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Review

Protein and Imaging Biomarkers in the Eye for Early Detection of Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is one of the most common causes of dementia worldwide. Although no formal curative therapy exists for the treatment of AD, considerable research has been performed to identify biomarkers for early detection of this disease, and thus improved subsequent management. Given that the eye can be examined and imaged non-invasively with relative ease, it has emerged as an exciting area of research for evidence of biomarkers and to aid in the early diagnosis of AD. This review explores the current understanding of both protein and retinal imaging biomarkers in the eye. Herein, primary findings in the literature regarding AD biomarkers associated with the lens, retina, and other ocular structures are reviewed.

Keywords: Alzheimer's disease, amyloid, cataract, crystalline, eye, lens, retina, vitreous

INTRODUCTION

Alzheimer's disease (AD) is a common neurodegenerative disorder of older adults and a leading global cause of dementia [1, 2]. It is estimated that among patients over age 70, roughly 1 in 10 experience substantial memory loss, with more than half of all cases attributable to AD [1]. The median cost to care for a patient with AD is believed to be in excess of 50,000 United States Dollars (USD) per individual, annually [1]. According to the Alzheimer's Association, government sponsored health care is expected to spend over 200 billion USD caring for those suffering from AD and dementia [3]. AD involves progressive non-reversible neuronal and synaptic degeneration throughout the cerebral cortex. Characteristic microscopic features are accumulation of amyloid plaques and neurofibrillary tangles [4]. Several theories have been proposed to explain the pathophysiology of AD including the amyloid propagation hypothesis [5] as well as the tau hypothesis [6]; however, the exact mechanism of action has not been fully elucidated. Currently, AD is a clinical diagnosis based on symptoms and neuropsychological testing; however, early detection via imaging and measurements of protein biomarkers is an active area of investigation, with the ultimate goal of detecting AD in its preclinical stages in order to maximize the potential for earlier treatment intervention and improved outcomes.

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The toll of this debilitating neurodegenerative disease on patients and their families has prompted exploration of methods for early detection via the use of biomarkers. Researchers have assessed the role that imaging with magnetic resonance (MRI) or positron emission tomography (PET) can play; however, the cost of these imaging modalities limits them as a diagnostic screening tool. Cerebrospinal fluid (CSF) biomarkers can play a role in diagnosing AD at earlier stages but are also limited by cost and their invasive nature. The use of blood to detect biomarkers such as neurofilament light chain (NfL), amyloid-β $(A\beta)_{42/40}$ ratio, and phosphorylated tau (p-tau)181 has the advantage of being relatively inexpensive and minimally invasive as a test, and recent studies show they may be clinically meaningful.

Over the last 20 years, the eye has emerged as a target of efforts geared toward early detection, as the eye is an extension of the central nervous system and offers the opportunity for non-invasive and multi-modal evaluation for early biomarkers of AD. Moreover, eye disease shares risk factors with AD and may therefore represent a vulnerable population deserving of additional screening [7–10]. This article is a review of biomarkers for early AD diagnosis that include promising protein and image-based intraocular targets. The focus on both proteins and biomarkers obtained by noninvasive imaging makes this review unique from prior reviews which have primarily emphasized either one or the other, and by doing so we offer a comprehensive assessment to address the possible applicability of both sources from the eye as distinct and potentially synergistic markers for early AD detection.

BRIEF REVIEW AND CURRENT STATE OF BIOMARKER RESEARCH

The detection of AD in patients through distinct biomarkers is an area of active study. Researchers have explored the role that noninvasive and invasive image-based modalities such as functional, structural, and amyloid PET imaging could play in identifying unique changes to the brain specific to AD [11–13]. Several studies have also analyzed patient serum or CSF samples for clinically meaningful AD biomarkers, often in combination with observed imaging changes [14–17]. It has been shown repeatedly that earlier intervention and therapy can have significant benefits for AD patients, further highlighting the importance and necessity of developing methods to identify biomarkers for early detection of this disease [18, 19].

Currently, some of the most promising and heavily investigated biomarkers for supporting the diagnosis of AD include low CSF levels of $A\beta_{42}$ or $A\beta_{42}$:$A\beta_{40}$ ratio, amyloid PET imaging, various biomarkers of tau deposition, including increased CSF total tau, and tau PET imaging [20]. In 2018, the NIA-AA issued updated guidelines for diagnostic testing to help researchers form a biological, biomarker-based definition of AD, framed within the ATN classification system established by Jack et al. in 2016 [21]. A – signifies changes on amyloid PET imaging and CSF levels of $A\beta_{1–42}$, T - indicates biomarkers of tau, including tau PET or CSF p-tau, while N stands for neurodegeneration as reflected by CSF total tau (t-tau), F-2-fluoro-2-deoxy-d-glucose PET (FDG-PET), and atrophy on magnetic resonance imaging (MRI). Ultimately, the recommendations put forth by the NIA-AA were for research purposes with the eventual goal of refining clinical practice. The authors deliberately did not include specific recommendations but created a general framework for better understanding the sequence of events that leads to AD, assisting with potential treatments and drug therapy [22], and creating a "common language" [23].

To date, there remains no clinical guidelines or recommendations on utilizing biomarker testing as part of routine screening or for early diagnosis of AD prior to symptom onset. Additionally, while the methods utilizing imaging modalities and CSF sampling have shown considerable promise in aiding probable or suspected clinical diagnosis, these tests are expensive, may involve radiation exposure, and are often invasive. The identification of cheaper, minimally-invasive methods for detecting both known biomarkers and new biomarkers in other organs and fluids remains a goal for researchers in the field.

THE HUMAN CRYSTALLINE LENS

The optically clear crystalline lens is located in the anterior segment of the eye, posterior to the iris [24]. Via accommodation, the lens, in tandem with the cornea, refract light onto the retina beginning the process of visual perception. The lens fibers are long, normally transparent cells that are known to accumulate misfolded protein aggregates over time [25–27]. The development of cataract is a common clinical condition that occurs with increasing age and can lead to vision loss over time. The removal of the lens

from cataract formation is the most common surgical procedure in the US, and each year approximately 3 million people in the US undergo cataract surgery.

In 2003, Goldstein et al. were among the first to demonstrate amyloid-β protein precursor (AβPP) and Aβ deposition was detectable in cadaveric human lens samples of patients with diagnosed AD. They reported, that via immunohistochemistry (using monoclonal antibodies against Aβ, specifically 4G8/βA4) and confirmed through tryptic digest, tandem liquid chromatography/mass spectrometry, that Aβ deposition could be identified at the level of the lens. The authors also reported a characteristic and distinct cortical cataract opacification in known AD patients, and additionally concluded that amyloid deposition was identifiable in the human lens as in the brain and that the molecular machinery necessary for the accumulation of Aβ was present in the lens itself, and not derived from the brain [28].

In 2010, Moncaster et al. expanded on their initial findings to analyze the lenses of patients suffering from trisomy 21, Down syndrome (DS) [29]. Chromosome 21 contains the gene encoding for AβPP [30], predisposing DS patients to accumulate amyloid deposition within the brain, developing dementia in a process much akin to AD [31]. Those suffering from this condition typically experience symptoms and onset of dementia and AD at an earlier age, compared to healthy patients [32]. Moncaster et al. utilized peptide sequencing, immunoblot analyses, and ELISA to demonstrate accumulation of Aβ amyloid aggregates within the lens. Additionally, the authors indicated that staining and slit lamp examination showed the same characteristic supranuclear opacifications previously described [29].

A subsequent study in 2012, by the same group of authors, aimed to identify genetic factors that linked the formation of age-related cataracts to AD. Jun et al. examined lens opacity imaging from 1,249 patients from the Framingham Eye Offspring Study and genome wide association studies and identified a statistically significant correlation of cortical cataracts with AD-related brain changes and suggested that genetic variations in Catenin Delta 2 gene (gene associated with both brain and eye development) may represent a link in cortical cataract formation and AD associated changes to the brain, identified by MRI [33].

A 2013 study by Kerbage et al. explored the use of a combination topical compound that binds to aggregated Aβ peptide and laser scanning device, called SAPPHIRE system, to examine *in vivo* human lenses and found a 2-fold increase in fluorescent signature in the thickest supranuclear region in their AD patients compared to controls [34]. The authors conducted a similar study in 2015, showing a statistically significant difference in fluorescent signature via the use of the fluorescent ligand eye scanning techniques and demonstrated that lens findings significantly correlated with florbetapir F18 PET amyloid brain analysis and imaging findings [35].

Given the safety, relative ease, and multitude of non-invasive techniques to examine the lens, the Goldstein, Moncaster, Jun, and Kerbage studies have sparked considerable interest from the scientific community to delve further into identifying neuropathological changes associated with AD in the lens. However, subsequent studies exploring their findings presented inconsistent results.

A study published in 2013 in *Experimental Eye Research*, by authors Michael et al., was unable to replicate the findings from Goldstein and Moncaster. The authors, analyzing the brains and lenses of 21 patients with AD and 15 without, could not successfully identify Aβ deposition in any of the cadaveric lenses from their study [36] nor the characteristic supranuclear opacification among their donor samples. The authors performed a follow up study in 2014, utilizing confocal Raman micro-spectroscopy and were unable to reveal the presence of Aβ in the cataracts of known AD patients [37]. In 2014, authors Ho et al. were unable to detect any Aβ deposition in the crystalline lens or retina of eleven neuropathologically diagnosed AD patients, four DS patients, and six age-matched controls [38]. In a 2017 study published in the *Journal of Neuropathology and Experimental Neurology*, Williams et al. were unable to identify evidence of deposits in any part of the globe after immunostaining (monoclonal antibody 6F/3D) [39].

Both the Ho article from 2014 and the Williams article from 2017 examined postmortem eyes of known AD patients for evidence of inclusions, or proteinaceous deposits of other known AD biomarkers such as tau. Neither study was able to demonstrate deposits of tau at any level or compartment in the eye as well. It should also be noted that the original articles from Goldstein and Moncaster asserted evidence of Aβ deposition at the lens and did not directly address deposition of other aggregates such as tau that are historically linked to AD in the brain [6, 40–42] (See Table 1 for summary of articles).

It is unclear why recent studies have been unable to replicate the findings of Goldstein and Moncaster. Some of the proposed theories include discrepancies

Table 1
Studies looking for evidence of Aβ in the lens

Title	Year, Authors [Ref]	Significant Finding
Cytosolic β-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer's disease.	2003, Goldstein et al. [28]	Identified Aβ in the supranuclear cortical lens fibers cells of pathology confirmed AD patients. Identified supranuclear cataracts in lenses of AD patients compared to controls.
Alzheimer's disease amyloid-β links lens and brain pathology in Down syndrome.	2010, Moncaster et al. [29]	Evaluation of DS lenses showed supranuclear opacification and supranuclear Aβ accumulation identical to the lens pathology identified in AD. Peptide sequencing, immunoblot analysis, and ELISA confirmed the identity and increased accumulation of Aβ in DS lenses.
δ-Catenin is genetically and biologically associated with cortical cataract and future Alzheimer-related structural and functional brain changes	2012, Jun et al. [33]	Genetic variation in Catenin Delta 2 identified as possible shared link between cortical cataract formation and MRI identified associated changes to the brain, in AD.
Alzheimer's disease diagnosis by detecting exogenous fluorescent signal of ligand bound to Beta amyloid in the lens of human eye: an exploratory study	2013, Kerbage et al. [34]	Fluorescent ligand and a laser scanning device, SAPPHIRE System, able to detect twofold difference in fluorescence signature in ligand bound Aβ in the supranuclear region of the lenses of AD compared to control patients.
Detection of amyloid β signature in the lens and its correlation in the brain to aid in the diagnosis of Alzheimer's disease	2015, Kerbage et al. [35]	Fluorescent Ligand Eye Scanning (FLES) technique measures significant difference in fluorescence signature from probable AD patient lenses compared to healthy controls and correlates with F18 amyloid brain imaging use F18.
Absence of beta-amyloid in cortical cataracts of donors with and without Alzheimer's disease	2013, Michael et al. [36]	Neither clinically diagnosed AD or control patient with cortical cataracts stain for Aβ with Congo red, thioflavin and Aβ immunohistochemistry staining. Authors conclude an absence of Aβ in AD lens or control lens with cortical cataracts.
Absence of amyloid-beta in lenses of Alzheimer patients: a confocal Raman microspectroscopic study	2014, Michael et al. [37]	Utilized confocal Raman microscopy to demonstrate absence of Aβ in lens and ultimately claim cortical lens opacification is not a hallmark of Aβ accumulation in the lenses of AD patients.
Beta-amyloid, phospho-tau and alpha-synuclein deposits similar to those in the brain are not identified in the eyes of Alzheimer's and Parkinson's disease patients	2014, Ho et al. [38]	No evidence of amyloid deposits detectable in the lens, retina, or other structures in the eyes of AD patients. Authors conclude Aβ either does not deposit in the eye like the brain or is present at lower levels or different forms.
Absence of Alzheimer disease neuropathologic changes in eyes of subjects with Alzheimer disease	2017, Williams et al. [39]	Immunohistochemistry for Aβ fails to demonstrate evidence of inclusions, deposits in any part of the globe in AD patients.

in staining technique, use of monoclonal antibodies with differing epitopes, as well as fixation modality [36, 43]. The Michael study acknowledges that their cadaveric samples were from patients with a clinical diagnosis of AD as opposed to the pathological confirmation used in the Goldstein and Moncaster studies [36]. This difference may explain the discrepancy in findings between Goldstein/Moncaster and Michael studies, but fails to apply to the Ho study, as their cadaveric samples had confirmed pathological diagnosis of AD [38]. Additionally, while the Ho, Michael, and Williams studies were unable to demonstrate clear evidence of Aβ in the lens, there are technical and procedural limitations to consider when evaluating their conclusions. One is the technical challenges of fixing, processing, and staining lens material compared to brain tissue. Secondly, the Ho, Michael, and Williams studies do not utilize a secondary analytical method, such as tandem liquid chromatography-mass spectrometry (LC-MS) to confirm their findings of an absence of Aβ in the analyzed tissues. Contrast this to Goldstein, Moncaster, and Jun who utilized tryptic digest sequencing with electrospray ionization LC-tandem mass

spectrometry to confirm the identity of Aβ in their lens samples. The lack of a specific secondary analytic technique to confirm findings weakens the challenges put forth by the authors in the Ho, Michael, and Williams studies. It should also be noted that confocal Raman microscopy has relatively poor sensitivity as well as low signal specificity when attempting to detect low levels of Aβ, as would be expected in the lens [44]. Alternately, LC-MS is specific in identifying a unique tryptic peptide sequence that corresponded to an internal peptide in AβPP, and sensitive in detecting levels of Aβ at levels several orders of magnitude less than that detectable by Raman microscopy. While Michael et al. attempted to confirm their findings with Raman microscopy, a negative finding utilizing this technique is not definitive evidence of a total absence of Aβ. The use of LC-MS in the Goldstein, Moncaster, and Jun studies confirms the presence of Aβ at the lens, and authors Michael, Ho, and Williams do not invalidate this finding. The question that now that remains is, are levels of Aβ in the lens ultimately associated with and potentially predictive of AD?

INTRAOCULAR FLUID

While examination of the anterior segment of the eye (cornea, anterior chamber, lens, ciliary body) has been of interest to researchers looking for early signs of AD, there also have been several studies that have explored the role of eye fluids (aqueous and vitreous humor) and posterior segment (retina and choroid) [45] to determine whether changes might predict the onset of AD. The aqueous humor is a transparent fluid composed primarily of water, amino acids, and electrolytes which fills both the anterior and posterior chambers of the anterior segment of the eye. It serves as a source of micronutrients and immunologic materials for avascular structures such as the cornea and provides intraocular pressure [24]. Studies have looked into sampling the aqueous for evidence of deposition of Aβ [46]. Indeed, authors Goldstein et al. were among the first to demonstrate that beta amyloid, specifically $A\beta_{1–40}$ and $A\beta_{1–42,}$ could not only be detected and measured in the aqueous humor, but that levels of such compounds were comparable to that of CSF samples [28].

The vitreous chamber accounts for 80% of the total volume of the globe [24] and is comprised of an optically clear non-reproducible gelatinous substance composed primarily of water, type II collage, hyaluronan, and glycosyaminoglycans, as well as other proteins. The vitreous serves an important role in supporting the posterior lens and providing shape and structure to the posterior segment of the eye [24]. Several studies have identified AD-associated pathological proteins in both the aqueous and the vitreous humor [46–52]. In 2019 our group published a study that was among the first to find an association of those levels with human cognitive function. Wright et al. sampled the vitreous humor of patients following vitrectomy surgery for eye disease, and found that higher levels of $A\beta_{40}$, $A\beta_{42}$, and total tau in the vitreous were associated with lower cognitive function, measured by Mini-Mental Status Examination (MMSE) [53]. Notably, these associations were preserved when adjusting for the patients' local eye disease. The findings for total tau were consistent with findings from CSF studies where high CSF levels of tau corresponded with worse cognitive function. On the other hand, Aβ showed an opposite relationship to cognitive function than that observed in the CSF, suggesting that Aβ accumulation in the vitreous follows a different dynamic than that in the CSF where it is inversely related to brain amyloid deposition. Our group subsequently identified NfL in the vitreous humor and reported a positive correlation between NfL levels and $A\beta_{40}$, $A\beta_{42}$, and total tau in 77 vitreous samples [54] obtained during surgery from patients with eye disease. The NfL levels noted in the vitreous was adjusted for systemic diseases such as diabetes and was not associated with the patients' clinical eye condition nor *APOE* genotype. These cumulative findings in the vitreous humor reaffirm other studies suggesting that patients with eye disease are an at-risk population for the development of AD and suggest that further investigation in patients with eye disease may yield results potentially generalizable to the population at large (Table 2).

RETINA

The neurosensory retina has been a particular tissue of interest for the detection of amyloid plaque deposition. AD and brain Aβ burden has been shown to be associated with Aβ deposits in the retina in animal models [54–59] and in human studies [60, 61]. Several studies have used curcumin, a fluorochrome, to label the presence of Aβ plaques at the retina [62, 63]. Hamaoui et al. reported Aβ in postmortem donor retinal tissue from AD patients and speculated that retinal Aβ plaques accumulated and progressed at

Table 2
Studies exploring presence of AD associated biomarkers in the vitreous humor

Title	Year, Authors [Ref]	Significant Finding
Association of cognitive function with amyloid-β and tau proteins in the vitreous humor	2019, Wright et al. [53]	Poor cognitive function, as determined by Mini-Mental State Exam, correlated with lower levels of vitreous $A\beta_{40}$, $A\beta_{42}$, and tTau. Vitreous biomarkers not associated with any underlying ophthalmic condition.
Neurofilament light chain in the vitreous humor of the eye	2020, Subramanian et al. [54]	Neurofilament light chain identified in vitreous samples, and significantly correlated with levels of $A\beta_{40}$, $A\beta_{42}$, and t-tau. NfL not found to be associated with any underlying ophthalmic condition.

the level of the retina before the brain [64]. A 2017 study by Koronyo et al. demonstrated histological evidence of neuronal loss and deposition of Aβ (via the use 12F4 mAb which targets the C terminus of $A\beta_{42}$) plaques within the inner layers of the retina of AD patients; this study was important because it was also able to noninvasively image Aβ plaques at the retina, via the use of curcumin staining and retinal imaging in live patients [63]. It should be noted, however, the curcumin binding and fluorescence is not specific for Aβ and not definitive evidence of retinal Aβ. Curcumin has been described as binding to dense amyloid structures and not specifically to Aβ peptide [65]. Additionally, several studies previously mentioned in this review have been unable to detect evidence of retinal amyloid deposition via immunostaining [38, 39]. Although these discrepancies may be related to staining technique or methodology, the limited and occasionally confounding data preclude any consensus conclusions on the topic [66].

Histopathological analyses from the late 1980s and early 1990s of cadaveric ocular tissue from AD patients revealed evidence of retinal ganglion cell loss [67, 68]. Similar methodology has been used to identify evidence of marked thinning of the retinal nerve fiber layer (rNFL), the innermost layer of the retina and an unmyelinated extension of the fibers of the optic nerve, in AD patients compared to controls [69, 70] (Table 3).

While the presence and associations of pathological proteins in eye fluid and retinal tissue is of interest, obtaining a biopsy of vitreous fluid and retina as a source of screening for AD is not practical because it requires an invasive needle injection or surgical intervention in the operating room. However, there is opportunity to access aqueous humor and lens material in the operative setting for patients undergoing cataract surgery, as it provides us with a mechanism to access eye fluid and tissue for possible screening purposes. Since this is a common procedure, the investigation of pathological proteins for AD in the eye may be more compelling than lumbar puncture for CSF procurement. Additionally, patients with cataracts and other eye diseases that require surgery are at higher risk for AD [7–10], therefore, selective screening of the eye during surgery may allow for early detection and follow up in this at-risk group.

NONINVASIVE IMAGING OF THE RETINA

More recently, current studies investigating the role of the eye in AD focus on retinal biomarkers imaged by optical coherence tomography (OCT). OCT is a noninvasive photographic test using light waves to take cross sectional images of the retina and macula, providing information on the retina's distinctive cell layers. For example, the rNFL is thinner, retinal volume is reduced, and the choroidal thickness of the eye is reduced in patients with mild cognitive impairment (MCI) and AD compared to cognitively normal (CN) controls [71–81]. A meta-analysis from 2017 by den Haan et al. looked at 25 studies involving 887 patients with AD, 216 with MCI, and 864 CN and demonstrated a statistically significant reduction in mean peripapillary rNFL layer thickness and macular thickness in patients with MCI and AD [82].

A 2017 study from the Netherlands compared retinal sublayer thickness, as determined by OCT, to brain MRI of 2124 patients. Mutlu et al. discovered that reduced gray and white matter volume was correlated with thinning of the inner retinal sublayers including the rNFL, the ganglion cell layer, and inner plexiform layer [83]. A similar study by Casaletto et al. used OCT and 3T Brain MRI to compare retinal sublayer thickness with medial temporal lobe volume (MTL) and like Mutlu found that reduction in MTL volume (which they reported as a hallmark of AD) was correlated with thinning of the rNFL as well as total macular and macular ganglion cell layer [84].

Table 3
Studies looking at evidence of retinal changes/retinal pathology associated with Alzheimer's disease

Title	Year, Authors [Ref]	Significant Finding
Retinal levels of amyloid beta correlate with cerebral levels of amyloid beta in young APPswe/PS1dE9 transgenic mice before onset of Alzheimer's disease	2020, Mei et al. [62]	Correlation between Aβ content in retina and cerebrum of APP mice. Curcumin can stain Aβ in the retina but found to suppress levels.
Retinal amyloid pathology and proof-of-concept imaging trial in Alzheimer's disease	2017, Koronyo et al. [63]	Aβ deposits found at the level of the retina using curcumin fluorochrome. Retinal Aβ could be imaged *in vivo* with solid-lipid curcumin and a modified scanning laser ophthalmoscope
Identification of amyloid plaques in retinas from Alzheimer's patients and noninvasive *in vivo* optical imaging of retinal plaques in a mouse model	2011, Koronyo-Hamaoui et al. [64]	Retinal Aβ plaques identified following systemic administration of curcumin in postmortem eyes of AD patients. Retinal plaques detectable earlier than in the brain and accumulated with disease progression.
Amyloid plaques in retina for diagnosis in Alzheimer's patients: a meta-analysis	2016, Jiang et al. [66]	Meta-analysis of 5 studies with small sample sizes fails to identify any conclusion in regard to pathological retinal Aβ detection as a diagnostic tool for AD.
Retinal thickness in Alzheimer's disease: A systematic review and meta-analysis	2017, den Haan et al. [75]	Significant reduction in mean peripapillary retinal nerve fiber layer thickness and macular thickness as identified by OCT, in patients with mild cognitive impairment and AD versus controls.
Retinal neurodegeneration and brain MRI markers: the Rotterdam Study	2017, Mutlu et al. [83]	Thinner RNFL, GCL, and inner plexiform layer identified by OCT, associated with MR identified smaller gray-matter and white-matter volume.
Retinal thinning is uniquely associated with medial temporal lobe atrophy in neurologically normal older adults	2017, Casaletto et al. [84]	Retinal nerve fiber thinning, reduced total macular and macular ganglion cell volumes identified via OCT, found to be associated with smaller MTL volumes
Decreased retinal thickness in patients with Alzheimer's disease is correlated with disease severity	2019, Kim et al. [85]	Both patients with severe AD and mild to moderate AD found to have evidence of significant rNFL thinning via OCT, compared to age matched controls.
Association of retinal nerve fiber layer thinning with current and future cognitive decline: a study using optical coherence tomography	2018, Ko et al. [86]	Evidence of thinned rNFL, associated with worse cognitive function in patients without neurodegenerative disease, and associated with increased likelihood of future cognitive decline.
The retinal vessel density can reflect cognitive function in patients with Alzheimer's disease: evidence from optical coherence tomography angiography	2021, Yan et al. [101]	rNFL and retinal vessel density significantly reduced in AD patients compared to controls. Retinal vessel density reduction associated with impairment in some cognitive function domains.
Retinal microvasculature dysfunction is associated with Alzheimer's disease and mild cognitive impairment	2020, Chua et al. [102]	Patients with AD found to have significant reductions in vessel density in superficial and deep capillary plexus compared to controls.
Retinal microvascular attenuation in mental cognitive impairment and Alzheimer's disease by optical coherence tomography angiography	2020, Wu et al. [103]	AD patients showed evidence of significant reductions in microvascular densities of the Deep retinal capillary plexus compared to matched controls, via OCT-A.
Retinal nerve fiber layer thinning in Alzheimer's disease: a case-control study in comparison to normal aging, Parkinson's disease, and non-Alzheimer's dementia	2016, Pillai et al. [105]	No significant difference in rNFL, GCL or macular volume on OCT in AD dementia versus healthy controls.
Usefulness of peripapillary nerve fiber layer thickness assessed by optical coherence tomography as a biomarker for Alzheimer's disease	2018, Sánchez et al. [106]	No significant difference in mean peripapillary rNFL thickness in AD or mild cognitively impaired patients compared to cognitively healthy patients.
Peripapillary retinal nerve fiber layer thickness in patients with Alzheimer's disease: a comparison of eyes of patients with Alzheimer's disease, primary open-angle glaucoma, and preperimetric glaucoma and healthy controls	2019, Zabel et al. [107]	While AD patients demonstrated significant decrease in rNFL thickness compared to healthy controls, there was no significant difference when compared to preperimetric glaucoma. Authors conclude changes observed on SD-OCT are non-specific.

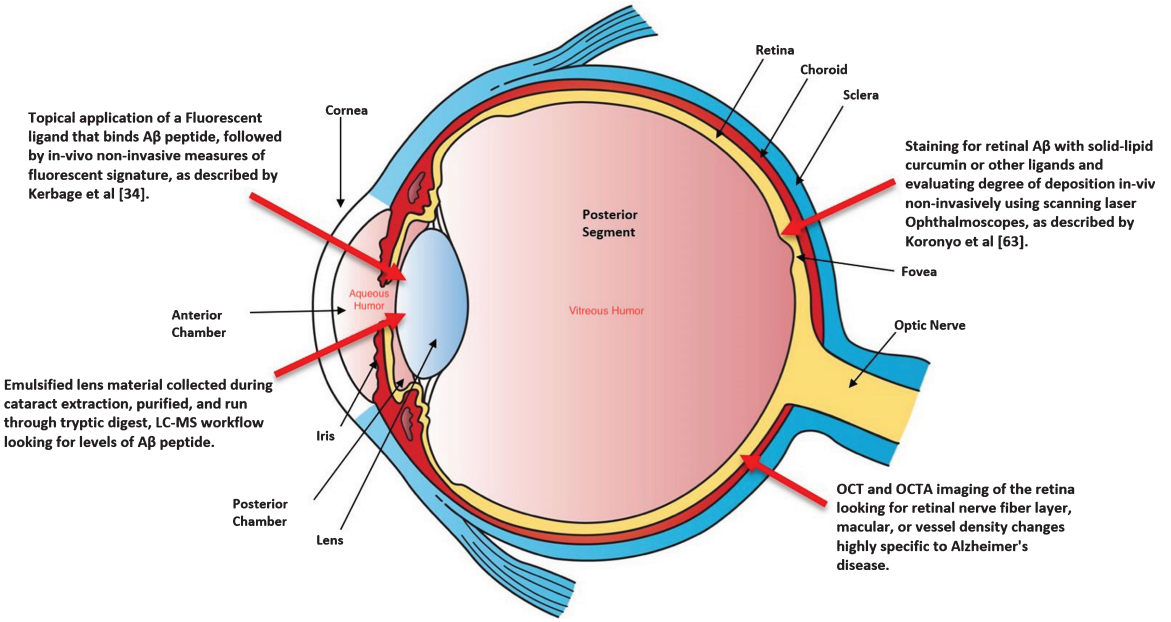

Fig. 1. Invasive and non-invasive theoretical approaches researchers have proposed for examining the eye, at all levels including the lens and retina, to look for changes specific to Alzheimer's disease [108]. This is original artwork by Holly Fischer. The authors have permission to use the figure per licensing requirements mentioned on Wikimedia common. Link to the license: (https://creativecommons.org/licenses/by/3.0/legalcode). The artwork was modified to include labels for major eye structures, and text boxes which feature theoretical descriptions of eye examination techniques to look for evidence of Alzheimer's disease.

A 2019 study by Kim et al. reports a similar reduction in thickness of the inner retinal layers in patients with severe AD compared to controls [85].

From a functional standpoint, a large prospective multicenter study based in the UK enrolled over 32,000 participants and performed baseline OCT imaging as well as a series of four cognitive tests on participants. Researchers discovered that those with rNFL thickness in the bottom 40% were nearly twice as likely to perform worse on one of the four cognitive tests, when compared to participants with relatively thicker rNFL, indicating that rNFL thickness could be used as a potential biomarker or early indicator of dementia and neurodegenerative disease [86].

In addition to retinal thinning and retinal sublayer changes, researchers have also explored potential changes in retinal vasculature in AD patients [87]. One of the earliest studies utilized laser doppler to show evidence of narrowing of venous blood column diameter and venous blood flow rate in AD patients compared to controls [88]. Given the relative complexity of the retinal vasculature, however, more precise imaging modalities and instrumentation were needed to highlight specific vascular changes within the retina of AD patients. More recent studies have thus implemented the use of OCT angiography (OCTA) to better discern the exact specific vascular changes occurring at the retina in AD patients [89–96]. OCTA is a relatively new technology that provides information of the retino-choroidal microvasculature in a non-invasive manner. It is an additional software enhancement to the commercially available OCT and provides supplementary vascular information similar to what can be imaged with an angiogram, but without the use of an invasive intravenous dye. It can capture images and quantify both the superficial vascular plexus (SVP) and deep vascular plexus (DVP) of the retinal microvasculature. Several studies have highlighted impairments of flow, as measured by either flow density or vessel density, of the SVP, a network of vessels that branch off of the central retinal artery [97] and supply the innermost layers of the retina, such as the rNFL and inner ganglion cell layer, in AD and cognitively impaired patients [95, 98–100]. A recent study by Yan et al. compared 37 patients with AD to 29 age matched controls and found a positive correlation between retinal vascular density and reduced rNFL thickness, measured by OCTA, in patients with mild AD [101]. Another study by Chua et al. found significantly reduced macular vessel density in both the SVP and DVP in patients with AD, while those with MCI

showed reduction in the SVP only [102]. Another study by Wu et al. showed similar results in the DVP in AD patients but a different result in MCI patients, which affected the DVP more than the SVP, from the Chua study [103].

While initial data shows promise for OCT imaging of the retina as a biomarker for early detection, there are several challenges to consider [104]. More recent studies utilizing higher resolution OCT, namely spectral domain OCT have not been able to consistently identify the same changes, or a significant difference in mean retinal thickness between AD and control patients as described earlier [105, 106]. Moreover, some studies have reported that a distinct retinal change unique to AD has not been identified, making it hard to discern AD related retinal thinning from other eye disease such as glaucoma and diabetic retinopathy [107]. Additionally, while several large studies and meta-analyses have supported the utility of measuring retinal cell layer thickness by OCT, OCTA is still a relatively new technology and individual studies investigating its role in MCI and AD have thus far been done in relatively small groups of patients, indicating that the use of OCTA as a tool to measure retinal vascular changes in those with MCI and AD is still in its early stages of investigation. Additionally, OCTA is now commercially available but not universally deployed in all practice settings primarily due to its cost and general uncertainty by clinicians if the advancement of this technology is a significant or incremental improvement in the diagnosis and management of eye disease over OCT alone, which is available universally in almost all eye physician and optometry offices. Lastly, measurements obtained by OCT and OCTA, because they are photographic tests, are unlikely to confirm AD pathology and at best can be used as a screening tool requiring further diagnostic confirmation. In short, larger, more extensive longitudinal studies are needed to identify more precise, specific patterns of retinal vascular changes, retinal thinning, and rNFL and GCL thinning changes during the course of dementia and AD, before any of these observed structural changes could be considered as a possible novel biomarker of AD.

CONCLUSION

The goal of uncovering biomarkers in the eye that may be used for early or preclinical detection of AD is possible but remains elusive. Research on the eye fluid and lens and other ocular structures to detect early signs of AD continues to be promising, particularly because it is accessible during cataract surgery, and while some early data has emerged with regard to Aβ deposition in the lens and retina that are potentially unique to AD, there is some confounding data and disagreement. Similarly, noninvasive imaging of the retina with OCT has shown promise and disagreement about distinct, identifiable AD-related changes in the retina. The more recent discovery of Aβ, tau, and NfL in eye fluids with cognitive association is compelling but more studies are needed to replicate these findings and clarify what role they may play in early AD detection. A future consideration may be a combined approach utilizing the eye that includes noninvasive imaging of the retina as a more sensitive marker, and the detection of proteins Aβ, tau, and NfL in eye fluid, lens, or retina as a more specific marker, which could resemble the combination of imaging by MRI/PET scanning and detecting proteins in CSF via lumbar puncture. What is clear is that considerably more research is needed in these particular areas of exploration. As examination and imaging techniques improve it is conceivable that larger meta-analyses and longitudinal studies exploring how the lens, retina or other parts of the eye change uniquely with dementia and AD, could identify key patterns or findings of biomarkers for early AD diagnosis.

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CONFLICT OF INTEREST

The authors do not have any conflicts of interest to report.

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