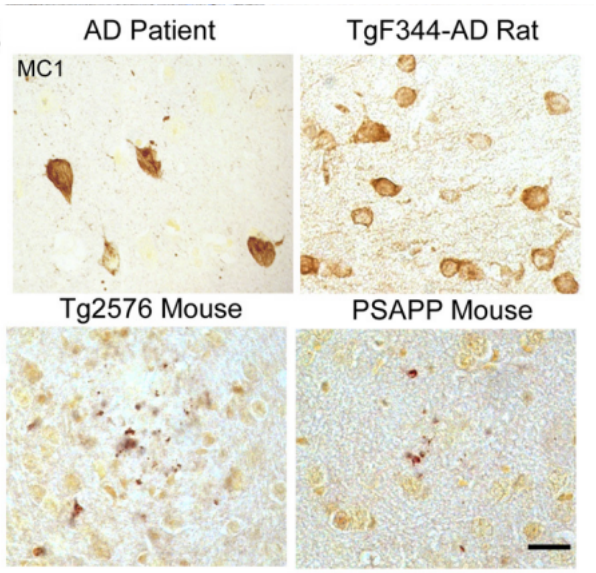
Supplementary Figure 5. OCT and Alzheimer´s disease. The image A show a control patient and the image B is a patient with AD Circular optical coherence tomography (OCT) taken in cylindrical section of tissue surrounding the optic disc shows a marked decrease of the RNFL reflection Bottom: The RNFL thickness in each clock position and the macular thickness in each region are reduced in the AD eye By Iseri P, 2016

Optic Nerve with Alzheimer´s Disease



Supplementary Figure 6. Optic Nerve with AD. Cross-section of part of the optic nerve showing the axon profiles. The upper picture shows axon profile corresponding to an elderly control patient and the lower picture corresponds to a patient with AD. By Armstrong Richard 2009

Animals models and Alzheimer´s Disease



Supplementary Figure 7. Comparison between different animal models. Comparison of tau pathology among AD patients, TgF344-AD rats, Tg2576, and PSAPP mice By Cohen R. 2013.

ABBREVIATION

ACh	Acetylcholine
AD	Alzheimer's Disease
Ad	Autosomal dominant
AICD	Amyloid Intracellular Domain
APP	Amyloid Precursor Protein
BDNF	Brain Derived Neurotrophic Factor
CIE-10	International Classification of Diseases
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
ACE-1	Angiotensin-Converting Enzyme-1
EPR	Retinal Pigment Epithelium
ERG	Electroretinogram
ERO	Reactive Oxygen Species
FDA	Food and Drug Administration
GCL	Ganglion Cell Layer
IPL	Inner Plexiform Layer
iPSCs	Induced Pluripotent Stem Cells
INL	Inner Nuclear Layer
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
mRGC	melanopsin Retinal Ganglion Cell
NIA-AA	National Institute on Aging and Alzheimer's Association
NFL	Retinal Nerve Fiber Layer
NFT	Neurofibrillary Tangles
OPL	Outer Plexiform Layer
OTC	Optical Coherence Tomography
PERG	Pattern Electroretinogram
PDT	Digital Perception Test
PET	Positron Emission Tomography
PHF	Matched Helical Filaments
PS1	Presenilin 1
PS2	Presenilin 2
RGC	Retinal Ganglion Cell
SF	Straight Filaments
SNP	Single Nucleotide Polymorphisms
SPECT	Single Photon Emission Computed Tomography
TBI	Traumatic Brain Injury
VA	Visual Acuity
WT	Wild Type
WHO	World Health Organization